JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Microbial Response to Bensulfuron-Methyl Treatment in Soil

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A laboratory incubation study was conducted to evaluate the effect of bensulfuron-methyl treatment on soil microbial biomass and N-mineralization of a loamy sand soil. The herbicide was applied at 0 (control), 0.01 (field rate), 0.1, and 1.0 μg g^{-1}, and soil microbial biomass carbon (C_{mb}), soil microbial biomass nitrogen (N_{mb}), and N-mineralization rate (k) were measured at different times after herbicide treatment. Compared to the untreated soil, C_{mb} and N_{mb} decreased significantly ($p \leq 0.05$) within the first 7 days after herbicide treatment at 0.1 and 1.0 μg g^{-1}, and the impact was greater for N_{mb} than for C_{mb}. Nitrogen mineralization was significantly suppressed during the first 5 days of incubation when the soil was treated with bensulfuron-methyl at 0.1 and 1.0 μg g^{-1}. The overall impact of bensulfuron-methyl to the soil microbial communities was closely related to the application rate in the range of 0.01−1.0 μg g^{-1}. This effect, however, was found to be transitory, and significant impact occurred only at high application rates.

KEYWORDS: Bensulfuron-methyl; sulfonylurea herbicides; microbial biomass; nitrification; ammonification; nitrogen mineralization

INTRODUCTION

Soil fertility depends closely on the size and activity of soil microbial biomass, because soil microbes actively participate in the biological cycles of major plant nutrients (*1*). There is often a close relationship between the size of soil microbial biomass and soil organic carbon content (C_{org}). The microbial biomass C (C_{mb}), and the ratio of C_{mb} over C_{org}, are considered more sensitive indicators for soil organic matter than C_{org} (*2*). Some studies have shown that soil microorganisms play an essential role in transforming immobile forms of N to bioavailable forms of N for crops (*3*, *4*). Olfs suggested that, under immobilizing conditions, the size of microbial biomass could be used for predicting N-mineralization rates (k) (*5*). Hassink et al. also observed that microbial activity was a good indicator for N-mineralization, particularly in sandy soils (*6*).

Application of pesticides has been shown to cause changes in soil microbial makeup and activity. For instance, Marsh and Davies found that N-mineralization and nitrification of soil were significantly inhibited for a prolonged time after treatment with dichlorprop and mecoprop (*7*). Perucci and Scarponi showed that rimsulfuron decreased soil C_{mb} during the first 10 days when the herbicide was applied at 10× or 100× the typical field rate (*8*). Ismail et al. observed that microbial biomass in a clay loam soil initially increased after metsulfuron-methyl treatment, but then decreased after 19 d and onward. In a sandy loam soil, microbial biomass was reduced at high herbicide application rates on the first day after treatment, although the effect quickly disappeared (*9*).

Sulfonylurea herbicides are used for controlling broad-leaved weeds and some grasses in a variety of cereal crops. Because of their exceptionally low application rates (10−40 g ha^{-1}), low mammalian toxicity, and high herbicidal activity, the use of these herbicides has steadily increased over the past few years (*10*). The low use rates also help to overcome some of the handling, application, and container disposal issues. Bensulfuron-methyl (2-[4,6-dimethoxypyrimidin-2-carbamoylsulfamoyl]-*o*-toluic acid methyl ester) is a highly active sulfonylurea herbicide, and it is used in California, southeast China, and many other regions as a rice herbicide (*11*, *12*). Bensulfuron-methyl has a pK_a of 5.2 and its water solubility is pH-dependent, being 2.9 and 120 mg L^{-1} at 25 °C at pH values of 5 and 7, respectively (*13*). In two nonflooded soils, Gigliotti et al. found that soil nitrification was significantly inhibited when bensulfuron-methyl was used at 160 μg kg^{-1} (*14*). However, the mechanism for this suppression was not investigated. The main objective of this study was to evaluate the interaction of bensulfuron-methyl treatment with several important soil microbial biomass indicators, including C_{mb}, N_{mb}, C_{mb}/N_{mb}, and k. This study was conducted in soil from a typical rice-growing region in southeastern China. The findings will be helpful for assessing the environmental risks of sulfonylurea herbicides when used in this and other similar regions.

