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The sagebrush steppe ecosystem of the northern Great Basin is severely degraded and continues to decline due in large part to the invasive, non-native annual grasses *Bromus tectorum* L. (cheatgrass) and *Taeniatherum caput-medusae* (L.) Nevski (medusahead). Restoration of invasive-dominated areas is difficult, but can be enhanced by adding a carbon source, which stimulates microbes to immobilize soil inorganic N and reduces yields of fast-growing ruderal plants. How much carbon is needed to induce this effect is uncertain, so our research objectives were to establish a response to increasing carbon doses and calculate the lowest dose where a significant response was observed for 1) biomass, density, and seed production of cheatgrass and medusahead; 2) soil microbial biomass C and N; and 3) inorganic soil N. In November 2005 we applied 12 carbon doses ranging from 0 to 2400 kg C/ha as sucrose to plots planted with cheatgrass and medusahead at two sites in the northern Great Basin. We measured aboveground plant biomass when plants matured in June and July 2006 and microbial biomass in November 2005, March 2006, and July 2006. Inorganic soil N was measured using mixed-bed resin capsules placed in situ for the duration of the study. For an increase in carbon dose of 100 kg C/ha, back-transformed mean medusahead biomass (g/m^2) at one site decreased 6.8%. This was the only significant response to carbon doses for both target species across both sites. In addition to cheatgrass and medusahead, other ruderal plants established in our plots, and the biomass of this entire ruderal community decreased approximately 6% for an increase in carbon dose of 100

kg C/ha at both sites. Microbial biomass C increased 2–4 mg/kg for an increase in carbon of 100 kg C/ha at the two earliest sampling dates at both sites, while microbial biomass N increased at only one site at the earliest sampling date, and this increase was 0.3 mg/kg for an increase in carbon of 100 kg C/ha. Soil NO_3^--N decreased at both sites with increasing carbon. For a significant reduction in ruderal biomass, we calculated lowest significant doses of 240-640 kg C/ha, but it remains to be determined if this reduction is sufficient to facilitate establishment of native perennials in northern Great Basin restoration projects.

Yield Responses of Invasive Grasses to Carbon Doses

by

Jessi L. Brunson

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jessi L. Brunson, Author

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Yield Responses of Invasive Grasses to Carbon Doses

INTRODUCTION

The sagebrush steppe ecosystem of the northern Great Basin is severely degraded and continues to decline due in large part to the invasive, non-native annual grasses *Bromus tectorum* L. (cheatgrass) and *Taeniatherum caput-medusae* (L.) Nevski (medusahead). These cool-season (C3 photosynthetic pathway) Eurasian grasses were introduced to the Intermountain West over a century ago, and decades of uncontrolled grazing and other disturbances in this region had set the stage for rapid invasion (Knapp 1996). Cheatgrass spread quickly to occupy its current range by 1930 (Mack 1981), while medusahead spread much more slowly and continues to replace cheatgrass in higher precipitation areas (Hironaka 1994). Both grasses can eventually displace the native cool-season grass- and shrub-dominated communities following wide-scale disturbances such as wildfire, where they maintain long-term dominance with positive feedback cycles such as a shortened fire return interval or altered N cycles (Whisenant 1990; D'Antonio & Vitousek 1992; Young 1992; West 1999, Evans et al. 2001, Sperry et al. 2006). By some estimates, 25% of what was once sagebrush steppe is now dominated by cheatgrass and medusahead (West 1999). Conversion of native shrublands to non-native annual grasslands reduces plant and wildlife diversity and decreases grazing and recreational values on public rangelands (Bolton et al. 1990; Norton et al. 2004).

Restoring native sagebrush communities in cheatgrass- or medusahead-dominated areas is difficult because these two invasives are prolific and their seedbanks are impossible to remove completely. Remaining seeds germinate at rates over 90% early in the growing season and their seedlings outcompete native seedlings for soil water and nutrients (Harris 1967; Melgoza et al. 1990; Goodwin et al. 1996; Clausnitzer et al. 1999; Miller et al. 1999; Mosley et al. 1999). Furthermore, conversion of *Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young (Wyoming big sagebrush) communities to invasive annual grasslands crosses ecological thresholds that are very difficult to reverse (Wisdom et al. 2005). These thresholds are changes in ecosystem processes that act as a barrier to restoration. They include shortened fire-return intervals, changes in inorganic N cycling, altered soil microbial communities

(Belnap & Phillips 2001; Evans et al. 2001; Belnap et al. 2006; Hawkes et al. 2006; Sperry et al. 2006), and the transition of conservative nutrient cycling to more "leaky" cycling (Norton et al. 2007). Norton et al. (2007:3) suggest that conservative nutrient cycling is the "key functional characteristic" that must be restored for successful reestablishment of native plant communities in sagebrush steppe. Our research focused on adding carbon to foster N immobilization, and thus a more conservative N cycle, in invasive-dominated communities.

Theoretically, carbon addition stimulates C-limited microbes to reproduce or grow and immobilize inorganic soil N, thus limiting inorganic N for plant uptake and reducing the competitive advantages of invasive annual species over late-seral native plants that have evolved in a more nutrient-limited system (McLendon & Redente 1992; Zink & Allen 1998; Blumenthal et al. 2003). This can have a disproportionate effect on ruderal or early-seral species, which assimilate nutrients quickly into biomass to maximize seed production, in contrast to slower uptake and nutrient storage in roots of stress-tolerating perennial grasses (Grime 1979; Lowe et al. 2003). Timing is also likely to be an important factor in the sagebrush steppe ecosystem. Limiting available soil N in the fall and early winter when cheatgrass and medusahead are actively germinating and growing and native perennial grasses tend to be dormant should have a disproportionate effect on cheatgrass and medusahead, regardless of which plants are better competitors under N-limited conditions.

Carbon as sucrose, sawdust, mulch, or a combination of these has been added to soils in attempts to decrease non-native plant yields across a variety of ecosystems and plant communities (McLendon & Redente 1992; Young et al. 1998; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Blumenthal et al. 2003). In some carbon addition studies, growth and establishment of late-seral native plants were facilitated in competition with non-natives (McLendon & Redente 1992; Zink & Allen 1998; Paschke et al. 2000; Perry et al. 2004), while a few carbon addition studies showed no benefit for native plants (Wilson & Gerry 1995; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Corbin & D'antonio 2004). Carbon addition has also been shown to decrease grass biomass while having a lesser effect on forbs (Alpert & Maron 2000;

Eschen et al. 2007), or to disproportionately suppress biomass of cool-season grasses compared to warm-season grasses (Bleier & Jackson 2007).

The amount of carbon applied in these studies varied widely, but in many experiments was applied at a high rate to increase the likelihood of a measurable response in biomass or seed production. In three separate carbon addition studies in annual-invaded sagebrush communities, application rates of readily available carbon were 470, 1600, and 1740 kg C ha^{-1} $year^{-1}$ (McLendon & Redente 1992; Young et al. 1998; Young et al. 1999). Adding large quantities of carbon may be necessary for a specific research question or to offset high site fertility, but high application rates might be unnecessary in the N-limited sagebrush steppe ecosystem, where a lower dose might be just as effective (Bilbrough & Caldwell 1995; Evans et al. 2001; Blumenthal et al. 2003).

Our primary research objective was to apply a range of increasing carbon doses to sites invaded by cheatgrass and medusahead in a field experiment to establish whether and by how much each species decreased in biomass, density, and seed production. This will help to determine if carbon applied as sucrose is practical for large-scale restoration projects. We were interested in these specific responses because decreased invasive plant biomass or density could create openings into which native perennial plants might be established. An effective reduction in biomass of either cheatgrass or medusahead, which can produce thousands of seeds per plant, must also include a concomitant seed reduction to reduce populations of invasives that will compete with native seedlings. Because species respond differently to carbon addition (Blumenthal et al. 2003; Eschen et al. 2006), we also predicted the dose responses between cheatgrass and medusahead would be different. Another research objective was to test the mechanism behind responses of these species to carbon addition by establishing the response of microbial biomass C and N to increasing carbon. We also tested the response of inorganic soil N to carbon doses over the entire experiment.

MATERIALS AND METHODS

Study Sites

We nonrandomly selected two study sites, Canyon Creek (Elmore County, Idaho) and Lincoln Bench (Malheur County, Oregon), located approximately 125 km apart on the Snake River Plain on Bureau of Land Management (BLM) parcels (Table 1, Appendix A). These sites had been approved previously by the BLM for other research. Both sites were historically dominated by Wyoming big sagebrush and *Pseudoroegneria spicata* (Pursh) A. Love ssp. *spicata* (bluebunch wheatgrass) communities, but both are currently dominated by cheatgrass and medusahead. Over time, the accumulation of litter and dense shading from near-complete cheatgrass and medusahead cover has eliminated the soil crust communities that were historically present on these sites. Canyon Creek is described as a loamy 10–12" ecological site (USDA–SCS 1991), and though there are no published soil surveys for Lincoln Bench, it was classified in 2003 as a non-sticky silty clay loam (Hempy-Mayer 2004). Both sites range in elevation from 900–1100 m, and receive ~300 mm of precipitation annually, mostly from winter storms. We also selected these sites because the different soil types—Canyon Creek with montmorillonitic silty clay and a 51–102 cm deep duripan layer (Bekedam 2004), and Lincoln Bench with non-sticky silty clay loam—represent some of the soil variability within the northern Great Basin.

Table 1. Site characteristics compiled from Hempy-Mayer (2004), Bekedam (2004), and NO_3^--N data from this study.

site	elevation (m)	annual precip. (cm/yr)	latitude longitude	soil texture	ambient NO_3^--N (mg/resin) from control plots (95% CI)
Canyon Creek	1060	30.7	43°17'37" N 115°44'48" W	silty clay	1.30 (0.20–2.40)
Lincoln Bench	927	28.5	43°54'25" N 117°6'20" W	silty clay loam	0.31 (0.20–0.42)

Experimental Design

We collected cheatgrass and medusahead seeds in June and July 2005 at each site. Seeds were machine-cleaned, and we determined germination rates to be between 70–84% by germinating 4 subsets of 50 seeds from each species collected at each site

(AOSA 2002). We used a randomized complete block design within each of two sites. Each site had three replicates of an 18 x 24–m treatment block established to account for topographic and soil variation within each site. Each block was divided into 36 3 x 4–m plots in a 6 x 6 pattern for a total of 216 plots across both sites. Each plot included a 2-m^2 sampling area with 1-m buffers (Appendix B). The 2-m^2 sampling area was divided further into a 1-m^2 area for soil sampling and a 1-m^2 area for vegetation sampling. Live plants were killed in each block with an application of the herbicide glyphosate in April 2005. This treatment was followed up with additional glyphosate applications or hand-weeding during summer 2005.

Experimental treatments were applied November 2005 by first raking the plots to scarify the soil surface and remove any remaining vegetation and litter. We then applied treatments in a factorial arrangement of 3 species (300 seeds/m^2 of cheatgrass or medusahead, and an unseeded control) crossed with 12 carbon doses as sucrose (granular white sugar). Sucrose is approximately 42% carbon, so all doses were applied at rates equivalent to kg C/ha: 0, 50, 100, 150, 200, 300, 400, 600, 800, 1200, 1600, and 2400 kg C/ha (Appendix B). This range of doses was selected to encompass the range of carbon applied in other similar experiments while providing more detail in the lower range of carbon doses. Species seeded and carbon doses were randomly assigned to each plot for a total of 36 different treatments (species x dose) per block. Sucrose for each plot was mixed with 0.45 kg of sterile fine sand (at least 75% of particles by volume < 0.5 mm) to help apply sucrose more evenly, and this mixture was hand-broadcasted across the plot. Sucrose was evenly distributed across the plot by raking lightly with the back of a landscape rake.

After we applied the sucrose, enough seeds by weight for approximately 300 pure live seeds/m^2 (number of actual seeds applied was adjusted to account for 70-84% germination rates) of either cheatgrass or medusahead were mixed with 500 mL of rice hulls and hand broadcasted across each plot as the species treatment. The soil surface was lightly raked again to incorporate the seeds into the soil surface. We used a lawn roller to pack down the surface, ensuring good seed-to-soil contact, and then we applied a small amount of water with a backpack sprayer to facilitate sucrose adhesion to the

topsoil (~1 L per 3 x 4–m treatment plot, equal to a 0.08 mm precipitation event). We installed 2-ft silt fencing around each 18 x 24–m block to prevent windblown seeds from moving onto plots and to help keep soil, sucrose, and seeding treatments in place.

Sample Processing

To determine plant biomass, density, and seed production, first we randomly selected 16 "target" cheatgrass or medusahead individuals from the 1–m^2 vegetation sampling area in June and July 2006, just prior to seed dispersal. Aboveground biomass (referred to hereafter as "biomass") from each of these plants was clipped and sealed in a separate paper envelope until seeds could be counted. The remaining cheatgrass or medusahead plants from each plot were counted, clipped, and placed into separate paper bags by species. Then all other vegetation from each 1–m^2 vegetation sampling area was clipped and composited together in paper bags. Because cheatgrass matures earlier than medusahead, cheatgrass was harvested up to 1 month earlier than medusahead. Plant biomass was dried for at least 48 hours at 60°C to a constant mass and was weighed to the nearest mg. Biomass from individual target plants collected for seed counts was summed and added to total plant biomass estimates for each plot. We calculated two separate measures of seed production: seeds/g and seeds/m^2. Seeds/g oven-dried plant tissue was determined by counting the number of mature seeds per each of 16 randomly selected target individuals and dividing that number by the biomass of each individual plant, including seed mass. Seeds/m^2 for each species was calculated by averaging the number of seeds per target plant and multiplying that number by the density of the species in each 1–m^2 vegetation sampling area. A few of the 16 randomly selected target plants were obviously missing inflorescences or had seeds that were still immature when harvested, and these plants were excluded from seed analyses.

