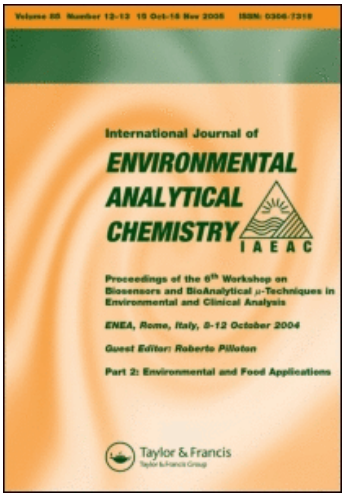

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Short-Time Effects of Pure and Formulated Herbicides on Soil Microbial Activity and Biomass

Cesare Accinelli[a]; Claudio Screpanti[a]; Giovanni Dinelli[a]; Alberto Vicari[a]
[a] Department of Agro-Environmental Science and Technology, Bologna, Italy

Online publication date: 17 September 2010

To cite this Article Accinelli, Cesare , Screpanti, Claudio , Dinelli, Giovanni and Vicari, Alberto(2002) 'Short-Time Effects of Pure and Formulated Herbicides on Soil Microbial Activity and Biomass', International Journal of Environmental Analytical Chemistry, 82: 8, 519 — 527
To link to this Article: DOI: 10.1080/03067310290009514
URL: http://dx.doi.org/10.1080/03067310290009514

Intern. J. Environ. Anal. Chem., Vol. 82, No. 8–9, pp. 519–527

Taylor & Francis
Taylor & Francis Group

SHORT-TIME EFFECTS OF PURE AND FORMULATED HERBICIDES ON SOIL MICROBIAL ACTIVITY AND BIOMASS

CESARE ACCINELLI*, CLAUDIO SCREPANTI, GIOVANNI DINELLI and ALBERTO VICARI

Department of Agro-Environmental Science and Technology, Via F. Re 6, 40126 Bologna, Italy

(Received 24 August 2001; In final form 11 January 2002)

The short-time of six pure herbicides (atrazine, terbuthylazine, rimsulfuron, primisulfuron-methyl, glyphosate and gluphosinate-ammonium) with respect to the corresponding commercial formulations on microbial activity and biomass of sandy loam soil were investigated. Application rates were: agricultural rate, 20 and 200 μg a.i. g^{-1} soil. Application at normal agricultural rates did not lead to significant effects on soil microbial activity, whereas soil microbial activity was markedly stimulated when pure and commercial formulations of the six herbicides were applied at 20 μg a.i. g^{-1} soil. The addition of 200 μg a.i. g^{-1} soil of four pure herbicides (atrazine, terbuthylazine, rimsulfuron, primisulfuron-methjyl) led to a significant decrease of soil microbial activity. Commercial formulations characterized by a higher relative a.i. concentration (atrazine and primisulfuron-methyl) approximately determined the same decreasing effect of the pure compound, whereas herbicide formulations with a lower relative a.i. concentration (terbuthylazine and rimsulfuron) produced a significant increase in soil microbial activity.

Keywords: Herbicides; Active ingredients; Commercial formulations; Adjuvants; Soil microbial activity; Soil microbial biomass

INTRODUCTION

Herbicides applied in agricultural systems have the potential for causing side-effects on soil microbial population [1]. Soil microorganisms have been recognised as the driving force behind nutrient transformation in soils and thus have an important role in soil fertility [2]. Several authors stressed the importance of preserving soil fertility and quality and consequently soil microbial population. According to this concept, measurements of soil microbial activity and biomass C have been widely used to asses the effect of herbicide applications on soil quality [3,4]. Several studies dealing with the effect of herbicides on soil microbial activity and biomass C have been conducted [5,6]. However, only few researches have separately investigated the effect of active ingredients with respect to the corresponding commercial formulations [7–9]. Besides the herbicide itself, a commercial formulation typically consists of solvents, diluents

*Corresponding author. Fax: +39 051 2091545. E-mail: accinel@agrsci.unibo.it

ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310290009514

and various adjuvants [10]. An adjuvant is defined as any substance in a herbicide formulation or added to the spray tank to modify herbicidal activity or application characteristics [11]. The effect on soil microorganisms of many active ingredients and their formulation preparations may differ [8].