MATERIALS AND METHODS

Soil and Chemical. The loamy sand soil used in this study was collected from the surface layer (0−20 cm) of a field near Hangzhou, Zhejiang Province, China (30°19′ N, 120°12′ E). The fresh soil was

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10.1021/jf010756x CCC: $22.00
Published on Web 11/22/2001

transported to the laboratory immediately after sampling, hand picked to remove discrete plant residues and large soil animals (e.g., earth worms), passed through a 2-mm sieve, and thoroughly homogenized. A subsample of the soil was air-dried, ground, and analyzed for various physical and chemical properties. Total N content was determined by the Kjeldahl method (*15*), and total organic carbon was determined by the Walkley−Black procedure (*16*). Soil clay (<2 μm), silt (2−20 μm), and sand (20−2000 μm) contents were 8, 10, and 82%, respectively. The total organic carbon content (C_{org}) was 1.76%, total organic nitrogen content was 0.16%, CEC was 10.6 meq (100 g)$^{-1}$ soil, and pH value was (1:2.5, soil/water) 6.3. Bensulfuron-methyl (purity >99%) was obtained from DuPont China (Shanghai, China).

Incubation Experiment. Spike solutions of bensulfuron-methyl were made in methanol at 0.5, 5, and 50 μg mL^{-1}. Soil (120 g, oven dry weight) in glass beakers was spiked with 48 mL of the herbicide solutions, or 48 mL of methanol (control treatment). After complete removal of the methanol by evaporation at room temperature, 5 g aliquots of the treated soil were weighed out and mixed with 95 g (oven dry weight) of fresh, untreated soil in a glass beaker. It was found that the residual methanol in the treated soil was less than 0.05 μg g^{-1}, which was not expected to influence the microbial biomass in our preliminary experiment. The treatment resulted in initial herbicide concentrations of 0 (control), 0.01, 0.1, and 1.0 μg g^{-1}. Assuming a uniform distribution of the herbicide in the top 20-cm soil layer with a 1.50 g cm^{-3} bulk density, these initial concentrations would correspond to 0, 1, 10, and 100 times of the recommended field application rate. The soil water content in each container was subsequently adjusted to 22.4% (w/w), i.e., 60% of the soil water-holding capacity, and then transferred to an incubator (25 ± 1 °C). The loss of water from each container was compensated daily with distilled water. At 1, 3, 5, 7, 10, 15, 25, and 45 d after the treatment, three replicates for each herbicide rate were removed and subjected to the following microbial biomass analysis and N-mineralization experiment.

Microbial Biomass Characterization. Soil samples for biomass organic C analysis were extracted by the chloroform-fumigation-extraction (CFE) method (*17*). Briefly, soil samples were fumigated with chloroform and then extracted with 0.5 M K_2SO_4 at 1:5 soil-to-solution ratio by shaking for 2 h on an end-over-end shaker. The organic carbon in the soil extracts was measured on a Shimadzu TOC-500 total organic carbon analyzer (Shimadzu, Japan). Microbial biomass carbon was calculated from the difference between fumigated and nonfumigated samples (*17*). Soil samples for microbial biomass N analysis were also prepared using the chloroform-fumigation-extraction method (*18*) and the total nitrogen in the soil extracts was measured after Kjeldahl digestion. Data were subjected to analysis of variance using completely randomized and Duncan's multiple range tests.

Determination of Nitrogen Mineralization. Soil samples treated with different rates of bensulfuron-methyl were simultaneously removed on the same sampling dates for nitrogen mineralization measurement. Briefly, anaerobic N-mineralization was determined in 10-g aliquots of spiked soil that were flooded with 25 mL of deionized water in 30-mL glass bottles. The bottles were gently tapped to remove air bubbles, sealed with a rubber stopper, and then incubated at 25 ± 1 °C for 7 days. After incubation, the samples were transferred to 125-mL extraction bottles and extracted with 25 mL of 4 M KCl by shaking for 1 h on a reciprocal shaker, followed by gravity filtering through pre-washed Whatman No. 5 filter paper. Ammonium concentration in the soil extracts was determined by indophenol blue colorimetric method (*19*). Another 10-g aliquot of soil was similarly extracted with 50 mL of 2 M KCl without incubation and analyzed for initial ammonium content. N mineralization was calculated as the difference in ammonium between incubated and initial (before anaerobic incubation) samples. Nitrogen mineralization rates (k) were expressed as N released per g of soil per day (μg g^{-1} d^{-1}). Data were subjected to variance analysis using completely randomized and Duncan's multiple range tests.

