Impact of Tebuthiuron on Biodiversity of High Elevation Mountain Big Sagebrush Communities

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Abstract—The objectives of this study were to determine tebuthiuron's (1) effectiveness at low application rates in thinning dense, high elevation stands of mountain big sagebrush, (2) impact on understory herbaceous plants and soil microflora, and (3) movement and stability in soil. Four study sites were established in the Fish Lake National Forest and adjacent Bureau of Land Management holdings. Tebuthiuron pellets were applied at one site in 1994, a second site in 1995, and two sites in 1996 at rates ranging from 0.2 to 0.7 pounds of active ingredient (AI) per acre (0.18 to 0.63 kg AI per ha). Data from the two sites treated in 1996 show that sagebrush canopy cover was reduced in annual increments from pretreatment levels of about 23 percent to11 percent (0.3 AI treatment), 6 percent (0.5 AI), and 4 percent (0.7 AI) by 1999 (control plots were 26 percent by 1999). Understory vegetation increased slightly or had no change over time depending on treatment and climatic factors. Tebuthiuron treatments at these rates did not have long-term adverse effects on soil microflora. Tebuthiuron was mobile and its concentration remained twice as high in the zero to 15 cm soil samples as compared to deeper soil samples (>15 to 30 cm). Overall, tebuthiuron levels dropped about six fold from the second to third year after application (to 0.1 ppm). The preliminary conclusion is that tebuthiuron applied at these low concentrations can maintain mountain big sagebrush cover at meaningful levels without damage to the understory and soil micoflora.

Introduction

Tebuthiuron, N[5-(1,1-dimethylethyl)-1,3,4-thidiazol-2-yl]-N-N'-dimethylurea, is an herbicide that is used to thin dense stands of woody plants in rangelands in order to increase access to forage plants in the understory. It has been used in many studies to control big sagebrush, *Artemisia* spp., (Britton and Sneva 1984; McDaniel and Balliette 1986; Whitson and Alley 1984). Tebuthiuron is applied to the soil surface as pellets and thus its movement through soil can be affected by soil type and moisture (Johnsen and Morton 1989). Studies have shown that tebuthiuron is highly mobile in soil and levels typically decrease rapidly in 1 to 4 years after treatment (Emmerich and others 1984; Johnsen and Morton 1989). Despite this initial rapid decrease, in one study Johnsen and Morton (1989) have shown that concentrations were detectable throughout the study and actually began to steadily increase 9 to 11 years after treatment (11 year study). It has been suggested that this may be due to slow release from soil particles or from slowly decaying litter (Garcia and Lee 1979). Long-term effects of residual tebuthiuron may be of particular importance in arid soils.

It is therefore important to determine if low doses of the herbicide can still be effective in thinning woody species while minimizing any possible detrimental effects on the community as a whole. This study examined the impact of tebuthiuron in low concentrations (0.18 to 0.63 kg AI per ha) on high elevation mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana*) communities. In addition to its effectiveness against the target species, this study sought to determine if tebuthiuron has an impact on understory plants or on soil microflora.

The functional composition and diversity of plant communities have been shown to be the principal factors controlling productivity (Hooper and Vitousek 1997; Tilman and others 1997). These findings suggest that management practices affecting plant diversity and composition can have a profound effect on ecosystem processes. Although compounds such as herbicides and insecticides are, in general, less detrimental to microbial activities, some soil microbial processes and properties may be affected (Hicks and others 1990; Nemes-Kosa and Cserhati 1995). This possible impact on soil microbial communities should be evaluated in view of their role in sustaining the global cycling of matter and their varied functions in supporting plant growth. Assessment of the impact of changes in plant communities on soil community structure is difficult because of the highly diverse nature of microbial communities in soil. This may be facilitated by investigation of specific groups of microorganisms or specific activities. Microorganisms involved in the nitrogen cycle are of particular concern since nitrogen is usually

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the limiting nutrient in soils and the rate of microbial activity is limited in arid ecosystems. Due to the limitations in scope and budget, efforts were concentrated on limited areas and methodologies. Hence, this study focused on determining the impact of tebuthiuron treatment on total heterotrophic microbial populations and aspects of nitrogen cycling.

Species abundance and diversity measurements are sensitive indicators of environmental conditions. However, the conventional methodologies investigating diversity of microbial communities have significant shortcomings. These include the time and resources required to quantify and identify microbial species of many different groups and extensive analysis of numerous samples from different treatments. Furthermore, a significant bias is invariably introduced by the selective cultivation, analysis, and interpretation of results. We acknowledge these shortcomings in this report.

The objectives of this study were (1) to evaluate the effectiveness of low application rates of tebuthiuron in thinning dense, high elevation stands of mountain big sagebrush, (2) to evaluate the impact of tebuthiuron on understory herbaceous plants and soil heterotrophic bacteria and fungi, ammonium oxidizing microflora, nitrifying/nitrate reducing (combined assimilatory and dissimilatory processes) microflora, photosynthetic microflora, and mycorrhizal associations, and (3) to examine the movement and stability of tebuthiuron in the soil.