Atrazine, terbuthylazine, rimsulfuron, primisulfuron-methyl, glyphosate and glufosinate-ammonium are widely used herbicides. These herbicides present different physico-chemical properties and differences in agricultural usage [12]. Atrazine and terbuthylazine are pre-emergence herbicides, used for weed control of several crops and are applied at rates ranging from 0.6 to 4.5 kg a.i. ha^{-1}. Rimsulfuron and primisulfuron-methyl are relevant members of the sulfonylures, an important class of herbicides, used in many crops and characterized by low application rates (ranging from 0.015 to 0.040 kg a.i. ha^{-1}) and low mammalian toxicity. Glyphoste and glufosinate-ammonium are non-selective, foliar applied herbicides. The increasing use of glyphosate or glufosinate-tolerant crops has increased the interest in these two herbicides, especially in their environmental aspects [13].

Side-effects of herbicides on soil microbial populations can be studied on a short or a long-term basis [14]. However, according to Haney *et al.* [13], experiments conducted on a short-term basis may provide a more realistic evaluation of the effect of herbicides on soil microorganisms.

The aim of the present study was to investigate, under laboratory conditions, the effects of six pure and formulated herbicides, namely atrazine, terbuthylazine, rimsulfuron, primisulfuron-methyl, glyphosate and gluphosinate-ammonium, on soil microbial activity and biomass. Herbicides were applied at a normal agricultural rate and at two additional higher rates. In addition, the effects of two common non-ionic adjuvants (Triton X 100 and Igepal CA 630) were evaluated.

EXPERIMENTAL

Soil and Chemicals

Soil samples were collected at the Experimental Farm of the University of Bologna at Ozzano (Bologna, Italy), from the top 20 cm of a field with no previous pesticide history. The soil is a sandly loam (Typic Ustochrepts) with 650 g kg^{-1} sand, 144 g kg^{-1} clay, 206 g kg^{-1} silt, organic C content 13 g kg^{-1}, pH (1 : 2.5 soil/water) of 6.5, and a water content of 22% at an applied pressure of 33 kPa. Soil was air-dried and passed through a 2 mm sieve. Twenty-five grams of soil (air-dry basis) were weighted in sterile culture tubes. Before the beginning of the experiment, soil moisture was adjusted to the field capacity by adding sterile ultrapure water. Field capacity was determined by porous-plate pressure apparatus at a suction pressure of 33 kPa. Soil samples were kept in a dark place in a climatic chamber at $25^{\circ}C \pm 0.5$ for 10 days. The conditioning period of 10 days allowed the soil to establish a steady-state level of microbial activity [15]. Since employed soil was air-dried and sieved, soil microorganism reactivity was assessed by glucose addition at a rate of 250 μg g^{-1} air-dried soil.

Herbicides employed in this experiment are reported in Table I. Active ingredients of rimsulfuron and primisulfuron-methyl were extracted from commercial formulations with dichloromethane in a Soxhlet extract, as reported by Dinelli *et al.* [16]. Mass spectral analysis was employed to confirm the identity of extracted sulfonylureas, as reported by Galletti *et al.* [17]. Remaining analytical grade chemicals (atrazine,

TABLE I Herbicides and application rates adopted in the experiment

Herbicide	*Chemical name (IUPAC)*	*Commercial formulation*	*Application rates*		
			Rate I	*Rate II*	*Rate III*
			μg a.i. g^{-1} *soil*		
Atrazine	6-Chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	Aatrex Nine-0 (90% a.i. WDG)	2 (1×)*	20 (10×)	200 (100×)
Terbuthylazine	N^2-*tert*-butyl-6-chloro-N^4-ethyl-1,3,5-triazine-2,4-diamine	Click 50 FL (50% a.i. SC)	2 (1×)	20 (10×)	200 (100×)
Rimsulfuron	1-(4,6-Dimethoxypyrimidin-2-yl)-3-(3-ethysulfonyl-2-pyridysulfonyl)urea	Titus (25% a.i. WG)	0.02 (1×)	20 (1000×)	200 (10,000×)
Primisulfuron-methyl	2-[4,6-Bis(difluoromethoxy) pyrimidin-2-yl carbamoysulfamoyl] benzoic acid	Tell (75% a.i. WG)	0.02 (1×)	20 (1000×)	200 (10,000×)
Glyphosate	*N*-(phosphonomethyl)glycine	Roundup Bioflow (31% a.i. SL)	10 (1×)	20 (2×)	200 (20×)
Glufosinate-ammonium	Ammonim 4-[hydroxy(methyl)phophinoyl]-DL-homoalaniate; ammonium DL-homoalanin-4-yl(methyl) phodphinate	Basta (11.33% a.i. SL)	10 (1×)	20 (2×)	200 (20×)

*× = multiple value with respect to the recommended agricultural rate.

terbuthylazine, glyphosate and glufosinate-ammonium) were provided by Dr. Ehrenstrofer (Augsburg, Germany).