We collected soil samples for microbial biomass in November 2005 to coincide with fall rains and thus sucrose incorporation into the soil, March 2006 to coincide with snow melt in the spring, and July 2006 at the end of the vegetation growing season. Four 10-cm deep, 2-cm diameter soil cores were collected from randomly selected points in each 1-m^2 soil sampling area and composited in sealed polyethylene bags. Samples were kept cool for transport to the lab and were refrigerated for up to 5 days

until each sample could be homogenized and sieved through a 2-mm mesh screen for further processing (see Appendix C for detailed field methods).

We used a modified chloroform fumigation extraction method to analyze soil samples for microbial biomass C and N (Horwath & Paul 1994; Saetre & Stark 2005; see Appendix D). Two 10 g subsets of field-wet sieved soil were weighed into plastic snap-cap containers, wetted to approximately 60% water holding capacity by visual estimation, capped, and incubated in the dark at room temperature for three days. One set was then extracted with 35 mL of 0.5 M K2SO4 on a shaker table for one hour as pre-fumigation samples. Samples were allowed to settle for 24 hours and the liquid was filtered through Whatman #42 filters, collected in scintillation vials, and frozen until analysis on a Shimadzu TOC-V CSH/CSN total organic carbon analyzer with TNM-1 total N measuring unit (Shimadzu Scientific Instruments, Columbia, MD). The other set of post-fumigation samples were fumigated for 5 days with ethanol-free chloroform in the dark inside a vacuum desiccator prior to extraction with K2SO4 and analysis as described above. Gravimetric soil moisture for each sample was determined by drying a third 10 g subset of soil at 105° C for 48 hours and then re-weighing the dry subset to calculate water lost. Microbial biomass C and N were determined by subtracting the soil moisture-corrected post-fumigation C or N values from the soil moisture-corrected pre-fumigation C or N values (Horwath & Paul 1994; Saetre & Stark 2005). We did not use a correction factor because we did not quantify extraction efficiencies.

To estimate plant-available inorganic N over the entire experiment, we buried PST-1 ion-exchange resin capsules (Unibest Inc., Bozeman, MT) 10 cm deep at three randomly selected locations in each 1-m^2 soil sampling area.. Resins were left in situ from November 2005 until June and July 2006, then were removed from the plots, rinsed with deionized water, and refrigerated until extraction with 60 mL of 2 M KCl for one hour on a shaker table. Samples were allowed to settle for 24 hours, then were decanted and filtered through Whatman #42 filters into scintillation vials and frozen until colorimetric analysis (QuickChem Methods 12-107-06-2-A and 12-107-04-1-B) on a Lachat QuickChem8000 autoanalyzer (Lachat Instruments, Milwaukee, WI). We experienced interference problems during colorimetric analysis of NH_4^+-N, most likely

due to an unknown substance in the mixed bed resins exchanging with KCl ions (Hart & Binkley 1984). Thus, we present only NO_3^--N data. In future experiments, rinsing resin capsules with 2M KCl prior to deployment, extraction with a different matrix such as 1M HCl, or colorimetric determination with non-salicylate chemistry might prevent similar interference problems.

Statistical Analyses

Sites were analyzed separately because they were not randomly selected and were not true replicates. Despite our attempts to control for non-target species, many other plants grew in our plots, mostly unseeded cheatgrass and medusahead and other non-native annuals. We summed biomass data from all species for analysis of total plant biomass. At Canyon Creek, medusahead established in every plot, while it was relatively unsuccessful at Lincoln Bench. Cheatgrass had the opposite success, establishing in every plot at Lincoln Bench, but failing to establish in most plots at Canyon Creek. Target species' biomass, seed production, density, and individual plant size (the biomass of each of the 16 target plants) were analyzed only from plots where that particular species was planted. Fourteen of the 216 plots from both sites were excluded from analyses because they had been destroyed by loose, blowing silt fence or were severely eroded. Microbial biomass was analyzed as C and N separately, but also as microbial biomass C:N. Only NO_3^--N (mg/L) was analyzed for inorganic N because of interference in the NH_4^+ samples.

The effect of carbon doses on our main responses of interest—total plant and target species biomass, target species seed production, microbial biomass C and N, and inorganic NO_3^--N—was modeled using linear regression in PROC MIXED in SAS v. 9.1 (SAS Institute, Cary, NC). We compared all possible regression models using the predictive, explanatory variables species seeded, dose, dose^2, and all their interactions, with blocks as a random effect. Dose and dose^2 were treated as continuous variables. Response variables were natural log transformed as necessary to equalize variance. Datasets were examined for outliers, and other model assumptions of normality and independence were determined to be met. Of all possible models, the model with the lowest Schwarz Bayesian Information Criterion (BIC) value was selected as the most

parsimonious and best-fitting model (Appendix E). We used BIC as our selection statistic over other information criteria because we believed there would be a correct model that would best fit our data, rather than selecting from a large set of explanatory variables that all contribute some information to our model (Ramsey & Schafer 2002). In some cases, we selected a model via BIC that included a non-statistically significant explanatory variable, and we presented estimates for that effect. If the null model was selected through BIC, we did not present estimates.

We calculated "lowest significant doses" for variables decreasing in response to carbon, including total plant biomass, target species biomass, individual plant biomass, seed production, and inorganic soil N, by calculating 95% confidence intervals around each regression line and finding the first confidence interval and associated point that was lower than the mean response of control plots where no carbon was applied. Using the y-value from this regression point, we solved for the corresponding carbon dose value (x) and called this the lowest significant dose. For microbial biomass C, N, and C:N, which increased in response to carbon, lowest significant doses were calculated for where the 95% lower confidence interval first exceeded the mean response at dose 0 kg C/ha.

RESULTS

Plant Biomass, Density, and Seed Production

More volunteer plants grew in our treatment plots than expected, and in some cases responses of the original target species were weak in comparison to all ruderal plants in the plot as a whole. These volunteers included *Lactuca serriola* L. (prickly lettuce) and *Helianthus annuus* L. (common sunflower) at Canyon Creek and *Sisymbrium altissimum* L. (tumblemustard) at Lincoln Bench and accounted for nearly all other species growing in addition to cheatgrass and medusahead. Mean relative medusahead biomass (medusahead biomass, g/m^2, divided by the oven-dried biomass of all plants in each plot, g/m^2) was 74–84% at Canyon Creek, depending on the species seeded (Figure 1a). Cheatgrass was the least successful plant at Canyon Creek, accounting for no more than 2% of mean relative biomass per plot. At Lincoln Bench, species other than the target species dominated the plots, comprising over 50% of the mean relative biomass, while cheatgrass and medusahead mean relative biomass per plot was 32–39% and 4–8%, respectively (Figure 1b).

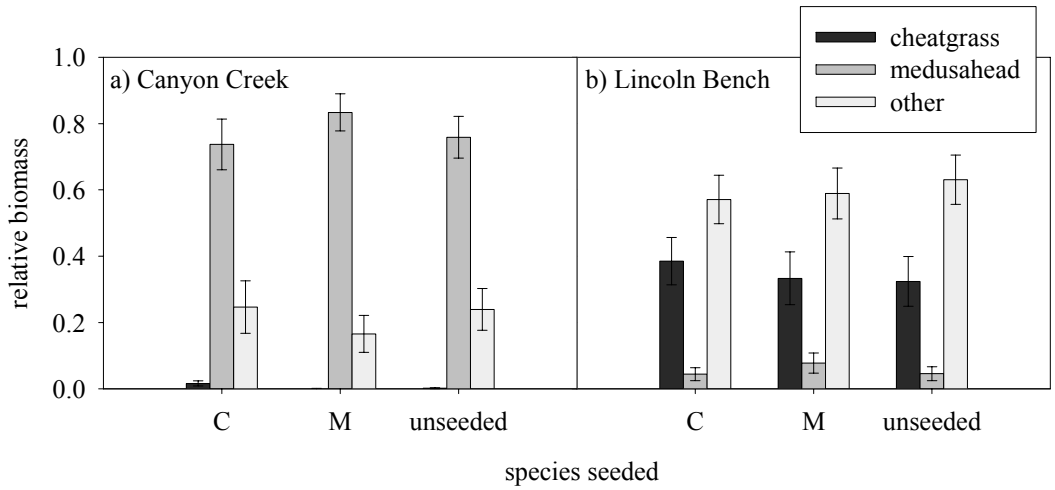

Figure 1. Success of seeding treatments at Canyon Creek and Lincoln Bench. Bars are mean relative oven-dried biomass of each species for each seeding treatment (C=cheatgrass, M=medusahead, unseeded). Error bars represent 95% confidence intervals.

Total plant biomass (g/m^2) incorporates the whole community of ruderal species growing in each plot, including both cheatgrass and medusahead when present. Total plant biomass decreased exponentially with increasing carbon at both sites. For an increase in carbon of 100 kg C/ha, the back-transformed mean total plant biomass decreased by 6.4% at Canyon Creek and 5.6% at Lincoln Bench (Table 2, Figure 2). Unexpectedly, when target species were examined individually, only medusahead biomass (g/m^2) at Canyon Creek decreased with increasing carbon (Figure 3). For an increase in carbon of 100 kg C/ha, back-transformed mean medusahead biomass (g/m^2) decreased 6.8% (Table 2, Figure 3). Carbon had no significant effect on cheatgrass at Canyon Creek or either species at Lincoln Bench. (Appendix E).

Back-transformed mean individual medusahead plant biomass (g/plant) decreased 7.1% at Canyon Creek and 2.7% at Lincoln Bench for an increase in carbon of 100 kg C/ha (Table 2, Figure 4a & 4b). Individual cheatgrass plant biomass (g) was not significantly affected by carbon at either site. Individual cheatgrass plant biomass at Lincoln Bench did appear to decrease when regressed on dose, but this was a weak relationship. The null model had better explanatory power and was selected as the preferred model based on the principle of parsimony (Appendix E).

Lowest significant carbon doses for total plant biomass g/m^2 were 240 kg C/ha at Canyon Creek and 640 kg C/ha at Lincoln Bench (Table 3). The lowest significant dose for medusahead biomass (g/m^2) was 690 kg C/ha at Canyon Creek, and the lowest significant dose for individual medusahead plant biomass (g/plant) at Canyon Creek was 300 kg C/ha (Table 3).

Seed production decreased with increasing carbon for only medusahead at Canyon Creek (Table 2). For an increase in carbon of 100 kg C/ha at Canyon Creek, back-transformed mean medusahead seeds/m^2 decreased 6.9% and seeds/g plant decreased by 0.7 seeds (Figure 4c & 4d). Cheatgrass seed production at both sites and medusahead seed production at Lincoln Bench were not significantly affected by carbon. Lowest significant doses for medusahead seeds/m^2 and seeds/g plant were 340 and 390 kg C/ha (Table 3).

Carbon had no effect on density/m^2 of either cheatgrass or medusahead at either site. Mean cheatgrass density from plots where it was the only species seeded was 9 and 109 plants/m^2 at Canyon Creek and Lincoln Bench. Mean medusahead density from plots where it was the only species seeded was 99 and 28 plants/m^2 at Canyon Creek and Lincoln Bench.

