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Adriano Sofo [a], Antonio Scopa [a], Stefano Dumontet [b], Angelo Mazzatura [a] & Vincenzo Pasquale [b]

[a] Department of Agriculture, Forestry and Environment, University of Basilicata, Potenza, Italy

[b] Department of Environmental Sciences, Parthenope University, Naples, Italy

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Toxic effects of four sulphonylureas herbicides on soil microbial biomass

ADRIANO SOFO[1], ANTONIO SCOPA[1], STEFANO DUMONTET[2], ANGELO MAZZATURA[1] and VINCENZO PASQUALE[2]

[1]*Department of Agriculture, Forestry and Environment, University of Basilicata, Potenza, Italy*
[2]*Department of Environmental Sciences, Parthenope University, Naples, Italy*

The effect of four triazinyl-sulfonylurea herbicides (cinosulfuron, prosulfuron, thifensulfuron methyl, triasulfuron) on soil microbial biomass, soil respiration, metabolic activity, metabolic quotient, and some enzymatic activities (acid and alkaline phosphatase, β-glucosidase, arylsulphatase, and fluorescein diacetate hydrolysis) were monitored under controlled conditions over 30 days. The herbicides were applied at the normal field dose (FD) and at ten-fold (10 FD) the field dose, in order to mimic a long term toxic effect. The measured soil microbial parameters showed that the FD had slight effects on soil microflora, while at 10 FD the tested herbicides exerted a stronger detrimental effect on soil microbial biomass and its biochemical activities.

Keywords: Herbicides, triazinyl-sulfonylurea, respiration, microbial biomass, metabolic quotient, soil enzymes.

Introduction

The modern agricultural practices are characterized by a high chemical input and a consequent high environmental impact on soil and on ecosystems at large. In particular, herbicides exert a detrimental effect on soil microbial biomass that spans from the impairment of plant promoting rhizobacteria[1,2] to the alteration of the community structure of soil microflora.[3] The impairment of soil ecosystemic functions caused by herbicides can have a long-lasting effect, as in the case of atrazine, representing a potential long-term threat to the environment and so affecting soil quality.[4] Soil quality is described by Doran et al.[5] as the capacity of soil to keep unaltered key ecological functions, such as decomposition and formation of soil organic matter. Soil quality grounds on chemical, physical or biological characteristics, the impairment of which lends to a decline of agricultural production.[6] Among the threats able to harm soil quality, xenobiotic compounds are of special concern: they can affect soil quality by altering key ecological functions, with the consequent impairment of the natural environmental balance.[7] Among the xenobiotic compounds, herbicides cause toxic effects on the living part of soil, even as root exudates, as the case of glyphosate released from soybean roots.[1] In addition, herbicides have been found to be able to affect the growth and the plant growth promoting rhizobacteria,[2] indoloacetic acid producers,[1] enzymatic activities, and soil respiration in addition to the known general detrimental effects on soil microbial biomass.[3, 7–10]

Researchers addressed several soil functions as early descriptors of detrimental soil modifications caused by xenobiotic compounds. As the soil is the terrestrial major C pool[11] and soil respiration the second-largest terrestrial carbon flux,[12] soil respiration has been widely used as a marker for environmental quality,[8, 13–16] and for assessing detrimental effects of toxic compounds on soil microflora.[17–19]

In this regard, microbial biomass is of particular interest in its role of active living matrix playing an essential role in soil. It represents an important fraction, with a rapid turnover, of the total amount of soil C, N and P stored in soil.[20] Changes in the number and activities of soil biomass components can lead to the disturbance of chemical and biological processes in agro-ecosystem, and consequently lead to impairment of soil nutrient balance.[21]

Sulfonylureas are a group of herbicides having a good selectivity, and characterized by broad-spectrum weed control for many cereal crops, such as rice, wheat, soybean, sugar beet, and maize. Solfonylureas are effective at very low application rates, and have shown a very low animal and human toxicity.[22] These agrochemicals affect the enzyme acetolactate synthase, inhibited the biosynthesis of branched-chain amino acids and impair cell division in

Address correspondence to Antonio Scopa, Department of Agriculture, Forestry and Environment, University of Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy; E-mail: antonio.scopa@unibas.it
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weeds.[23] Their chemical structure is generally constituted by three distinct parts: an aryl group, a sulfonylurea bridge, and a heterocyclic rings (dia or triazinic). Literature data demonstrated that these molecules are readily degraded in soil by chemical hydrolysis and/or microbial breakdown, and their metabolites may persist in the environment showing a strong residual phyto-toxicity.[24,25]

The purpose of this work was to describe the influence of four herbicides belonging to the triazinyl-sulfonylurea group, at two different doses (field rate, and 10-fold the field rate), on soil microbial biomass and on some enzymatic activity in controlled laboratory conditions. The sulphonylureas herbicides selected for this experiment were cinosulfuron, prosulfuron, thifensulfuron methyl, and triasulfuron, commonly used for weed control.