Methods

Study Sites

Four study sites were established in the Fish Lake National Forest and adjacent Bureau of Land Management holdings: three were on the Monroe Mountain portion of the Sevier Plateau, designated as the Plateau, Big Flat and Burnt Flat treatment sites. The fourth site was on the nearby Fish Lake Plateau and is referred to as the Fish Lake treatment site. The elevations of the sites are: Plateau, 8,125 ft (2,508 m); Fish Lake, 8,800 ft (2,716 m); Burnt Flat, 9,300 ft (2,870 m); and Big Flat, 9,400 ft (2,901 m). All sites were dominated by mountain big sagebrush. Soils were characterized as loams with 40 to 48 percent sand, 20.56 to 26.56 percent clay, and 29.16 to 33.44 percent silt in all but the upper depths (zero to 15 cm) at Plateau, which was a clay loam (29.56 percent clay and 26.42 to 28.16 percent silt). Soil pH ranged from 5.9 to 6.6. Upper soils typically had more organic matter than did corresponding deeper soil (>15 to 30 cm). Overall percentages of soil organic matter are: Plateau, 2.34 to 3.88 percent; Fish Lake, 3.57 to 9.36 percent; Big Flat, 4.39 to 5.32 percent; and Burnt Flat, 9.46 to 10.70 percent.

Tebuthiuron Application

Study plots measuring 50 x 100 ft (15.24 x 30.48 m) were established in a random manner and tebuthiuron was applied as pellets in the following concentrations and times at the four sites: Plateau site, 0.0, 0.3, 0.5, and 0.7 pounds active ingredient (AI) per acre (0.0, 0.27, 0.45, and 0.63 kg AI per ha) applied Sept. 29, 1994; Fish Lake site: 0.0, 0.2, 0.4, and 0.6 pounds AI per acre (0.0, 0.18, 0.36, and 0.54 kg AI per ha) applied Sept.13, 1995; Big Flat site 0.0, 0.3, 0.5, and 0.7 pounds AI per acre (0.0, 0.27, 0.45, and 0.63 kg AI per ha) applied July17, 1996; and Burnt Flat site 0.0, 0.3, and 0.5 pounds AI per acre (0.0, 0.27, and 0.45 kg AI per ha) applied July17, 1996. Big Flat and Burnt Flat sites also included replicate plots.

Vegetation Study

Plant ecological data were collected in the summers prior to soil sampling. In 1996, vegetation surveys were conducted before tebuthiuron was applied to the Big Flat and Burnt Flat treatment sites. Vegetation data were collected over a 4-year period from 1996 to 1999.

Control and treatment plots (about 0.4 ha each) were set up in areas of level terrain and homogeneous vegetation and soil. Within each plot a circular 0.01 ha subplot was established in a random manner and centered on a steel rebar post. Subplot perimeters were at least two meters from the plot boundary. Data collected in the subplots included vascular plant species present and percent cover partitioned among shrubs, grasses, forbs, bare soil, liter, rock (>2 cm), and cryptogams (Daubenmire 1959; McArthur and Sanderson 1992, 1995). Density of plant species was determined from 4 m^2 quadrats located 1 m in each cardinal direction from the center of the circular subplots. Sagebrush cover was monitored along two 45 m diagonal line transects in each treatment and control plot. Means separations of data were performed using Proc Means procedures of the SAS statistical package (SAS 1989).

Soil Sampling

Soil sampling was done during site visits at the four sites in August 1996, 1997 and 1998. In 1996, soil samples were taken at two depths (0 to 15 cm and >15 to 30 cm) at the base of two selected healthy looking plants (healthy) in the control plots, and one healthy, two slightly unhealthy looking plants (medium affected), and one unhealthy looking plant (affected) in each of the treated plots at the Fish Lake and Plateau sites. The plants were tagged and samples were taken at the base of the same plants in subsequent years. In addition, pooled (composite) samples from each plot were taken each year. In 1996 only composite samples were taken at the Big Flat and Burnt Flat sites. In the subsequent 2 years, 1997 and 1998, a full sampling approach (healthy, medium affected, affected and composite) was taken at all treatment sites.

Two to four pounds of soil were collected per sample and put into plastic airtight bags. Each sample was then mixed thoroughly and split into two separate labeled bags and transported on ice to the laboratory. One set was used for soil chemical analysis and the other for microbial analysis. Soil samples were kept refrigerated until tests were conducted.

Tebuthiuron and Soil Analysis

Tebuthiuron and its metabolites were extracted from soil samples with acidified methanol. It was quantified using a

Waters HPLC (Loh and others 1980). Soil classification (texture class) was made based on percent sand, clay, and silt. In addition, percent organic matter, pH, and electrical conductivity were determined.

Microbial Analysis of Soil

Total Heterotrophic Bacteria and Mold Counts—Serial dilutions of soil samples were made and surface plated on Trypticase Soy Agar (TSA) and Sabouraud Dextrose Agar (SAB). Plates were incubated at 30 °C for 72 hours. Total bacterial and fungal counts were made. The plates were reincubated and checked again after 5 days to observe slow colony forming organisms. The counts were amended as required.

