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# The influence of nitrogen on atrazine and 2,4-dichlorophenoxyacetic acid mineralization in grassland soils

**James A. Entry**[1], **Kim G. Mattson**[2], **William H. Emmingham**[1]

[1] Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA
[2] Department of Forest Resources, University of Idaho, Moscow, ID 83843, USA

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**Abstract.** The influence of fertilizer N on the mineralization of atrazine [2-chloro-4(ethylamino)-6(isopropylamino)-s-triazine] and 2,4-D (2,4-dichlorophenoxyacetic acid) in soils was assessed in microcosms using radiometric techniques. N equivalent to 0, 250, and 500 kg N as $NH_4NO_3$ $ha^{-1}$ was added to three grassland soils. Compared to the control, the 250- and 500-kg treatments suppressed mineralization of atrazine by 75 and 54%, respectively, and inhibited mineralization of 2,4-D by 89 and 30%, respectively. Active fungal biomass responded to the N treatments in an opposite manner to herbicide mineralization. Compared to the control, the 250- and 500-kg treatments increased the active fungal biomass by more than 300 and 30%, respectively. These results agree with other observations that N can suppress the decomposition of resistant compounds but stimulate the primary growth of fungi. The degree of suppression was not related to the amount of N added nor to the inherent soil N levels before treatment. The interaction between the N additions and the active fungal biomass in affecting herbicide mineralization suggests that N may alter microbial processes and their use of C sources and thus influence rates of herbicide degradation in the field.

**Key words:** Grassland soils – Microbial biomass – Nitrogen fertilization – Atrazine – 2,4-D – Radiometric techniques – Herbicide mineralization

The ability of soil microorganisms to degrade aromatic herbicides seems to be associated with lignin-degrading enzyme systems (Hammel 1989; Aust 1990; Lamar et al. 1990; Donnelly 1991). White-rot fungi are noted for their ability to degrade both lignin (Kirk and Farrel 1987) and aromatic hydrocarbons (Bumpus 1989) to $CO_2$. These fungi secrete $H_2O_2$ and a family of peroxidases that catalyze the free radical oxidation and depolymerization of lignin (Kersten and Kirk 1987) The C-centered free radicals thus produced are responsible for the non-specific cleavage of the lignin polymer and probably the mineralization of other aromatic hydrocarbons as well (Aust 1990).

The ligninolytic enzyme system in several, but not all, species of fungi is stimulated by N limitation (Kirk and Farrel 1987; Reid 1991). Therefore, high concentrations of N may be expected to reduce the ligninolytic enzyme system and thus the mineralization of aromatic herbicides. Such results have been reported by Donnelly (1991), who found that atrazine and 2,4-D mineralization by *Phanerochaete chrysosporium* and *Trappea darkeri* was inhibited by additional N in vitro. The objective of the present sutdy was to extend the findings of Donelly (1991) by examining the effects of two levels of added N on herbicide mineralization in three pasture soils containing resident soil microbial populations.

## Materials and methods

### Site descriptions

The top 10 cm of mineral soil was sampled in the Willamette Valley near Corvallis, Oregon, from riparian grassland sites along Oak Creek (44° 38′N, 123° 15′E), Jackson Creek (44° 38′N, 123° 18′E), and Soap Creek (44° 41′N, 123° 15′E). The Oak Creek site was in Oregon State University McDonald Forest on a 3% slope with a grass community dominated by *Festuca arundinacea* Schreb., *Trifolium pratense* L., and *Lolium perenne* L. The soil is a Fluvaquentic Haplaquoll, fine mixed mesic, in the Waldo series (Knezevich 1975).

The Jackson Creek site was in McDonald State Forest on a 5% slope with vegetation consisting mainly of the grasses *F. arundinacea, T. subterraneum, T. pratense,* and *L. perenne.* The soil is a Vertic Haploxeroll, fine montmorillonitic, mesic, in the Witham series (Knezevich 1975).

