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# Atrazine impacts on shortgrass prairie microcosms

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## Abstract

**Alterations in shortgrass ecosystem structure and function following long-term use of atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine,2,4-diamine] for increased secondary productivity raised concerns about sustainability of the practice. A microcosm approach was designed to 1) model the direction and temporal features of blue grama [*Bouteloua gracilis* H.B.K.) Lag. ex Steud.] biomass accumulation and tissue N, nitrate reductase activity, chlorophyll, total nonstructural carbohydrate, and phosphorus (P) levels following atrazine applications of 0.84 and 2.24 kg ha$^{-1}$ and hydroxyatrazine (6-hydroxy-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine,2,4,-diamine) at 1.12 kg ha$^{-1}$, and 2) to relate plant growth and metabolism changes to possible short- and long-term modifications of soil microbial abundance and activities associated with C and N transformations. Atrazine applied to dormant plant-soil microcosms reduced below-ground (crown plus root) biomass and shoot total nonstructural carbohydrate levels during regrowth. Atrazine application increased shoot chlorophyll content, N levels, and nitrate reductase activity, but not total plant N content. Decreased belowground biomass accumulation, and increased shoot N and nitrate reductase activity levels were linked to decreased total nonstructural carbohydrate availability. Total plant P levels were highest at the intermediate atrazine rate. Differences in soil microbial biomass and activities, and chemical properties resulted primarily from presence of blue grama and duration of plant regrowth. Soil nitrifying activity was depressed in soil previously exposed to atrazine whether or not blue grama was present. Hydroxyatrazine was not identified as an important factor in observed plant or soil changes. Atrazine may alter shortgrass system structure and function by immediate impacts on primary producers and long-term impacts on soil microbial processes.**

**Key Words: blue grama, 6-chloro-*N*-ethyl-*N'*-(1 methylethyl)-1,3,5-triazine,2,4-diamine, hydroxyatrazine, sublethal effects, shortgrass steppe, long-term herbicide effects**

The *s*-triazine herbicide atrazine (6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine,2,4-diamine) became an important range management tool after the rangeland label was obtained in 1976. Atrazine in sublethal doses reportedly induces growth and increases protein content of desirable plant species on California rangeland (Kay 1971) and shortgrass range dominated by blue grama [*Bouteloua gracilis* (H.B.K.) Lag. ex Steud.] in northeastern Colorado (van der Sluijs 1972, Tapia 1973, Houston and van der Sluijs 1975, Houston and Hyder 1976). Atrazine also reportedly reduces soil moisture depletion of blue grama sods and protects blue grama from drought stress, when combined with N fertilization (Brakken 1976, Hyder et al. 1976). A long-term study (1977-1985) established at the Central Plains Experimental Range, Weld County, Colo. demonstrated that increased blue grama yield and beef production were maintained after unwanted species were eliminated from blue grama sods and results from soil analyses suggest long-term atrazine application altered C and N cycling (Shoop, personal communication)[1].

Controlled environmental investigations have shown that immediate soil N concentration changes accompany atrazine application but the changes are temporary, an indirect result of changes in aboveground plant growth and metabolism, not alterations in soil microbial processes (Morgan and Knight 1991, Knight et al. 1993). Although these studies have enhanced understanding of atrazine effects on blue grama physiology and soil microbial processes, from the perspective of shortgrass ecosystem structure and function, several issues have not been investigated that influence whether or not repeated use of atrazine, or herbicides with similar mode of action, is a sustainable practice. These issues include 1) nature of belowground plant response and interaction with soil microbial processes, 2) relationship between timing of atrazine application and expression of the plant response, and 3) cumulative nutrient cycling impacts of repeated atrazine applications.

We designed a controlled microcosm approach to simultaneously study the relationship between aboveground and belowground blue grama growth and development, and microbially-mediated soil N and C transformations in shortgrass soils. This relationship was studied for soil that had a history of long-term atrazine exposure and soil never exposed to the herbicide. Our hypothesis was that alteration in soil C and N status following repeated sublethal atrazine applications to blue grama sods results from an interaction between the plant and associated soil microbial community. Objectives of the study were 1) to model the

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direction and temporal features of alterations in blue grama biomass accumulation and C and N dynamics induced by sublethal atrazine applications to plant-soil microcosms and, 2) to relate these alterations to possible short- and long-term changes in soil microbial abundance and activities associated with C and N transformations.

## Materials and Methods

### Experimental Design

Treatments were arranged factorially in a randomized complete-block design (Steel and Torrie 1980) in a plant growth chamber with a controlled environment. Treatments included plant-soil and soil-only microcosms treated with atrazine at 0, 0.84, and 2.24 kg $ha^{-1}$, and 1.12 kg $ha^{-1}$ hydroxyatrazine. Each treatment was applied to microcosms established from soil that had been previously treated with atrazine (treated soil) and soil that had no prior history of atrazine treatment (untreated soil). Treatments with plants present were harvested at 4 regrowth stages following atrazine application. Soil-only microcosms were studied at the first and last regrowth stages. Treatments were replicated over time in 4 blocks.

### Soil and Plant Collection

Ascalon sandy loam (fine-loamy, mixed, mesic Aridic Argiustoll) soil samples were collected from adjacent atrazine-treated and untreated pastures at the Central Plains Experimental Range. Pastures were components of a long-term study concerned with the feasibility of N fertilizer use for increased beef production on shortgrass range. Atrazine was aerially applied to treated pastures at 1.12 kg $ha^{-1}$ in October 1976, 1978, 1979, 1981, 1983, 1985, and 1987. An untreated pasture served as the control. Neither pasture had been fertilized. Both pastures were stocked on 1 June of each year with 12 to 15 month-old Hereford steers (*Bos tarus*) with an average weight of 226 kg. The pastures were grazed until October of each year by which time the herbage level had been reduced to approximately 400 kg $ha^{-1}$.

