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Influence of chlorsulfuron herbicide on size of microbial biomass in the soil

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Abstract: A laboratory incubation experiment was conducted to study the effect of chlorsulfuron herbicide on the size of the microbial in loamy sand soil. The herbicide was applied, at four levels that were control, field rate 0.01 (FR), 0.1 (10FR) and 1 (100FR) μg/g. Determinations of microbial biomass-C content 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 microbial biomass carbon and biomass nitrogen decreased significantly in soil treated with herbicide in levels 10FR and 100FR within the first 10 days incubation. A more considerable increase in the microbial biomass C:N ratio was observed in the herbicide treated soil than the non-treated control. This effect was transitory and only at the higher rates of chlorsulfuron was significant.

Key words: chlorsulfuron; microbial biomass C; microbial biomass N; biomass C/N

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Introduction

The use of chemicals, including pesticides, has become an integral and economically essential part of modern agriculture. Pesticides are often applied several times during one growing season, and a portion of the substances applied always reaches the soil. As they are designed to be biologically active, it is of concern that repeated application of pesticides may affect the soil microflora and so impairs the soil fertility. In recent years, two revolutionary classes of herbicides have been put on the market, the sulfonylureas and imidazolinones, which are employed at rates ranging from 10 to 100 times less of the traditional herbicides (Perucci, 1996).

When herbicides are applied to soil, they may exert certain side effects on non-target organisms. Therefore, there has been considerable interest on the influence of herbicides on the soil microflora and microbially mediated processes (Simon-Sylvester, 1979; Greaves, 1980).

The majority of studies on this subject have focussed on herbicide effect on microbial activities, including nitrification, denitrification, soil respiration and soil enzyme activity (Carlisle, 1986; Yeomans, 1987) and little on microbial biomass. Although the microbial biomass has been largely neglected in herbicide studies, it plays an important role in the soil ecosystem in acting as a highly labile pool of nutrients, which are rapidly cycled and are directly responsible for regulating the nutrient levels available for plants (Anderson, 1980; Jenkinson, 1981; Stevenson, 1986).

Chlorsulfuron is member of the sulphonylurea group of herbicides and is used for the control of annual weeds in autumn and spring-sown cereals (Beyer, 1987). Chlorsulfuron is highly active in soil at the recommended rates of 13 to 30 g/hm^2. Many crops are sensitive to chlorsulfuron and may be severely injured by chlorsulfuron 1 year after application (Zimdahl, 1983). Walker and Brown (Walker, 1983) found chlorsulfuron to have a half-life, which depends on temperature.

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The half-life varied from 64d at 10℃ to 9d at 30℃ in a sandy loam. They also reported increase in chlorsulfuron persistence when water content decreased.

The purpose of the present investigation was to assess the influence of chlorsulfuron on size of microbial biomass carbon (C_{mic}), microbial biomass nitrogen (N_{mic}), and the ratio of C_{mic}/N_{mic} in loamy sand soil.

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 the collection, hand picked to remove discrete plant residues and large soil animals (earth warms, etc.), passed through a 2 mm sieve and homogenized thoroughly. A sub-sample of the soil was taken, air-dried, ground, and analyzed for various physico-chemical propertied listed in Table 1.

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 other were treated with chlorsulfuron herbicide.

Table 1 Some physico-chemical properties of the soil used in the experiment

Coarse sand, %	9	Soil water % at 33 kPa	22.4
Fine sand, %	73	pH	6.27
Silt, %	10	CEC, meq/100g soil	10.55
Clay, %	8	Total organic carbon, %	1.76
Soil texture	Loamy sand	Total nitrogen, %	0.158

Methanol solutions of chlorsulfuron were prepared at three different concentration: 0.0005, 0.005 and 0.05 mg/ml, respectively. The conversion of field-rate application to milligrams of chlorsulfuron/100g of soil was calculated assuming an even distribution of the herbicide in the 0 to 20 cm layer and a bulk density 1.5 g/cm^3.

The herbicide was incorporated into the soil sub-sample as follows: forty-eight ml of the methanolic solution of chlorsulfuron was added to 120g of air-dried soil previously for each sub-sample. Forty-eight ml of methanol was added 120g of air-dried soil for the control soil.

After complete removal of the methanol by evaporation at room temperature, each of the 120g soil was divided to 24 portion each one having 5g and transferred it into the beaker containing 95g fresh soil (even dry basis) and homogenized.

With this procedure three application rates corresponding to FR, 10FR and 100FR were obtained.

Soil moisture was adjusted to 60% water content at 33 kPa and incubated in the dark at 24℃. The beakers were removed from the incubator every day and brought to the original weight by adding the required amount of distilled water.

Three beakers each with the a control and three for each treated soil sample were removed and submitted to analysis 1, 3, 5, 7, 10, 15, 25 and 45 days after chlorsulfuron treatment.

