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# Dynamic response of chlorsulfuron herbicide to nitrogen mineralization and the ratio of microbial biomass nitrogen to nitrogen mineralization in the soil

A. M. EL-Ghamry, HUANG Chang-yong * , XU Jian-ming

(College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310029, China)

**Abstract**: A laboratory incubation experiment was conducted to elucidate the effect of chlorsulfuron herbicide on nitrogen mineralization and the ratio of microbial biomass nitrogen to nitrogen mineralization ($N_{mic}/N_{min}$ ratio) in loamy sand soil. The herbicide was applied at four levels that were control, field rate 0.01 (FR), 10 times of field rate 0.1(10FR), and 100 times of field rate 1.0 (100FR) μg/g soil. Determinations of N-mineralization and microbial biomass-N content were carried out 1, 3, 5, 7, 10, 15, 25 and 45 days after herbicide application. In comparison to untreated soil, the N-mineralization decreased significantly in soil treated with herbicide at levels 10FR and 100FR within the first 5 days incubation. A more considerable reduction in the $N_{mic}/N_{min}$ ratio was observed in the herbicide treated soil than the non-treated control. Among the different treatment of chlorsulfuron, 100FR displayed the greatest biocidal effect followed by 10FR and FR, showing their relative toxicity in the order of 100FR > 100FR > FR. The results indicated that the side effect of this herbicide on N-mineralization is probably of little ecological significance.

**Key words**: chlorsulfuron; N-mineralization; $N_{mic}/N_{min}$ ratio; loamy sand soil

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## Introduction

Herbicides are now used extensively in crop production. The increasing use of herbicides with nitrogen fertilizers has created serious concern about the potential adverse effect of these pesticides on non-target organisms and the environment, and it has emphasized the need for information concerning the effects of herbicides on nitrogen transformations in soil (Yeomans, 1985). It is becoming increasingly clear that the mismanagement of many toxic substances, including pesticides, can cause serious ecological problems to non-target species. Many research works have been done to study the fate and distribution of toxic substances in the environment (Giesy, 1980). But little information is available about effect of sulfonylurea herbicide on soil environment particularly N-transformation.

Ammonification is a key process in soil supplying readily available nitrogen to plants and microorganisms. Without it, nitrification will be severely limited and $NO_3^-$ ions, the preferred form of nitrogen, will also be limited. The widespread utilization of a variety of toxic chemicals in agriculture and industry has led to the contamination of soils with toxic residues. Any pesticide that has the potential to induce physical, chemical, or microbial changes can affect the population of ammonifiers in soil and the final organic compounds produced during the degradation of proteinaceous material. This will have a corresponding effect on soil fertility and plant nutrition. Hence the potential danger of such chemicals and their residues to these non-target ammonifiers has to be assessed (Rup, 1988).

The effect of herbicides on ammonification varies, ranging from no effect or inhibition to

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* Corresponding author

stimulation. The major groups like phenoxy acids, ureas and triazines are generally positive. Frequently nitrification seems to be more sensitive resulting in the accumulation of $NH_4^+$ -N. This is different from stimulation and supports the fact that one has to take total mineral nitrogen into consideration. Usually the adverse effects are manifested only at higher concentrations, while at recommended field levels they merely of an ephemeral nature (Rup, 1988). Few investigators have directed their attention toward the effects of toxic substances on ecosystem level properties such as changes in the dynamics of nutrient cycling in the presence of a toxicant (National Research Council, 1981).

Our purpose of this study was to observe the dynamic response of chlorsulfuron herbicide on nitrogen mineralization and the ratio of microbial biomass to nitrogen mineralization in loamy sand soil. Chlorsulfuron is one of the initial sulfonylureas developed, with soil activity persisting for 518 ± 30d when applied in the Central Great Plains at 35g/h (Anderson, 1983). Chlorsulfuron has a half-life of 1 to 2 months (Palm, 1980; Walker, 1982; 1983).

The herbicide studied is highly active in soil at the recommended rates of 13 to 30 g/hm$^2$. Many crops are sensitive to chlorsulfuron (Zimdahl, 1983).

# 1 Materials and methods

## 1.1 Sampling and preparation of soil

A laboratory incubation experiment was conducted using the loamy sand soil collected from the surface layer (0—20 cm) from Hangzhou, Zhejiang Province, China. The field fresh soil was brought to the laboratory immediately after collecting, the discrete plant residues and large soil animals (earth warms, etc.), were removed by hand and then the soil was passed through a 2 mm sieve and homogenized thoroughly. A sub-sample of the soil was taken, air-dried, ground, and analyzed for various physical and chemical properties listed in Table 1.