Soil collections were made on 4 Aug. 1987 and 4 Aug. 1988 at sites equidistant and perpendicular to the fenceline of the atrazine-treated and untreated pastures; a different location along the fence was used for soil collection at each date. Soil was removed from each pasture to a depth of 20 cm, coarse-sieved through a 5-mm mesh screen to remove surface plant material, air-dried and stored in polyethylene bags at 4°C. A sufficient quantity of soil was collected from each pasture at each date to establish blue grama greenhouse microsods (sixteen 8-liter pots with 6 kg soil each) and microcosms (thirty-two 4-liter pots with 2.7 kg soil each) for each of 2 treatment blocks. Chemical and physical soil analyses were performed each year (Table 1).

**Table 1. Properties of Ascalon Sandy Loam soil samples collected 4 Aug. 1987 and 4 Aug. 1988 from long-term atrazine-treated and untreated pastures at Central Plains Experimental Range, Weld County, Colo.**

| | Pasture | | | |
|---|---|---|---|---|
| | At-treated | | Untreated | |
| Property | 1987 | 1988 | 1987 | 1988 |
| Texture | SL[1] | SL | SL | SL |
| $pH_w$ (30 g soil:60 ml water) | 7.2 | 7.1 | 7.0 | 6.8 |
| Organic carbon, g $kg^{-1}$ | 7.2 | 9.0 | 7.8 | 9.4 |
| Total nitrogen, g $kg^{-1}$ | 0.63 | 0.76 | 0.82 | 0.88 |
| Cation exchange capacity, cmol $kg^{-1}$ | 9.0 | 9.8 | 10.0 | 10.1 |

[1]SL = sandy loam

**Table 2. Microcosm establishment, treatment, and harvest schedule for all blocks.**

| Days | Activities/Environmental conditions |
|---|---|
| 1-74 | -Microcosm establishment<br>--16 with 2.7 kg soil from treated pasture + 12 blue grama tillers<br>--16 with 2.7 kg soil from untreated pasture + 12 blue grama tillers<br>--Microcosms maintained in greenhouse |
| 75-99 | -Growth chamber acclimation<br>--Microcosms moved to growth chamber on day 75<br>--30/13°C day/night temperature cycle; 14-hour photoperiod; average photon flux density at top of canopy = 1,000 µmol $m^{-2}$ $s^{-1}$; irrigated at 3-day intervals with 355 ml water (MPa at 9 cm = -0.010 to -0.012) |
| 100-114 | -Dormancy-induction period<br>--day temperature decreased to 26°C on day 100 then by 1°C/day<br>--night temperature decreased to 10°C and photoperiod to 12 hours on day 108 |
| 115-133 | -Dormancy period<br>--12/10°C day/night temperature cycle<br>--plants clipped to 5 cm on day 122<br>--atrazine and hydroxyatrazine applied on day 129 |
| 134-147 | -Dormancy-release period<br>--day/night temperature cycles reverse of dormancy induction period (above) |
| 148-185 | -Regrowth period<br>--30/13°C day/night temperature cycle |
| 186-246 | -Destructive harvests<br>--day 186 = 0-day harvest<br>--day 206 = 20-day harvest<br>--day 226 = 40-day harvest<br>--day 246 = 60-day harvest |

Uniform blue grama sods were collected from an exclosure at the Central Plains Experimental Range on the same days that soil collections were made. The exclosure had not been treated with atrazine. Microsods were established in the greenhouse by planting 48 bare-root tillers obtained from the field sods into 8-liter plastic pots that contained either the treated or untreated soil samples. Microsods were planted in such a manner that division into quarters yielded 12 tillers for establishment of each microcosm. Microsods were placed in a greenhouse and exposed to a combination of natural and artificial light and watered every 3 days until needed for microcosm establishment.

### Microcosm Establishment, Treatment, and Harvest

Microcosms were established, treated, and harvested according to the schedule outlined in Table 2. The schedule included incubation in a temperature regime designed to induce blue grama dormancy. Technical grade atrazine or hydroxyatrazine, dissolved in 200 ml distilled water, were added to the microcosm soil surface to achieve atrazine application rates of 0.84 and 2.24 kg $ha^{-1}$ and a hydroxyatrazine level of 1.12 kg $ha^{-1}$; an equal volume of distilled water was added to each control (0 kg $ha^{-1}$) microcosm. Microcosms were destructively harvested by clipping

shoot regrowth at the soil surface and separating regrowth from stubble. Crown and root tissues were washed over a fine screen after separation from the soil; roots were removed from crowns immediately. Fresh weight was recorded for all tissues prior to storage in polyethylene bags at 4°C. Soils were stored without drying at 4°C in polyethylene bags.

### Plant Analysis

At each harvest, fresh tissues were cut into 3-mm pieces within 24 hours and subsampled for shoot dry weight (g per microcosm), and crown and root dry weights (g per microcosm); these were determined on samples oven-dried for 24 hours at 105°C. Chlorophyll a, chlorophyll b, and total chlorophyll concentrations (mg $kg^{-1}$ fresh weight) were estimated for fresh tissue as described by Hiscox and Israelstam (1979). Shoot and root nitrate reductase activities (*m*mol $NO_2^-$-N $kg^{-1}$ 2 $hours^{-1}$) were calculated as the difference in activity measured in vivo for endogenous and induced substrates (Bar-Akiva et al. 1970) with the following modifications. Fresh tissues were infiltrated with a solution (Hunter et al. 1982) containing 0.1 *M* phosphate buffer (pH 7.5), 1.0% (v/v) propanol, and 0.1% Triton X-100 for the determination of endogenous nitrate reductase activity; the endogenous solution was made to 0.1 *M* $KNO_3$ for the induced nitrate reductase activity assay. Shoot and root tissues were incubated at 30°C in each substrate. Fresh tissues were oven-dried for 24 hours at 60°C, ground, and subsampled for further analyses. Tissue N and P concentrations (g $kg^{-1}$ dry weight) were determined by the micro-Kjeldahl method and as molybdovanadophosphoric acid, respectively. Total nonstructural carbohydrate concentrations (g $kg^{-1}$ dry weight) were assayed by extraction with 0.2 *N* $H_2SO_4$ (Smith et al. 1964). An iodometric titration developed by Heinz and Murneek (1949), but using modified reagents (Association of Official Analytical Chemists 1965), was then used to determine the extractable total nonstructural carbohydrate concentration, which was expressed on a glucose-equivalent basis. Nitrogen, phosphorus, total nonstructural carbohydrate, and chlorophyll concentrations were multiplied by the appropriate plant tissue weight to express tissue contents on a microcosm basis.