Materials and methods

Chemicals and apparatus

Cinosulfuron (*N*-[[(4,6-dimethoxy-1,3,5-triazin-2-yl) amino]carbonyl]-2-(2-methoxyethoxy) benzenesulfonamide); prosulfuron (*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino] carbonyl]-2-(3,3,3-trifluoropropyl) benzenesulfonamide); thifensulfuron methyl (3-[[[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)amino]carbonyl]amino] sulfonyl] -2-thiophenecarboxylic acid); triasulfuron (2-(2-chloroethoxy)-*N*-[[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) amino] carbonyl] benzenesulfonamide) (purity >96 %) supplied by Riedel-de Haën, Sigma Aldrich (Milan, Italy). Fluorescein diacetate (3',6'-diacetyl-fluorescein, FDA) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals, RPE-ACS analytical grade, were furnished from Carlo Erba (Milan, Italy). Ultrapure water was obtained with a Milli-Q purification system from Millipore (Bedford, MA, USA) and was used to prepare all aqueous solutions, and to establish the proper soil moisture content. A Beckman DU 640 UV-Vis spectrophotometer (Beckman Inc., Palo Alto, CA, USA) was utilized for enzyme determination.

Characterization and fortification of soil

The soil utilized in the present study was collected from the surface layer (0–20 cm) of a field at Guardia Perticara (Agri basin - Southern Italy) (40°37′N, 16°08′E; elevation 720 m a.s.l.), and pedologically classified as *Vertic Ustorthens*, according to the USDA classification. No herbicides have been added to the soil in the five years prior to beginning the sampling. The soil sample was carefully air dried in the laboratory to obtain suitable moisture content for sieving at 2-mm mesh to remove plant residues, soil macrofauna and stones, and stored in sealed plastic bags at 4°C until analyzed. A subsample of the soil was analyzed for the physical, chemical and hydraulic properties that were determined according to ASA-SSSA methods,[26,27] and are reported in Table 1. For the determination of soil microbial biomass and enzyme activity the sample was preventively homogenised for 1 h in a rotary cylinder. The soil sample was pre-conditioned and incubated at 21°C for 5 days (60 % water holding capacity) to stabilize the microbiological activities disturbed during soil sampling, subdivided into different equilibrated portions, and then placed in a 750 mL glasses container for the microbiological and enzymatic analyses. One soil sub-sample was used as a control while the others were treated with the single herbicides at different concentrations. Stock standard solutions containing each herbicide were prepared by dissolution in water-methanol solutions (90:10, v:v), and stored at 4°C. Soil samples were spiked by adding, by spraying, of 2 mL of water-methanol solution of herbicide on 0.5 g talcum, and mixing to 10 g portion of soil subsample. The soil was then homogenized by shaking for 1 h, and the solvent was removed by evaporation at room temperature. The herbicide-spiked soil samples were carefully mixed with 90 g of soil subsample to obtain field doses (FD), and ten-fold (10 FD) higher field dose of herbicides, and then homogenised for 1 h in a rotary cylinder. The field doses of herbicide used in this experiment were 350, 55, 10 and 37 g ha^{-1} for cinosulfuron, prosulfuron, thifensulfuron methyl and triasulfuron, respectively. The conversion of rate application of each xenobiotic compound to mg kg^{-1} of soil was calculated assuming an even distribution of the herbicides in the 0–20 cm layer (bulk density 1.28 g cm^{-3}). The control samples received 2 mL of water-methanol solution (90:10, v:v) without herbicides on 0.5 g talcum, and underwent the same procedure as described above.

Table 1. Physical-chemical characteristics of soil.