Estimation of Nitrifying and Nitrate-Reducing Microbial Populations—Soil samples were taken from the base of healthy plants in control plots, and healthy and affected plants in the highest treatment plot at each site. The MPN method described by Alexander (1982) was used and an estimation of nitrifying populations was made using the method described by Schmidt and Belser (1982). A total of 25 tubes of each medium, ammonium oxidizer and nitrite oxidizer media, for each soil sample were used. Samples were incubated at room temperature with light.

The presence of ammonium was determined in incubated samples using Nessler's reagent, nitrite and nitrate were detected using modified Griess-Ilosvay reagents, and diphenylamine, respectively (Schmidt and Belser 1982). A sample was considered positive for ammonium oxidation if nitrite and/or nitrate were detected after incubation in ammonium medium. A sample was considered positive for nitrite oxidation when all nitrite was exhausted from the medium or nitrate was detected in the total absence of nitrite in the nitrite medium. Complete oxidation to nitrate and subsequent removal of nitrate (assimilatory reduction by incorporation into cell material and/or dissimilatory reduction to N2 gas) could be concluded by the absence of nitrite and nitrate in nitrite medium.

Estimation of Photosynthetic Microbial Populations (Algae and Cyanobacteria)—During examination of plates and MPN tubes in 1996 and 1997, green colonies with mixed bacterial and photosynthetic microorganisms were seen on some plates and green-pigmented growth was observed in several MPN tubes. The most vigorous growth was found in nitrite medium when incubated in light. This provided an opportunity to compare the photosynthetic microbial communities in samples from control and the highest treated plots at each site using the MPN method. This was done using samples taken in 1998.

Mycorrhizal Association—In the first year (1996) root samples of more than 20 plant species from control plots at the Fish Lake and replicate Big Flat sites were collected and examined for the presence of mycorrhizal associations. Each root sample was washed thoroughly with tap water through a 1 mm sieve, retaining the roots on top of the sieve. The washed roots were preserved in FAA (5 percent Formalin, 5 percent Acetic acid, 40 percent Ethanol, 50 percent H_2O) until needed.

Ectomycorrhizae: Each root sample was spread out in a petri dish with enough FAA to keep roots immersed. Small pieces of root tips were stained in lacto-phenol-cotton blue, transferred to clear lacto-phenol, mounted on a slide in clear lacto-phenol under a coverslip, and examined under a microscope for the presence of ectomycorrhizal associations.

Endomycorrhizae and Vesicular-Arbuscular Mycorrhizae (VAM): Small pieces of root tip were placed in perforated microfuge tubes. The roots were placed in a beaker containing 10 percent KOH and autoclaved at 121 °C for 10 minutes. Roots were then rinsed in tap water, bleached in 30 percent hydrogen peroxide for 10 minutes, and rinsed with tap water three times. The roots were covered in 1 percent HCl for 3 to 4 minutes without rinsing and stained with Trypan Blue for 20 minutes in a hot water bath (90 °C). Roots were then placed in 40 percent glycerin and examined microscopically. Photomicrographs were taken for documentation.

In 1997 and 1998, the above procedure was used to examine the mycorrhizal associations in selected plant species in control and the highest treatment plots at all four sites.

Results

Soil Concentrations of Tebuthiuron

Soil chemical analyses from the Plateau and Fish Lake sites were pooled, as were those from the Big Flat and Burnt Flat sites (table 1). Tebuthiuron was detected up to 100 ft (30.48 m) from the areas of application after the first year. Tebuthiuron concentrations remained significantly higher (at least double) in the upper 15 cm of soil as compared with deeper soil samples throughout the duration of the study. The final measured tebuthiuron concentrations (2 years after application) were significantly (80 to 83 percent) less than those measured in the previous year. There was no significant difference in tebuthiuron concentrations among treatments.

Vegetation Study

Plant community sampling spanned a 4-year period (1996 to 1999). Results from the Big Flat and Burnt Flat sites are presented. Similar trends were seen at the other sites. The percent cover of mountain big sagebrush (table 2) was not

Table 1—Tebuthiuron concentrations (ppm); treatment is in pounds of active ingredient per acre.

Location	Time frame	Soil depth 0 to 15 cm		Soil depth >15 to 30 cm		Significance[a]
Plateau and Fish Lake	All years	0.40		0.16		NS
	1997–1998	0.50		0.09		*
Treatment						NS
Big and Burnt Flat	All years	0.54		0.27		*
	1997–1998	0.69		0.11		*
Treatment		0	0.3	0.5	0.7	
		0.14	0.38	0.38	0.33	NS

[a] * = $P < 0.05$, NS = not significantly different.

Table 2—Percent cover of mountain big sagebrush after tebuthiuron treatment at Big Flat and Burnt Flat treatment sites.

	Year[b]			
Treatment level[a]	1996	1997	1998	1999
0	22.9 A	24.6 A	24.9 A	25.9 A
0.3	23.3 A	15.2 B	12.3 B	11.0 B
0.5	24.9 A	13.1 B	7.2 B	6.3 B
0.7	19.2 A	7.0 B	5.1 B	4.3 B

[a]Active ingredient in pounds per acre.
[b]Different letters in rows and columns indicate significantly different mean values, $P < 0.05$.

significantly different among plots prior to herbicide application. In each subsequent year after tebuthiuron application sagebrush cover decreased with increasing tebuthiuron concentration, while remaining almost constant in control plots. Treated plots had significantly less sagebrush cover than did control plots.