### Soil Analysis

All analyses were performed or incubations initiated within 4 days of soil collection except for pH and nitrate reductase activity measurements that were made using air-dried soil. Gravimetric soil water content (g $kg^{-1}$) was determined for samples dried to constant mass (105°C). Soil microbial biomass C (mg C $kg^{-1}$) and biomass N (mg N $kg^{-1}$) were determined by chloroform fumigation-incubation (Jenkinson and Powlson 1976). The equations of Jenkinson and Powlson (1976) and Voroney and Paul (1984) were used to calculate biomass C and biomass N, respectively with $k_C = 0.45$ (Shan-Min et al. 1987) and $k_N = 0.41$ (Paul and Clark 1989). Soil respiration rates (μmol $CO_2$ $kg^{-1}$ $hour^{-1}$) were measured for samples after 6- and 12-day incubations (Anderson 1982). Potential N-mineralization (mg $NH_4^+$-N $kg^{-1}$) was determined as the difference between $NH_4^+$-N concentration at the end of a 14-day incubation (water-logged conditions at 30°C) and the initial $NH_4^+$-N concentration in the soil before incubation (Keeney 1982). Potential rates of nitrification (μg $NO_2^-$-N $kg^{-1}$ $hour^{-1}$) were determined using the chlorate inhibition technique described by Berg and Rosswall (1987). Soil nitrate reductase activity (mg $NO_2^-$-N $kg^{-1}$ 24 $h^{-1}$) was determined by the procedure of Abdelmagid and Tabatabi (1987). The modified Griess-Ilosvay method (Keeney and Nelson 1982) was used to determine accumulated $NO_2^-$-N for both the nitrification and nitrate reductase activity procedures. Ammonium-N and nitrate-N concentrations (mg $kg^{-1}$) were determined with a Lachat Flow Injection Autoanalyzer (Lachat Instruments, Mequon, Wisc.) for 2 *M* KCl soil extracts. Soil pH in water ($pH_w$) was obtained electrometrically (McLean 1982) for 30 g soil:60 ml distilled water. Detailed descriptions of all protocols are found in Miller (1990).

### Data Analysis

Data were analyzed using multivariate analysis of variance procedures (SPSS-X User's Guide 1988). For both plant and soil parameters, orthogonal polynomials were used to partition the atrazine application rate treatment sum of squares into linear, and quadratic components; interactions were partitioned and analyzed separately for atrazine and hydroxyatrazine. For plant parameters, the harvest day treatment sum of squares was partitioned into linear, quadratic, and cubic components. Orthogonal polynomial equations were developed with multiple regression to summarize the variables responsible for significant plant parameter responses and to facilitate 3-dimensional illustration of these responses. Since our purpose in using regression techniques was for trend summary and description rather than to find the 'best possible fit' of the data for predictive purposes (Mosteller and Tukey 1977), regression models were developed through the use of main effects, orthogonal polynomials, and interaction effects with significant ($P<0.05$) F test values following multivariate analysis of variance. Separate models were developed when effects caused by previous soil exposure to atrazine were significant for a particular plant parameter.

## Results and Discussion

### I Plant Parameters

Regression models developed from main effects were highly significant ($P<0.001$) in all cases except for root P content ($P<0.05$) (Table 3). These models allowed concise summary and illustration of key plant responses to atrazine application rate and harvest date or both. Previous soil exposure to atrazine was rarely identified as an important factor in modeling the plant responses. Proportionate reduction in error sums of squares ($R^2$) were generally lowest for parameters that involved crown tissue measurements and highest for shoot tissue measurements. Histograms and normal probability plots of the standardized residuals approximated normal distributions for all parameters studied.

Hydroxyatrazine had no significant effect on any measured plant parameters. Plants treated with hydroxyatrazine appeared morphologically similar to plants in control microcosms. Eastin and Davis (1967) applied hydroxyatrazine to 3 atrazine-tolerant species and found no consistent effect on plant dry weight or any of several plant N fractions studied. Leaf blades of atrazine-treated plants appeared longer and thinner than those of control or hydroxyatrazine-treated plants, and this effect was most pronounced at the 2.24 kg $ha^{-1}$ rate. Van der Sluijs (1972) reported a similar effect for atrazine-treated blue grama grown from seed in a greenhouse study.

#### *Biomass*

Blue grama shoot dry weight was not significantly affected by increasing rate of applied atrazine (Table 3). In contrast, root

Table 3. Regression models, F-test statistics, coefficients of determination ($R^2$) and standard errors (SE) for biomass, nitrogen (N), chlorophyll (CHL), nitrate reductase activity (NRA), phosphorus (P), and total nonstructural carbohydrate (TNC) contents or concentrations of blue grama tissue grown in atrazine-treated microcosms harvested at 4 regrowth stages (0, 20, 40, and 60 days).