Soil samples were separately treated with pure active ingredients and commercial formulations of the six herbicides. Both commercial formulations and active ingredients were added as a water solution. Herbicides were applied at the normal agricultural rate and at 20 and 200 μg a.i. g^{-1} air-dried soil, as reported in Table I. Normal agricultural rates were calculated considering a soil layer of 5 cm for atrazine, terbuthylazine, rimsulfuron and primisulfuron-methyl [18,19]. Because of the high adsorptivity and the low leachability of glyphosate and glufosinate-ammonium, a soil layer of 1 cm was considered for these herbicides [20].

To evaluate the effect of adjuvant on soil microbial activity and biomass, a set of soil samples was separately treated with a water solution of two common non-ionic adjuvants, namely Triton X 100 (*t*-Octylphenoxypolyethoxyethanol) and Igepal CA 630 (Octylphenoxypolyethoxyethanol), at four different rates: 20, 50, 100 and 500 μg g^{-1} air-dried soil. The two adjuvants were provided by Sigma Ltd.

Soil Microbial Activity

Soil microbial activity was estimated by soil respiration and by soil dehydrogenase activity (DH). Soil respiration was measured according to the method proposed by Anderson [21]. Treated and untreated soil samples were placed in 500 mL sealed glass cylinders containing 15 mL of NaOH 0.5 M solution in separate vials. Soil samples were incubated in the dark at 25°C ± 0.5. Soil moisture content was

constantly maintained at the field capacity throughout the incubation by weighing and correcting for any weight loss, using sterile ultrapure water. Soil CO_2-evolution was regularly (4-days interval period) estimated during the 20-days incubation period. Carbon dioxide recovered in each NaOH solution was measured by titration, following addition of $BaCl_2$ [21]. Preliminary tests showed that headspace volume of the employed sealed cylinder did not represent a limiting factor for soil respiration, adopting a 4-days incubation interval. Respiration rate was expressed as the average daily rate (g CO_2 kg^{-1} of air-dried soil day^{-1}) of the control (blank samples without soil).

Soil DH-activity was measured at the same sampling intervals previously described. Measurements were made according to the iodonitrotetrazolium chloride (INT) method [22]. Subsamples of 5 g (air-dried basis) were weighed into test-tubes and mixed with a 7.5 mL aqueous solution of INT (10 mg L^{-1}). Test-tubes were sealed and incubation in the dark at 40°C ± 0.5 for 2 h. During incubation, test-tubes were slightly shaken. Developed iodonitrotetrazolium formazan (INTF) was extracted by keeping the test-tubes in a dark place for 1 h, shaking them vigorously every 20 min and finally by filtering the solution. The INTF was measured specrophotometrically at 464 nm, after extraction with 10 mL of *N*,*N*-dimethylformamide/ethanol (50/50 v/v) solution.

For soil respiration and DH-activity, all soil treatments were performed in triplicate.

Soil Biomass C

Soil biomass was estimated, using a slight modification of the fumigation–incubation method proposed by Jenkinson and Powlson [23]. Soil biomass C was estimated in treated and untreated soil samples incubated as previously described. Soil samples were sampled at 0, 7 and 20 days after treatment (DAT). For each herbicide and adjuvant treatment, four replicates were adopted. Two replicates were subjected to the fumigation treatment and the remaining two replicates represented the unfumigated control soil. For the fumigation treatment, moisture content of soil samples was adjusted to 50% of the field capacity and fumigated with alcohol-free $CHCl_3$ for 24 h. After removal of $CHCl_3$ vapour, soil moisture was adjusted to the field capacity by adding an equivalent volume of distillated water plus a small volume (0.3 mL) of a liquid obtained by the filtration of a peat sludge through a 0.4 μm size filter [19]. Unfumigated soil samples were moistened to the field capacity using ultrapure sterile water. Fumigated and unfumigated soil samples were incubated in 500 mL sealed glass cylinders containing 15 mL of NaOH 0.5 M in separate vials, as previously described. Five cylinders without soil samples were used as blanks. Soil samples were kept in a dark place in a climatic chamber at 25°C ± 0.5. Cylinders were opened at 18, 42, 66, 90 and 120 h after incubation and NaOH solutions removed. CO_2–C recovered in each NaOH solution was measured by titration following addition of $BaCl_2$. Biomass C (Bc) was calculated from a modification of the expression proposed by Jenkinson and Powlson [23]: Bc = Fc/kc where Fc = (CO_2–C evolved by fumigated soil during 0–5 days) − (CO_2–C evolved by non-fumigated soil during 0–5 days). A kc factor of 0.45 was used for converting the CO_2–C flush to biomass C content [24].