The density of perennial grasses (table 3) was not significantly different with increased tebuthiuron concentrations. Overall, the density was highest in 1998, but there was no significant difference between years. The density of native perennial grasses also peaked in 1998, but again there was no significant difference among years (McArthur and Sanderson, data on file at Shrub Sciences Laboratory). There were, however, significantly fewer native perennial grasses in the highest herbicide treatment plots as compared with control plots. The densities of native perennial forbs and native annuals were not significantly different among treatments (McArthur and Sanderson, data on file at Shrub Sciences Laboratory). The density of native perennial forbs increased significantly while the density of native annuals decreased significantly in later years (1998 an 1999) as compared with earlier years.

Soil Microbial Study

Results from all four sites were similar. Total soil heterotrophic bacterial counts ranged from 10^6 to 10^7 as seen on TSA with the exception of 1998 (zero to 15 cm) at Big Flat, where all plots produced 10^4 to 10^5 bacteria (data not shown). Total soil mold counts ranged from 10^4 to 10^6. Data from Fish Lake will be presented and are representative of the other sites. Total heterotrophic bacterial counts (fig. 1) and total mold counts (fig. 2) did not differ significantly among treatments, years, or soil depth.

There was essentially no significant difference in the MPN of nitrite oxidizers (fig. 3) or in the MPN of nitrate reducers (fig. 4) isolated from soil near plants in control and heavily treated plots or in samples collected in different years. There were slightly more nitrite oxidizers and nitrate reducers in the soil samples from the base of healthy plants in the treated plot as compared with that of affected plants in the same plot and controls. There was virtually no difference in the detected MPN of ammonium oxidizers among treatments or between healthy and affected plants (fig. 5). The detected MPN of ammonium oxidizers varied slightly between years, but the results were very consistent among treatments and plants.

Table 3—Density of perennial grasses after tebuthiuron treatment at Big Flat and Burnt Flat treatment sites.

	Year[b]			
Treatment level[a]	1996	1997	1998	1999
0	180 B	287 AB	328 A	308 A
0.3	147 B	210 A	235 A	228 A
0.5	163 A	208 A	228 A	206 A
0.7	74 A	136 A	139 A	131 A
Year	155 A	225 A	251 A	236 A

[a]Active ingredient in pounds per acre.
[b]Different letters in rows indicate significantly different mean values, $P < 0.05$.

Mycorrhizal Study

There were essentially no differences found in the quantity or type of mycorrhizal associations found on root samples among control and treated plots or in different years (data not shown). Virtually all root samples examined had endomycorrhizal associations.

Photosynthetic Soil Microflora

In the last year of the study (1998), the MPN, based on green-pigmented growth in nitrite tubes, was used to compare and quantify photosynthetic soil populations. The MPN of control and the highest treated plots from all sites showed no persistent effect among treated and untreated plots on photosynthetic microbial populations.

Discussion

Tebuthiuron was shown to be mobile in the soils in this study. This concurs with a previous study by Whisenant and Clary (1987), which found that tebuthiuron is dispersed over greater lateral distances in loam soils of low organic content (such as those in this study) than those of high organic content. The results of this study are consistent with several other reports (Bovey and others 1978; Garcia and Gontarek 1975; Johnsen and Morton 1989; Whisenant and Clary 1987) showing that tebuthiuron is found in higher concentrations in upper soil levels. Emmerich and others (1984) found that tebuthiuron applied at 0.84 kg AI per ha decreased by 62 percent after 21 months. The results of the present work showed a similar trend, an 80 to 83 percent decrease 2 years after treatment. Johnsen and Morton (1989) showed that tebuthiuron can persist in the soil for many years (particularly in a semiarid environment) and exert herbicidal activity on vegetation for several years, it is therefore necessary to determine minimum dosages required to achieve the desired effects while avoiding over-dosing to prevent undesirable prolonged effects on an ecosystem.

The present study showed that low levels of tebuthiuron were extremely effective in decreasing the cover of mountain big sagebrush for at least 3 years. Overall, sagebrush cover