Table 2. Estimates of carbon dose effect on all plant responses (95% confidence intervals in parentheses), degrees of freedom (numerator, denominator) for the best fit model, and that model's F-ratio and p-value. Regression model: back-transformed mean $y=\beta_0 * \exp\beta_1(\text{dose})$.

	total plant biomass g/m^2					target plant biomass g/m^2					
site	β_0	β_1	*df*	F-ratio	p-value	species	β_0	β_1	*df*	F-ratio	p-value
Canyon Creek	162.57	-0.0006	1, 98	162.32	<0.0001	medusahead	125.35	-0.0007	1, 30	33.81	<0.0001
	(119.47–233.88)	(-0.0007 to -0.0005)					(74.54–210.78)	(-0.0009 to -0.0004)			
Lincoln Bench	74.93	-0.0006	1, 94	83.57	<0.0001						
	(34.08–164.76)	(-0.0007 to -0.0004)									

	seeds/g plant						seeds/m^2					
site	species	β_0	β_1	*df*	F-ratio	p-value	species	β_0	β_1	*df*	F-ratio	p-value
Canyon Creek	medusahead	105.4	-0.0071	1, 30	15.89	0.0004	medusahead	13,750	-0.0007	1, 30	23.56	<0.0001
		(98.14–112.67)	(-0.0108 to -0.0035)					(7,601–24,877)	(-0.0010 to -0.0004)			

	individual medusahead plant biomass g/plant				
site	β_0	β_1	*df*	F-ratio	p-value
Canyon Creek	0.88	-0.0007	1, 555	107.09	<0.0001
	(0.53–1.44)	(-0.0009 to -0.0006)			
Lincoln Bench	0.11	-0.0003	1, 519	21.77	<0.0001
	(0.04–0.30)	(-0.0004 to -0.0002)			

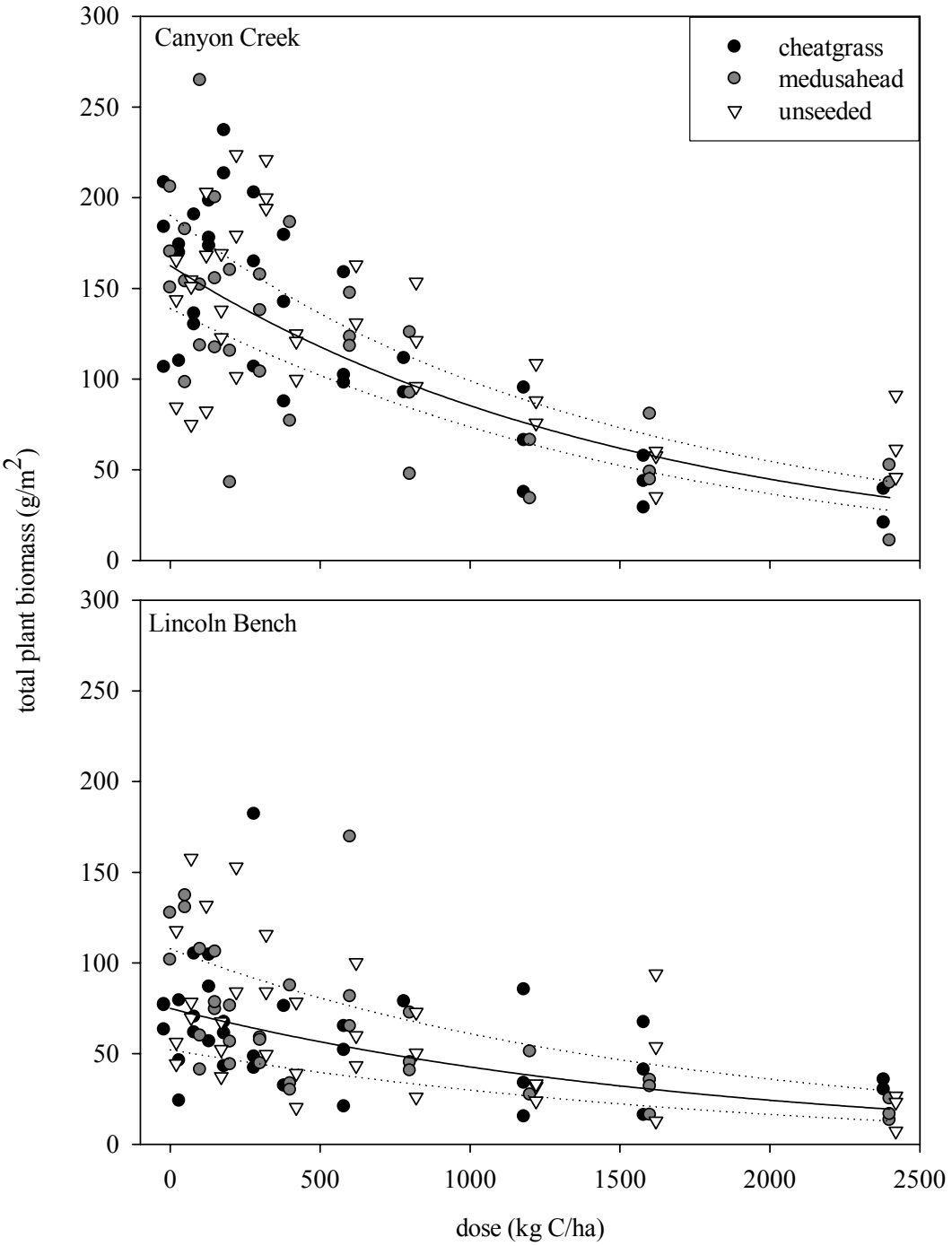

Figure 2. The effect of carbon doses on total plant biomass (g/m^2) at Canyon Creek and Lincoln Bench. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals. Cheatgrass, medusahead, and unseeded indicate species seeded in each plot, and data points are jittered on the x-axis for display.

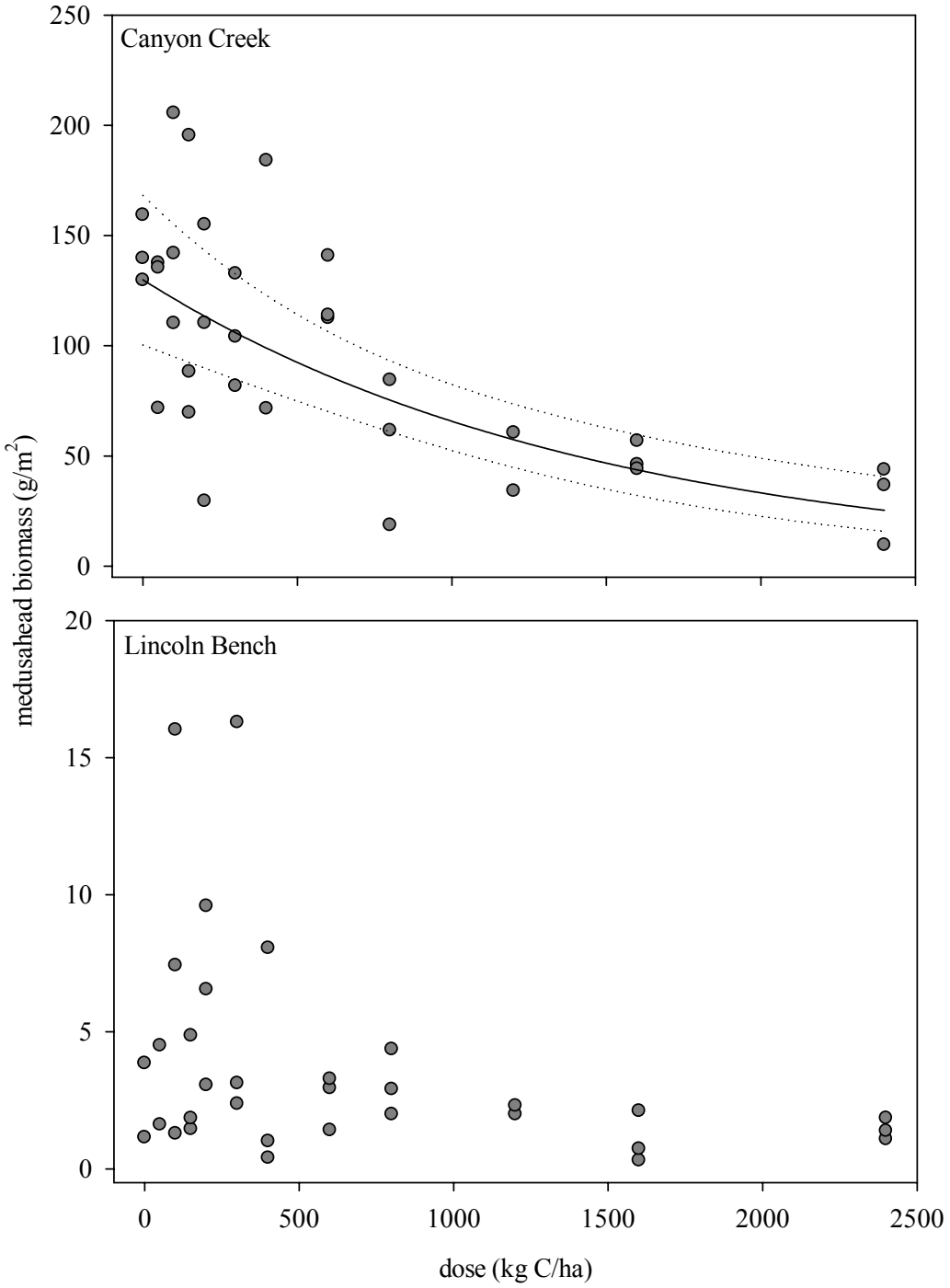

Figure 3. The effect of carbon doses on back-transformed mean medusahead biomass (g/m^2) at Canyon Creek and Lincoln Bench. Only medusahead at Canyon Creek was affected by dose. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals.

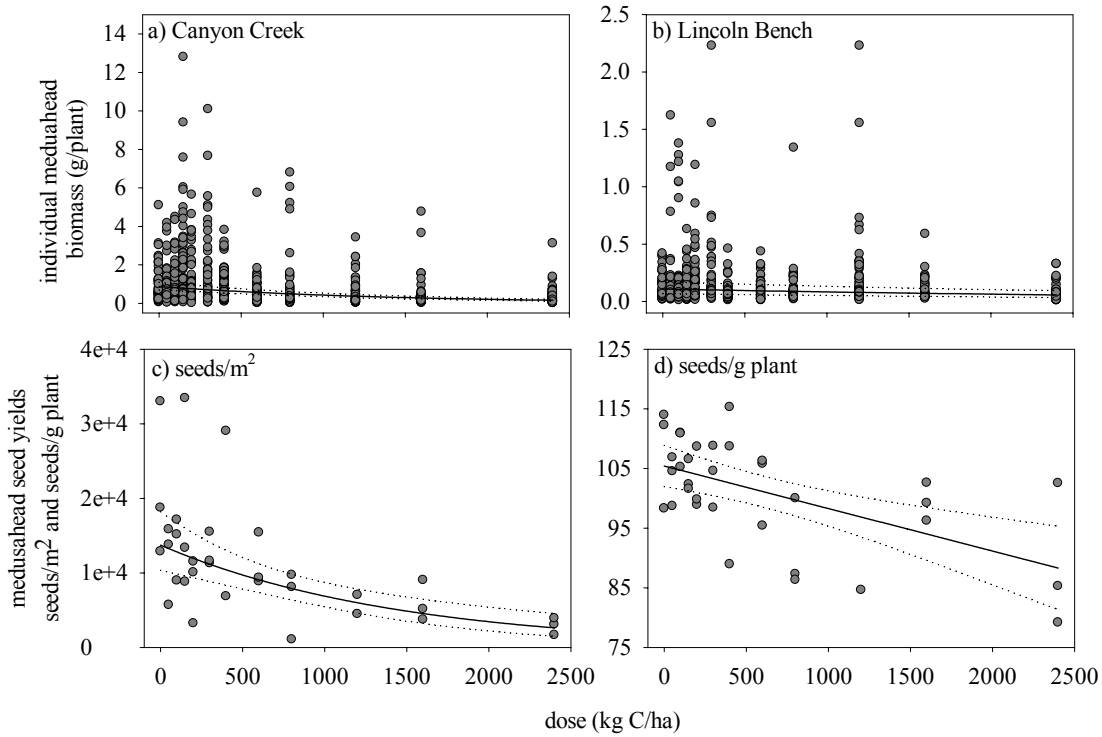

Figure 4. The effect of carbon doses on back-transformed individual medusahead biomass (g/plant) at a) Canyon Creek and b) Lincoln Bench and c) back-transformed mean seeds/m^2 and b) mean seeds/g plant for medusahead at Canyon Creek. Note different scales on Y axes. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals.

Table 3. Lowest significant carbon doses calculated from regression of each response on dose.

response	site	date	species seeded	lowest significant dose (kg C/ha)
total biomass g/m^2	Canyon Creek	—	—	240
	Lincoln Bench	—	—	640
medusahead biomass g/m^2	Canyon Creek	—	—	690
medusahead seeds/g plant	Canyon Creek	—	—	390
medusahead seeds/m^2	Canyon Creek	—	—	340
individual medusahead plant biomass g	Canyon Creek	—	—	300
	Lincoln Bench	—	—	1800
microbial biomass C mg/kg soil	Canyon Creek	NOV	cheatgrass	560
			medusahead	560
			unseeded	550
		MAR	cheatgrass	550
			medusahead	550
			unseeded	550
	Lincoln Bench	NOV	cheatgrass	440
			medusahead	430
			unseeded	430
		MAR	cheatgrass	550
			medusahead	520
			unseeded	510
microbial biomass N mg/kg soil	Lincoln Bench	NOV	cheatgrass	900
			medusahead	890
			unseeded	890
microbial biomass C:N	Canyon Creek	NOV	cheatgrass	470
			medusahead	460
			unseeded	450
		MAR	cheatgrass	350
			medusahead	350
			unseeded	350
	Lincoln Bench	NOV	—	300
		MAR	—	970
inorganic NO_3^--N mg/resin	Canyon Creek	—	cheatgrass	540
		—	medusahead	540
		—	unseeded	540
	Lincoln Bench	—	cheatgrass	550
		—	medusahead	560
		—	unseeded	560

Microbial Biomass and Inorganic Nitrogen

At both sites sampled in November 2005 and March 2006, microbial biomass C (mg/kg soil) increased linearly with increasing carbon dose, while species seeded had no significant effect (Table 4). However, species seeded remained in all microbial biomass C models as this effect had some explanatory power and was selected by BIC (Table 4, Appendix E). We present figures for only Lincoln Bench because microbial biomass C responses were similar between sites (see Appendix F for Canyon Creek figures). In November 2005, for an increase in carbon of 100 kg C/ha, mean microbial biomass C increased 4.0 mg/kg soil at Canyon Creek and 3.4 mg/kg soil at Lincoln Bench (Figure 5a). In March 2006, for an increase in dose of 100 kg C/ha, mean microbial biomass C increased 2.9 mg/kg soil at Canyon Creek and 2.2 mg/kg soil at Lincoln Bench (Figure 5c). Microbial biomass C sampled in July 2006 was not significantly affected by either dose or species seeded, although the BIC-specified model included species seeded as an explanatory variable (Table 4, Figure 5e).