Fine sand (%)	3.8
Sand (%)	39.1
Loam (%)	24.6
Clay (%)	32.5
$CaCO_3$ (%)	7.0
pH (H_20)	7.25
Organic matter (%)	1.29
Total N (%)	0.11
P-Olsen (ppm)	48
K_2O (ppm)	340
Bulk density (g cm^{-3})	1.28
C.E.C. (meq 100 g^{-1} of soil)	25.6
Field capacity (cm^3 water cm^{-3} soil)	0.28

Microbiological and enzymatic activities

Soil respiration was monitored at 22°C for 30 days according to Dumontet and Mathur.[28] The CO_2 evolved during the experiment was trapped in 20 mL of sodium hydroxide solution (0.2 M). The NaOH solution was treated with

2 mL of 1.5 M of $BaCl_2$. NaOH residue in excess was titrate with 0.05 M of HCl, and Phenolphthalein was used as indicator of the titration end point.

Microbial Biomass-C (MBC) and Microbial Biomass-N (MBN) at the end of the experiment were determined following the fumigation extraction (FE) method according to Sparling and West.[29] Microbial biomass was calculated from the difference between fumigated and not-fumigated C using a correction factor of 0.45 according to Wu et al.[30]

Soil respiration and biomass C data were used to calculate the specific respiration activity (qCO_2), which represents the amount of C-CO_2 produced per unit of biomass-C per day (expressed as mg C-CO_2/mg C mic day^{-1} kg^{-1} d. s.), assuming that the angular coefficient of the linear equation fitting the respiration data represented the daily soil respiration. The hydrolysis rate of fluorescein diacetate (FDA hydrolysis) was calculated according to Adam and Duncan,[31] and the data obtained were utilized to estimate the specific hydrolytic activity (qFDA) according to Perucci et al.[32] to express the amount of FDA hydrolyzed per unit of microbial biomass-C. Acid and alkaline phosphatase, β-glucosidase, and arylsulphatase activities were measured according to Page et al.[26] All measured parameters were calculated on soil dry matter bases.

Statistical analysis

All treatments were carried out in triplicate, and differences between all collected data were treated statistically by one-way analysis of variance using Statistix 8 software program (Analytical software, USA). The differences among mean value were ranked by Duncan's Multiple Range test and were considered significant at $P \leq 0.05$.

Results

Soil respiration

Table 2 reports the cumulated respiration of all samples at 30rd day of incubation. Taking into account the respiratory of soil microbial biomass exposed to the FD of cinosulfuron, prosulfuron, thifensulfuron methyl and triasulfuron, the control soil showed an intermediary response regarding the tested herbicides. The samples treated with thifensulfuron methyl produced more CO_2, while those treated with prosulfuron and triasulfuron produced less CO_2 than the control. The CO_2 evolved from samples spiked with cinosulfuron, over the incubation period, was not statistically different from the control. On the contrary, all the samples spiked with a 10-fold dose of herbicides produced more CO_2 than the control.

The soil respiration data along the 30 days incubation period, as affected by the studied herbicides, are reported in Figures 1a (FD) and 1b (10 FD). The control samples, the cinosulfuron, and triasulfuron treatments increased the CO_2 production over the time along the incubation both at FD and 10 FD doses. The CO_2 evolved from the samples treated with prosulfuron, on the contrary, seemed to start to decline from the 23rd day of incubation, both at FD and 10-FD.

Soil microbial biomass C and N

The MBC and MBN data clearly showed a detrimental effect of herbicides over the soil microbial biomass (Table 2). The amount of microbial C ranked as follows: control > herbicides at FD > herbicides at 10 FD, being prosulfuron and thifensulfuron methyl at 10 FD the compounds exerting the strongest detrimental effect on this parameter. The MBC reduction spanned from 25 % of the control for

Table 2. Soil respiration, microbial biomass C (MBC), microbial biomass N (MBN), qCO_2, qFDA, and enzymatic activities in soil treated with sulphonylurea herbicides.