| Y[1] | Regression model[2] | F | $R^2$ | SEy.x |
|---|---|---|---|---|
| TISSUE CONTENT | | | | |
| *Biomass, g DW* | | | | |
| Shoot | $\hat{Y}$= 1 89+0.0383HD | 107.1*** | 0.62 | 0.732 |
| Crown | $\hat{Y}$= 4.75-0.3702AT+0.0150HD | 8.59*** | 0.20 | 1.011 |
| *N, mg DW* | | | | |
| Shoot | $\hat{Y}$= 26.7+3.98AT | 18.9*** | 0.22 | 7.07 |
| Crown+root | $\hat{Y}$= 48.3+0.30HD | 15.4*** | 0.20 | 16.1 |
| Total | $\hat{Y}$= 78.7+0.31HD | 14.2*** | 0.19 | 16.4 |
| *Shoot N:Crown+Root N* | $\hat{Y}$= 534+91.3AT-2.29HD | 9.18*** | 0.23 | 209.7 |
| *Shoot TNC:N* | $\hat{Y}$= 8.46-2.66AT+0.02211HD+0.03803ATHD | 22.6*** | 0.46 | 2.47 |
| *P, mg DW* | | | | |
| Shoot | $\hat{Y}$= 6.29-0.5127AT+0.07242HD | 36.7*** | 0.53 | 1.82 |
| Root | $\hat{Y}$= 2.59+1.03AT-0.5459$AT^2$+0.01150HD | 4.67* | 0.20 | 0.745 |
| Total | $\hat{Y}$= 13.7+2.68(AT)-1.55$AT^2$+0.0900HD+0.0010$ATHD^2$ | 9.19*** | 0.41 | 2.57 |
| TISSUE CONCENTRATION | | | | |
| *N, g $kg^{-1}$ DW* | | | | |
| Crown | $\hat{Y}$= 6.71+0.8587AT-0.00675ATHD | 7.59*** | 0.14 | 1.62 |
| Root, U-Soil | $\hat{Y}$= 8.06+0.6088AT-0.00375$HD^2$+0.00006$HD^3$ | 9.32*** | 0.28 | 1.63 |
| *NRA, mmol (kg FW)$^{-1}$ (2 h)$^{-1}$* | | | | |
| Shoot | $\hat{Y}$= 446+54.0AT-14.7HD+0.1487$HD^2$ | 21.9*** | 0.55 | 183.2 |
| *CHL, mg $kg^{-1}$ FW* | | | | |
| CHL a | | | | |
| T-Soil | $\hat{Y}$= 840+606AT-9.82HD+0.0363$HD^2$-20.4ATHD+0.1861$ATHD^2$ | 154.7*** | 0.96 | 125.1 |
| U-Soil | $\hat{Y}$= 1013+287AT-22.7HD+0.2110$HD^2$-3.80ATHD | 65.4*** | 0.88 | 164.8 |
| CHL b | $\hat{Y}$= 443+148AT-8.66HD+0.0747$HD^2$-2.12ATHD | 103.0*** | 0.83 | 82.5 |

[1] Weights without denominators refer to total microcosm content. DW=dry weight, FW=fresh weight, T=soil with previous atrazine exposure, U=soil with no previous exposure to atrazine.
[2] HD=days after 38-day regrowth period; AT=atrazine application rate, kg $ha^{-1}$.
***, *significant at the 0.001 and 0.05 probability levels, respectively.

(Fig. 1a) and crown (Table 3) dry weight decreased linearly with increasing atrazine rate. This reduction in root and crown biomass influenced total microcosm biomass throughout the regrowth period for treated and untreated soil (Fig. 1b). All biomass components were significantly affected by harvest date. There were linear increases in shoot and crown dry weights with harvest date (Table 3), while the root biomass response was quadratic (Fig. 1a). Variation included in response models for all fresh weight biomass measurements were similar to those for dry weight (data not shown).

Crown and root biomass accumulations of blue grama treated with 2.24 kg atrazine $ha^{-1}$ were reduced to 61% and 73% of the control, respectively, at the earliest harvest date. Total crown plus root biomass accumulation, however, recovered to 90% of the control by the 40-day harvest date. Atrazine-induced disruption in below-ground biomass accumulation during early regrowth has important implications for long-term use of this herbicide on semi-arid shortgrass range. These perennating structures are important to plant vigor and competitive ability, especially in response to drought and grazing. Maintenance of a large root system for competitive ability may not be critical, since atrazine kills competing forbs and cool-season grasses. However, a deficiency in early-season root biomass could lead to critical reductions in both root exudates and sloughing which provides substrates for rhizosphere microorganisms (Biondini et al. 1988). Root exudates that maintain a rhizosphere microflora of low C:N are thought to be a controlling factor of nitrogen-cycle dynamics in the shortgrass ecosystem dominated by blue grama (Milchunas et al. 1985). Reduced root C inputs in grazed grasslands decrease N immobilization and increase net N mineralization and can limit C availability to decomposers (Holland and Detling 1990). This link may explain the 2-fold increase in ammonium- and nitrate-N concentrations in shortgrass soil and lower soil organic C levels observed following long-term atrazine application to blue grama pastures (Shoop, personal communication).

### *Nitrogen and Total Nonstructural Carbohydrates*

Atrazine application increased shoot N concentration (Fig. 1c). Shoot N concentration responses changed through time; the greatest difference from the control occurred at the first harvest date. Crown N concentration increased with increasing rate of applied atrazine during early regrowth (Table 3). Root N concentration was not influenced by atrazine applied to soil with previous exposure to atrazine, but increased with increasing atrazine rate during early regrowth in soil that had never been exposed to the herbicide (Table 3).