RESULTS AND DISCUSSION

Effect on Microbial Biomass Carbon. The carbon associated with microbial biomass, C_{mb}, in the untreated soil represented 1.3−1.5% of the total organic carbon (C_{org}). This was within the 1−4% range found by other researchers (*2*, *20*). Application of bensulfuron-methyl affected C_{mb} of the soil, and the effect was clearly dependent on the herbicide treatment rate, and it was significant throughout the incubation period at high herbicide rates (Table 1). When the soil was treated with bensulfuron-methyl at 0.01 μg g^{-1} (typical field application rate), C_{mb} decreased only during the first few days after treatment and then was restored to a level that was no different from that of the untreated soil (Table 1). When the herbicide rate was increased to 0.1 or 1.0 μg g^{-1}, the decrease in C_{mb} significantly increased and the effect also lasted longer. The decrease was especially significant during the first 7 days after treatment. After 10 d, only the 1.0 μg g^{-1} treatment still had a C_{mb} value significantly ($P < 0.05$) smaller than that of the control treatment (Table 1).

Table 1. Effect of Bensulfuron-Methyl Treatment on Microbial Biomass Carbon Content (μg g^{-1}) in a Loamy Sand Soil

days after treatment	herbicide rate (μg g^{-1})			
	0	0.01	0.1	1.0
1	263.3 ± 6.1 a[a]	244.4 ± 0.7 b	220.6 ± 0.7 c	205.2 ± 3.7 d
3	255.4 ± 3.6 a	236.2 ± 6.7 b	209.3 ± 0.1 c	189.6 ± 4.8 d
5	236.4 ± 5.9 a	225.0 ± 5.8 a	193.8 ± 4.0 b	166.5 ± 3.2 c
7	232.6 ± 4.4 a	220.6 ± 2.0 a	197.7 ± 3.5 b	185.9 ± 4.2 b
10	224.4 ± 6.7 a	220.9 ± 5.5 a	205.3 ± 2.7 ab	190.1 ± 2.9 b
15	225.0 ± 5.8 a	224.8 ± 6.1 a	209.6 ± 6.2 ab	194.3 ± 5.0 b
25	223.1 ± 6.1 a	219.5 ± 2.7 a	212.0 ± 5.6 ab	203.9 ± 4.5 b
45	220.0 ± 2.0 a	216.7 ± 4.6 ab	212.5 ± 5.0 ab	209.6 ± 0.7 b

[a] Means with different letters in the same row are significantly different at the 0.05 probability level.

The negative effect of bensulfuron-methyl on soil microbial biomass C may be attributed to the toxicity of the herbicide to the indigenous soil microorganisms. Bensulfuron-methyl may be toxic to soil microorganisms at higher application rates. This mechanism may be also used to explain the time-dependent impact. In freshly spiked soil, the exposure of microorganisms to the herbicide would be the greatest. However, as time increases, degradation and adsorption to soil would reduce the fraction of herbicide that is still biologically active. Degradation may detoxify the parent compound, while the sorbed chemical may not have the herbicidal activity. It is generally believed that adsorption of organic chemicals such as pesticides to soil renders them unavailable for soil microorganisms (*21*). Bensulfuron-methyl, like other sulfonylurea herbicides, was found to degrade rapidly in soil, and the half-life ranged from less than 1 week to about 3 weeks (*14*). Gigliotti et al. also found that microbial population size and activities were influenced by the concentration of bensulfuron-methyl (*14*). A transitory reduction in microbial biomass C was also observed during the initial 10 days after application of rimsulfuron to a clay loam soil at 1.5 and 15 μg g^{-1} (*8*). However, Dinelli et al. reported that when three sulfonylurea herbicides were applied to soil, soil microbial activities (soil respiration and dehydrogenase activity) were initially and transitorily enhanced for an initial concentration of 5 μg g^{-1}, and then decreased afterward (*22*).

Effect on Microbial Biomass Nitrogen. The fraction of N associated with soil microbial biomass, or N_{mb}, was 1.8−2.7% of the total organic N in the untreated soil. The effect of bensulfuron-methyl on N_{mb} generally followed a pattern similar to that for C_{mb}, but the magnitude of impact seemed to be greater for N_{mb} than for C_{mb} (Table 2). Application of bensulfuron-methyl at 0.01 μg g^{-1} caused a temporary decrease in N_{mb} from 3 to 7 d after herbicide treatment, and the impact became