The Soap Creek site was on an 8% slope in the Dunn State Forest with vegetation consisting of the grasses *L. perenne, F. arundinacea, T. subterraneum* L., and *Alopecurus pratensis* L. The soil is a Cumulic Ultic Haploxeroll, fine silty mixed mesic, in the McAlpin series (Knezevich 1975).

*Correspondence to:* J.A. Entry

All three sites were within 15 km of each other. Annual precipitation averaged 100–150 cm $year^{-1}$. Annual air temperatures ranged from 10 °C to 12 °C.

## *Experimental design*

The experiment was arranged in a random block design (Kirk 1982) with the three soil sampling sites as blocks. The three treatments were no additions of N (control), additions of 250 kg N $ha^{-2}$, and additions of 500 kg N $ha^{-2}$. Mineralization rates of atrazine and of 2,4-D were quantified after a 30-day incubation. Microbial biomass was determined before the N additions and again after the incubations. Soil chemical properties were mesured at each site.

## *Sampling procedures*

Soil samples were collected to a depth of 10 cm on May 26, the sampling date coinciding with the peak season of active bacterial and fungal biomasses found at a nearby site (Ingham et al. 1991). Nine samples were collected from each treatment on each site (total of 81 samples) for chemical and microbial biomass analyses. Twenty-seven samples collected from each treatment on each site (total of 243 samples) were analyzed for atrazine and 2,4-D mineralization. Except for the chemical analyses the soil was not sieved; it was pre-incubated at 4 °C with moisture conditions similar to those in the field and prepared for microbial assays within 24 h to minimize the effects of storage on microbial activity (West et al. 1986).

## *Microbial biomass measurements*

Total and active bacterial and fungal biomasses were estimated before and after the herbicide mineralization assays, using methods described by Ingham and Klein (1984). A suspension of 1.0 g soil diluted in 1 liter of distilled, deionized $H_2O$ was further diluted in 4 ml 60 m*M* phosphate buffer. One milliliter of this solution was incubated with 1 ml filter-sterilized (0.22 mm pore size) 20 mg $liter^{-1}$ fluorescein diacetate solution for 3 min at 20 °C and passed through a polycarbonate filter (25 mm diameter, 0.22 mm pore size). The filters were removed and 1 ml 3% agar was added to the soil suspension, mixed, then 0.1 ml was transferred to a microscope slide containing a cavity of known volume (Ingham and Klein 1984). The slides were examined for the fluorescein diacetate-stained hyphal length immediately after preparation by epifluorescent microscopy (Stamatiadis et al. 1990). Three fields per slide were examined with phase-contrast microscopy for the total hyphal length, and three transects were examined for the fluorescein diacetate-stained (active) hyphal length at 100× total magnification.

We used iodonitrotetrazolium stain to count live bacteria (Stamatiadis et al. 1990). A suspension of 1.0 g soil diluted in 1 liter distilled, deionized $H_2O$ was further diluted to 0.2 mg soil in 4 ml buffer. The soil suspension was incubated in the dark with 4 ml filtered iodonitrotetrazolium buffer (MacDonald 1980) for 60 min at room temperature. The incubated solution was examined two slides per sample and 10 fields per slide with epifluorescence microscopy for iodonitrotetrazolium-stained (active) bacteria at approximately 1000× magnification. Total bacteria reported are stained (active) bacteria plus non-stained bacteria.

We computed the bacterial volume from the number of soil bacteria per gram of soil with the assumption that bacterial spheres were 1 μm in diameter (Jenkinson and Ladd 1981). A C to volume conversion factor of 102 μg C $mm^{-3}$ was used for both bacteria and fungi, assuming 1.1 g $cm^{-3}$ wet density, 0.25 dry matter content, and a 0.37 C content in the bacterium or fungus (Jenkinson and Ladd 1981).