When plant N dynamics were examined for the microcosms, shoot N content was positively linearly related to atrazine rate while crown plus root N content increased with harvest date (Table 3). Increased shoot N content elevated total plant N content approximately 9% above the control with 2.24 kg atrazine $ha^{-1}$. However, since crown plus root biomass was depressed by increasing atrazine rate, and shoot biomass was not significantly

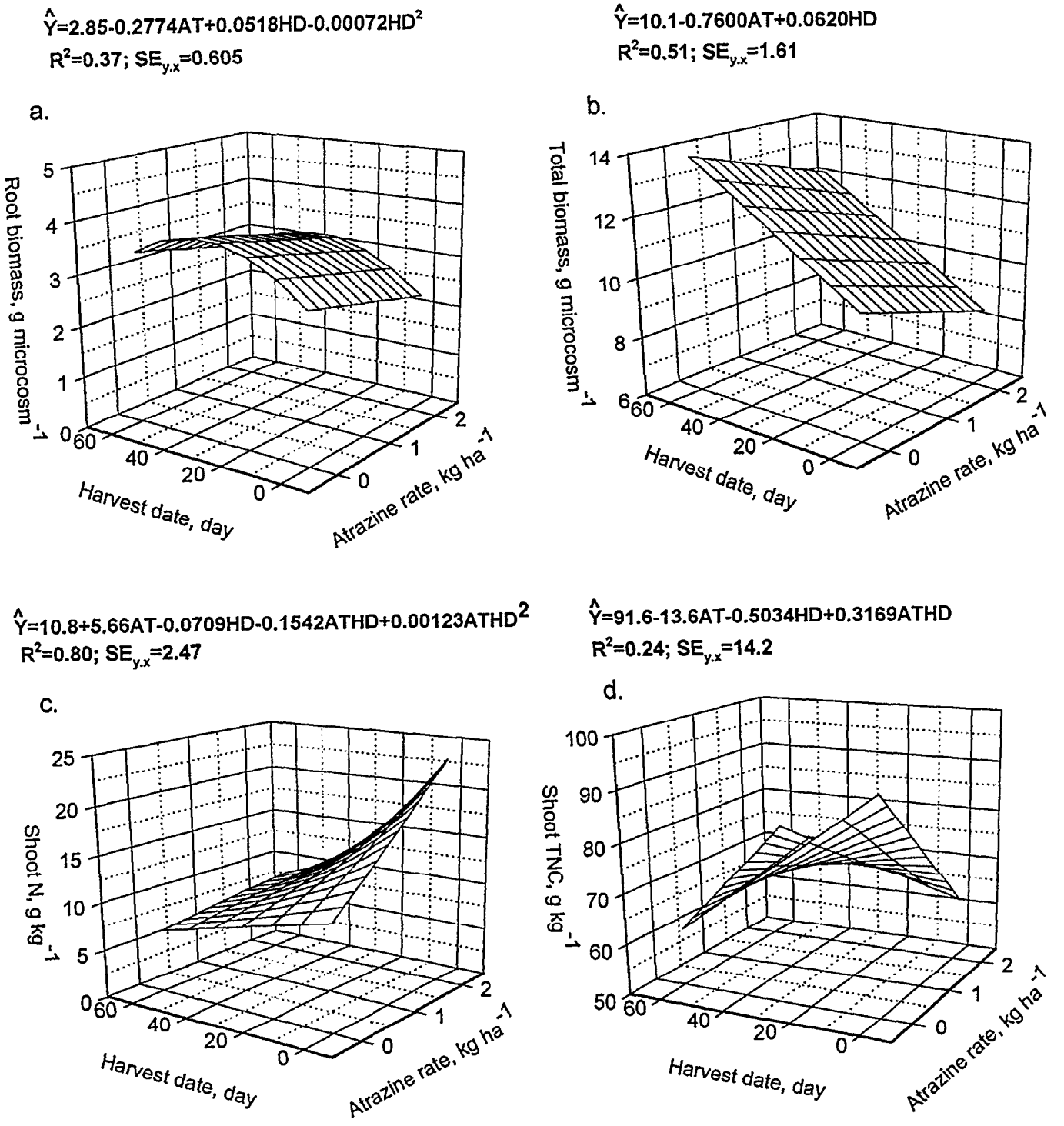


Fig. 1. Response surface models for prediction of the effect of atrazine rate (AT), harvest date (HD), and atrazine rate x harvest date interaction (ATHD) on blue grama a. root biomass; b. total biomass; c. shoot N concentration; d. total nonstructural carbohydrate (TNC) concentration.

affected, the increase in total plant N content was not significant ($P>0.13$). Total plant N content responded linearly to harvest date only, and increased shoot N content was explained by atrazine-induced alteration of the aboveground to belowground ratio of plant N (Table 3). The 2.24 kg ha$^{-1}$ atrazine rate increased total plant N aboveground 33% compared to the control. Further evidence of aboveground N accumulation was increased nitrate reductase activity in shoots of atrazine-treated plants (Table 3). This may be explained by continued N uptake and increased nitrate accumulation in the shoots.

Forage crude protein has traditionally been calculated from total shoot tissue N concentration. This study supports previous evidence that total shoot N may not be an accurate indicator of protein content in atrazine-treated blue grama or other atrazine-tolerant forages because the N accumulates as mineral forms in leaf tissue, particularly during early regrowth. Kay (1971) measured increases in the protein fraction of range forage treated with atrazine at 1.12 kg ha$^{-1}$ but not following treatment with atrazine at 2.24 kg ha$^{-1}$. However, plant nitrate concentration increased after treatment with atrazine at 2.24 kg ha$^{-1}$ to near-toxic levels for livestock (Kay 1971).

Atrazine-induced elevation in shoot N content was coincident with changes in plant carbohydrate status. Atrazine depressed blue grama shoot total nonstructural carbohydrate concentration (Fig. 1d). However, a significant atrazine rate × harvest date interaction occurred with an increase in shoot total nonstructural carbohydrate concentration with maturity in plants treated with the highest atrazine rate (Fig. 1d). Total nonstructural carbohydrate contents decreased at the 0- and 20-day harvests and increased at 40 and 60 days in plants receiving 2.24 kg ha$^{-1}$ of atrazine versus the control (Table 4). Even with these increases, the ratio of shoot total nonstructural carbohydrate to shoot total N (Table 3) decreased in relation to increasing atrazine rate because shoot N content or concentration was elevated throughout the regrowth period. Atrazine-induced inhibition of photosynthetic C fixation is the likely cause of reduced total nonstructural carbohydrate availability, and, subsequently, reductions in crown and root biomass accumulation. Morgan and Knight (1991) observed depressions in carbon exchange rates of greenhouse-grown blue grama 9 days after application of 0.32 and 0.64 kg atrazine ha$^{-1}$ to 60-day old seedlings but did not quantify the impact on belowground biomass.