Table 2. Effect of Bensulfuron-Methyl Treatment on Microbial Biomass Nitrogen Content (μg g^{-1}) in Soil

days after treatment	herbicide rate (μg g^{-1})			
	0	0.01	0.1	1.0
1	47.4 ± 1.4 a[a]	44.8 ± 1.3 a	36.1 ± 2.1 b	28.5 ± 2.3 c
3	38.4 ± 0.8 a	33.4 ± 1.6 b	24.9 ± 0.8 c	21.2 ± 0.7 d
5	34.8 ± 0.4 a	30.3 ± 0.7 b	25.8 ± 1.3 c	19.9 ± 1.6 d
7	33.5 ± 1.5 a	29.1 ± 0.8 b	24.4 ± 2.7 c	20.8 ± 1.5 d
10	30.3 ± 1.5 a	28.9 ± 0.6 a	25.8 ± 1.3 b	23.5 ± 1.8 b
15	32.1 ± 0.8 a	31.2 ± 1.3 a	27.6 ± 2.0 b	25.4 ± 1.3 b
25	34.1 ± 0.9 a	33.3 ± 0.8 a	31.5 ± 0.1 ab	28.7 ± 2.1 b
45	33.3 ± 1.6 a	33.0 ± 0.7 a	31.6 ± 1.5 a	28.5 ± 1.2 b

[a] Means with different letters in the same row are significantly different at the 0.05 probability level.

Table 3. Effect of Bensulfuron-Methyl Treatment on the Ratio of Soil Microbial Biomass Carbon/Nitrogen (C_{mb}/N_{mb}) in Soil

days after treatment	herbicide rate (μg g^{-1})			
	0	0.01	0.1	1.0
1	5.55 ± 0.22 c[a]	5.46 ± 0.17 c	6.12 ± 0.36 b	7.24 ± 0.50 a
3	6.66 ± 0.33 b	7.07 ± 0.13 b	8.42 ± 0.26 a	8.94 ± 0.32 a
5	6.79 ± 0.17 c	7.42 ± 0.36 b	7.53 ± 0.49 b	8.42 ± 0.85 a
7	6.95 ± 0.18 b	7.52 ± 0.20 b	8.20 ± 0.40 ab	8.98 ± 0.89 a
10	7.44 ± 0.62 b	7.63 ± 0.25 ab	7.98 ± 0.51 a	8.09 ± 0.82 a
15	7.01 ± 0.39 a	7.22 ± 0.52 a	7.59 ± 0.17 a	7.67 ± 0.41 a
25	6.54 ± 0.30 b	6.60 ± 0.16 b	6.74 ± 0.33 b	7.14 ± 0.65 a
45	6.61 ± 0.30 b	6.58 ± 0.19 b	6.74 ± 0.29 b	7.36 ± 0.35 a

[a] Means with different letters in the same row are significantly different at the 0.05 probability level.

insignificant as time further increased. Treatment at higher herbicide rates, however, resulted in substantial and prolonged decreases in N_{mb}, and the reduction was much more substantial at the beginning of incubation. For instance, the averaged reduction in N_{mb} over the control treatment was 28% for the 0.1 μg g^{-1} treatment, and 41% for the 1.0 μg g^{-1} treatment. As for C_{mb}, the negative effect on N_{mb} was the greatest 3 to 5 d after the treatment, and then quickly leveled off. For the 0.1 μg g^{-1} treatment, soil N_{mb} recovered to a level similar to that in the control after 25 d. However, for the 1.0 μg g^{-1} treatment, a complete recovery in N_{mb} was not reached even by the end of the experiment.

It is also evident that the impact of bensulfuron-methyl treatment imposed a greater impact on N_{mb} than on C_{mb} (Tables 1 and 2). The averaged reduction in N_{mb} for the first 7 days was consistently greater than that in C_{mb} by >1.6 times for both the 0.1 and 1.0 μg g^{-1} treatments. The unbalanced effect on soil microbial biomass C and N was also reflected in the change of the C_{mb}/N_{mb} ratio over time (Table 3). Compared to the untreated soil, treatment of bensulfuron-methyl generally enhanced C_{mb}/N_{mb}, and the enhancement was proportional to the initial herbicide concentration. This enhancement was caused by a greater reduction in N_{mb} than in C_{mb} after the herbicide treatment. A possible explanation is that bensulfuron-methyl might partially inhibit amino acid formation and protein synthesis, resulting in low N content in microbial cells.

Effect on Nitrogen Mineralization. Compared to the untreated soil, herbicide treatment at 0.01 μg g^{-1} showed no significant effect on soil N-mineralization rate (Table 4). When the soil was treated with the herbicide at 0.1 or 1.0 μg g^{-1}, significant suppression of N-mineralization occurred during the first 1−5 d of incubation, and the greatest reduction (19.6−23.5%) was observed 5 d after treatment. However, this effect quickly diminished, and no difference in k was observed for any of the treatments from 7 d after treatment and onward (Table 4).