## *Herbicide mineralization assay*

Three N treatments were imposed on soils collected from each site. For the 250-kg treatment, 1 ml distilled deionized $H_2O$ containing 0.025 g $NH_4NO_3$ was added to 15 g (equivalent dry weight) moist soil (equivalent to 250 kg N $ha^{-1}$). For the 500-kg treatment, 1 ml distilled deionized $H_2O$ containing 0.050 g $NH_4NO_3$ was added to moist soil (equivalent to 500 kg N $ha^{-1}$). For the control, 1 ml distilled deionized $H_2O$ was added to moist soil.

Ring-labeled $^{14}C$ herbicides were added to the N-treated soils and mineralization was assayed via $^{14}CO_2$ production over a 30-day interval. Atrazine with radiochemical purity > 98% (Ciba-Geigy Corp., Greensboro, North Carolina, USA) and ring-labeled 2,4-D with radiochemical purity > 99.5% (Sigma Chemical Co. St. Louis, MO) were dissolved in 10 ml 95% ethanol. The mixture was brought to 100 ml volume with deionized water. The N-treated soils received 1.0 ml of either a 1.0 m*M* solution containing 1995 Bq of ring-labeled $^{14}C$ atrazine or 1.0 ml of a 1.0 m*M* solution containing 2557 Bq ring-labeled $^{14}C$-2,4-D and were thoroughly mixed. The soils were then placed in 0.9-liter containers with vials containing 10 ml 1 *M* NaOH and vials containing 10 ml deionized water (to maintain humidity) and incubated for 30 days (Entry et al. 1987). Previous studies have shown that soil microflora contained in the amount of soil used in this system does not alter the $O_2$ content inside the container relative to the $O_2$ content outside the container (Entry et al. 1987). We ran one blank to account for background radiation for each set of 27 samples. The blanks were treated as above, but without radio-labeled herbicides added to the soil.

After incubation, 0.5 ml of the NaOH was removed from each vial and mixed with a 1.0-ml deionized $H_2O$ and 17-ml scintillation cocktail (Bio-Safe II, Research Products International Corp., Mount Prospect, Illinois, USA). The samples were counted for 10 min with a Beckman LS 7500 autoscintillation counter.

## *Soil chemical measurements*

The analyses included total N, extractable $NH_4^+$, extractable $NO_3^-$, extractable P, and total C. Concentrations of total N in soils were determined using methods described by Bremner and Mulvaney (1982) extractable concentrations of $NH_4^+$ and $NO_3^-$ by a microdiffusion method (Keeney and Nelson 1982), and extractable P with Bray techniques (Olsen and Sommers 1982). Total C was estimated by dry-ashing, assuming C equal to 50% of loss on ignition (Nelson and Sommers 1982). The C : N ratio was calculated by dividing total C by total N.

## *Statistical analysis*

All dependent variables were tested for normality with univariate procedures. Data were then analyzed by means of analysis of variance procedures for a random block design with Statistical Analysis Systems (SAS Institute, Inc. 1982). Residuals were equally distributed with constant variances. All digits reported are the sample values minus control values. Differences were judged significant at $\alpha = 0.05$, as determined by Fisher's Protected Least Significant Difference test.

Because the analyses of variance for active and total fungal and bacterial biomass, and for atrazine and 2,4-D mineralization, did not indicate significant differences among sites, only differences among treatments are discussed (Snedecor and Cochran 1980).

# Results

Compared to the control, the 250- and 500-kg treatments suppressed mineralization of atrazine by 75 and 54%, respectively and suppressed mineralization of 2,4-D by 89 and 30%, respectively (Table 1). Both atrazine and 2,4-D mineralization were suppressed to a greater extent by the 250-kg treatment than by the 500-kg treatment. There were no differences among sites in the degree of mineralization of either herbicide or in the effect of added N.

Before the N additions, there were site differences in active microbial biomass (Table 2). After the addition of N and incubation, the active microbial biomass increased and the site differences were reduced. The active and total fungal biomass was greatest in the 250-kg treatment while the active bacterial biomass was greatest in the 500-kg treatment.