For soil respiration, dehydrogenase activity and microbial C, results were expressed as means on a air-dried weight soil basis. Three-way ANOVA was employed to test at, each time interval, the significance of soil microbial activity and biomass C in soil samples receiving separately different application rates of the six pure and formulated herbicides with respect to the untreated soil.

RESULTS AND DISCUSSION

Soil Microbial Activity

The effect of pure and formulated herbicides on soil respiration are reported in Figs. 1–3. Application rates corresponding to the normal agricultural rates of both pure and commercial formulations, which were different for the six herbicides (Table I), did not lead to a significant effect on the CO_2 evolution with respect to the untreated control soil. Obtained results are in agreement with literature [18,25–27], where are reported for herbicides applied at the respective agricultural rates.

On the contrary, soil microbial activity was markedly stimulated by the addition of both pure and commercial formulations of the six herbicides at 20 μg a.i. g^{-1} soil. Soil samples treated with both pure and formulated atrazine, terbuthylazine (Fig. 1), primisulfuron-methyl and with commercial formulations of rimsulfuron (Fig. 2), glyphosate and glufosinate-ammonium (Fig. 3) showed a peak of CO_2 evolution 16 DAT. At the end of the incubation period (20 days), soil respiration was significantly higher ($p < 0.01$) in soil samples treated with these chemicals with respect to the untreated soil. Pure rimsulfuron, glyphosate and glufosinate-ammonium led to a more rapid increase of soil respiration, showing a peak to CO_2 evolution 8 DAT. After the peak occurrence, CO_2 evolution of soil samples treated with pure rimsulfuron approximately returned to the background level. On the contrary, 20 DAT, CO_2 evolution of soil samples treated with pure glyphosate and glufosinate ammonium was significantly higher ($p < 0.01$) than the untreated soil. A rapid increase of soil microbial activity, following pure glyphosate application, was also reported by Carlisle and Trevors [7] and by Haney *et al.* [13]. In addition, Haney *et al.* [13], observed that the application