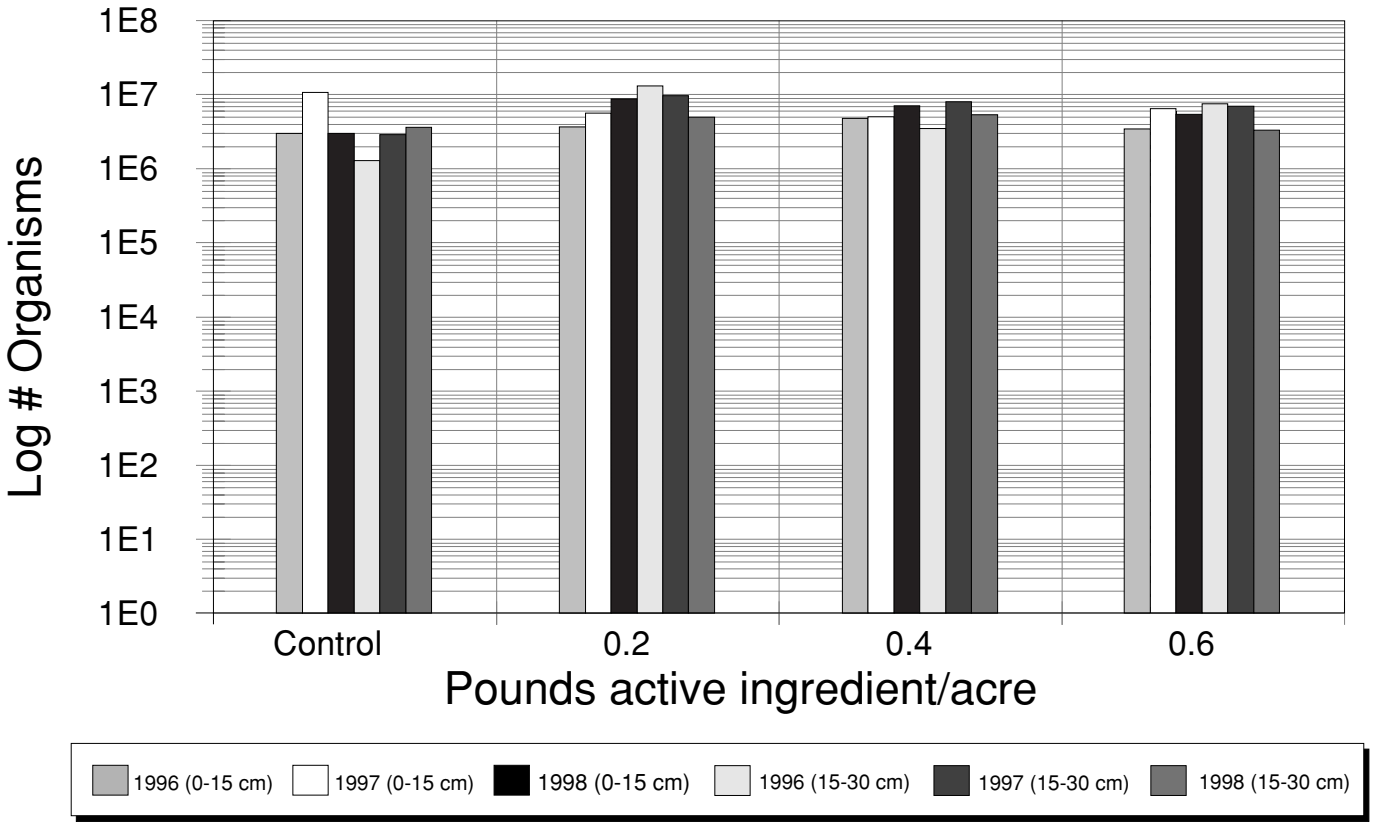

Figure 1—Total soil heterotrophic bacterial counts after tebuthiuron treatment at the Fish Lake treatment site. Data from soil near healthy, medium affected, and affected plants were similar and are pooled.

