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Combined effects of two sulfonylurea herbicides on soil microbial biomass and N-mineralization

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Abstract: The interaction effect of two sulfonylurea herbicides, bensulfuron-methyl (B) and metsulfuron-methyl (M), were tested on microbial biomass C, N, N-mineralization and C/N ratio in a loamy sand soil. The herbicides were applied at various levels of: control (B0M0), 0.01 and 0.01 (B1M1), 0.01 and 0.1 (B1M2), and 0.01 and 1.0 (B1M3) μg/g soil. Determinations of soil microbial biomass-C, N and N-mineralization contents were carried out at 1, 3, 5, 7, 10, 15, 25 and 45 days after herbicides application. The results showed that the soil microbial biomass-C (C_{mic}) and microbial biomass-N (N_{mic}) decreased consistently with the increasing rates of herbicides. The results further indicated that B1M1 and B1M2 caused a significant reduction in C_{mic} and N_{mic} within first 10 and 7 days of incubation, respectively, as compared with the control. These reductions in C_{mic} and N_{mic} were also significant ($P = 0.05$) with B1M3 application especially within first 15 days of incubation. A significant reduction in N-mineralization (N-min) was observed with high doses (B1M2, B1M3) of herbicides within first 5 days of incubation, while low rate (B1M1) failed to produce any significant effect. An increase in the soil microbial biomass C:N ratio was also noted.

Key words: bensulfuron-methyl; metsulfuron-methyl; C_{mic}; N_{mic}; N-mineralization; loamy sand soil

Introduction

Agrochemicals have been acted a very important role in agricultural production. For avoiding damage of crops, many kinds of pesticides are applied for the reason of insect, diseases and weed control in China. Scientists have done a lot of work on the fate and metabolism of pesticides, and got a lot of useful data (Waiwright, 1978; Moorman, 1989). Yet most of the studies were focused on a single application for a short period, and knowledge of pesticide effects on soil properties, especially repeated long-term applications, has been still limited. Microorganisms are an important component of soil ecosystem. The effects of agrochemicals on soil microbial activity are of great concern to biologists (Hang, 1993; Tu, 1990).

In a laboratory study, cellulose decomposing activity and microbial biomass in a sandy loam soil were stimulated by low or normal rates of herbicides, insecticides and fungicides used typically in winter wheat and in orchard fruit crops. Higher rates or the use of combinations or sequences of several compounds, however, could lead to reduced microbial activity and populations (Nowak, 1990). A sandy loam soil was treated with Afalon (50% linuron), Gramoxone (20% paraquat) and a mixture of both, all applied at 1 - 1000 mg/kg soil, and incubated at 10% - 90% maximum water capacity. Morobial biomass, determined after 7, 14 and 21d, was stimulated by the lower concentration of Gramoxone alone and in combination with Afalon while itself was inhibitory. Increasing doses increased the inhibitory effects of the herbicides (Nowak, 1992).

Several herbicide combinations with other pesticides have been shown to increase crop injury compared to either pesticide applied alone. Efficacy data on herbicide-pesticide mixtures are limited because of the number of potential combinations (Alan, 1995).

The concept of concentration addition has been shown to be applicable for mixtures of substances with independent modes of action or similar modes of action and common target sites, e.g. anticholinesterase compounds such as organophosphates or carbamates. The effect is additive if the same effect can be obtained by replacing one of the two combined substances totally or in part, with an equi-effective amount of the other, e.g. by acting like a dilution of the other (Boedecker, 1993; Faust, 1993; 1994).

The present investigation aimed at assessing the influence of bensulfuron-methyl and metsulfuron-methyl herbicides on size of microbial biomass carbon (C_{mic}), microbial biomass nitrogen (N_{mic}), the ratio of C_{mic}/N_{mic}, and N-mineralization in a loamy sand soil.

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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 physical and chemical properties. The soil used contains 1.76% total organic carbon, 0.158% total nitrogen, 22.4% soil moisture at – 33 kPa, and pH value of 6.27.

1.2 Herbicide treatment

After sampling and preparation of soil, the soil sample was subdivided into four sub-samples (120g each). One sub-sample was used as a control, and the others were treated with various combinations of bensulfuron-methyl and metsulfuron-methyl herbicides.