**Table 4. Crown and root total nonstructural carbohydrates (TNC) concentrations and contents and total plant TNC content of blue grama as affected by atrazine rate (AT) and harvest date (HD).**

| | | TNC Concentration | | TNC Content | | |
|---|---|---|---|---|---|---|
| AT | HD[1] | Crown | Root | Crown | Root | Total |
| kg ha$^{-1}$ | | - (g kg$^{-1}$) - | | --- -(g microcosm$^{-1}$) - - - - | | |
| 0 | 0 | 81.1 | 23.8 | 0.409 | 0.088 | 0.792 |
| | 20 | 67.6 | 41.5 | 0.386 | 0.152 | 0.799 |
| | 40 | 62.0 | 29.5 | 0.319 | 0.100 | 0.646 |
| | 60 | 59.9 | 25.0 | 0.284 | 0.071 | 0.619 |
| 0.84 | 0 | 77.3 | 24.7 | 0.336 | 0.088 | 0.636 |
| | 20 | 61.2 | 34.8 | 0.289 | 0.164 | 0.802 |
| | 40 | 62.1 | 24.8 | 0 277 | 0.086 | 0.638 |
| | 60 | 59.1 | 30.8 | 0.302 | 0.094 | 0.640 |
| 2.24 | 0 | 69.5 | 20.0 | 0.275 | 0.056 | 0.517 |
| | 20 | 61.7 | 35.5 | 0.266 | 0.110 | 0.665 |
| | 40 | 65.7 | 29.0 | 0.319 | 0.126 | 0.766 |
| | 60 | 65.2 | 33.6 | 0.306 | 0.091 | 0.666 |
| $LSD_{0.05}$ (n=4) | | 15.7 | 8.4 | 0.116 | 0.038 | 0.191 |
| $F_{(AT\backslash HD)}$ | | 4.32* | 6.26* | 5.20* | 4.18* | 5.97* |

[1]HD: harvest date = days after end of 38-day regrowth period.
* Significant at $P<0.05$.

*Chlorophyll*

Changes in chloroplast anatomy, morphology, and biochemistry accompany changes in N metabolism following partial inhibition of photosynthesis in atrazine-tolerant species such as blue grama (Ebert and Dumford 1976). Chlorophyll a and chlorophyll b concentrations increased with increasing rates of atrazine and decreased with harvest date. This age-related decrease was decelerated as atrazine rate increased and was further modified for chlorophyll a concentration by previous soil atrazine exposure (Table 3). For example, on the first harvest day, the response for plants grown in soil with previous exposure to atrazine applications was a 1,333 mg chlorophyll a kg$^{-1}$ mean difference between the 0 and 2.24 kg ha$^{-1}$ atrazine rates. This difference was 530 mg chlorophyll a kg$^{-1}$ for plants grown in the soil with no previous atrazine treatment. Total chlorophyll content (chlorophyll a + chlorophyll b) of blue grama treated with 0.84 and 2.24 kg ha$^{-1}$ increased 1.5 and 2 times, respectively, over that of the control. Total microcosm chlorophyll content was negatively linearly related to harvest date (Fig. 2). There was no significant atrazine rate effect on the chlorophyll a: chlorophyll b (data not shown).

*Phosphorus*

Atrazine rate effects on shoot, and root and total phosphorus content were dominated by negative linear and negative quadratic responses, respectively (Table 3). Highest total plant phosphorus content occurred at the 0.84 kg ha$^{-1}$ rate. Phosphorus status of atrazine-treated plants has not been investigated as intensively as photosynthesis and N metabolism. Low-level atrazine applica-

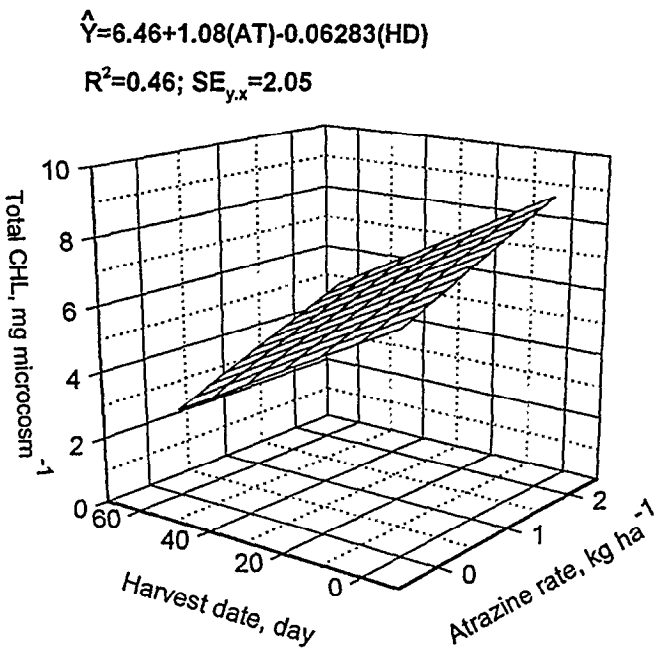


**Fig. 2. Response surface model for prediction of the effect of atrazine rate (AT) and harvest date (HD) on blue grama total chlorophyll (CHL) content.**

tions are known to stimulate metabolic activity in tolerant plants (Janjic et al. 1979). Quadratic response of total plant phosphorus to atrazine rate (Table 3) may reflect increased rates of phosphate uptake associated with higher metabolic activity at the 0.84 kg $ha^{-1}$ rate.