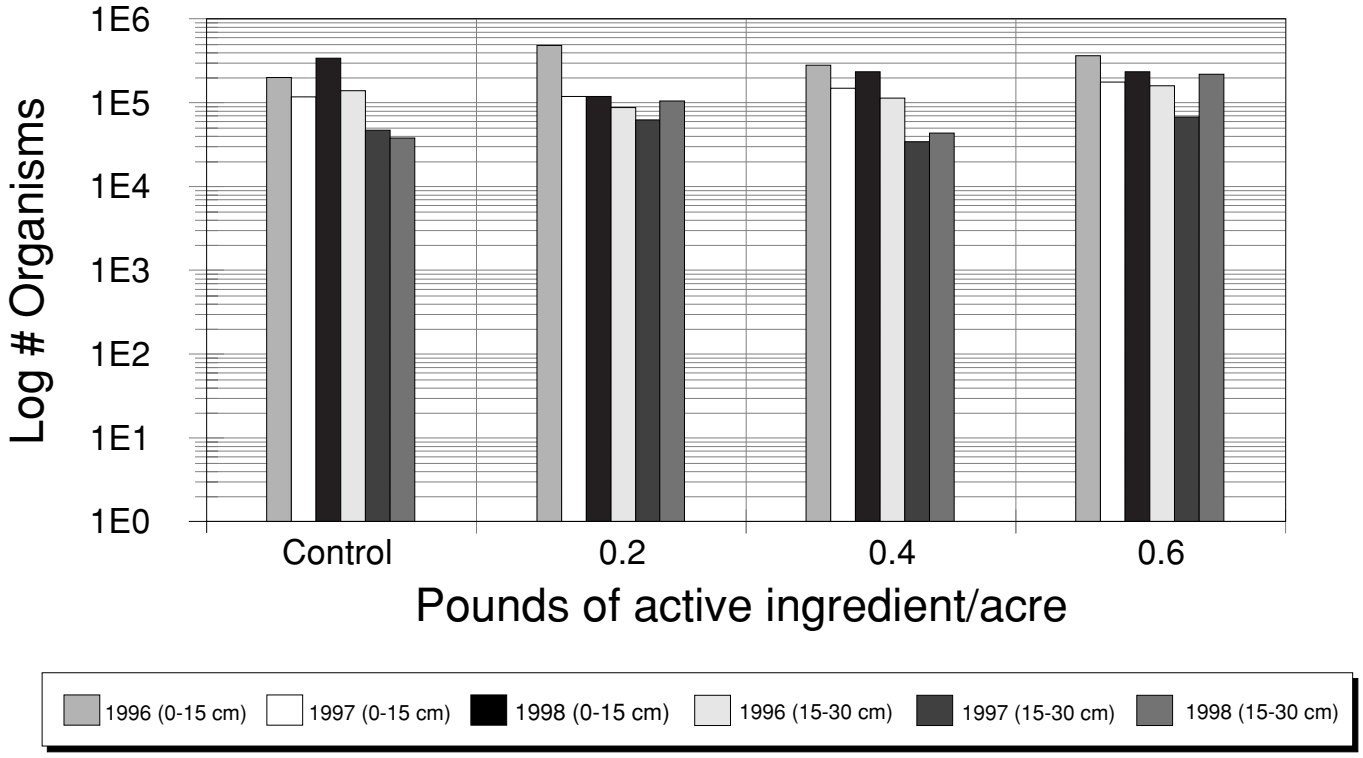

Figure 2—Total soil mold counts after tebuthiuron treatment at the Fish Lake treatment site. Data from soil near healthy, medium affected, and affected plants were similar and are pooled.

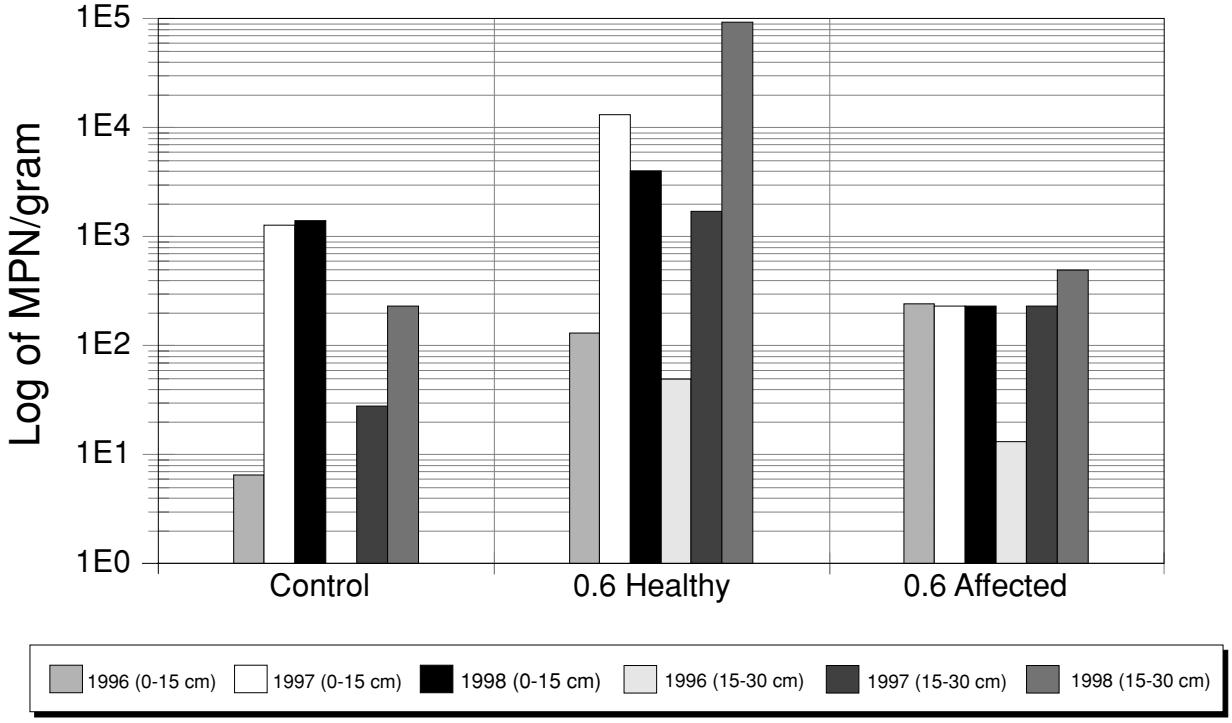

Figure 3—Most Probable Number (MPN) of nitrite oxidizers after tebuthiuron treatment at the Fish Lake treatment site. Soil samples were taken from the base of healthy control plants and healthy and affected plants in the highest treatment plot (0.6 pounds active ingredient per acre).