**Table 1 Some physical and chemical properties of the soil used in the experiment**

| | | | |
|---|---|---|---|
| Soil texture | Loamy sand | Soil water % at 33 kPa | 22.4 |
| Coarse sand, % | 9 | pH | 6.27 |
| Fine sand, % | 73 | CEC, meq/100g soil | 10.55 |
| Silt, % | 10 | Total organic carbon, g/kg | 17.6 |
| Clay, % | 8 | Total nitrogen, g/kg | 1.58 |

## 1.2 Herbicide treatment

After sampling and preparation of soil, the soil sample was subdivided into four sub-samples. One sub-sample was used as a control, and the others were treated with chlorsulfuron herbicide. Methanol solutions of chlorsulfuron were prepared in three different concentrations of 0.05, 0.5 and 5.0 $\mu$g/ml, respectively. The conversion of field-rate application to milligrams of chlorsulfuron/100g of soil was calculated assuming an even distribution of herbicide in 0 to 20 cm layer with a bulk density of 1.5 g/cm$^3$.

The herbicide was incorporated into the soil sub-sample as follows: 6 ml of the methanolic solution of chlorsulfuron of each concentration was added to 15g of air-dried soil previously for each sub-sample. Six ml of methanol was added to 15g of air-dried soil for the control.

After complete removal of the methanol by evaporation at room temperature, each of the 15g treated soil was transferred into the beaker containing 285g fresh soil (oven dry basis) and homogenized. From each beaker 27 soil portions (9 incubation stages × 3 replicates), each of 10g, were weight and transferred into 30 ml glass bottles.

With this procedure three application rates corresponding to FR, 10FR and 100FR were obtained. Incubated in the dark at 24℃. Three bottles from each treatment were removed and submitted to analysis at 0, 1, 3, 5, 7, 10, 15, 25 and 45 days after chlorsulfuron treatment.

### 1.3 Determination of nitrogen mineralization

Anaerobic N-mineralization was determined in 10g soil, the sample was flooded with 25 ml deionized water in a 30 ml glass bottle. The bottles were gently tapped for 30s to remove air bubbles, sealed with a rubber stopper and then kept for the incubation period at 24℃. After incubation, the samples were transferred to 125 ml extraction bottles and extracted with 25 ml 4 mol/L KCl with shaking for 1h at 150 reciprocation $min^{-1}$ and subsequent gravity filtering using prewashed Whatman No. 5 paper (Barrios, 1996). Ammonium was determined colorimetrically (Anderson, 1993).

### 1.4 Microbial biomass determination

Soil samples for the determination of microbial biomass N were extracted by a fumigation-extraction (FE) method (Brookes, 1985b) and the total nitrogen in the soil extracts was measured after Kjeldahl digestion (Brookes, 1985a).

### 1.5 Soil analysis

Water contents at an applied pressure of 33 kPa (0.33 bar) were determined using a pressure membrane system similar to that described by Heining (Heining, 1963). The pH of the soils (in water, 1:2.5) was measured with a pH meter. Total N was determined by Kjeldahl method and total organic carbon by Walkley-Black procedure (Jackson, 1958).

### 1.6 Statistical analysis

Data were examined by analysis of variance completely randomized and Duncan's multiple range tests using statistic software (CoStat statistical software, 1990).

## 2 Results

### 2.1 The effect of chlorsulfuron on N-mineralization

The dynamic responses of the N-mineralization to the herbicide treatments are shown in Table 2. The data exhibited that the N-mineralization was not significantly modified at the FR. When chlorsulfuron was applied at 10FR and 100FR, the decrease in the N-mineralization was significant especially within the first 5 days.

**Table 2 The effect of chlorsulfuron on N-mineralization**

| Incubation, | Herbicide treatment, μg/g | | | | LSD |
|---|---|---|---|---|---|
| period/d | Control(0) | FR(0.01) | 10FR(0.1) | 100FR(1) | 0.05 |
| 1 | 21.35 a* | 19.75 a | 16.43 b | 13.14 c | 3.281 |
| 3 | 23.82 a | 21.35 ab | 18.07 ab | 14.78 b | 6.959 |
| 5 | 25.46 a | 22.17 a | 20.35 ab | 16.43 b | 5.187 |
| 7 | 28.75 a | 27.92 a | 23.82 a | 20.53 a | 8.146 |
| 10 | 35.32 a | 33.67 a | 30.40 a | 28.70 a | 11.52 |
| 15 | 36.96 a | 34.49 a | 32.85 a | 30.39 a | 12.42 |
| 25 | 38.60 a | 37.78 a | 36.14 a | 33.67 a | 16.24 |
| 45 | 40.20 a | 39.40 a | 37.78 a | 36.96 a | 14.61 |

* Means with different letters, within rows, differ significantly according to LSD ($P<0.05$)

The result indicated that chlorsulfuron treatment at FR reduced the N-mineralization by 7.49%, 10.37%, 12.92%, 2.89%, 4.67%, 6.68%, 2.12% and 1.99% at 1, 3, 5, 7, 10, 15, 25 and 45 days of incubation respectively compared with the control. However, when applied at 10FR the decrease in N-mineralization were 23.04%, 24.14%, 20.07%, 17.15%, 13.93%, 11.12%, 6.37% and 6.02%, respectively, compared to the control in the same incubation period. The soil treated with 100FR resulted in 38.45%, 37.95%, 35.47%, 28.59%, 18.74%, 17.78%, 12.77% and 8.06% respectively in the same incubation period compared to the control.