Methanol solutions of metsulfuron-methyl were prepared at three different concentrations: 1, 10, and 100 μg/ml and bensulfuron-methyl solution was prepared at one concentration of 1 μg/ml.

1.3 Herbicides treatments for assay of microbial biomass C and N

The herbicides were incorporated into the soil sub-sample as follows: 24 ml of the metsulfuron-methyl methanolic solution was taken from each of the three concentrations and added to each sub-sample, respectively. 24 ml of methanolic bensulfuron-methyl solution was also added to each sub-sample. 48 ml of methanol was added to 120g of air-dried soil used as control. After complete removal of the methanol by evaporation at room temperature, each of the 120g soil was further divided to 24 portions of 5g each and transferred them into the beakers containing 95g fresh soil (oven dry basis) and homogenized. With this procedure four application rates corresponding to 0.0 + 0.0 (B0M0), 0.01 + 0.01(B1M1), 0.01 + 0.10 (B1M2) and 0.01 + 1.00 (B1M3) μg/g soil (bensulfuron-methyl + metsulfuron-methyl, respectively) were obtained.

Soil moisture was adjusted to 60% water content at – 33 kPa and incubated in dark at 25 ± 1℃. The beakers were removed from the incubator every day and brought to the original weight by adding the required amount of distilled water. At 1, 3, 5, 7, 10, 15, 25 and 45 days after the addition of metsulfuron-methyl and bensulfuron-methyl three beakers were taken out from each treatment and submitted to analysis for microbial biomass C and N.

1.4 Herbicides treatments for assay of N-mineralization

Methanolic solution of herbicides were prepared by the same procedure as described above.

The herbicides were mixed into the soil sub-sample as follows: 3 ml of methanolic solution of bensulfuron-methyl was added to 15g of air-dried soil for each sub-sample. 3 ml of the metsulfuron-methyl methanolic solution of each concentration (1, 10 and 100 μg/ml) was also added to the same sub-samples, respectively. 6 ml of methanol was added to 15g of air-dried soil used as 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 weighed and transferred into 30 ml glass tubes. With this procedure three application rates corresponding to B1M1, B1M2 and B1M3 were obtained.

The soil was then incubated in dark at 25 ± 1℃. Three tubes from each treatment were removed and submitted to analysis for N- mineralization at 1, 3, 5, 7, 10, 15, 25 and 45 days after the addition of bensulfuron-methyl + metsulfuron-methyl.

1.5 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).

1.6 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 calorimetrically (Anderson, 1993).

1.7 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).

1.8 Statistical analysis

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

2 Results

2.1 Combined effect of bensulfuron-methyl and metsulfuron-methyl on C_{mic}

The combined addition of bensulfuron-methyl and metsulfuron-methyl reduced the soil microbial biomass-C (Table 1). The microbial biomass-C (C_{mic}) decreased consistently with the increasing levels of combined herbicides in the soil. Results indicated that B1M1 treatment of herbicides caused a significant reduction within first 10 days of incubation and from the day 15th it became non-significant as compared with the control.

Table 1 The combined effect of bensulfuron-methyl and metsulfuron-methyl on microbial biomass C (μg/g soil) in a loamy sand soil

Incubation		Microbial biomass C, μg/g soil			Reduction in C_{mic}, %			LSD
period, d	B0M0**	B1M1	B1M2	B1M3	B1M1	B1M2	B1M3	(0.05)
1	251.00 a*	220.98 b	197.69 c	177.56 d	11.96	21.24	29.26	9.08
3	248.12 a	216.02 b	193.63 c	168.32 d	12.94	21.96	32.16	11.90
5	235.06 a	208.69 b	190.00 c	171.09 d	11.22	19.17	27.21	15.90
7	230.23 a	205.03 b	188.98 b	170.01 c	10.95	17.92	26.16	17.36
10	220.20 a	207.11 b	198.12 b	178.04 c	5.94	10.03	19.15	11.52
15	212.82 a	204.14 ab	200.09 b	188.83 b	4.08	5.98	11.27	15.28
25	212.99 a	210.11 a	205.18 a	196.21 a	1.35	3.67	7.88	18.77
45	213.69 a	210.89 a	207.84 a	200.72 a	1.31	2.74	6.07	12.29