	mg C–CO_2 kg^{-1} dm	*mg C or N kg^{-1} dm*					*μ mol PNP g^{-1} d.m. h^{-1}*			
	Respiration	*MBC*	*MBN*	*qCO_2*	*% FDA*	*qFDA*	*Alkaline Phosphatase*	*Acid Phosphatase*	*Arylsulfatase*	*β-Glucosidase*
Control	338c	221g	86h	0.0431a	20d	0.0890a	246b	124b	64f	34c
CI - FD	343c	165f	48c	0.0645d	17c	0.1009b	249bc	124b	56de	26b
CI - 10 FD	475h	124c	58de	0.1126h	15a	0.1209d	204a	106a	42a	22a
PR - FD	254a	133d	73g	0.0488b	15a	0.1153cd	246b	123b	54cd	24ab
PR - 10 FD	381e	106a	36a	0.0851f	23e	0.2129g	262d	130b	53c	23a
TH - FD	361d	131d	60ef	0.0770e	17c	0.1326e	272e	142c	65f	35cd
TH - 10 FD	447g	101a	43b	0.1239i	23e	0.1660f	270e	130b	65f	35cd
TR - FD	308b	150e	54d	0.0589c	16b	0.1088bc	254c	128b	44b	24ab
TR - 10 FD	437f	117b	63f	0.0971g	19d	0.1619f	263d	137c	58e	38d

Values in the same column followed by the same letter are not statistically different ($P < 0.05$).

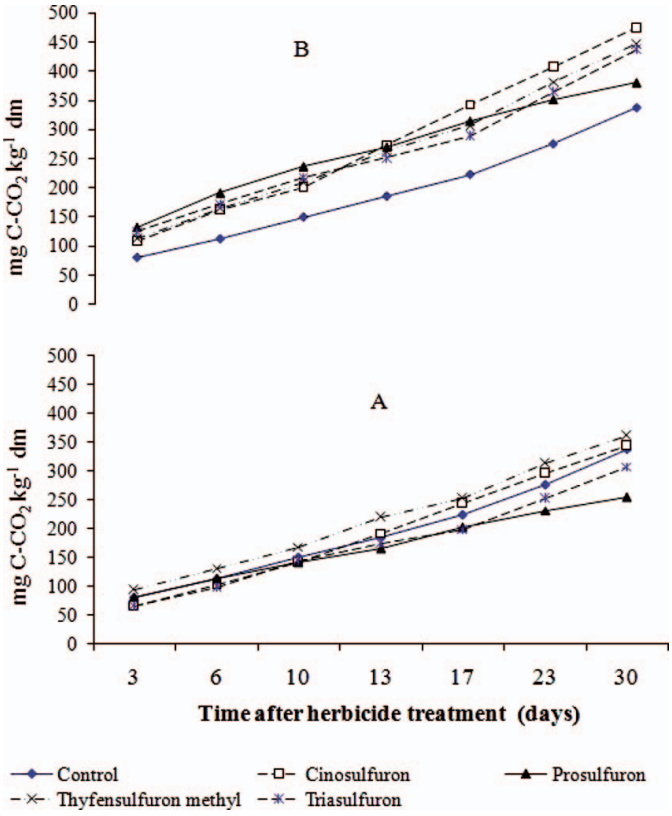

Fig. 1. Cumulate microbial soil respiration as affected by herbicidal treatments at filed dose (A) and ten-fold (10 FD) higher field dose (B) (color figure available online).

cinosulfuron FD to 54 % of reduction in the case of thifensulfuron methyl 10 FD. The MBN did not follow the same trend of the MBC, as cinosulfuron FD and triasulfuron FD seemed to have a less toxic effect than the corresponding 10-fold doses. The herbicide exerting the stronger detrimental effect was prosulfuron 10 FD, which reduced MBN to 58 % of the control value, whereas the same herbicide at the FD showed the less toxic effect reducing MBN to 15 % of the control.

qCO₂

The metabolic quotient (Table 2), expressed as mg C-CO_2 kg^{-1} C_{mic} day^{-1}, showed an inverse trend, in terms of absolute value, if compared to MBC and MBN, as it increased along the increase in herbicides concentration. For both the FD and 10 FD treatments the effect on qCO_2 ranked as follows: control < prosulfuron < triasulfuron < cinosulfuron < thifensulfuron methyl. The lowest detrimental effect was exerted by prosulfuron at FD, which increased the control value by 13 %, whereas the highest effect was shown by thifensulfuron methyl at 10 FD, which increased the control value by 187 %.

Enzymatic activities

Alkaline phosphatase, acid phosphatase, arylsulfatase and β-glucosidase (Table 2) were affected substantially less than soil respiration, MBC and MBN. All these parameters seemed to be less sensitive towards the potentially xenobiotic effect of tested herbicides. As for alkaline phosphatase, cinosulfuron FD and prosulfuron FD showed values not statistically different from the control, whereas, with the exception of cinosulfuron 10 FD, all the other studied herbicides showed values higher than the control. Cinosulfuron 10 FD was the only treatment in which the alkaline phosphatase was lowered to 17 % of the control value.