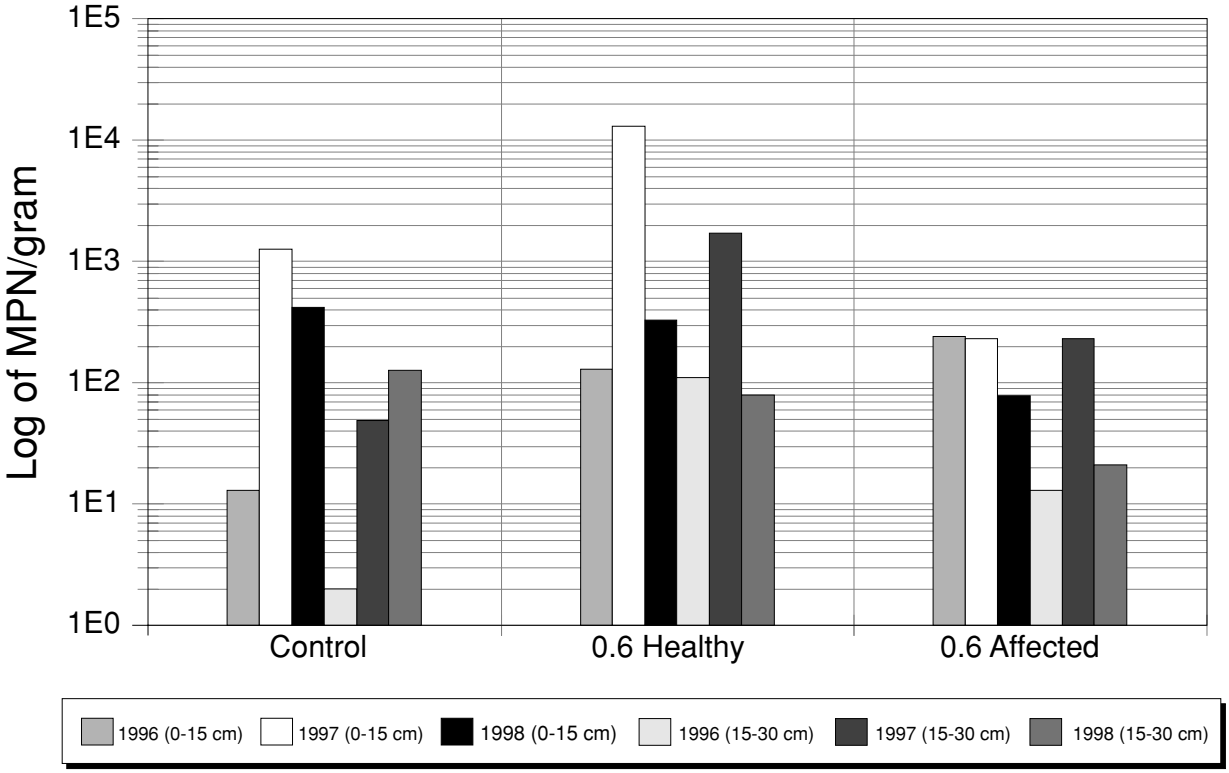

Figure 4—Most Probable Number (MPN) of nitrate reducers after tebuthiuron treatment at the Fish Lake treatment site. Soil samples were taken from the base of healthy control plants and healthy and affected plants in the highest treatment plot (0.6 pounds active ingredient per acre).