Microbial biomass N (mg/kg soil) increased in response to carbon only at Lincoln Bench in November 2005; for an increase in carbon of 100 kg C/ha, microbial biomass N increased 0.3 mg/kg soil (Figure 5b). For all sampling dates, species seeded did not significantly affect microbial biomass N, but this variable remained in all models as specified by BIC (Table 4, Appendix E). Microbial biomass C:N increased with carbon at both sites in November and March, but not in July (Figure 6, Table 5). For an increase in carbon of 100 kg C/ha, microbial biomass C:N increased approximately 0.1 for both sites in November and March. Cheatgrass-seeded plots had higher microbial C:N in control plots compared to other seeding treatments at Canyon Creek in March and July. At Lincoln Bench, species seeded had no significant effect on microbial C:N, although this variable remained in the regression model for July samples (Table 5).

Inorganic NO_3^--N (mg/resin) decreased at both sites with increasing carbon, and initial amounts were different depending on species seeded and site, but there was no interaction between species seeded and dose (Table 5, Figure 7). For an increase in carbon of 100 kg C/ha, back-transformed mean NO_3^--N decreased 16% at Canyon Creek and 10% at Lincoln Bench.

The lowest significant carbon doses for microbial biomass C ranged from 430–560 kg C/ha depending on the species seeded (Table 3). The lowest significant dose for microbial biomass N sampled in November at Lincoln Bench was 890–900 kg C/ha, depending on the species seeded. The lowest significant doses for microbial biomass C:N at Canyon Creek were 350-470 kg C/ha depending on species seeded and date, and 300 and 970 kg C/ha at Lincoln Bench for November and March. The lowest significant doses for NO_3^--N across both sites were 540–560 kg C/ha.

Table 4. Estimates of species seeded and carbon dose effects on microbial biomass C and N in mg/kg soil (95% confidence intervals in parentheses), degrees of freedom (numerator, denominator) for the best fit model, and that model's F-ratio and p-value. Regression model: mean $y=\beta_0 + \beta_1$*dose.

	microbial biomass C mg/kg soil									
site	sample date	species seeded	β_0	*df*	F-ratio	p-value	β_1	*df*	F-ratio	p-value
Canyon Creek	November 2005	cheatgrass	134.62 (110.60-158.64)	2, 54	2.17	0.12	0.040 (0.027-0.053)	1, 54	37.20	<0.0001
		medusahead	123.60 (99.53-147.66)							
		unseeded	107.60 (84.13-131.08)							
	March 2006	cheatgrass	144.84 (126.90-162.78)	2, 54	0.27	0.77	0.029 (0.019-0.040)	1, 54	30.12	<0.0001
		medusahead	140.56 (122.73-158.39)							
		unseeded	136.74 (118.73-154.75)							
	July 2006	cheatgrass	153.62 (124.69-182.55)	2, 57	3.55	0.04				
		medusahead	137.83 (108.90-166.76)							
		unseeded	132.90 (103.86-161.95)							
Lincoln Bench	November 2005	cheatgrass	113.28 (97.70-128.85)	2, 56	0.59	0.56	0.034 (0.027-0.041)	1, 56	98.79	<0.0001
		medusahead	114.56 (99.22-129.90)							
		unseeded	120.46 (105.13-135.80)							
	March 2006	cheatgrass	111.29 (98.08-124.51)	2, 53	0.18	0.83	0.022 (0.014-0.029)	1, 53	32.76	<0.0001
		medusahead	115.97 (103.42-128.53)							
		unseeded	112.79 (100.46-125.11)							
	July 2006	cheatgrass	117.74 (106.52-128.97)	2, 56	0.74	0.48				
		medusahead	124.43 (113.64-135.23)							
		unseeded	117.23 (106.43-128.02)							

	microbial biomass N mg/kg soil									
site	sample date	species seeded	β_0	*df*	F-ratio	p-value	β_1	*df*	F-ratio	p-value
Lincoln Bench	November 2005	cheatgrass	19.53 (16.84-22.22)	2, 56	0.21	0.81	0.003 (0.002-0.004)	1, 56	19.09	<0.0001
		medusahead	19.10 (16.46-21.74)							
		unseeded	19.93 (17.29-22.57)							

Table 5. Estimates of species seeded and carbon dose effects on microbial biomass C:N and inorganic NO_3^--N (95% confidence intervals in parentheses), degrees of freedom (numerator, denominator) for the best-fitting model, and that model's F-ratio and p-value. Microbial C:N regression model: mean $y=\beta_0 + \beta_1*dose$. NO_3^--N regression model: back-transformed mean $y=\beta_0*\exp\beta_1(dose)$.

	microbial biomass C:N									
site	sample date	species seeded	β_0	*df*	F-ratio	p-value	β_1	*df*	F-ratio	p-value
Canyon Creek	November 2005	cheatgrass	6.13 (5.62–6.65)	2, 53	0.93	0.40	0.0010	1, 53	41.57	<0.0001
		medusahead	5.77 (5.28–6.28)				(0.0007–0.0013)			
		unseeded	6.15 (5.66–6.64)							
	March 2006	cheatgrass	7.33 (6.87–7.79)	2, 54	3.25	0.05	0.0012	1, 54	79.41	<0.0001
		medusahead	6.67 (6.21–7.13)				(0.0009–0.0015)			
		unseeded	6.73 (6.27–7.19)							
	July 2006	cheatgrass	7.21 (5.64–8.79)	2, 57	4.23	0.02				
		medusahead	6.44 (4.86–8.01)							
		unseeded	6.49 (4.91–8.07)							
Lincoln Bench	November 2005	—	6.09 (5.59–6.59)				0.0007	1, 58	42.86	<0.0001
		—	—	—	—	—	(0.0005–0.0008)			
		—	—							
	March 2006	—	6.24 (4.91–7.56)				0.0006	1, 55	30.58	<0.0001
		—	—	—	—	—	(0.0004–0.0008)			
		—	—							
	July 2006	cheatgrass	6.85 (6.49–7.21)	2, 56	1.31	0.28				
		medusahead	6.66 (6.32–7.00)							
		unseeded	6.44 (6.10–6.79)							

	NO_3^--N mg/resin								
site	species seeded	β_0	*df*	F-ratio	p-value	β_1	*df*	F-ratio	p-value
Canyon Creek	cheatgrass	0.90 (0.37–2.18)	2, 266	4.40	0.01	-0.002	1, 266	244.23	<0.0001
	medusahead	0.63 (0.26–1.53)				(-0.0018 to -0.0014)			
	unseeded	1.03 (0.42–2.50)							
Lincoln Bench	cheatgrass	0.15 (0.09–0.26)	2, 288	5.48	<0.01	-0.001	1, 288	153.26	<0.0001
	medusahead	0.10 (0.06–0.17)				(-0.0011 to -0.0008)			
	unseeded	0.12 (0.07–0.20)							

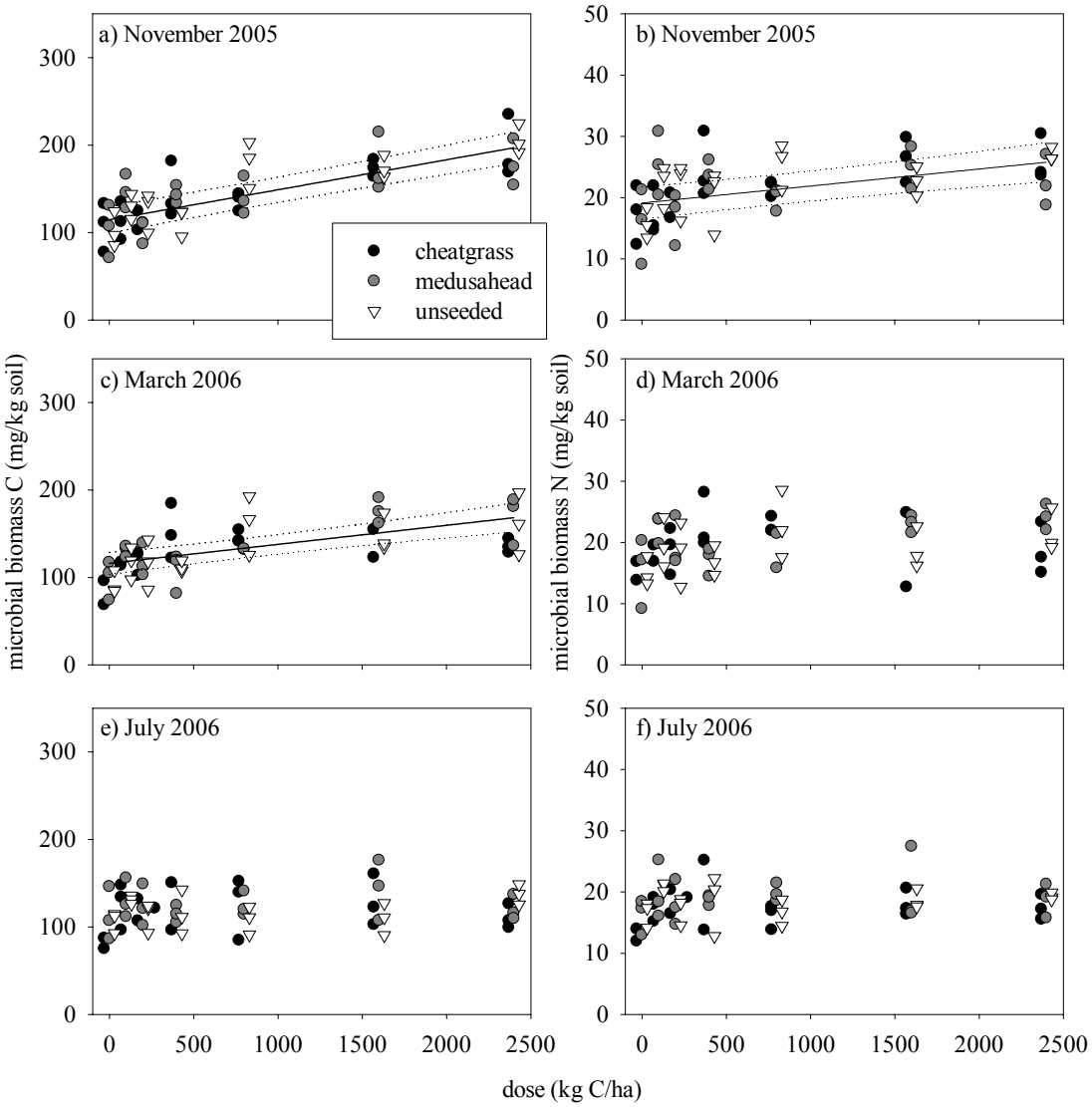

Figure 5. The effect of carbon doses on microbial biomass C (a, c, e) and N (b, d, f) in mg/kg soil at Lincoln Bench by date. Microbial biomass at Canyon Creek is not presented. Cheatgrass, medusahead, and unseeded refer to species seeded in each plot, and this effect remained in each model from the model selection process, although differences were not significant. Regression lines shown are from medusahead-seeded plots only, but are representative of plots seeded with cheatgrass and unseeded plots. Dotted lines indicate 95% confidence intervals, and data points are jittered on the x axis for display.