Table 4. Effect of Bensulfuron-Methyl Treatment on Nitrogen Mineralization Rate (μg g^{-1} day^{-1}) in Soil

days after treatment	herbicide rate (μg g^{-1})			
	0	0.01	0.1	1.0
1	2.67 ± 0.23 a[a]	2.60 ± 0.20 a	2.33 ± 0.11 ab	2.13 ± 0.23 b
3	2.93 ± 0.22 a	2.80 ± 0.12 ab	2.47 ± 0.12 b	2.33 ± 0.12 b
5	3.40 ± 0.20 a	3.20 ± 0.19 a	2.73 ± 0.10 b	2.60 ± 0.19 b
7	4.00 ± 0.29 a	3.93 ± 0.31 a	3.73 ± 0.22 a	3.53 ± 0.12 a
10	4.73 ± 0.20 a	4.67 ± 0.22 a	4.52 ± 0.23 a	4.47 ± 0.22 a
15	5.13 ± 0.11 a	5.07 ± 0.23 a	4.93 ± 0.18 a	4.73 ± 0.21 a
25	5.27 ± 0.31 a	5.20 ± 0.01 a	5.13 ± 0.09 a	5.07 ± 0.22 a
45	5.40 ± 0.24 a	5.33 ± 0.21 a	5.20 ± 0.18 a	5.06 ± 0.10 a

[a] Means with different letters in the same row are significantly different at the 0.05 probability level.

Nitrogen mineralization is an important process in soil N cycles and a critical indicator of soil fertility and health. Transformation of organic N to inorganic N is a complex process that is mediated by consortia of different microorganisms. In general, herbicides have been shown to have little influence on ammonification of soil when used at field rates. Our study showed that soil N-mineralization was not affected by bensulfuron-methyl at the field rate, and was only temporarily suppressed at rates that were 10× or 100× the field rate. On the other hand, oxidation of ammonium to nitrate, or nitrification, was reported to be affected by herbicide applications. Nitrifying bacteria are commonly considered more sensitive to herbicides (*23*).

The cause for the decrease in N-mineralization by bensulfuron-methyl at high rates may be also attributed to the toxic effect of the herbicide. However, in general, effects of pesticide application on soil N-mineralization under field conditions are often inconclusive or contradictory among different pesticides or studies. For example, Hendrix and Parmelee reported that paraquat and glyphosate treatment reduced the decomposition of crop residues (*24*). In field trials, Kasper and Fischbeck showed that N mineralization rate and potentially mineralizable N were not affected by 2,4-D amine or ester at rates of 1.12 kg ha^{-1}, but urease activity was temporarily depressed by both formulations. In the same study, nitrification was also inhibited temporarily by 2,4-D ester (*25*). Harper et al. showed that nitrogen in a clover crop increased until anthesis, and then declined slightly prior to desiccation after paraquat application (*26*). Hart and Brookes investigated the effects of 19 years of consecutive application of five pesticides on the mineralization of soil organic matter, and they concluded that mineralization of soil organic N to ammonium or nitrate was mostly unaffected by these pesticide treatments (*27*). Therefore, more studies, such as impact of bensulfuron-methyl and other sulfonylurea herbicides on soil N mineralization under field conditions, should be conducted to validate the laboratory observations.

From the above discussion, the overall effect of bensulfuron-methyl treatment on soil microbial biomass and C/N cycling is expected to be minimal when the herbicide is used at typical field rates. However, this conclusion was drawn based on a uniform herbicide distribution in the top 20-cm soil layer. In real practices, herbicides distribution in soil may be far from uniform, especially for pesticides such as bensulfuron-methyl that are used at very low rates. As a result, some spots may contain pesticides at a higher concentration than other spots. Microorganisms in the spots that receive the higher-than-normal rate may be more vulnerable to pesticide effect. The potential

effect of bensulfuron-methyl on microbial biomass C, N, and N-mineralization should be evaluated under field conditions. Moreover, the effect after repeated treatments of bensulfuron-methyl to the same soil should also be evaluated in order to gain a better understanding of the long-term ecological effect by this group of herbicides.

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Received for review June 11, 2001. Revised manuscript received October 10, 2001. Accepted October 11, 2001. This work was supported by the Teaching and Research Award Program for Outstanding Young Teachers in Higher Education Institutions of China, the National Natural Science Foundation of China (Grant 49871044) and the Natural Science Foundation of Zhejiang Province, China (Grant RC99032).

JF010756X