Acid phosphatase was enhanced in triasulfuron 10 FD and thifensulfuron methyl FD treatments, whereas its value was not statistically different from the control for cinosulfuron FD, prosulfuron FD, prosulsuforon 10 FD, thifensulfuron methyl 10 FD and triasulfuron FD. Also in this case the only recordable detrimental effect was exerted by cinosulfuron 10 FD, which lowered the control value by 14 %.

The arylsulphatase activity confirmed the low sensitivity of this enzymatic parameters regarding the effect of the selected herbicides on soil microbial biomass. The statistical analysis allowed identifying two sets of data. The first one comprised values that were not statistically different from the control (thifensulfuron methyl FD and 10 FD), the second set comprised data statistically lower than the control (triasulfuron FD, cinosulfuron FD, prosulfuron FD, prosulfuron 10 FD, triasulfuron FD and cinosulfuron 10 FD), cinosulfuron 10 FD being the most toxic effect. The results regarding triasulfuron and prosulfuron were inconsistent, as their overall toxicity appears to be higher or non statistically different at FD than at 10 FD, respectively.

β-glucosidase data pointed out an increase of activity in samples spiked with triasulfuron 10 FD, whereas thifensulfuron methyl FD and 10 FD were not statistically different from the control. The lowest toxic effect on this enzymatic activity was shown by cinosulfuron FD, whereas prosulfuron FD and triasulfuron FD, and prosulfuron 10 FD and cinosulfuron 10 FD gave results not statistically different from each other. Also in this case an inconsistent result was obtained for triasulfuron, as the FD dose appeared to exert a toxic effect on microbial biomass, whereas the 10 FD dose seemed to enhance the β-glucosidase activity.

The fluorescein diacetate hydrolysis (FDA) (Table 2) seemed enhanced by the prosulfuron 10 FD treatment, while the triasulfuron 10 FD treatments gave an activity not statistically different from the control. All the other treatments exerted a low decrement on this activity. Inconsistent results were obtained for triasulfuron FD, thifensulfuron methyl FD and prosulfuron FD that showed higher values than their 10 FD doses.

In order to sum up the obtained results, Table 3 shows the effect of selected solfonylureas on studied microbial parameters ranking the effect as: a) high toxicity (the

Table 3. Effect of selected sulfonylureas on studied microbial parameters (see text for explanation).

	Toxicity		
Microbial parameter	*Non- toxic*	*Low*	*High*
Respiration	CI-FD CI-10 FD TH-10 FD PR-10 FD	TR-FD	PR-FD
MBC	none	CI-FD	TH-10 FD PR-10 FD
MBN	none	PR-FD	PR-10 FD
qCO_2	none	PR-FD	TH-10 FD
FDA	PR-10 FD TR-10 FD	TR-FD	CI-10 FD PR-FD
qFDA	none	CI-FD	TH-10 FD
Alkaline phosphatase	CI-10 FD PR-FD PR-10 FD TH-FD TH-10 FD TR-FD TR-10 FD	none	CI-10 FD
Acid phosphatase	CI-FD PR-FD PR-10 FD TH-10 FD TR-FD	TH-FD TR-10 FD	CI-10 FD
Arylsulphatase	TH-FD TH-10 FD	TR-10 FD	CI-10 FD
β-glucosidase	TH-FD TH-10 FD TR-10 FD	CI-FD	CI-10 FD PR-FD PR-10 FD TR-FD

highest detrimental effect observed); b) low toxicity (the lowest detrimental effect observed) and c) non-toxic (treatments giving the same results of the control, or performing better). The parameters MBC, MBN, qCO_2 and qFDA are more sensitive to the toxic effect of studied herbicides, always showing responses falling into the high or low toxicity and never into the non-toxic case.

Discussion

Soil respiration seemed unable to depict a clear-cut effect of the studied herbicides, as the 10 FD treatments produced more CO_2 than both the control and FD samples and they were classified as not toxic (Table 3).

MBC and MBN both were strongly affected by the 10 FD of thifensulfuron methyl, being that MBC was also affected by prosulfuron 10 FD, which were classified as more toxic for this parameter among the studied herbicides.