## II Soil Parameters

*Immediate Response*

Alterations in soil microbial biomass and activities, and chemical properties resulted primarily from variation in harvest date and presence of blue grama. Hydroxyatrazine application increased mean microbial biomass C from 408 to 463 mg C $kg^{-1}$ ($LSD_{0.05}$=37; n= 31). No other chemical treatment had immediate ($P \leq 0.05$) effects on microbial biomass C or any other microbial or chemical property of the soils. An immediate soil microbial response to *s*-triazine herbicides has been absent in several studies (Freney 1965, Theodorou and Sands 1980, Knight et al. 1993). Thus, plant growth alterations appear to be a direct result of *s*-triazine herbicide effects on plant metabolism and not attributable to any immediate impact the chemical may have on soil microbial activities.

Lack of soil microbial response to rate of atrazine in this study was unexpected given the alterations in plant growth and metabolism (Figs. 1 and 2), particularly decreases in below-ground blue grama growth. Due to lack of significance, data were pooled over chemical application rates to simplify presentation of blue grama and harvest date effects on soil microbial processes and chemical properties (Table 5). In general, soil microbial biomass was greater at the 0-day than the 60-day harvest, and microbial activities were greater when blue grama was present. Concentrations of soil mineral N were not influenced by harvest date but blue grama presence increased ammonium-N levels between 26 and 51% and decreased soil nitrate-N levels to between 2 and 5% of levels in unplanted microcosms.

*Long-term Response*

Soil nitrification rates and $pH_w$ of atrazine-treated microcosms varied with the presence of blue grama and harvest date, but differences were also detected in response to previous soil exposure to atrazine (Table 6). Trends in nitrification rates and soil $pH_w$ were generally the same for hydroxyatrazine-treated microcosms (data not shown). Nitrifying activity at the 60-day harvest was higher in unplanted microcosms than in microcosms with blue grama. At both harvests, soil $pH_w$ was lower in unplanted microcosms for soil never exposed to atrazine prior to this study. In contrast, when blue grama was present, $pH_w$ reduction did not occur. Soil $pH_w$ levels were also lower in unplanted microcosms within a harvest date, regardless of previous soil exposure to atrazine.

Immediate inhibition of nitrification by atrazine was not detected in our study. However, nitrification rates were depressed in soil with long-term atrazine exposure versus soil with no previous atrazine exposure at the 0-day harvest whether or not blue grama was present. Simon-Sylvestre and Fournier (1979) reported depressed nitrification after 1 atrazine application to soil, and others after repeated applications (Voets et al. 1974) to soil. Thus, responses of microbial nitrifier populations to atrazine may be cumulative in this soil, and inhibition may only be detected after repeated herbicide application. A cumulative inhibitory effect of atrazine on nitrifier populations after repeated soil treatment may decrease losses of $NO_3^-$-N by delaying production early in the season as blue grama breaks dormancy and initiates growth. This is a period when increases in microbial activity, especially ammonification and nitrification, accompany increases in soil temperature and favorable soil moisture conditions. Potential for loss of $NO_3^-$-N by denitrification is high during this period for the soil studied (Knight et al. 1993).

We could not link alterations in nitrifier activity to soil $NO_3^-$-N level changes since neither immediate nor long-term atrazine treatment resulted in significantly different mineral N levels in this study. However, when averaged over all chemical rates, an interesting, but nonsignificant trend was observed that indicated lower $NO_3^-$-N in long-term atrazine treated soil compared to soil never exposed to the herbicide (18.9 versus 23.8 mg $kg^{-1}$, respectively). This trend supports our suggestion that long-term atrazine

**Table 5. Soil microbiological and chemical properties of atrazine- and hydroxyatrazine-treated microcosms as affected by harvest date and presence of blue grama.**

| Harvest Date[1] | Blue grama Presence[2] | Property: Microbial biomass C | Microbial biomass N | Microbial biomass C:N | Respiration 6-day | Respiration 12-day | Nitrate reductase activity | Potential N mineralization | Mineral N $NH_4^+$-N | Mineral N $NO_3^-$-N |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ----- (mg $kg^{-1}$) ----- | | | ($\mu molCO_2$ $kg^{-1}$ $hour^{-1}$) | | ($mgNO_2$--N $kg^{-1}$ 24 $hours^{-1}$) | ($\Delta mgNH_4^+$-N $kg^{-1}$) | -- (mg $kg^{-1}$) -- | |
| | | | | | | | Atrazine-treated Microcosms | | | |
| 0-day | w | 400 | 47.9 | 9.3 | 43.0 | 24.4 | 1.44 | 10.5 | 1.54 | 1.0 |
| | w/o | 431 | 47.6 | 9.3 | 19.9 | 13.1 | 1.10 | 10.7 | 0.77 | 34.3 |
| 60-day | w | 373 | 41.5 | 9.0 | 46.2 | 38.1 | 1.51 | 12.0 | 1.64 | 2.60 |
| | w/o | 397 | 39.1 | 10.3 | 20.6 | 14.8 | 0.86 | 11.2 | 1.13 | 47.6 |
| | $LSD_{0.05}$ | 36 | 3.2 | 0.8 | 6.3 | 6.0 | 0.20 | NS[3] | 0.46 | 6.8 |
| | n | 24 | 21 | 21 | 20 | 18 | 24 | 24 | 24 | 24 |
| | | | | | | | Hydroxyatrazine-treated Microcosms | | | |
| 0-day | w | 449 | 49.6 | 10.0 | 49.6 | 28.1 | 1.48 | 11.4 | 1.46 | 0.7 |
| | w/o | 460 | 48.6 | 9.82 | 18.9 | 12.1 | 1.06 | 10.1 | 0.80 | 36.3 |
| 60-day | w | 423 | 44.2 | 9.5 | 48.5 | 42.7 | 1.58 | 13.5 | 1.57 | 2.3 |
| | w/o | 409 | 40.6 | 10.5 | 21.6 | 16.5 | 0.90 | 12.1 | 1.16 | 45.5 |
| | $LSD_{0.05}$ | NS | 4.3 | NS | 7.0 | 7.6 | 0.19 | 2.0 | 0.55 | 7.4 |
| | n | 16 | 16 | 16 | 14 | 12 | 16 | 16 | 16 | 16 |

[1] days after 38-day regrowth/incubation period
[2] w, w/o = with and without blue grama present, respectively.
[3] NS = no significant differences detected.