There were significant differences among sites in soil chemical properties (Table 3). The soils from Oak Creek had the lowest concentrations of all nutrients analyzed. The Jackson Creek soils had the highest total N and total C, whereas the Soap Creek soils had the highest extractable N and extractable P.

## Discussion

N additions to pasture soils stimulated the microbial biomass but suppressed the mineralization of herbicides. Active and total fungal biomass were greatest in the N treatments that displayed the greatest suppression of herbicide mineralization. Inherent site differences in soil N had no effect on the mineralization of the herbicides.

In studies of pure fungal cultures, added N has been shown to induce fungal growth of almost all species and suppress degradation of aromatic C compounds by several species. Typically, degradation ability is suppressed in those species most capable of degrading these resistant compounds. For example, ammonium tartarate suppressed the degradation of atrazine and 2,4-D by *Phanerochaete chrysosporium* (Donelly 1991). Amino acids suppressed ligninolytic activity in *Phanerochaete chrysosporium, Phlebia brevispora, Coriolus versicolor,* and *Pholiota mutabilis* (Leatham and Kirk 1983). The ability of *Phanerochaete chrysosporium* to degrade lignin (Kirk and Farrel 1987) and other aromatic compounds (Bumpus 1989; Aust 1990) is thought to be dependent on the same enzyme system and this system is stimulated by low N levels. The low N levels initiate a secondary metabolism in the fungus (Kirk and Farrel 1987), during which several biochemical changes occur, including stimulation of the ligninolytic system.

In a review, Fog (1988) observed that the empirical evidence indicated that when N was added to organic matter that was relatively easily decomposable, such as fresh litter, or when N was actually incorporated into the organic matter, enhanced rates of decomposition were often observed, particularly during the initial stages of decay. In instances where N was added to recalcitrant organic matter such as wood, soil, humus, or lignin, and during the later stages of litter decay, suppressed rates of decay were more often observed. N suppression of late stages of decay of coniferous litter was also reported in a review by Berg (1986).

Our findings are consistent with the two lines of evidence presented above. Added N, while stimulating total microbial biomass of the soils, also acts to suppress the enzyme system of certain resident decomposers capable of degrading the recalcitrant aromatic structures of the herbicides.

There were no interactions between herbicide mineralization and sites, even though inherent soil N levels among the three sites varied considerably. Lack of an inherent soil N effect on herbicide mineralization is most likely a result of the total soil N being largely unavailable and the highest extractable N levels being sufficiently low to stimulate the ligninolytic system of the resident fungi. We added 292 $\mu g\ N\ g^{-1}$ soil as either $NH_4^+$ or $NO_3^-$ in our 250 treatment compared to normal concentrations of less than 17 $\mu g\ N\ g^{-1}$ soil.

Why the 250 treatment suppressed herbicide degradation to a greater degree than the 500 treatment is not apparent. The fungal biomass was highest in the 250 treatment also. Donelly (1991) observed the same pattern in *Phanerochaete chrysosporium* grown at three N levels with 2,4-D, with the greatest suppression of 2,4-D degra-

**Table 1.** Atrazine and 2,4-dichlorophenoxyacetic acid mineralization in the top 10 cm of pasture soil with three levels of N addition

| Treatment | Atrazine mineralization (% $^{14}C$ recovered) | | | | 2,4-D mineralization (% $^{14}C$ recovered) | | | |
|---|---|---|---|---|---|---|---|---|
| | Jackson Creek | Oak Creek | Soap Creek | $\bar{X}$ | Jackson Cree | Oak Creek | Soap Creek | $\bar{X}$ |
| 0 (control) | 1.98a | 1.96a | 1.69a | 1.88a | 13.85a | 13.83a | 17.02a | 14.90a |
| 250 kg N $ha^{-1}$ | 0.40c | 0.48c | 0.54c | 0.47b | 1.81c | 1.45c | 1.44c | 1.57c |
| 500 kg N $ha^{-1}$ | 0.83b | 0.94b | 0.82b | 0.86c | 10.33b | 11.65b | 9.27b | 10.42b |