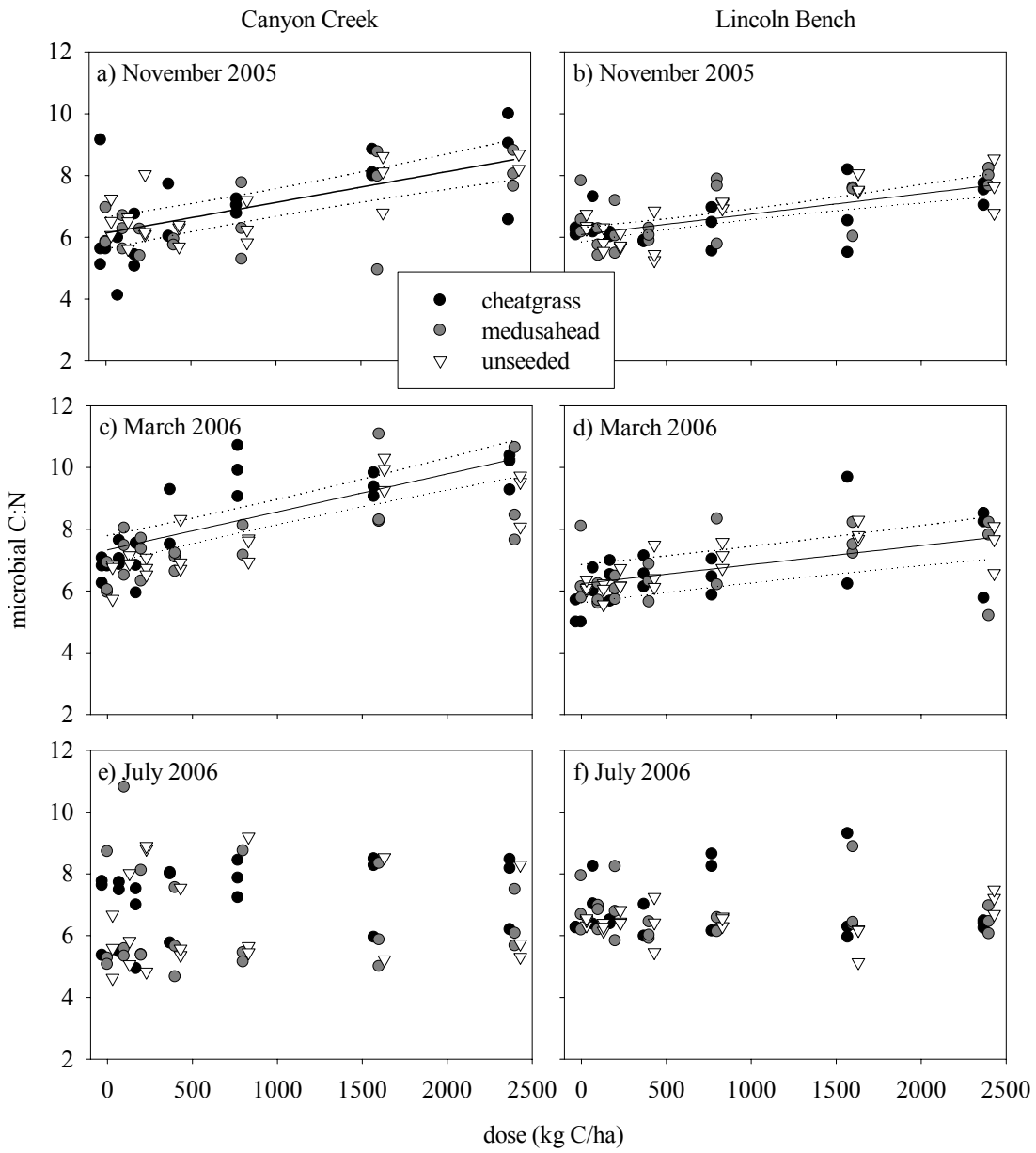

Figure 6. The effect of carbon doses on microbial C:N at Canyon Creek (a, c, e) and Lincoln Bench (b, d, f). Regression lines shown for Canyon Creek are from cheatgrass-seeded plots only, but are also representative of plots seeded with medusahead and unseeded plots. Regression lines shown for Lincoln Bench represent all seeding treatments combined. Cheatgrass, medusahead, and unseeded refer to species seeded in each plot. Dotted lines indicate 95% confidence intervals.

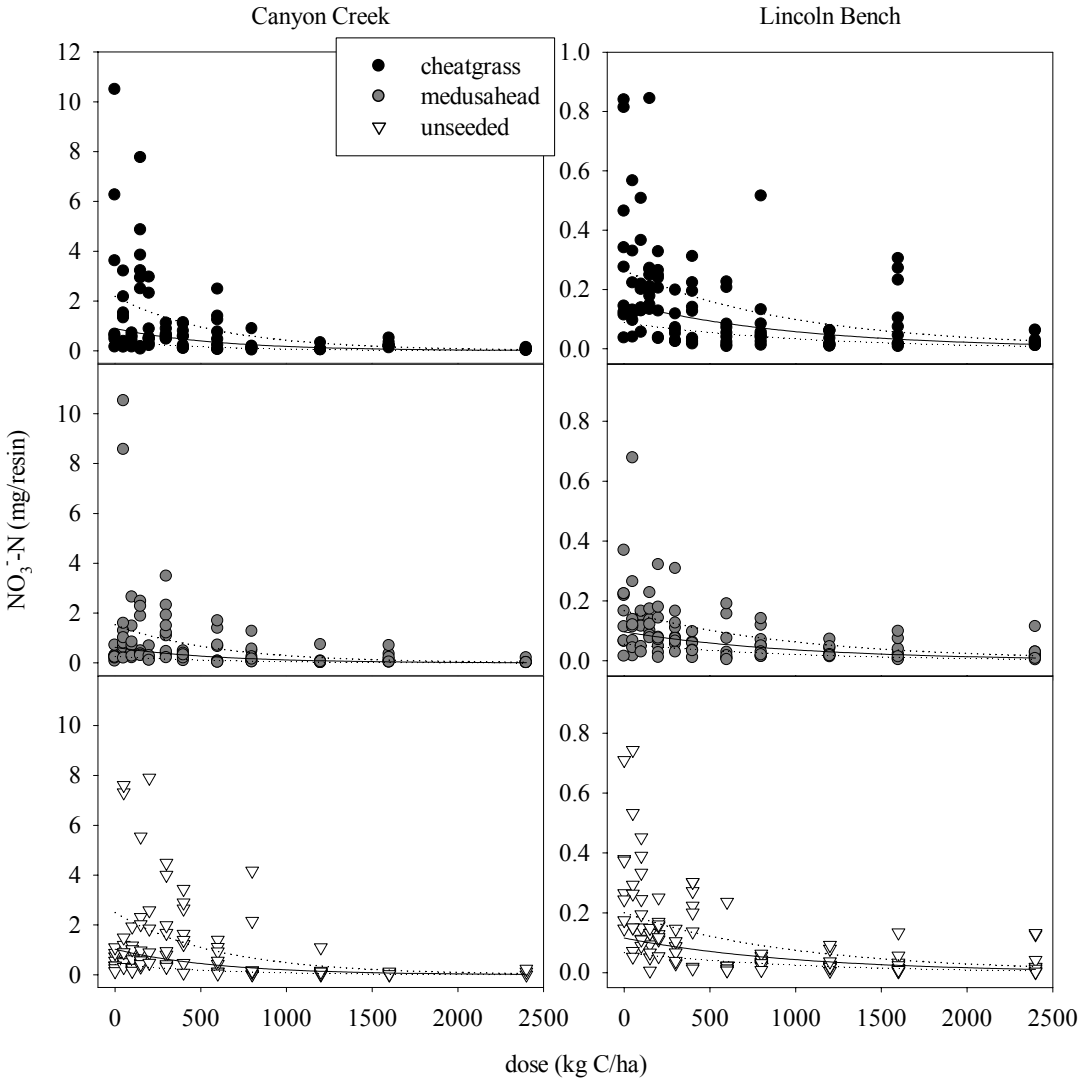

Figure 7. The effect of carbon doses on resin-extracted back-transformed inorganic NO_3^--N (mg/resin) at Canyon Creek and Lincoln Bench. Cheatgrass, medusahead, and unseeded refer to species seeded in each plot and are presented separately. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals. Note different scales on Y axes.

DISCUSSION

Medusahead at Canyon Creek was the only target species that decreased in biomass as we predicted with increasing carbon dose. However, it is likely we were unable to detect a carbon dose effect on medusahead at Lincoln Bench and cheatgrass at both sites because of the strong response of all other ruderals that established in the plots. The total plant biomass response incorporates this whole community of ruderal species, including both cheatgrass and medusahead when present, and competition among all plant species and soil organisms for the limited resources in each plot. Total plant biomass decreased with increasing carbon dose as we predicted, which is similar to other studies where carbon addition has been shown to reduce biomass of various ruderal species (Reever Morghan & Seastedt 1999; Blumenthal et al. 2003). Because the total plant biomass response to carbon addition incorporates the response of ruderal species as a whole in invaded sagebrush steppe, the calculated lowest significant doses from this study might reduce total plant biomass across a variety of ruderal plant communities, including the problematic invasives cheatgrass and medusahead. Although it appears that cheatgrass seeding was not successful at the medusahead-dominated site, Canyon Creek, and the opposite was true for medusahead seeding, the simplest explanation is that the plants that established at each site substantiated the extant plant community before we attempted to remove the seed banks.

We predicted that adding carbon would increase microbial biomass C most strongly soon after adding it to the soil and that this effect would decrease over time. We found that carbon addition did increase microbial biomass C at both sites in November and March, but this effect did not last through the end of the growing season as there was no dose effect on microbial biomass C in July 2006. Blumenthal et al. (2003) found reductions in N over two years with a one-time carbon application, but only at high doses using sawdust mixed with sucrose, a more recalcitrant source of carbon. Eschen et al. (2007) also observed a decrease in NO_3^- that persisted for more than a year after carbon addition, but this was also with a mixture of sawdust and sucrose. Our microbial biomass C results indicate that the microbial community had most likely consumed all of the added carbon by July and had returned to pretreatment

levels by the end of one growing season. Whether this one-season effect is enough to influence plant community succession would require further testing.

Surprisingly, changes in microbial biomass N did not parallel increases in microbial biomass C as we expected if microbes were actively acquiring and immobilizing inorganic N. Only samples collected in November 2005 at Lincoln Bench exhibited an increase in microbial biomass N with increasing carbon dose. There are two possible explanations for these responses. First, an increase in microbial C:N as we observed in our experiment could indicate a shift toward a more fungal-dominated soil community as soil fungi have a higher C:N than bacteria (Zink & Allen 1998; Bleier & Jackson 2007). Another possible explanation is that our microbial biomass estimation technique might be insufficient in capturing changes in microbial biomass N response. The chloroform fumigation-extraction (CFE) method to estimate microbial biomass has been shown to be less effective in extracting biomass from dry soils compared to wet soils, so we rewetted soils to 60% field capacity and incubated them in the lab for 3 days prior to fumigation (Sparling & West 1989; Gallardo & Schlesinger 1992; Zagal 1993). Indeed, our modified-CFE technique produced values for both microbial biomass C and N that are comparable to other studies (Chen & Stark 2000). However, Chen and Stark (2000) found that re-wetting cheatgrass soils released a small, labile pool of C, most likely composed of N-rich microbial cytoplasm and cells, which was used up within 2 days by the active microbial biomass. After the first day, net N mineralization declined while microbial biomass remained stable. In our experiment, after the 3-day lab incubation the microbial community could have become more N-limited or shifted in composition, thus increasing in C:N.

Although we were mostly unable to detect changes in microbial biomass N, we did find that NO_3^--N decreased with increasing carbon dose, which is consistent with other studies (Blumenthal et al. 2003; Witwicki 2005; Eschen et al. 2007). Our microbial biomass N samples were instantaneous measurements, while NO_3^--N was measured cumulatively over the entire growing season, so we did not expect the soil nitrogen pools we measured to be equivalent between these two responses. Regardless, it is likely that the decreasing inorganic N pool would affect both native perennials and

non-native annuals in this system, as there are some indications that NO_3^- is preferred over NH_4^+ in sagebrush plant communities (Davies et al. 2007), and native perennial bunchgrasses have been shown to respond more strongly to NO_3^- addition relative to NH_4^+ addition compared to cheatgrass and medusahead (Monaco et al. 2003). However, native perennial bunchgrass seedlings are less negatively impacted by low N availability compared to faster-growing ruderal species (Monaco et al. 2003), and exploiting this difference by limiting NO_3^- pools with carbon additions is key to restoring native plant communities.

Despite observed differences in soil types and ambient NO_3^- as measured in control plots across both sites, we observed similar dose effects across sites, with a 6.4% decrease in total plant biomass for an increase in carbon of 100 kg C/ha at Canyon Creek and 5.6% decrease for the same at Lincoln Bench. Soils at Canyon Creek were observed to have higher initial NO_3^--N in control plots over the growing season compared to Lincoln Bench and also supported more total plant biomass per plot than Lincoln Bench. Although the total plant biomass response to carbon was observed to be similar at both sites, the lowest significant doses for total plant biomass g/m^2 were somewhat different at each site: 240 kg C/ha at Canyon Creek and 640 kg C/ha at Lincoln Bench, which correspond with a 13.4% and 31.9% reduction in total plant biomass g/m^2. The lower significant dose of carbon at Canyon Creek suggests that these soils were initially more carbon limited than Lincoln Bench soils.

We were able to calculate lowest significant doses statistically, but we do not know whether these values are biologically significant with respect to establishing native plants. Some studies indicate a minimum of 25–50% reduction in cheatgrass or medusahead field densities are necessary (Rafferty & Young 2002; Monaco et al. 2005). Using biomass as a proxy for density, a 25% reduction in total plant biomass in our study corresponds with carbon doses of approximately 480 kg C/ha at each site. This dose is reflected in our calculated lowest significant doses for microbial biomass C, microbial biomass N, and inorganic NO_3^--N, all of which ranged from 430–560 kg C/ha. For comparison purposes, a recommended minimum carbon dose that incorporates nearly all of the calculated lowest significant doses is 600 kg C/ha, which is equivalent

to 143 g sucrose/m^2. Blumenthal et al. (2003) found a minimum carbon dose of 394 g C m^{-2} (~1000 g sucrose and sawdust/m^2) decreased weed biomass in tallgrass prairie, but only carbon applications exceeding 1000 g C/m^2 (~2500 g sucrose and sawdust/m^2) facilitated native plant growth. They suggested high site fertility necessitated high carbon doses in their study and speculated that sites with lower fertility might not need as much carbon to facilitate native establishment (Blumenthal et al. 2003). We found this not to be true for biomass reduction of annual ruderal plants as Lincoln Bench, the site with lower ambient NO_3^-, required more sucrose for a significant dose response than did Canyon Creek. Furthermore, at both sites a carbon dose of 480 kg C/ha caused a 25% reduction in total plant biomass, indicating that initial site fertility measured as NO_3^- from control plots might not matter with respect to ruderal—and in our case mostly non-native—plant biomass reduction. Further exploration on how much carbon would be needed to facilitate native species is necessary.