*. Means with different letters, within rows, differ significantly according to LSD ($P = 0.05$); **. B0M0, B1M1, B1M2, and B1M3 = control, 0.01 + 0.01, 0.01 + 0.1, 0.01 + 1.0 μg/g soil of bensulfuron-methyl and metsulfuron-methyl, respectively

The combined addition of bensulfuron-methyl and metsulfuron-methyl (B1M2) herbicides to the soil also resulted in a significant decline in the soil microbial biomass-C within first 15 days of incubation. While, the addition of B1M2 caused a non-significant reduction in the C_{mic} at 25th day of incubation, as compared with the control. The B1M3 treatment exhibited significant reductions within first 15 days of incubation, but it caused non-significant reductions in the C_{mic} from 25th day of incubation, as against the control.

2.2 Combined effect of bensulfuron-methyl and metsulfuron-methyl on N_{mic}

The microbial biomass-N was decreased by the combined additions of bensulfuron-methyl and metsulfuron-methyl to the soil (Table 2). The B1M1 treatment resulted in significant declines in N_{mic} within first 7 days of incubation, and it changed to non-significant reductions from the day 10th of incubation onward, as compared to the control.

Table 2 The combined effect of bensulfuron-methyl and metsulfuron-methyl on microbial biomass N(μg/g) soil in a loamy sand soil

Incubation		Microbial biomass N, μg/g soil			Reduction in N_{mic}, %			LSD
period, d	B0M0**	B1M1	B1M2	B1M3	B1M1	B1M2	B1M3	(0.05)
1	44.92 a*	33.31 b	30.40 c	25.41 d	25.85	32.32	43.43	2.40
3	36.18 a	28.26 b	23.52 c	19.68 d	21.89	34.99	45.61	3.77
5	30.75 a	23.66 b	21.78 b	17.23 c	23.06	29.17	43.97	2.90
7	28.94 a	23.12 b	20.20 bc	16.70 c	20.11	30.20	42.29	3.80
10	29.94 a	26.91 ab	23.59 ab	21.08 b	10.12	21.21	29.59	6.44
15	32.06 a	30.15 ab	27.90 ab	24.65 b	5.96	12.98	23.11	5.74
25	31.10 a	29.62 a	28.33 a	25.39 a	4.76	8.91	18.36	5.66
45	31.60 a	30.25 a	29.70 a	26.68 a	4.27	6.01	15.57	5.39

* Means with different letters, within rows, differ significantly according to LSD ($P = 0.05$); ** B0M0, B1M1, B1M2, and B1M3 = control, 0.01 + 0.01, 0.01 + 0.1, 0.01 + 1.0 μg/g soil of bensulfuron-methyl and metsulfuron-methyl, respectively

A considerable decrease in the N_{mic} was observed by B1M2 application to the soil. The herbicides treatment B1M2 caused significant reductions in the N_{mic} within first 7 days of incubation, it became non-significant at 10th day of incubation, as compared with control.

These reductions were also found significant ($P = 0.05$) with B1M3 application especially within first 15 days of incubation, and it became non-significant at 25th day and onward incubation against the control.

2.3 Combined effect of bensulfuron-methyl and metsulfuron-methyl on N-mineralization

The combined application of herbicides to the soil slightly reduced the N-mineralization (Table 3). A non-significant decline in the N-mineralization occurred at B1M1 level of herbicides at all incubation periods of 1, 3, 5, 7, 10, 15, 25 and 45 days, compared to the control. A significant decrease in N-min. was observed at B1M2 level of herbicide especially within first 5 days of incubation, which became non-significant after 7th day of incubation, as compared with the control. The applications of B1M3 exhibited significant reductions in N-min. especially within first 5 days of incubation, then it became non-significant, as compared with the control.