The criticism on the use of soil respiration alone as a tool for assessing ecotoxicological effects of synthetic organic compounds is based on the Odum theory of ecosystem succession.[33] This theory was the conceptual ground on which Anderson and Domsch[34] theorized the microbial metabolic quotient (qCO_2), an index which increases under ecosystem disturbance (including the toxic effect of xenobiotic compounds). The metabolic quotient was extensively used as tool for assessing ecotoxicological behaviors of xenobiotic compounds towards soil microbial biomass and activities.[32, 35–37] The parameter qCO_2 quantitatively measures the C flux through the microbial biomass and was able to point out, in this work, a coherent trend in toxicity exerted by the studied herbicides, as it is able to integrate the data obtained from the soil respiration under controlled conditions, and the data of microbial biomass C. The evaluation of qCO_2 values seemed to be able to point out a disturbance upon the soil microbial biomass, which is forced to divert metabolic energy to repairing processes in order to withstand the toxic effect of herbicides.

Even though highly variable results were obtained for less toxic and non-toxic herbicide for the enzymatic activities, including FDA hydrolysis, all of them resulted to be strongly affected by the 10 FD of cinosulfuron (Table 3). It is noteworty that both the specific enzymatic activities (alkaline and acid phosphatase, arylsulphatase and β-glucosidase), and the more general FDA hydrolysis activity, were all impaired at the maximum level by the same herbicide at 10 FD. FDA is hydrolyzed by a number of different enzymes, such as proteases, lipases, and esterases and is a general indicator of the activity of soil microflora, through the general hydrolase activity.[38]

The hydrolysis of FDA was mostly used to monitor the effect of soil amendments on soil microbial biomass,[39–41] but it revealed itself as a useful tool for observing the effect of xenobiotic compounds on soil microflora.[32–42] A lack of correlation between CO_2 evolution and FDA was found by Son et al.,[43] after conversion of agricultural lands to natural vegetation, calling into question the FDA soundness as index of general microbial activity. The qFDA index, as proposed by Perucci et al.,[32] should overcome this inconsistency by relating the FDA hydrolysis to the amount of MBC, expressing this way the general hydrolytic activity as function of the C stored in the microbial biomass. In Table 3 is shown that a general consistency of results was obtained regarding the strongest herbicide effect among MBC, qCO_2 and qFDA, all of them being more sensitive to thifensulfuron methyl 10 FD.

Conclusions

In this work an interesting consistency of results for MBC, qCO_2 and qFDA was observed concerning the toxic effect exerted by the 10 FD of thifensulfuron methyl. At the same time was observed the same response of specific enzymatic activities (alkaline and acid phosphatase, arylsulphatase and β-glucosidase) to the cinosulfuron 10 FD, that revealed

itself as the more toxic among the studied sulphonylureas for these parameters.

As shown in Table 3, besides the mentioned consistency of results regarding MBC, qCO_2 and qFDA, and the studied specific enzymatic activities, was also recorded that there are inconsistencies about the less toxic and non toxic herbicides, as they were scattered, lacking of a clear trend, among the studied microbial parameters. All this lends support to the hypothesis that only the strongest toxic effect of xenobiotic compounds can be correctly recorded.

In addition, the results pointed out two different sorts of toxicity: the first is related to metabolic quotients (qCO_2 and qFDA), which were strongly affected by the higher dose of thifensulfuron methyl, and the second was related to MBC and MBN, which were influenced by the prosulfuron 10 FD, and the third was related to the studied specific enzymatic activities, which were affected by the higher dose of cinosulfuron. All this points out that microbial metabolic activity are affected in different ways by different herbicides and that we need more than an unique index to highlight the toxic effect of xenobiotic compounds on soil microbial biomass and its activities. All this is in agreement with Smith et al.[6] who pointed out that in the assessment of "health" status of soil, considered as soil quality, no single parameter can be used as a reliable and unique indicator.

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References

[1] Zobiole, L.H.S.; Kremer, R.J.; Olivera, R.S.; Constantin, J. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. J. Appl. Microbiol. **2010**, *110*, 118–127.

[2] Ahemad, M.; Khan, S. Toxicological effects of selective herbicides on plant growth promoting activities of phosphate solubilizing *Klebsiella* sp. strain PS19. Curr. Microbiol. **2011**, *62*, 532–538.