At current wholesale refined sugar prices, cost of a sucrose treatment equivalent to 600 kg C/ha is approximately $800/ha. Glyphosate, an herbicide currently approved for use on BLM land to control invasive weeds, costs approximately $9/ha, whereas imazapic costs approximately $46/ha (Link et al. 2006). Although much less expensive per hectare than sucrose, imazapic has not yet been approved for use on BLM land, and widespread herbicide use on public lands can be controversial. Furthermore, herbicide treatments are not intended to directly restore the altered N cycles of invaded sagebrush steppe and may actually increase N availability through disturbance (Norton et al. 2007). Sucrose treatments, on the other hand, directly affect soil N pools in a way that could potentially favor native reestablishment, and could prove to be a useful alternative to pesticides in sagebrush steppe restoration.

We specifically chose sucrose for our carbon treatments because it was a labile carbon source that was easy to apply. Other more cost-effective and recalcitrant carbon source alternatives could include sawdust or mulch, but these materials could contain secondary plant chemicals and might have other effects on N-cycling besides stimulating the microbial community to immobilize N, and they should be explored further. Carbon additions have also been shown to have a “priming” effect on soil

microorganisms, releasing previously unavailable sources of soil organic matter (De Nobili et al. 2001; Fierer & Schimel 2003). This priming could cause long-term changes in site fertility that might negatively impact native plant establishment over time. Furthermore, the current paradigm of nitrogen cycling indicates that plants are not always outcompeted by soil microorganisms and that too much importance has been placed on inorganic N mineralization, neglecting organic N pools in N-limited systems (Schimel & Bennett 2004). It is important to better characterize both inorganic and organic sources of potentially plant-available N in Great Basin systems before we can predict how adding any particular carbon source might ultimately affect plant communities.

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APPENDIX

Appendix A. Site Information

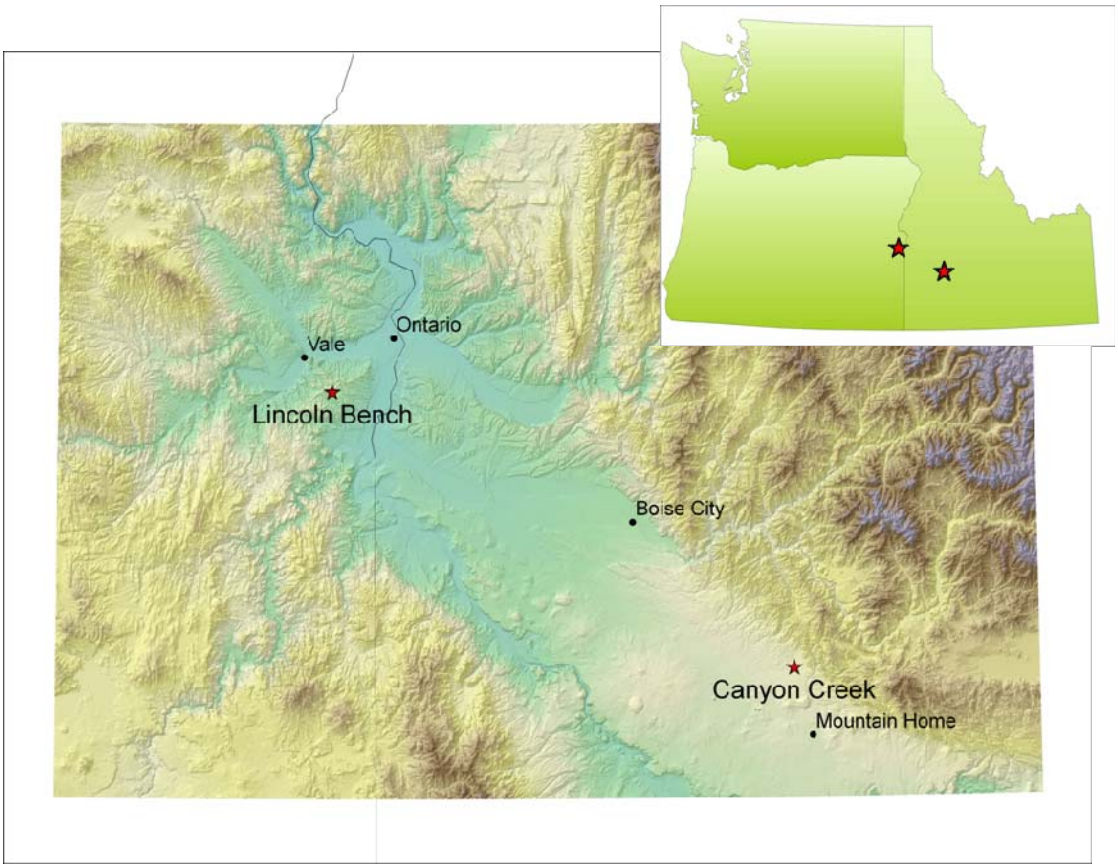

Figure 1. Topographic overview of field sites: Canyon Creek, Idaho, and Lincoln Bench, Oregon. Small map in upper right corner is overview of Washington, Oregon, and Idaho.

Figure 2. Overview of field sites, June 2005. On left, Canyon Creek, Idaho, looking east toward exclosure in foreground and Danskin mountains in background. On right, Lincoln Bench, Oregon, looking north from the exclosure toward remnant sagebrush communities. A treatment block (after herbicide application) is visible in the foreground in each photo.

Driving directions:

Canyon Creek (Elmore County, Idaho: 43°17’37” N, 115°44’48” W)

1. From Boise, Idaho, travel east on I-84 approx. 16 miles and exit at Simco Road.
2. Turn left and travel NE on Simco road approximately 0.5 miles to Old Highway 30.
3. Turn right on Old Highway 30 and follow for approx. 7 miles. Road will jog left at Squaw Creek Rd. and right on Ditto Road; stay on main road.
4. At Martha Ave., turn left and travel east for approx. 5.5 miles. The roads from this point on are unpaved.
5. At Mayfield Rd., turn left and travel north for approx. 2 miles. You will be able to see the large USGS exclosure from Mayfield just before you turn off.
6. At Helen Rd., turn left. This is an undeveloped road that connects with Squaw Creek Road and will take you to the exclosure gate, after a few hundred feet or so. Field plots for this study are located in the far southwest corner of the exclosure.

Lincoln Bench (Malheur County, Oregon: 43°54’25” N, 117°6’20” W)

1. From Vale, Oregon, travel south on Lytle Blvd.
2. After approximately 8 miles, you will crest Keeney Pass. Keeney Pass is on the Oregon National Historic trail and you will see a BLM kiosk and trail to your right. Continue past this point approx. 0.5 miles. You will parallel a ridgeline to your east; near the end of this ridge and at a slight bend in the road, you will make a left turn.
3. You are now on an undeveloped and unnamed jeep road, traveling north. Four wheel drive is recommended for this road if wet or extremely dry. At the first intersection, in ~1500 feet, turn right. After another 1000 feet, bear right and cross an ephemeral stream bed.
4. Travel east approx. 0.5 miles up a steep hill until the road flattens out on top of a ridge. The exclosure will be to your left on the north side of the road.

Appendix B. Experimental Design and Treatments

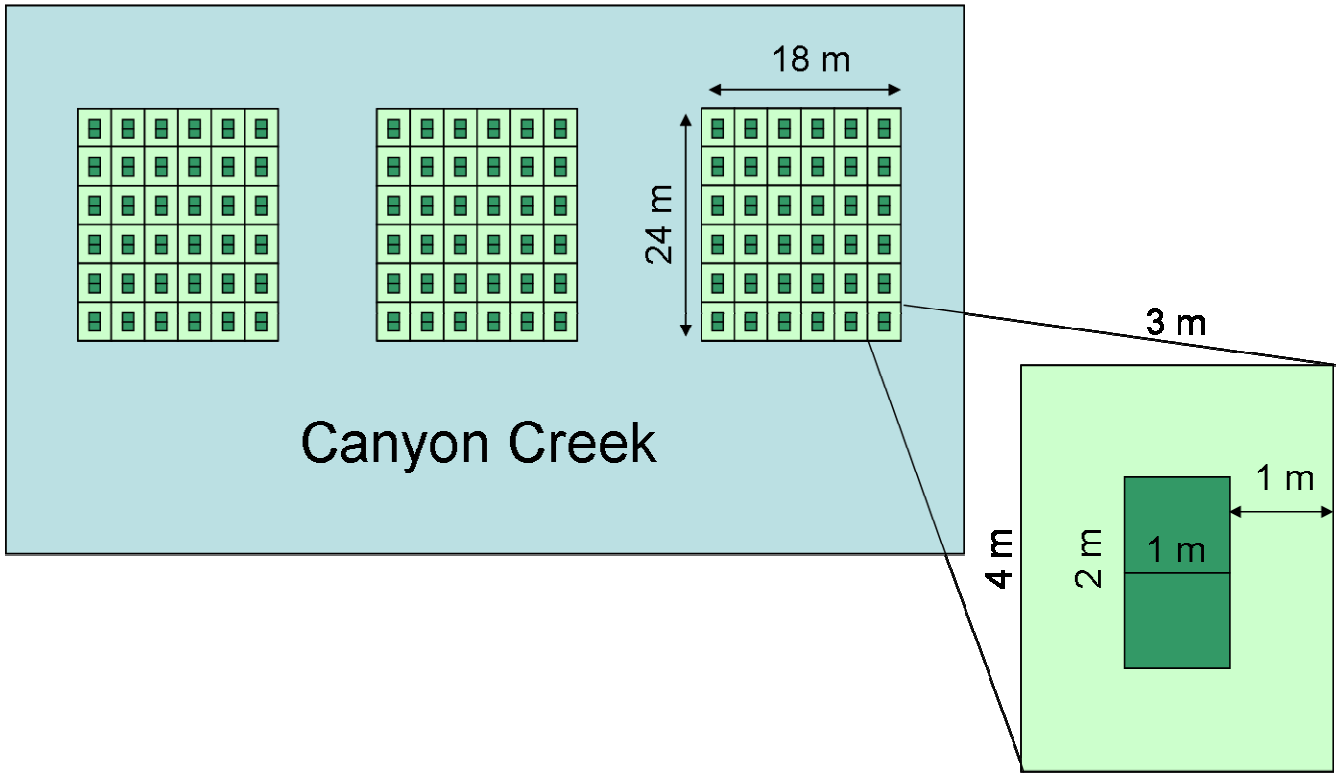

Figure 3. Overview of treatment design. Dark green rectangle in center of each plot is the 2-m^2 sampling area composed of a 1-m^2 area for soil sampling and a 1-m^2 area for vegetation sampling, surrounded by a 1-m buffer (light green). This entire plot was treated with one of 36 combinations of species x dose treatments. Plots were arranged in three 18 x 24–m blocks at each site, with each block containing the full array of 36 treatment combinations.

Table 1. Carbon doses in kg C/ha and equivalent dose in g of sucrose/m^2.

carbon doses	
kg C/ha	g sucrose/m^2
0	0
50	12
100	24
150	36
200	48
300	71
400	95
600	143
800	190
1200	286
1600	381
2400	571

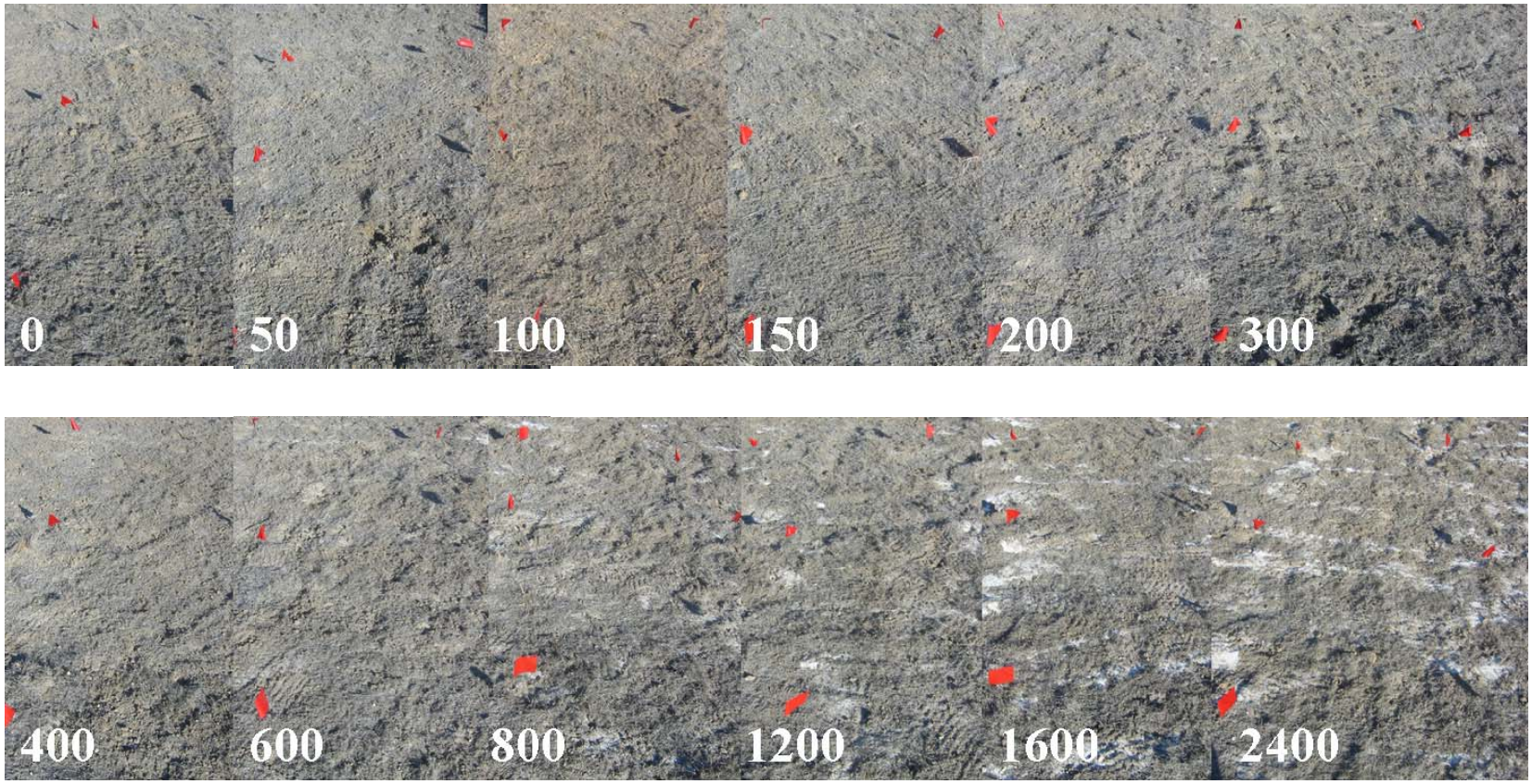

Figure 4. Photos of sucrose applied to plots, from lowest to highest dose. Number in lower left corner refers to carbon dose in kg C/ha.