Table 3 The combined effect of bensulfuron-methyl and metsulfuron-methyl on N-mineralization (μg/g soil) in a loamy sand soil

Incubation	Microbial biomass, μg/g soil				Reduction in C_{mic}, %			LSD
period, d	B0M0**	B1M1	B1M2	B1M3	B1M1	B1M2	B1M3	(0.05)
1	18.75 a*	16.34 ab	14.80 b	13.94 b	12.85	21.07	25.65	3.12
3	20.19 a	18.27 ab	16.63 b	15.30 b	9.51	17.63	24.22	2.93
5	21.25 a	20.19 ab	18.46 b	18.27 b	4.99	13.13	14.02	2.34
7	23.56 a	22.59 a	20.67 a	21.15 a	4.12	12.27	10.23	2.83
10	28.36 a	27.40 ab	26.44 ab	25.48 b	3.39	6.77	10.16	2.72
15	32.69 a	32.21 a	30.76 a	30.28 a	1.47	5.90	7.37	2.72
25	33.17 a	32.69 a	31.72 a	31.24 a	1.45	4.37	5.82	2.22
45	35.10 a	34.61 a	33.84 a	33.26 a	1.40	3.59	5.24	2.65

*. Means with different letters, within rows, differ significantly according to LSD ($P = 0.05$); **. B0M0, B1M1, B1M2, and B1M3 = control, 0.01 + 0.01, 0.01 + 0.1, 0.01 + 1.0 μg/g soil of bensulfuron-methyl and metsulfuron-methyl, respectively

2.4 Combined effect of bensulfuron-methyl and metsulfuron-methyl on C_{mic} : N_{mic} ratio

An increase in the soil microbial biomass C : N ratio was noted due to combined application of bensulfuron-methyl and metsulfuron-methyl to the soil (Fig. 1). The increase in the ratio was significant at the 1st day of incubation with B1M1 level of herbicides, but with B1M2 it was significant up to 3rd day of incubation, and with B1M3 the significance increased up to 5th day of incubation.

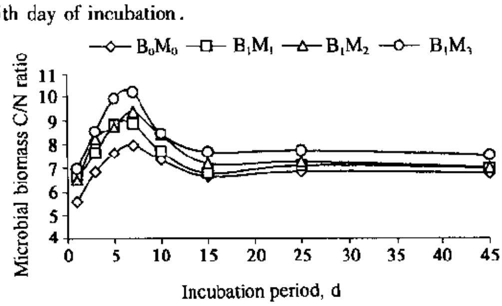

Fig. 1 Combined effect of bensulfuron-methyl and metsulfuron-methyl herbicides on microbial biomass C/N ratio in a loamy sand soil

The biomass C : N ratio of 5.59 observed in the control increased significantly to 6.63, 6.50 and 6.99 with B1M1, B1M2, B1M3 respectively at the 1st day of incubation. A considerable increase in the biomass C : N ratio of 6.86 was found with the addition of herbicides to the soil at the 3rd day of incubation, the biomass C : N ratio observed in the control was improved to 7.64, 8.23 and 8.55 with the same levels of herbicides, respectively. The treatments of herbicides applied at B1M1, B1M2 and B1M3 resulted in the increased biomass C : N ratios of 8.82, 8.72 and 9.93, respectively, at the 5th day of incubation as compared to the control (7.64).

At 7th day of incubation, C_{mic} : N_{mic} ratios were enhanced to 8.87, 9.36 and 10.18 as compared with the control (7.96). But at 10th day of incubation the increase in the ratio was 7.70, 8.40 and 8.45 with increasing levels of herbicides against the control (7.35). Results indicated that herbicides applied at the three different levels caused an increase in the ratio from 6.64, found in the control, to 6.77, 7.17 and 7.66 at the 15th day of incubation, respectively. With the same levels of herbicides, at 25th day of incubation, the increase was 7.09, 7.24 and 7.73 respectively, against to the control (6.85). But the increase in the C_{mic} : N_{mic} ratio was 6.97, 7.00 and 7.52, respectively, compared to the control (6.76) at 45th day of incubation.