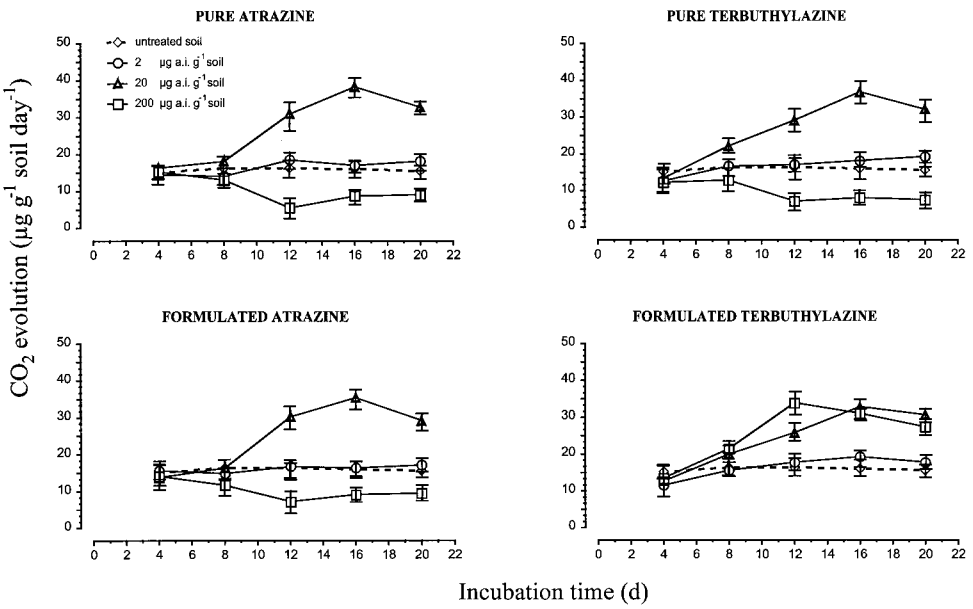

FIGURE 1 Respiration rate of untreated soil and soil samples treated with pure and formulated atrazine and terbuthylazine, at three different rates: normal agricultural rates (○), 20 μg a.i. g^{-1} soil (Δ) and 200 μg a.i. g^{-1} soil (□). Vertical bars represent standard error ($n = 3$).

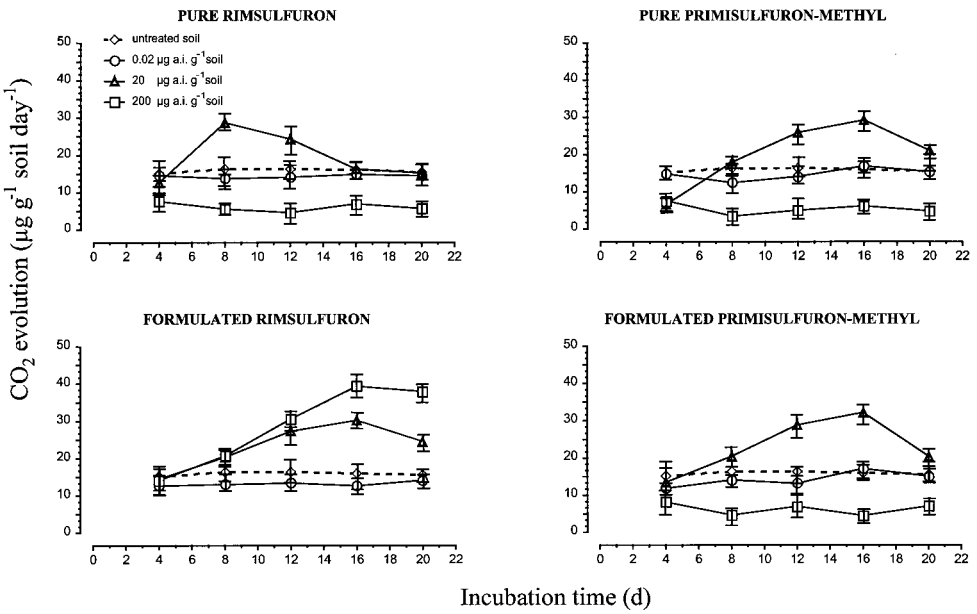

FIGURE 2 Respiration rate of untreated soil and soil samples treated with pure and formulated rimsulfuron and primisulfuron-methyl, at three different rates: normal agricultural rates (○), 20 µg a.i. g^{-1} soil (Δ) and 200 µg a.i. g^{-1} soil (□). Vertical bars represent standard error ($n = 3$).