Appendix C. Field Methods

Seeding and Sugaring

Materials

50 lb bag of rice hulls

measuring cup marked to 500 mL

seed in packets at quantities of 300 live seeds/sq. m

6, 2L plastic containers for mixing and broadcasting seed/rice hulls

36" landscape rake plastic rake might work better in the future

metal tags and pins for plot labeling

900 lbs of sugar

216 lbs of sugar-sized, sterile sand (playbox sand)

buckets and measuring cups (for sugar mixing)

5-gallon backpack sprayer

water

wind barrier

Procedure

1. Prior to seeding, remove any extra vegetation that may have blown in or germinated, including weeds, bunchgrasses, and other conspicuous perennials.
2. Rake the plot to scarify the soil and create 0.5 to 1.0 cm deep furrows.
3. Place a wind barrier around the plot if necessary.
4. Measure the appropriate amount of sugar for the plot. Mix in a container with 1 lb of sterile sand.
5. Evenly distribute the sugar/sand mixture across the plot and rake it in using the back of the landscape rake or a plastic yard rake.
6. Mix the appropriate seed combination with approximately 80g (500 mL) of rice hulls. Broadcast the mix uniformly over the plot.
7. Rake again to incorporate seed and sugar into soil surface.
8. Pack the seeds and sugar into the soil with a roller packer.

9. With the backpack sprayer, evenly distribute 1L of water across each plot to ensure sugar incorporation into the soil.
10. Label plots with metal tags as follows: Lincoln Bench, block 1, plot C10: "LB1C10".

Resin Deployment

Materials

650 Unibest resin capsules

30-lb test fishing line

blue painter's masking tape

centrifuge tubes (15mL) for capsule storage

soil corer marked for burial at 10 cm depth

latex gloves

heavy duty sewing needles

scissors

Procedure

1. Prior to placing in the field, string each capsule separately onto a length of fishing line marked with a colored tape flag at the surficial end. Should be long enough to reach surface but not so long that it blows in the wind. 14–16 inches is best.
2. Most efficient method of stringing is to thread 100 or so capsules onto the same line, then tie them off one by one. Wear gloves during this process so as to not contaminate the capsules. We worked as a team, with one person cutting fishing line, another poking holes into the resin capsules individually and stringing them, another tying them, and finally taping over the knot and placing into a tube.
3. Randomly select three locations in each plot to place the resin capsules. We selected which half of the plot by giving a centrifuge tube a spinning toss, and whichever way it pointed was the half of the plot in which we placed the capsules.

4. Using the soil corer, remove an intact core of soil at a 45 degree angle.
5. Remove the capsule from the tube and gently shake down into the hole left by the soil corer. Take care not to touch any part of the capsule with hands or any metal object. Capsule should fall into place easily, but if it does not, it can be coaxed into place with a twig. Also, replacing the column of the soil from the soil corer into the hole on top of the capsule helps to push it into place.
6. Remove the soil from the corer in one intact column and replace as follows: using a finger, push up on the soil column through the hole in the bottom of the corer; tip the corer sideways and let the column fall gently into an outstretched hand or on the ground; slide the column back into the hole and pat into place.
7. Be sure the fishing line and tag is still visible at the soil surface for future removal.

Soil Collection

Materials

2 small diameter soil corers (2 cm)

Ziploc bags (or polyethylene bags)

portable hanging balance

large cooler

ice packs

permanent marker

2 mm sieve

Procedure

1. From three or four random locations in each plot, collect soil cores using a 2 cm inner diameter soil corer to 10 cm deep.
2. Remove the top litter layer from the cores and composite them in a Ziploc bag. Total soil weight should be approximately 200 g; a portable hanging balance can be used to double check this amount—3–4 cores is sufficient.

3. Label each bag with date, site, block, and plot (e.g. "21 Sept. 2005, LB1 P4, microbial").
4. Soils should be chilled, but not frozen, until they can be processed in the lab. They should be processed within 24 hours, but up to 3 days is acceptable.
5. Mix the soils thoroughly within the bag.
6. In the lab, pass the soils through the 2 mm sieve.

Plant Biomass Collection

Materials

small paper bags

labels

staplers and staples

clippers, shears, scissors

permanent markers

1 m^2 biomass frame

large boxes for sample storage

Procedure

1. After seed maturity but before seed dispersal, randomly select 16 plants from the 1 m^2 destructive sampling plot as follows: divide the 1 m^2 plot into 8 equal sections by placing the biomass frame tied with string to divide into subplots over the plot. In one of the subplots, drop a pin flag from above without looking. Whichever plant the flag stem lands closest to should be harvested. Do this 2 times in each subplot for a total of 16 plants.
2. Harvest the aboveground biomass of these 16 plants and place each separately in a pre-labeled envelope.
3. Harvest all remaining aboveground biomass and place in a labeled paper bag, writing the number of plants on the outside of the bag. Separate all other plants from the cheatgrass and medusahead and place into a separate labeled paper bag.
4. Store all bags from a single block in cardboard boxes for transport back to lab.

Appendix D. Lab Methods

Microbial Biomass: CFE Method for dry grassland soils

Materials

sieved soils
balance
scoopula
aluminum weigh dishes
120 mL sample cups with lids
folded Whatman 42 filter paper (12.5cm circles)
6.5 cm funnels
filtering racks
20 mL scintillation vials
computer labels
squirt bottle with K_2SO_4
shaker table
autopipetter
vacuum pump
bungee cords
boxes (for shaker table)
vacuum desiccators
vacuum pump
vacuum grease
fume hood
45 mL snap cap containers
50 mL Erlenmeyer flasks
boiling chips
dark-colored garbage bags
ethanol-free chloroform
0.5 M K_2SO_4
repipetter with 3 L bottle set to dispense 35 m

Procedure

1. Soil pre-treatment: sieved through a 2 mm mesh screen, stored at 2°C (see sieving notes). Process as soon as possible. The ideal storage time is not more than 3 days, but I have stored soils for up to two weeks prior to processing.
2. For each soil, weigh out the following aliquots (wet weight). Weigh 10 g soil (within +/- 0.02 g) into each of two 45 mL snap cap containers. Weigh 10 g soil into an aluminum weigh dish after recording the number and weight of the dish and taring. Record the weight of the soil, then place the aluminum dishes in a drying oven at 105° C for 48 hours. Label the snap cap containers with the sample number and one for PRE and one for POST with pencil or industrial strength permanent marker since regular permanent marker will run with chloroform.

3. Steps 4 and 5 can be skipped if soil is already wet.
4. Using an auto-pipetter or a squeeze bottle with nanopure DI water, wet soils in each of the snap cap containers to 2/3 WHC. This amount may need to be adjusted visually; soils should be visibly wet but not saturated (see methods below for determining WHC).
5. Close containers and incubate in the dark at room temperature (incubation room in Pyke clean lab works well for this) for three days.
6. After incubation, pipet 35 mL 0.5 K_2SO_4 into each of the "PRE" labeled snap cap containers with soil and three blanks. Seal and shake on a shaker table set at 180 rpm for 1 hour.
7. TIPS FOR SHAKING: The containers may need to be hand shaken once or twice prior to putting on the shaker to loosen soil at the bottom—I found it easiest to bang them on the counter top 2 or 3 times prior to shaking. Use foam or bubble wrap to help secure the samples in a box for shaking. Place the containers lengthwise in the direction of the shaking to facilitate complete sample mixing. A wide, flat box will work better on the table than a tall box. Use a bungee cord to secure the box to the shaker table.
8. While the pre extraction is shaking, begin the chloroform fumigation. Line the bottom of an empty vacuum dessicator with several wet paper towels. Open the "POST" samples and place in the dessicator. Up to 35 snap cap containers can be placed into one dessicator at a time. Be sure to include three empty snap cap containers as blanks.
9. Add 30 mL ethanol-free chloroform and plenty of boiling chips to a 50 mL Erlenmeyer flask and place in the dessicator.
10. Seal dessicator (relube if necessary) and place in fume hood; apply vacuum pump (Perakis lab has a good, strong one) until chloroform boils rapidly. Maintain boil for 1 minute. Be sure the pump is vented into the hood.
11. Remove the vacuum hose and allow the dessicator to vent (suck in air) for up to a minute.

12. Repeat chloroform and evacuation 2 more times. On the last evacuation, do not vent. Rather, after 1 minute of boiling, turn the top cap on the dessicator to close off the vent hole so the dessicator is sealed, then remove the vacuum hose.
13. Place dark colored garbage bag over desiccators and incubate for 5 days in a hood (at ~25˚ C).
14. After the pre samples have settled for 24 hours, place folded Whatman 42 paper filters in 6.5 cm funnels.
15. Rinse filters with 10-20 mL K_2SO_4 from the squirt bottle and allow to sit for 5 minutes.
16. Pour off the liquid from the samples into the funnel, taking care not to disturb the soil or pour it into the filter (while this is not a disaster if it happens, it will make the filtering very slow). Collect the filtrate into labeled 20 mL scintillation vials, letting the vials fill to the shoulder (extra filtrate can be poured off).
17. Samples to be run on both the Lachat and the Shimadzu should be split into two separate scint vials (this is typically done only on the pre samples). Samples can be stored frozen or very cold for long time periods prior to analysis (up to two years in some cases).
18. After the 5-day incubation for the post samples, release the vacuum and remove the chloroform from the dessicator. Evacuate the dessicator for one minute using the pump (again, make sure it vents into the hood). Remove the tube and allow to vent to the atmosphere.
19. Repeat two more times for a total of three evacuations. Open the dessicator and allow to vent to the atmosphere for 10 minutes.
20. Add K_2SO_4, shake, and filter these samples as described for the pre samples above (steps 5, 12-15).
21. DON'T FORGET: autopipetter should be calibrated for an average volume dispensed to use in calculations.

Medusahead Seed Cleaning

1. Run sample through coarse debearder with #7 cylinder, gate ½" open, up to three times (fraction out the end usu. three times, out the bottom usually twice).
2. Sieve seed on 1200 sec. burst with sieves in order from top to bottom:
    a. #8 circular
    b. 4 x 36 rectangular
    c. bottom sieve
3. Air blower at setting 28 for 30 seconds, (equivalent to 3.8 M/S)

Cheatgrass Seed Cleaning

1. Coarse debearding at slowest speed setting with #16 screen, gate ½" open.
2. Run sample through as quickly as possible.
3. Most seed runs out the end and chaff out the bottom.
4. Sieve seed on 1200 sec. burst with sieves in order from top to bottom:
    a. 16 x 16 square (0.0515)
    b. 4 x 34 rectangle (0.0174)
    c. Bottom sieve
5. Air blower at setting 24 for 15-30 seconds (equivalent to 3.6 M/s volume).

Note: Keep all extra fractions—may need extra seeds at the end.