### 2.2 The effect of chlorsulfuron on $N_{mic}/N_{min}$ ratio

The addition of chlorsulfuron to the soil caused reduction in the $N_{mic}/N_{min}$ ratio (Table 3). The data revealed that the decrease in $N_{mic}/N_{min}$ ratio during first 10 days of incubation was due to increase in the level of herbicide.

**Table 3 The effect of chlorsulfuron on the $N_{mic}/N_{min}$ ratio**

| Incubation, | Herbicide treatment, μg/g | | | |
|---|---|---|---|---|
| period/d | Control (0) | FR(0.01) | 10FR(0.1) | 100FR(1) |
| 1 | 2.314 | 2.299 | 2.200 | 2.085 |
| 3 | 1.682 | 0.556 | 1.396 | 1.352 |
| 5 | 1.397 | 1.371 | 1.102 | 1.093 |
| 7 | 1.184 | 0.968 | 0.826 | 0.786 |
| 10 | 0.909 | 0.822 | 0.619 | 0.530 |
| 15 | 0.906 | 0.838 | 0.711 | 0.623 |
| 25 | 0.907 | 0.858 | 0.818 | 0.745 |
| 45 | 0.849 | 0.777 | 0.759 | 0.655 |

The data indicated that herbicide application at FR decreased $N_{mic}/N_{min}$ ratio by 0.63%, 7.50%, 1.91%, 18.21%, 9.48%, 7.50%, 5.47% and 8.49%, while at 10FR the ratio was decreased by 4.91%, 16.99%, 21.13%, 30.19%, 31.86%, 21.53%, 9.78% and 10.56%, respectively against the control in the same incubation periods. At 100FR, it reduced by 9.88%, 19.64%, 21.82%, 33.58%, 41.71%, 31.27%, 17.83% and 22.86% at 1, 3, 5, 7, 10, 15, 25 and 45 days incubation respectively, compared to the control.

## 3 Discussion

Our study showed that the application of chlorsulfuron herbicide at field rate had no significant effect on N-mineralization at different incubation periods. In general, herbicides have little influence on ammonification and nitrification when added at field rates (Domsch, 1974; Wainwright, 1977). Many studies on the effect of pesticides on microorganisms have focused on nitrogen transformations in soils. Coring (Coring, 1982) showed a range of effects of different pesticides on nitrogen mineralization, immobilization, nitrification, denitrification and fixation. Nitrifying bacteria are most sensitive to herbicide application (Edwards, 1989). Hegazi (Hegazi, 1979) observed that nitrogenase activity as well as numbers of a symbiotic nitrogen-fixing bacteria declined in soils dosed with a group of pesticides.

Our results further revealed that the application at 10 and 100 field rates significantly reduced the soil N-mineralization within first 5 days after treatment as compared to the control. The reason for the decrease in N-mineralization by chlorsulfuron may be related to the toxic effect of the herbicide as the effect of herbicides on soil fauna is either a direct toxic effect or through the influence on predator-prey interactions (Roper, 1995). Hendrix and Parmelee (Hendrix, 1985) also reported that paraquat and glyphosate treatment reduced the decomposition of crop residues. There are numerous difficulties in the interpretation of the data available from various research programs carried out on effect of herbicides on N-mineralization in soils. In field trials, Kasper and

Fischbeck (Kasper, 1989) indicated that N mineralization rate and potentially mineralizable N are not affected by formulations such as 2,4-D amine or ester at rates of 1.23 kg/hm$^2$, but urease was temporarily depressed by both formulations. Nitrification is also reduced temporarily by the ester formulation. Harper et al. (Harper, 1995) showed that nitrogen in the clover crop increase until anthesis, and then declined slightly prior to desiccation with herbicides.

Hart and Brookes (Hart, 1996) investigated the effects of 19 years of cumulative annual field application of five pesticides applied at the recommended rates in 25 combinations, on the mineralization of soil organic matter in UK. They observed that the mineralization of soil organic N to ammonium and then nitrate was mostly unaffected by the pesticide treatments.

The noted decrease of $N_{mic}/N_{min}$ ratio was related to the decrease of microbial biomass N and N-mineralization, due to increase in the level of chlorsulfuron and subsequent increase in toxicity caused greater reduction in $N_{mic}$ in treated soil than in the control. The decrease in $N_{mic}/N_{min}$ ratio can give a highlight of changes in microbial populations and N-mineralization.

Still there is further scope to investigate the effect of sulfonylurea herbicides on $N_{mic}/N_{min}$ ratio.

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