3 Discussion

The combination effect of bensulfuron-methyl and metsulfuron-methyl was observed more pronounced as compared to control at the different levels of application on soil microbial biomass C and N at different incubation periods. The microbial biomass C and N decreased with the increasing levels of herbicide applications. The decrease in microbial biomass C was significant during the first 10, 15 and 15 days of incubation at B1M1, B1M2 and B1M3 applications, respectively, as compared with the control. However, the decrease in microbial biomass N was significant only during the first 7, 7 and 15 days of incubation at the different levels of treatments, respectively as compared to the control. The decreased toxicity of herbicides after few/some days of incubation might be their short half lives. Gigliotti *et al*. (Gigliotti, 1998) found that bensulfuron-methyl, like other sulfonylureas herbicides, is rapidly degraded in soil. The half-lives ranged from less than 1 week to about 3 weeks, which places bensulfuron-methyl at the lower end of the range (1 – 8 weeks). Also, the half-life for metsulfuron-methyl at different soil water content and temperatures is 8 to 36 days (James, 1995).

The reason for the decrease of microbial biomass as affected by combination of herbicides may be related to the toxic effect of herbicides. The behavior of microbial numbers and activities in the soils in the presence of bensulfuron-methyl could be related to the observed persistence of the herbicide (Gigliotti, 1998), also Junnila *et al*. (Junnila, 1994) reported that metsulfuron persisted in the soil. They also reported that the decrease in some microbial numbers may be related to the fact that microflora in this soil had not adapted to bensulfuron-methyl.

The fate of sulfonylurea in soils is directly related to their chemical structure and mainly to the ionization of the sulfonylurea bridge. They are weak acids with pKa from 3 – 5 (3.3 for metsulfuron-methyl) and in soils they are mainly in the ionized form. This explains their low sorption coefficients, which are pH-dependent (Brown, 1990; Walker, 1989).

Some research workers found that herbicides such as MCPA and simazine has caused no detectable effects to the microflora, but repeated paraquat application significantly lowered the soil microbial biomass. Their results indicated that there might be substantially different effects on soil biomass produced by single or repeated applications of pesticides (Yentum, 1986).

Our results demonstrated that the N-mineralization was non-significantly effected with the addition of combination herbicides. Much of the research on the effect of pesticides on microorganisms was focused on nitrogen transformations in soils. Transformation of nitrogen, a complex process brought about by succession of different microorganisms, is obviously related to soil fertility. Each form of mineral nitrogen is determined separately in order to obtain a measure of nitrification, the oxidation of ammonium to nitrite and eventually nitrate. This reaction is one which many herbicides are known to influence (Audus, 1970). Nitrifying bacteria are most sensitive to herbicide application (Edwards, 1989). Hegazi *et al*. (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 applications at B1M2 and B1M3 rates significantly ($P = 0.05$) reduced the soil N-mineralization within first 5 days after treatment as compared to control. Gigliotti *et al*. (Gigliotti, 1998) found that the differences in nitrifies activity attributed to an inhibition by bensulfuron-methyl were always statistically significant. However, only for the highest dose of herbicide in soil the inhibition was notable. The reason for the decrease in N-mineralization by bensulfuron-methyl at high rates 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). 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 1.12 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 increase in C_{mic}/N_{mic} ratio is related to the decrease of microbial biomass C and N and increase in the level of herbicides application. The increase in the levels of combined herbicides, causing subsequent increase in toxicity of the herbicide, caused greater decline in N_{mic} in treated soil than the control. The ratio of biomass C_{mic}/N_{mic} was therefore in favor of C_{mic} than N_{mic}. The increase in biomass C/N ratio can give a highlight of changes in microbial populations (Khan, 1998). They also suggested that the changes in the biomass C/N ratio could be a good indicator of the changes in the microbial community structure. On the basis of these findings the increases in C:N ratio may be explained by the increase in fungal population due to the herbicides toxicity. Anderson (Anderson, 1980) found that C:N ratio of the fungal biomass was higher than of the bacteria.

We observed that combined application of herbicides caused a temporary increase of microbial biomass C/N ratio. It is concluded that change in microbial biomass only occurred at herbicide concentrations of much higher than that which occurs following lower application, the side effect of these chemicals is probably of little ecological significance. Those concentrations of herbicides, which are higher than those recommended, although, not relevant for agricultural management practices, may be useful for assessing the environmental risk of herbicides in cases of long-term application. Although the present investigation involved studies under laboratory conditions, the results could be extrapolated to field conditions.

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