Appendix E. BIC Tables from SAS

Total Plant Biomass

Canyon Creek		Lincoln Bench	
model	BIC	model	BIC
dose	106	dose	141
species dose	110	species dose	146
species dose species*dose	132	$dose^2$	164
dose $dose^2$	137	species $dose^2$	170
$dose^2$	138	dose $dose^2$	172
species dose $dose^2$	140	species dose species*dose	175
species $dose^2$	142	species dose $dose^2$	177
species dose $dose^2$ species*dose	163	null	184
dose $dose^2$ dose*$dose^2$	174	species	188
species dose $dose^2$ dose*$dose^2$	178	species dose $dose^2$ species*dose	206
null	185	dose $dose^2$ dose*$dose^2$	215
species	189	species dose $dose^2$ dose*$dose^2$	220
species $dose^2$ species*$dose^2$	195	species $dose^2$ species*$dose^2$	230
species dose $dose^2$ species*$dose^2$	196	species dose $dose^2$ species*$dose^2$	237
species dose $dose^2$ species*dose dose*$dose^2$	200	species dose $dose^2$ species*dose dose*$dose^2$	249
species dose $dose^2$ species*dose species*$dose^2$	221	species dose $dose^2$ species*dose species*$dose^2$	263
species dose $dose^2$ species*$dose^2$ dose*$dose^2$	232	species dose $dose^2$ species*$dose^2$ dose*$dose^2$	280
species dose $dose^2$ species*dose species*$dose^2$	259	species dose $dose^2$ species*dose species*$dose^2$	307
species|dose|$dose^2$	338	species|dose|$dose^2$	389

Target biomass

Canyon Creek				Lincoln Bench			
B. tectorum		*T. caput medusae*		*B. tectorum*		*T. caput medusae*	
model	BIC	model	BIC	model	BIC	model	BIC
null	156	dose	68	null	66	null	90
dose	159	null	75	dose	79	dose	98
$dose^2$	176	$dose^2$	88	$dose^2$	95	$dose^2$	116
dose $dose^2$	185	dose $dose^2$	98	dose $dose^2$	108	dose $dose^2$	126
dose|$dose^2$	221	dose|$dose^2$	139	dose|$dose^2$	148	dose|$dose^2$	166

seeds/g plant

Canyon Creek				Lincoln Bench			
Cheatgrass		Medusahead		Cheatgrass		Medusahead	
model	BIC	model	BIC	model	BIC	model	BIC
null	275	dose	241	null	342	null	302
dose	279	null	243	dose	348	dose	306
$dose^2$	294	$dose^2$	259	$dose^2$	364	dose $dose^2$	321
dose $dose^2$	299	dose $dose^2$	263	dose $dose^2$	370	$dose^2$	324
dose|$dose^2$	327	dose|$dose^2$	299	dose|$dose^2$	403	dose|$dose^2$	340

seeds/m^2

Canyon Creek				Lincoln Bench			
Cheatgrass		Medusahead		Cheatgrass		Medusahead	
model	BIC	model	BIC	model	BIC	model	BIC
null	160	dose	80	null	86	null	71
dose	164	null	82	dose	99	dose	84
$dose^2$	181	$dose^2$	99	$dose^2$	114	$dose^2$	100
dose $dose^2$	190	dose $dose^2$	108	dose $dose^2$	127	dose $dose^2$	111
dose|$dose^2$	226	dose|$dose^2$	149	dose|$dose^2$	167	dose|$dose^2$	152

density

Canyon Creek				Lincoln Bench			
Cheatgrass		Medusahead		Cheatgrass		Medusahead	
model	BIC	model	BIC	model	BIC	model	BIC
null	114	null	89	null	64	null	81
dose	121	dose	104	dose	79	dose	92
$dose^2$	138	dose2	119	dose2	95	dose2	110
dose $dose^2$	149	dose dose2	131	dose dose2	107	dose dose2	118
dose|$dose^2$	189	dose|dose2	172	dose|dose2	149	dose|dose2	155

individual plant biomass

Canyon Creek				Lincoln Bench			
Cheatgrass		Medusahead		Cheatgrass		Medusahead	
model	BIC	model	BIC	model	BIC	model	BIC
null	656	dose	1781	null	2071	dose	1426
dose	662	dose $dose^2$	1810	dose	2083	null	1430
$dose^2$	679	$dose^2$	1812	$dose^2$	2097	$dose^2$	1437
dose $dose^2$	690	dose|$dose^2$	1847	dose $dose^2$	2111	dose $dose^2$	1451
dose|$dose^2$	722	null	1861	dose|$dose^2$	2153	dose|$dose^2$	1492

NO_3^- mg/resin

Canyon Creek		Lincoln Bench	
model	BIC	model	BIC
spp dose	875	spp dose	852
dose	880	spp dose dose^2	853
spp dose dose^2	901	dose	858
dose dose^2	906	dose dose^2	861
spp dose dose^2 spp*dose	927	spp dose spp*dose	880
spp dose^2	928	spp dose dose^2 spp*dose	881
dose^2	933	spp dose dose^2 dose*dose^2	887
spp dose dose^2 dose*dose^2	942	dose dose^2 dose*dose^2	895
spp dose dose^2 spp*dose^2	958	spp dose^2	912
spp dose spp*dose	958	spp dose dose^2 spp*dose^2	913
dose dose^2 dose*dose^2	958	spp dose dose^2 spp*dose dose*dose^2	915
spp dose^2 spp*dose^2	958	dose^2	917
spp dose dose^2 spp*dose dose*dose^2	969	spp dose dose^2 spp*dose spp*dose^2	937
spp dose dose^2 spp*dose spp*dose^2	983	spp dose dose^2 spp*dose2 dose*dose^2	947
spp dose dose^2 spp*dose2 dose*dose^2	999	spp	958
spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	1024	null	962
spp	1032	spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	970
null	1038	spp dose^2 spp*dose^2	972
spp|dose|dose^2	1096	spp|dose|dose^2	1051

Microbial Biomass C:N, Canyon Creek

November 2005		March 2006		July 2006	
model	BIC	model	BIC	model	BIC
spp dose	180	spp dose	175	spp	182
dose	181	dose	180	null	197
spp	196	spp dose dose^2	193	spp dose	197
null	197	dose dose^2	198	dose	204
spp dose^2	197	spp dose spp*dose	203	spp dose^2	213
dose^2	197	spp dose^2	207	dose^2	220
spp dose spp*dose	208	spp	210	spp dose spp*dose	224
spp dose dose^2	209	dose^2	211	spp dose dose^2	225
dose dose^2	209	null	213	dose dose^2	232
spp dose dose^2 spp*dose	236	spp dose dose^2 spp*dose	221	spp dose dose^2 spp*dose	252
spp dose dose^2 dose*dose^2	248	spp dose dose^2 dose*dose^2	234	spp dose dose^2 dose*dose^2	267
dose dose^2 dose*dose^2	249	dose dose^2 dose*dose^2	239	spp dose^2 spp*dose^2	272
spp dose^2 spp*dose^2	256	spp dose dose^2 spp*dose^2	252	dose dose^2 dose*dose^2	273
spp dose dose^2 spp*dose^2	267	spp dose dose^2 spp*dose dose*dose^2	262	spp dose dose^2 spp*dose^2	284
spp dose dose^2 spp*dose dose*dose^2	275	spp dose^2 spp*dose^2	266	spp dose dose^2 spp*dose dose*dose^2	293
spp dose dose^2 spp*dose spp*dose^2	288	spp dose dose^2 spp*dose spp*dose^2	273	spp dose dose^2 spp*dose spp*dose^2	305
spp dose dose^2 spp*dose^2 dose*dose^2	307	spp dose dose^2 spp*dose^2 dose*dose^2	293	spp dose dose^2 spp*dose^2 dose*dose^2	325
spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	328	spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	315	spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	346
spp|dose|dose^2	407	spp|dose|dose^2	388	spp|dose|dose^2	425

Microbial Biomass C:N, Lincoln Bench

November 2005		March 2006		July 2006	
model	BIC	model	BIC	model	BIC
dose	144	dose	153	spp	147
spp dose	145	spp dose	155	null	147
null	160	null	161	spp dose	163
dose^2	161	spp	162	dose	163
spp	161	dose dose^2	175	spp dose^2	178
spp dose^2	162	dose^2	176	dose^2	179
dose dose^2	173	spp dose dose^2	177	spp dose spp*dose	191
spp dose spp*dose	173	spp dose^2	178	spp dose dose^2	192
spp dose dose^2	174	spp dose spp*dose	184	dose dose^2	192
spp dose dose^2 spp*dose	202	spp dose dose^2 spp*dose	206	spp dose dose^2 spp*dose	220
dose dose^2 dose*dose^2	213	dose dose^2 dose*dose^2	215	spp dose dose^2 dose*dose^2	233
spp dose dose^2 dose*dose^2	215	spp dose dose^2 dose*dose^2	217	dose dose^2 dose*dose^2	234
spp dose^2 spp*dose^2	222	spp dose dose^2 spp*dose^2	238	spp dose^2 spp*dose^2	237
spp dose dose^2 spp*dose^2	234	spp dose^2 spp*dose^2	238	spp dose dose^2 spp*dose^2	251
spp dose dose^2 spp*dose dose*dose^2	243	spp dose dose^2 spp*dose dose*dose^2	246	spp dose dose^2 spp*dose dose*dose^2	262
spp dose dose^2 spp*dose spp*dose^2	256	spp dose dose^2 spp*dose spp*dose^2	261	spp dose dose^2 spp*dose spp*dose^2	269
spp dose dose^2 spp*dose^2 dose*dose^2	275	spp dose dose^2 spp*dose^2 dose*dose^2	277	spp dose dose^2 spp*dose^2 dose*dose^2	292
spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	296	spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	301	spp dose dose^2 spp*dose spp*dose^2 dose*dose^2	311
spp|dose|dose^2	376	spp|dose|dose^2	381	spp|dose|dose^2	387

Appendix F. Canyon Creek Microbial Biomass Results

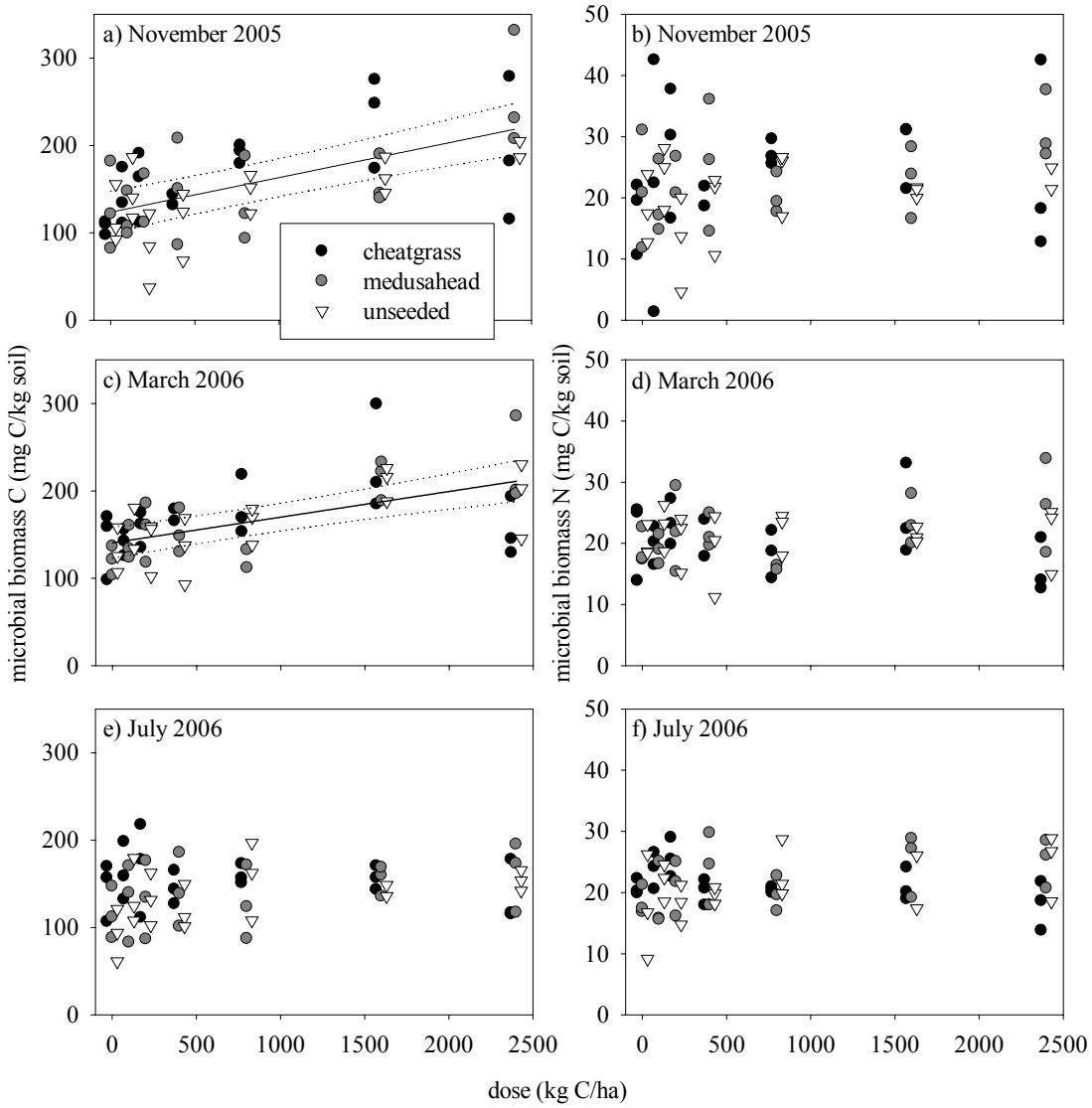

Figure 5. The effect of carbon doses on microbial biomass C and N (mg/kg soil) at Canyon Creek by date. Cheatgrass, medusahead, and unseeded refer to species seeded in each plot, and this effect remained in each model from the model selection process, although differences were not significant. Regression lines shown are from medusahead-seeded plots only, but are representative of plots seeded with cheatgrass and unseeded plots. Dotted lines indicate 95% confidence intervals, and data points are jittered on the x axis for display.