1.3 Microbial biomass determination

Soil samples for the determination of microbial biomass C were extracted by a fumigation-extraction (FE) method (Vance, 1987) and the organic carbon in the soil extracts was measured using an automated total organic carbon analyzer (Wu, 1990). 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).

1.4 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 (in water, 1: 2.5) of the soils was measured with a pH meter. Total N was determined by Kjeldahl method and total organic carbon by Walkley-Black procedure (Jackson, 1958).

1.5 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 microbial biomass carbon (C_{mic})

The responses of the microbial biomass-C content to the herbicide treatments are shown in Table 2. The data confirmed that the microbial biomass-C content was not significantly modified at the FR. When chlorsulfuron was applied at 10FR and 100FR the decrease in the microbial biomass C content became significant especially within the first 10 days.

Table 2 The effect of chlorsulfuron herbicide on microbial biomass-C

Incubation period/day	Herbicide treatment, μg/g Control(0)	FR(0.01)	10FR(0.1)	100FR(1)	LSD 0.05
1	270.5 a*	256.9 a	232.4 b	199.6 c	21.58
3	261.5 a	234.7 b	212.4 b	179.0 c	22.73
5	240.1 a	222.2 ab	192.2 bc	165.4 c	32.53
7	234.1 a	208.5 ab	-180.2 bc	161.4 c	35.49
10	212.6 a	206.7 a	176.7 b	153.3 c	18.75
15	224.9 a	216.8 ab	196.3 bc	173.7 c	23.59
25	227.0 a	223.7 a	207.4 b	192.1 c	10.87
45	223.2 a	219.8 ab	203.6 bc	188.2 c	18.83

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

The result indicated that chlorsulfuron treatment at FR reduced the microbial biomass-C content by 5.04%, 10.23%, 7.74%, 10.91%, 2.76%, 3.63%, 1.48% and 1.5% at 1, 3, 5, 7, 10, 15, 25 and 45 days of incubation respectively compared with the control. However, with applied 10FR the decrease in microbial biomass C were 14.1%, 18.8%, 19.9%, 23%, 18.9%, 12.7%, 8.6% and 8.8% respectively, compared with the control and the same incubation period. The treated soil with 100FR resulted in 26.2%, 31.5%, 31.1%, 31.0%, 27.9%, 22.8%, 15.4% and 15.7% respectively and with the same incubation period compared with the control.

2.2 The effect of chlorsulfuron on microbial biomass nitrogen (N_{mic})

Data regarding the effects of chlorsulfuron treatments in incubation trials are shown in Table 3. When chlorsulfuron was applied at the field rate, there was no significant change on soil microbial biomass N content. The 10FR dosage caused significant decrease in microbial biomass N content, and with 100FR the decrease in microbial biomass N became highly significant within first 15 days after incubation.

Table 3 The effect of chlorsulfuron herbicide on microbial biomass-N

Incubation	Herbicide treatment, μg/g				LSD
period/day	Control(0)	FR(0.01)	10FR(0.1)	100FR(1)	0.05
1	49.40 a*	45.41 a	36.15 b	27.40 c	6.645
3	40.07 a	33.22 b	25.23 c	19.98 c	5.326
5	35.58 a	30.39 a	22.43 b	17.95 b	5.256
7	34.03 a	27.03 b	19.68 c	16.14 b	6.554
10	32.09 a	27.69 a	18.82 b	15.20 b	6.525
15	33.48 a	28.90 a	23.35 b	18.92 b	5.090
25	35.02 a	32.40 a	29.58 ab	25.10 b	5.849
45	34.12 a	30.60 ab	28.68 ab	24.20 b	6.214

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

The result indicated that chlorsulfuron applied at FR resulted in 8.1%, 17.1%, 14.6%, 20.6%, 13.7%, 7.5% and 10.3% decrease in biomass N. A decrease in microbial biomass N, was also observed at 10FR whereby it was reduced by 26.8%, 37.0%, 36.96%, 42.17%, 41.4%, 30.3%, 15.5% and 15.9%. At 100FR on the other hand, the microbial biomass N decreased by 44.5%, 50.14%, 49.6%, 52.6%, 52.6%, 43.5%, 28.3% and 29.1%, compared with the control at 1, 3, 5, 7, 10, 15, 25 and 45 days after incubation respectively.