In each column, values followed by the same letter are not significantly different as determined by Fisher's protected least significant difference (LSD) test (a = 0.05), $n = 27$

**Table 2.** Active and total bacterial and fungal biomass in the top 10 cm of pasture soil before and after the addition

| Treatment | Fungal biomass ($\mu g\ C\ g^{-1}$ soil) | | | | | | Bacterial biomass ($\mu g\ C\ g^{-1}$ soil) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jackson Creek | | Oak Creek | | Soap Creek | | Jackson Creek | | Oak Creek | | Soap Creek | |
| | Active | Total | Active | Total | Active | Total | Active | Total | Active | Total | Active | Total |
| Before N | 200b | 239b | 77b | 325c | 11c | 395b | 274b | 359b | 161b | 227b | 152c | 210c |
| After N | | | | | | | | | | | | |
| 0 (control) | 177b | 332b | 94b | 344c | 30c | 360b | 315b | 448b | 311b | 165b | 256b | 316bc |
| 250 kg N $ha^{-1}$ | 350a | 805a | 263a | 707a | 324a | 987a | 316b | 324b | 277b | 234b | 295b | 333b |
| 500 kg N $ha^{-1}$ | 193b | 209b | 96b | 510b | 132b | 369b | 592a | 643a | 677a | 793a | 540a | 590a |

In each column, values followed by the same letter are not significantly different as determined by Fisher's Protected Least Significant Difference (LSD) test ($P \leq 0.05$, $n = 9$. Values are averaged over herbicide treatment

**Table 3.** Some chemical properties of soils

| Site | Total N | $NH_4$ | $NO_3$ | P | C (%) | C:N |
|---|---|---|---|---|---|---|
| | ($\mu g\,N\,g^{-1}$) | | | ($\mu g\,P\,g^{-1}$) | | |
| Oak Creek | 2071c | 2.23c | 0.91c | 2.10c | 2.48c | 12b |
| Jackson Creek | 3039a | 3.88b | 6.33b | 10.48b | 4.67a | 15a |
| Soap Creek | 2642b | 16.05a | 16.19a | 22.74a | 3.43b | 13ab |

For explanation of letters within columns, see Table 2

dation and highest growth at medium levels of N additons (0, 1, and 10 m*M* ammonium tartarate). However, in tests with atrazine, Donelly (1991) observed that the greatest suppression of degradation occurred at the highest N levels when *Phanerochaete chrysosporium* grew at the slowest rate. Estimates of microbial biomass are inherently variable and should probably be used only as coarse indices for interpreting growth or activity. The suppressive effect of added N does not appear to be linear over wide ranges of N concentrations, and under some conditions may be correlated with the degree of stimulation of fungal biomass.

The herbicides used in the present study were labeled only in the ring structure and, therefore, are conservative values compared to those from studies that have examined the disappearance of only the parent compound in the soil. These studies have estimated only the amount of the herbicide and, in some cases, compounds generated as a result of the first or second alteration of side chains attached to the ring structure of atrazine or 2,4-D. Side-chain degradation of atrazine could have occurred, but would have gone undetected. Our methods detected only the amount of atrazine or 2,4-D degraded to $CO_2$ and did not measure the amount of intermediate compounds. The degradation pathways of atrazine and 2,4-D have been investigated and intermediate compounds are well known (Giardina et al. 1982; Chaudhry and Chapalamadugu 1991). Atrazine and 2,4-D could have been degraded to a variety of intermediate compounds, some of which are toxic to plants (Van der Tweel et al. 1987; Kaufman and Blake 1970).

Improved knowledge of how N additions modify microbial physiology as well as the soil environment is necessary if we are to understand the interactions of soil management practices. Degradation of atrazine and 2,4-D, as well as of other herbicides, may vary depending on the specific chemical and the impact of soil management practices on the soil microbial community.

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