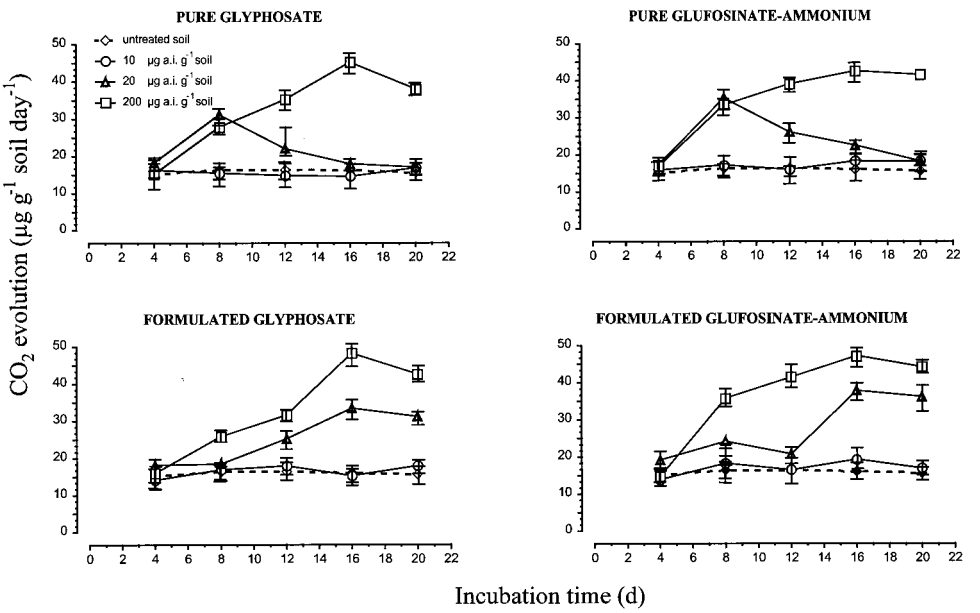

FIGURE 3 Respiration rate of untreated soil and soil samples treated with pure and formulated glyphosate and glufosinate-ammonium, at three different rates: normal agricultural rates (○), 20 µg a.i. g^{-1} soil (Δ) and 200 µg a.i. g^{-1} soil (□). Vertical bars represent standard error ($n = 3$).

of glyphosate commercial formulation at high rates caused a relevant delay in the stimulation of soil microbial activity with respect to the pure compound.

In spite of difference in physico-chemical properties of the six tested herbicided, soil microbial activity reached similar peak values, ranging from 28.8 to 38.3 μg CO_2 g^{-1} soil $days^{-1}$.

Different effects were observed when herbicides were applied at the highest rate. Except for glyphosate and glufosinate-ammonium (Fig. 3), the addition of remaining pure herbicides at 200 μg a.i. g^{-1} caused a significant decrease of soil CO_2 evolution (Figs. 1–2). Similar trend was found for commercial formulations characterized by a high a.i. content (atrazine and primisulfuron-methyl). On the contrary, commercial formulations of terbuthylazine and rimsulfuron, characterized by a low a.i. content, caused an increase of CO_2-flush with respect to corresponding pure compound and to the untreated soil. Rimsulfuron (25% a.i.) caused a higher stimulation of soil respiration than terbuthylazine (50% a.i.). For both formulated rimsulfuron and terbuthylazine, CO_2 peak was reached at 16 DAT. At 20 DAT, for both herbicides, CO_2-flush was significantly higher ($p < 0.01$) than for the untreated soil. Stimulatory effects of applied herbicides at application rates significantly higher than recommended agriculture rates are reported in literature [18,28,29]. However, in some cases, a decrease of microbial activity due to high application rates was reported [6,30].

The effect of the highest application rate (200 μg a.i. g^{-1} soil) of glyphosate and glufosinate-ammonium on soil microbial activity was in contrast with results obtained for the other herbicides. Both pure and formulated glyphosate and glufosinate-ammonium determined a rapid and significant increase of soil respiration compared with the untreated soil (Fig. 3). Carlisle and Trevors [7] found that commercial formulations caused a more significant increase of soil respiration compared with pure chemicals. The stimulatory effect of glyphosate on soil microbial activity was also reported by Haney *et al.* [13].

DH-activity and soil respiration data were well correlated. A significant correlation between soil DH-activity and CO_2-flush in soil samples treated with different rates of rimsulfuron and primisulfuron-methyl was also reported by Dinelli *et al.* [18]. In our experiment, a good correlation was obtained for all tested herbicides. The coefficients of correlation ranged from 0.893 to 0.931 (data not shown). In our study, the two employed methods for measuring soil microbial activity were consequently equivalent and soil DH-activities confirmed the results obtained by soil respiration.

Soil microbial population was markedly stimulated by glucose addition (Fig. 4). The availability of a readily metabolizable carbon source (i.e. glucose) cause a high rate of microbial activity, as expected in natural soil conditions [31]. A significant increase of soil respiration was also achieved by the addition of high rates (100 and 500 μg g^{-1}) of the two adjuvants (Fig. 4). The effect was significantly ($p < 0.05$) lower with respect to glucose addition. No significant differences were observed between the two adjuvants. This findings suggests that the high rate of the two adjuvants caused a stimulatory effect on soil microganisms, probably reducing detrimental effects of some a.i., especially when applied at high rates.