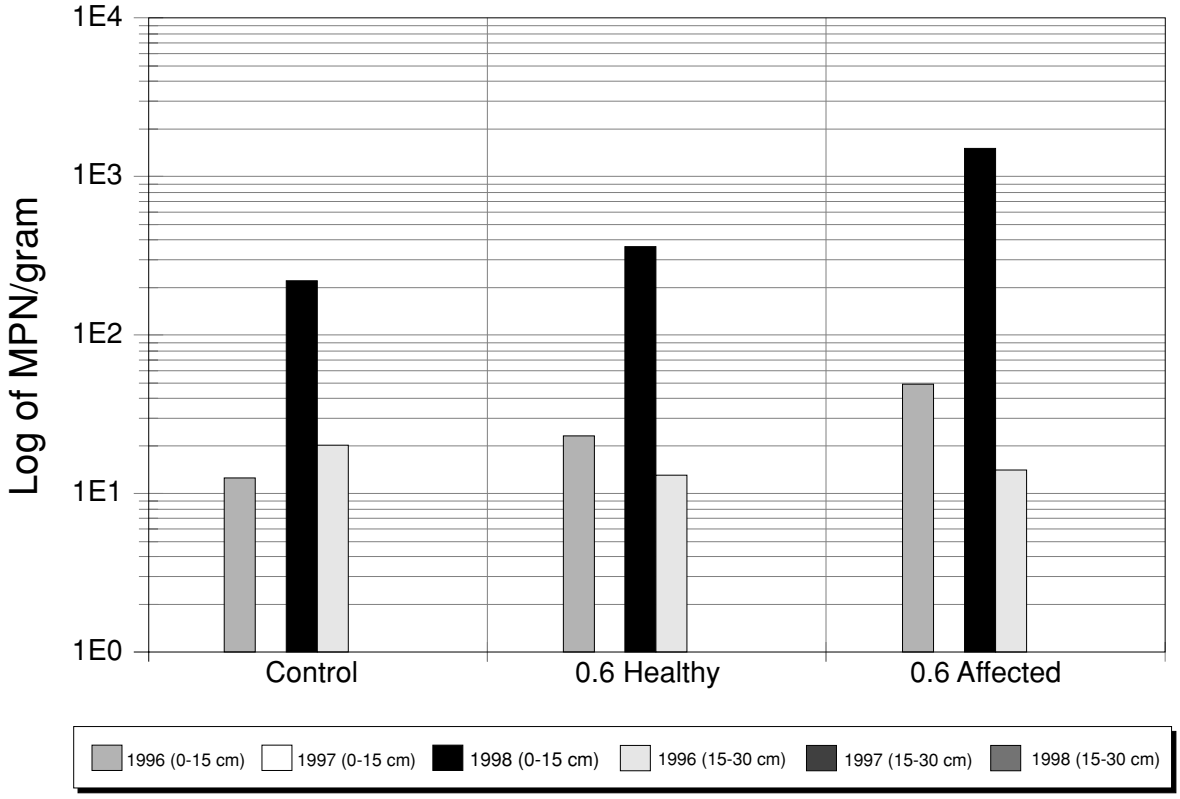

Figure 5—Most Probable Number (MPN) of ammonium oxidizers after tebuthiuron treatment at the Fish Lake treatment site. Soil samples were taken from the base of healthy control plants and healthy and affected plants in the highest treatment plot (0.6 pounds active ingredient per acre).

was less in treated plots as compared with controls. There was a trend, although not significant, for sagebrush cover to decrease with increased herbicide concentration. Considering the long-term study mentioned above it could therefore be a concern that tebuthiuron may continue to exert its effects for a long time and may decrease target species to lower levels than necessary or desired. The study also showed that concentrations above the lowest level used here may not be necessary and concentrations lower than those used in this study may actually achieve the desirable results.

The densities of perennial grasses and native perennial grasses were greater after treatment, though not significantly so. The concentration of tebuthiuron had no significant effect on these groups except that there were significantly fewer native perennial grasses in the highest treatment level. In the second and third years after treatment there were significantly more perennial forbs and significantly fewer native annuals than in earlier years. The level of tebuthiuron did not have any significant effect on these groups. It is probable that the annuals declined as competition with perennial understory plants increased. These results suggest that tebuthiuron concentrations as low as 0.27 kg AI per ha could be effective in reducing big sagebrush cover, while having very little adverse effects on herbaceous plants in the community.

It is widely accepted that analysis of changes in the composition of a community can be used to characterize its response to stress. In order to observe effects by quantifying changes in microbial biomass or in the general metabolic activities of microbial communities, a variety of tests have been recommended (Gerber and others 1991) and employed. Some may argue that species abundance and diversity are the most sensitive indicators of environmental conditions and that a community's composition and diversity are most significantly represented by the nucleic acid profiles of organisms present in the soil (Engelen and others 1998). We acknowledge that comprehensive studies employing the most advanced and proven methodologies are required to derive the most meaningful and accurate conclusions. However, in the absence of studies on the impact of tebuthiuron on microbial populations and activities, and a limited scope and budget, results generated from the present study are a step in the right direction.

Our work indicates that neither heterotrophic microbial populations (bacteria and mold) nor nitrifying activities at these sites were affected by tebuthiuron. This is in agreement with the general view that herbicides are less detrimental to microbial activity and, under certain conditions, may even stimulate certain activities (Hicks and others 1990).

The low ammonium oxidizer populations detected in the present study could be attributed to inhibition of ammonium-sensitive fractions (Phillips and others 2000; Suwa and others 1994) in the media used to determine MPN and the low number of ammonium oxidizers in semiarid land in general. Hence, our data do not represent total ammonium oxidizers, but only the populations that are not adversely affected by the 0.5 g/l ammonium used in the medium (Schmidt and Belser 1982). The apparent absence of ammonium oxidizers in some samples determined by MPN in ammonium medium could be misleading. It is likely that the removal of nitrate, as it is produced from further oxidation of nitrite, is responsible for these results. The consistency of data obtained from control and the highest treated plots indicate that tebuthiuron does not affect ammonium oxidizers.

Although the present study did not determine microbial composition of samples or a profile of microbial diversity, the results of the comparison of total heterotrophic counts and nitrifying activity contribute to the available data on the possible ecological impacts of tebuthiuron.

Due to the fact that tebuthiuron is an herbicide, there is reason to suspect that it may have possible adverse effects on photosynthetic microbial populations in treated soil. The role of cyanobacteria and algae in nitrogen fixation and the cryptogamic crust structure is well known. However, there is limited information regarding the impact of tebuthiuron on green algae. Price and others (1989) studied its effects on green algae found in playa lakes. This study indicated that runoff from agricultural fields treated with tebuthiuron concentrations greater than 2.24 kg AI per ha may adversely affect the green algae communities of playa lakes. To our knowledge, there are no studies addressing the effect of tebuthiuron on photosynthetic microorganisms in the soil. There were no significant differences between photosynthetic microbial population sizes at each site when the MPN of the highest treated and control plots were compared. The results may even suggest a slight increase in treated sites by the end of the third year. Therefore, in the absence of data to assess photosynthetic microbial populations in all 3 years, we could only conclude that tebuthiuron does not have a lasting effect at concentrations used in this study.

The results indicate that understory vegetation is not affected after 3 years and that all plant species sampled show mycorrihzal associations. Therefore, one could conclude that mycorrhizal fungi in the soil and their associations are not affected by tebuthiuron application.

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