Table 6. Soil nitrifying activity and $pH_w$ of atrazine-treated microcosms as affected by harvest date, blue grama presence, and previous soil exposure to atrazine.

| | Harvest Date[1] | | | |
|---|---|---|---|---|
| | 0-Day | | 60-Day | |
| Parameter | With Blue grama | Without Blue grama | With Blue grama | Without Blue grama |
| Nitrifying Activity ($\mu g\ NO_3^-$-N $kg^{-1}$ $hour^{-1}$) | | | | |
| Treated soil[2] | 35.3 | 36.7 | 50.7 | 98.6 |
| Untreated soil | 75.6 | 89.3 | 49.0 | 80.4 |
| $LSD_{0.05}$ (n=7) | 29.7 | | | |
| $pH_w$ | | | | |
| Treated soil | 7.1 | 7.0 | 7.2 | 7.0 |
| Untreated soil | 7.1 | 6.7 | 7.2 | 6.7 |
| $LSD_{0.05}$ (n=7) | 0.2 | | | |

[1] Harvest date = days after 38-day regrowth/incubation period.
[2] Treated soil = soil with previous long-term atrazine exposure; untreated soil = soil with no previous exposure to atrazine.

application may produce cumulative effects on soil microbial processes not detectable immediately following one application. In contrast, Knight et al. (1993) reported a significant, but temporary increase in $NO_3^-$-N levels of an Ascalon soil 16 days following application of 0.64 kg $ha^{-1}$ to 60-day old blue grama seedlings grown in a greenhouse, but no $NO_3^-$-N increase for laboratory-incubated soil. The authors linked increase in soil $NO_3^-$-N levels to alterations in blue grama leaf tissue N status. However, given reported severe reductions in blue grama seedling survival associated with soil-applied atrazine at 1.1 kg $ha^{-1}$ (Bahler et al. 1984), and our finding that atrazine application immediately suppresses blue grama root biomass, we cannot link nitrification depression solely to immediate, atrazine-induced shoot responses. Furthermore, we observed depression of nitrification rates in soil with long-term atrazine exposure whether blue grama was present or not.

### III Relationship to Long-term Field Study

Reported increases in end-of-season blue grama above-ground standing crop (Shoop, personal communication) appear related to atrazine-induced delay of senescence observed in this study. Atrazine-treated plants remained green longer with higher shoot total nonstructural carbohydrate concentrations, and chlorophyll, shoot N, and total plant P contents than the control plants. Differences in above-ground biomass may have been apparent if harvest periods were extended, because untreated plants had senesced by the 60-day harvest. Delays detected in senescence of blue grama plants grown in microcosms might help explain the 28% increase in steer grazing days reported for pastures treated with 1.12 kg $ha^{-1}$ atrazine at the Central Plains Experimental Range and the 53% increase in beef production noted for those pastures (Shoop, personal communication).

Estimates from this study suggest atrazine applications of 1.12 kg $ha^{-1}$ may delay early-season crown and root biomass proliferation compared with untreated plants. Blue grama vigor was not adversely impacted by applications of 1.12 kg $ha^{-1}$ atrazine in the fall of alternate years as long as grazing was closely managed (Shoop, personal communication). However, indirect, delayed, or cumulative impacts of atrazine-induced alterations in blue grama biomass distribution and metabolic function may take longer to detect in the field. Mineralization of easily decomposable organic N compounds from root exudates, exfoliates, and short-lived unsuberized roots is one of 4 important N-supplying mechanisms in the shortgrass system (Clark 1977). The loss of these more labile sources of N may cause an imbalance in N cycle dynamics through a decrease in 'fast' versus 'slow' substrate decomposition pathways (Biondini et al. 1988) thus linking reduced root inputs to changes in soil mineral N levels.

Connections between elevated mineral N levels in soil treated repeatedly with atrazine and atrazine impacts on soil microbial processes in microcosms remain less apparent. We did not detect soil mineral N level changes following atrazine application to dormant plant-soil or soil-only microcosms. However, depression of nitrification rates following dormancy in the long-term treated soil with or without plants present suggests that $NO_3^-$-N accumulation may be the result of protection from loss, particularly from denitrification. This protection may partially explain increased soil mineral N levels observed following repeated atrazine applications to shortgrass pastures dominated by blue grama.

## Conclusions

Modeled changes in blue grama growth and development, and modifications of microbial activities observed in this study indicate that, despite measured benefits for secondary production, repeated atrazine application could result in alteration of shortgrass system structure and function. Chronic atrazine-induced belowground biomass reduction at the individual plant level could result in changes at the ecosystem level through modification of interactions among plants and between plants and the edaphic environment (Levin and Kimball 1984). Depressed nitrification rates during early blue grama regrowth in soils from pastures repeatedly treated with atrazine may be associated with such changes. Subtle changes in soil processes are of great concern because they are more difficult to detect before widespread, irreversible changes have occurred (Weinstein and Birk 1989). This is especially true in agroecosystems such as rangelands where monitoring is more extensive in nature than is characteristic of intensive crop monocultures.

The rangeland label was removed from atrazine in the late 1980s and will not likely be renewed. Nor is it likely that a label would be obtained for forage quality and quantity improvement on shortgrass range. However, these results have increased understanding of possible risks to shortgrass ecosystem structure and function that could occur when unknown or undetectable effects of intensive management practices have chronic effects on dominant plant physiology or soil microbial activities.

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