[3] Zhang, X.; Li, X.; Zhang, C.; Li, X.; Zhang, H. Ecological risk of long-term chlorimuron-ethyl application to soil microbial community: an in situ investigation in a continuously cropped soybean field in Northeast China. Environ. Sci. Pollut. Res. **2011**, *18*, 407–415.

[4] Jablonowski, N.D.; Schäffer, A.; Burauel, P. Still present after all these years: persistence plus potential toxicity raise questions about the use of atrazine. Environ. Sci. Pollut. Res. Int. **2011**, *18*, 328–331.

[5] Doran, J.W.; Sarrantonio, M.; Liebig, M.A. Soil health and sustainability. Advances in Agronomy **1996**, *56*, 1–54.

[6] Smith, J.L.; Halvorson, J. L.; Papendick, R.I. Using multiple-variable indicator kriging for evaluating soil quality. Soil Sci. Soc. Am. J. **1993**, *57* (3), 743–749.

[7] Gianfreda, L.; Rao, M.A. Interactions between xenobiotics and microbial and enzymatic soil activity. Crit. Rev. Env. Sci. Tec. **2008**, *38*, 269–310.

[8] Araújo, A.S.F.; Monteiro, R.T.R.; Abarkeli, R.B. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere **2003**, *52*, 799–804.

[9] Dumontet, S.; Perucci, P.; Bufo, S.A. Les amendements organiques et traitements avec les herbicides. Influence sur la biomasse microbienne du sol. In *Actes du XXVII Congrès du Groupe Français des Pesticides*, Orléans, France, Mai 21–22, 1977; Mouvet, C., Guillet, B., de Las Heras, J., Eds.; BRGM Editions, 1998, 211–213.

[10] Chowdhury, A.; Pradhan, S.; Saha, M.; Sanyal, N. Impact of pesticides on soil microbiological parameters and possible bioremediation strategies. Indian J. Microbiol. **2008**, *48*, 114–127.

[11] Post, W.M.; Emanuel, W.R.; Zinke, P.J.; Stangenberger, A.G. Soil carbon pools and world life zones. Nature **1982**, *298*, 156–159.

[12] Bond-Lamberty, B.; Thomson, A. Temperature-associated increases in the global soil respiration record. Nature **2010**, *464*, 579–582.

[13] Brendecke, J.; Alexson, R.D.; Pepper, I.L. Soil microbial activity as an indicator of soil fertility: long-term effects of municipal sewage sludge on an arid soil. Soil Biol. Biochem. **1993**, *25*, 751–758.

[14] Kennedy, A.C.; Smith, K.L. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil. **1995**, *170* (1), 75–86.

[15] Franzluebbers, J.; Zuberer, D.A.; Hons, F.M. Comparison of microbiological methods for evaluating quality and fertility of soil. Biol. Fert. Soils. **1995**, *19*, 135–140.

[16] Paul, E.A.; Clark, F.E. *Soil microbiology and biochemistry;* Academic Press: San Diego (CA), USA, 1996; pp 340.

[17] Dumontet, S.; Dinel, H.; Lévesque, M. The distribution of pollutant heavy metals and their effect on soils respiration and acid phosphatase activity in mineral soils of the Rouyn-Noranda region Québec. Sci. Total Environ. **1992**, *121*, 231–245.

[18] Bååth, E. Effects of heavy metals in soil on microbial processes and populations (a review). Water Air Soil Pollut. **1989**, *47*, 335–379.

[19] Busse, M.D.; Ratcliff, A.W.; Shestak, C.J.; Powers, R.F. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biol. Biochem. **2001**, *33* (12–13), 1777–1789.

[20] Amato, M.; Ladd, J.N. Studies of nitrogen immobilization and mineralization in calcareous soils. V. Formation and distribution of isotope labelled biomass during decomposition of ^{14}C and ^{15}N labelled plant material. Soil Biol. Biochem. **1980**, *12*, 405–411.

[21] Nannipieri, P.; Ascher, J.; Ceccherini, M.T.; Landi, L.; Pietramellara, G.; Renella, G. Microbial diversity and soil functions. Eur. J. Soil Sci. **2003**, *54*, 655–670.

[22] Tomlin, C. *The pesticide manual*, 10th ed.; British Crop Protection Council: Cambridge, UK, 1994; 1341 pp.

[23] Brown, H.M. Modes of action, crop selectivity, and soil relations of the sulfonylurea herbicides. Pestic. Sci. **1990**, *29*, 263–281.