Soil Biomass C

In spite of the described effect on soil microbial activities, soil biomass was not influenced by herbicide and adjuvant applications, even at the highest application rates

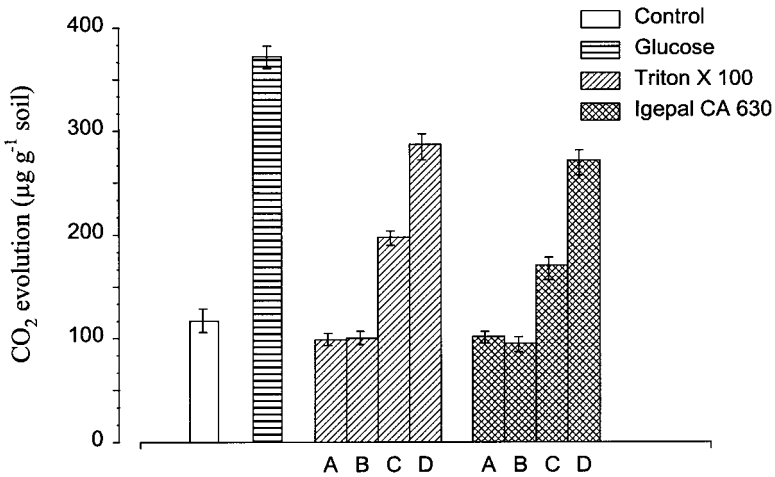

FIGURE 4 Effect of glucose (250 μg g^{-1} soil) and increasing rates of Triton X 100 and Igepal CA 630 (A: 20, B: 50, C: 100, D: 500 μg g^{-1} soil) on cumulated CO_2 evolution of soil samples incubated for 20 days. Vertical bars represent standard error ($n = 3$).

(data not shown). These findings suggests that, in the employed experimental conditions, soil microbial activity was a more sensitive parameter to study short-time response of soil microorganisms to applied herbicides than soil biomass C, as also reported by Haney *et al.* [13].

The stimulatory effect produced by high rates (100 and 500 μg g^{-1} soil) of the two tested adjuvants on soil microbial activity can partially explain results from herbicide treatments. As previously described, the addition of 200 μg g^{-1} soil of herbicides characterised by a high a.i. content (atrazine and primisulfuron-methyl) was followed by a detrimental effect on soil microbial activity. On the contrary, herbicides with a low a.i. content (terbuthylazine and rimsulfuron) produced a stimulatory effect. Presumably, this effect was consequence of the high amount of adjuvant, included in the commercial formulation, applied to the soil. When terbuthylazine and rimsulfuron were applied at 200 μg a.i. g^{-1} soil, the adjuvant addition to soil was respectively of 200 and 600 μg a.i. g^{-1} soil, which, presumably, represented a sufficient amount to stimulate soil microbial activity. In contrast, the low adjuvant addition of 22.2 and 66.7 μg g^{-1} soil, followed by the application of atrazine and primisulfuron-methyl, respectively, at 200 μg a.i. g^{-1} soil, was not enough to overcome the detrimental effect of the corresponding active ingredients. The rate and a.i. concentrations of commercial formulations of herbicides are consequently important factors which have to be considered to better predict the potential side-effects on soil microbial activity and biomass on a short-time basis. Our results further support the absence of adverse effects of glyphosate and glufosinate-ammonium on soil microbial population, as previously reported by other authors [7,13,14].

Even considering that localized pockets of high herbicide concentrations may occur following conventional application [32], it is unlikely that they could be greater than the highest rates employed in this experiment. Obtained results consequently suggest that tested herbicides, if used as recommended, do not cause short-time effects on soil microbial activity and biomass.