2.3 The effect of chlorsulfuron on (C_{mic}/N_{mic}) ratio

The addition of chlorsulfuron to the soil showed increase in the C_{mic}/N_{mic} ratio (Fig.1). Results indicated that the increase in C_{mic}/N_{mic} ratio was due to increase in the level of herbicide within first 10 days. The data indicated that herbicide application at FR increased C_{mic}/N_{mic} ratio by 3.30%, 8.28%, 8.33%, 12.16%, 12.69%, 11.64%, 6.49% and 9.83% at 1, 3, 5, 7, 10, 15, 25 and 45 days incubation respectively, compared to the control. At 10FR the ratio was increased by 17.39%, 29.00%, 26.99%, 33.13%, 41.74%, 25.13%, 8.17% and 8.52%, while at 100FR it increased by 33.04%, 37.32%, 36.54%, 45.38%, 52.24%, 36.66%, 18.0428% and 18.91% at 1, 3, 5, 7, 10, 15, 25 and 45 days incubation respectively, compared to the control.

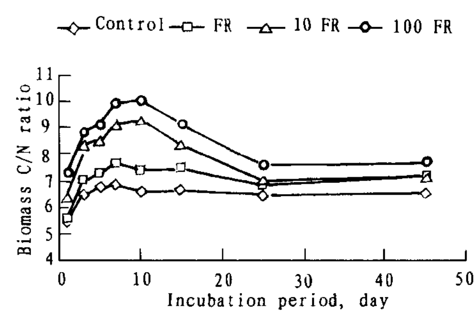

Fig.1 The effect of chlorsulfuron on C_{mic}/N_{mic} ratio

3 Discussion

The application of chlorsulfuron herbicide at field rate had no significant effect on microbial biomass. However the application at 10 and 100 field rates significantly reduced the soil microbial biomass within first 15 days after incubation. The reason for the decrease of microbial biomass as affected by chlorsulfuron may be related to the toxicity effect of the herbicide because chlorsulfuron is a new generation of herbicides that are toxic even at low rates, and injury to rotational crops may occur up to 3 years following application (Sweetser, 1982; Duah, 1986). Ismail *et al*. (Ismail, 1996) observed a decrease in microbial biomass with increase of herbicide concentration on day 1, and decreased bacterial population as the concentration increased in sandy loam soil during first 9 days. The use of remsulfuron decreased microbial biomass-C levels at 10 field rate and 100 time of field rate within the first 10d (Perucci, 1996).

For this reason, the decrease of microbial biomass content was negatively correlated with dosage of herbicides. So it can be inferred that a portion of the soil microflora was killed by chlorsulfuron herbicide. Similarly Tyuryukanova *et al*. (Tyuryukanova, 1987) showed a negative correlation between herbicide dose and microbial biomass, which become more marked as temperature and moisture conditions become unfavorable.

Significant decrease in microbial biomass during early incubation period with high concentration of chlorsulfuron can possibly be explained by the degradation rate, the half-life and the adsorption of herbicide. Like other sulfonylureas, chlorsulfuron is rapidly degraded in soil either by chemical hydrolysis or microbial processes (Brown, 1990; Palm, 1989). The half-life of chlorsulfuron has been found to be approximately 1 to 2 month (Walker, 1983). This means that this herbicide that does not usually cause carryover problems, and the residual levels are low. Thirunarayanan *et al*. (Thirunarayanan, 1985) reported that disappearance of chlorsulfuron is rapid in the first 15 days after treatment after which there is a slower rate. With decreasing moisture and temperature, chlorsulfuron degraded more slowly. They also reported that adsorption of chlorsulfuron on four soils having different pH values (6.2 to 8.1) was low. We also observed similar trend in our study that during the first 15 days of incubation there was a sharp decrease in the microbial biomass carbon and nitrogen contents and then an increase was noticed afterwards with the incubation period and become stagnant at the final stage of incubation compared to the control.

The noted increase in C_{mic}/N_{mic} ratio was related to the decrease of microbial biomass C and N and due to increase in the level of chlorsulfuron and subsequent increase in toxicity caused greater decline in N_{mic} in treated soil than in the control. Decline in C_{mic} was relatively lower. The increase in biomass C/N ratio can give a highlight of changes in microbial populations. Khan and Huang (Khan, 1998) concluded that increase in biomass C/N ratio, in chemically polluted soil was a resulted have increased fungal biomass in the soil. They suggested that the changes in the biomass C/N ratio could be a good indicator of the changes in the microbial community structure.

There still a lack of information on the effect of sulfunylurea herbicides on microbial biomass C, N and the biomass C/N ratio. Future experiments should try to focus on this group of herbicides.

4 Conclusion

We have observed that temporary reduction of microbial biomass C, N, and increase in the C_{mic}/N_{mic} ratio followed application of chlorsulfuron. This result is consistent with other earlier reports that have shown that this herbicide did not have any detrimental effect on soil microflora when used at recommended rate. But the effect is highly transitory and is significant at 10 and 100 times of the field rates. These concentrations of herbicides 10FR, and 100FR which are higher than those recommended, although, not relevant for agricultural managemental practices, may be useful for assessing the environmental risk of herbicides in cases of long-term application. Although the present experiment involved studies under laboratory conditions, the results could be extrapolated to field conditions.

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