[24] Black, I.D.; Pederson, R.N.; Flynn, A.; Moerkerk, M.; Dyson, C.B.; Kookana, R.; Wilhelm, N. Mobility and persistence of three sulfonylurea herbicides in alkaline cropping soils of south-eastern Australia. Aust. J. Exp. Agr. **1999**, *39*, 465–472.

[25] Sarmah, A.K.; Kookana, R.S.; Alston, A.M. Degradation of chlorsulfuron and triasulfuron in alkaline soils under laboratory conditions. Weed Res. **1999**, *39*, 83–94.

[26] Page, A.L.; Miller, R.H.; Keeney, D.R. *Methods of Soil Analysis, Part 2*; Am. Soc. Agron.: Madison, WI, USA, 1982; pp 1159.

[27] Klute, A. *Methods of Soil Analysis, Part 1*, 2nd ed. Am. Soc. Agron.: Madison, WI, USA, 1986; pp 1358.

[28] Dumontet, S.; Mathur, S.P. Evaluation of respiration-based methods for measuring microbial biomass in metal-contaminated acidic mineral and organic soils. Soil Biol. Biochem. **1989**, *21*, 431–436.

[29] Sparling, G.P.; West, A.W. Modifications to the fumigation-extraction technique to permit simultaneous extractions and estimation of soil microbial C and N. Commun. Soil Sci. Plan. **1988**, *19*, 327–344.

[30] Wu, J.; Joergensen, R.G.; Pommerening, B.; Chaussod, R.; Brookes, P.C. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. Soil Biol. Biochem. **1990**, *22*, 1167–1169.

[31] Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biol. Biochem. **2001**, *33*, 943–951.
[32] Perucci, P.; Dumontet, S.; Bufo, S.A.; Mazzatura, A.; Casucci, C. Effects of organic amendment and herbicide treatment on soil microbial biomass. Biol. Fert. Soils. **2000**, *32*, 17–23.
[33] Odum, E.P. The strategy of ecosystem development. Science **1969**, *164*, 262–270.
[34] Anderson, T.H.; Domsch, K.H. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol. Biochem. **1993**, *25*, 393–395.
[35] Fliessbach, A.; Martens, R.; Reber, H.H. Soil microbial biomass and microbial activity in soils treated with heavy metal contaminated sewage sludge. Soil Biol. Biochem. **1994**, *26*, 1201–1205.
[36] Leita, L.; De Nobili, M.; Muhlbachova, G.; Mondini, C.; Marchiol, L.; Zerbi, G. Bioavailability and effects of heavy metals on soil microbial biomass survival during laboratory incubation. Biol. Fert. Soils. **1995**, *19*, 103–108.
[37] Zhang, N.L.; Wan, S.Q.; Li, L.H.; Bi, J.; Zhao, M.M.; Ma, K.P. Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China. Plant Soil. **2008**, *311*, 19–28.
[38] Inbar, Y.; Boehm, M.J.; Hoitink, H.A.J. Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Soil Biol. Biochem. **1991**, *23*, 479–483.
[39] Vekemans, X.; Godden, B.; Penninckx, M.J. Factor analysis of the relationships between several physico-chemical and microbiological characteristics of some Belgian agricultural soils. Soil. Biol. Biochem. **1989**, *21*, 53–58.
[40] Perucci, P.; Dumontet, S.; Casucci, C.; Schnitzer, M.; Dinel, H.; Monaci, E.; Vischetti, C. Moist olive husks addition to a silty clay soil: influence on microbial and biochemical parameters. J. Environ. Sci. Health B **2006**, *41* (6), 1019–1036.
[41] Sánchez-Monedero, M.A.; Mondini, C.; Cayuela, M.L.; Roig, A.; Contin, M.; De Nobili, M. Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils. Biol. Fertil. Soils. **2008**, *44*, 885–890.
[42] Vischetti, C.; Perucci, P.; Casucci, C.; Monaci, E.; Dumontet, S. Biochemical parameter changes in urban-waste compost used as biofilter for pesticide decontamination. Int. J. Environ. Anal. Chem. **2006**, *86*, 195–205.
[43] Son, Y.; Seo, K.Y.; Kim, R.H.; Kim, J. Soil respiration and FDA hydrolysis following conversion of abandoned agricultural lands to natural vegetation in central Korea. J. Plant. Biol. **2006**, *49*, 231–236.