References

[1] D.A. Wardle and D. Parkinson, *Plant Soil*, **122**, 21–28 (1991).
[2] J.S. Smith and E.A. Paul, In: J.M. Bollag and G. Stotzky (Eds.), *Soil Biochemistry*. Marcel Dekker, New York, pp. 357–369 (1990).
[3] D.A. Wardle and D. Parkinson, *Soil Boil. Biochem.*, **24**, 185–186 (1992).
[4] L. Tessier, E.G. Gregorich and E. Topp, *Soil Boil. Biochem.*, **30**, 1369–1377 (1998).
[5] P. Nannipieri, S. Greco and B. Ceccanti, In: J.M. Bollag and G. Stotzky (Eds.), *Soil Biochemistry*. Marcel Dekker, New York, pp. 357–369 (1990).
[6] A.G.S. Prado and C. Airoli, *Pest Manag. Sci.*, **57**, 640–644 (2001).
[7] S.M. Carlisle and T.J. Trevors, *Water Air Soil Poll.*, **27**, 391–401 (1986).
[8] H.P. Malkomes, *Z. Pflanenkz Pflanzen.*, **107**, 781–789 (2000).
[9] J. Nowak, A. Nowak, D. Klodka and M. Turos-Biernacka, *Z. Pflanzenkz. Pflanzen.*, **107**, 769–774 (2000).
[10] P. Kudsk, In: J.E. Jensen, J.C. Streibig and C. Andreasen (Eds.), *Weed Science Compendium*. KVL, Copenhagen, pp. 199–214 (1998).
[11] Weed Science Society of America, *Herbicide Handbook*, 7th edn. Weed Science Society of America, Champaign, IL, p. 313 (1994).
[12] C.D.S. Tomlin (Ed.), *The Pesticide Manual*. British Crop Protection Council, Farnham, UK, p. 1606 (1997).
[13] R.L. Haney, S.A. Senseman, F.M. Hons and D.A. Zuberer, *Weed Sci.*, **48**, 89–93 (2000).
[14] M.R. Hart and P.C. Brookes, *Soil Biol. Biochem.*, **28**, 1614–1649 (1996).
[15] A.J. Franzluebbers, R.L. Haney, F.M. Hons and D.A. Zuberer, *Soil. Sci. Soc. Am. J.*, **60**, 1133–1139 (1996).
[16] G. Dinelli, A. Vicari, A. Bonetti and P. Catizone, *J. Agric. Food Chem.*, **45**, 1940–1945 (1997).
[17] G.C. Galletti, A. Bonetti and G. Dinelli, *J. Chromatogr.*, **692**, 27–37 (1995).
[18] G. Dinelli, A. Vicari and C. Accinelli, *J. Environ. Qual.*, **27**, 1459–1464 (1998).
[19] C. Accinelli, G. Dinelli, A. Vicari and P. Catizone, *Biol. Fert. Soils*, **33**, 495–500 (2001).
[20] P. Sprankle, W.F. Meggitt and D. Penner, *Weed Sci.*, **23**, 229–234 (1975).
[21] J.P.E. Anderson, In: A.L. Page and R.H. Miller (Eds.), *Methods of Soil Analysis*, Part 2. ASA Madison, WI, pp. 831–871 (1982).
[22] W. Von Mersi and F. Schinner, *Biol. Fert. Soils*, **11**, 1133–1138 (1991).
[23] D.S. Jenkinson and D.S. Powlson, *Soil Biol. Biochem.*, **8**, 209–213 (1976).
[24] D.S. Jenkinson and J.N. Ladd, In: E.A. Paul and J.N. Ladd, (Eds.), *Microbial Biomass*: *Measurement and Turnover*. Dekker, New York, pp. 415–471 (1981).
[25] T.B. Moorman, *J. Crop. Prod.*, **2**, 14–23 (1989).
[26] R.J. Hicks, G. Stotzky and P. Van Vorris, *Adv. Appl. Microbiol.*, **35**, 195–253 (1990).
[27] J.P.E. Anderson, In: *Proc. Int. Symp. on Environmental Aspects of Pesticide Microbiology*, *Sigtuna*, *Sweden*, *17–21 Aug. 1992*. Dep. of Microbiology, Swedish Univ. of Agric. Sci., Uppsala, Sweden, pp. 31–34 (1993).
[28] M.T. Ceccherini, N. Miclaus, C. Piovanelli and A. Sasso, In: *Proc. 5th Eur. Weed Res. Soc. Mediterranean Symp.*, *Perugia*, *Italy*, *6–8 June 1994*. Università degli Studi di Perugia, Perugia, Italy, pp. 89–96 (1994).
[29] C. Piovanelli, N. Miclaus and M.T. Ceccherini, *Agricoltura e Ricerca*, **160**, 63–70 (1995).
[30] L.B. Taiwo and B.A. Oso, *Agr. Ecosyst. Environ.*, **65**, 59–68 (1997).
[31] N. Barros, I. Gomez-Orellana, S. Feijoo and R. Balsa, *Themochim. Acta*, **249**, 161–168 (1995).
[32] J.A.P. Marsh, H.A. Davies and E. Grossbard, *Weed Res.*, **17**, 77–82 (1977).