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Glyphosate effect on soil biochemical properties under conservation tillage

M. Panettieri *, L. Lazaro, R. López-Garrido, J.M. Murillo, E. Madejón

Instituto de Recursos Naturales y Agrobiología de Sevilla, IRNAS-CSIC, PO Box 1052, 41080 Sevilla, Spain

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ABSTRACT

In conservation tillage (CT) the use of herbicides is often required. Glyphosate ($C_3H_8NO_5P$, *N*-phosphonomethylglycine) is one of the most used herbicides. Since biochemical parameters are often used as soil quality indexes, the stimulation effects on these indexes induced by CT could be misinterpreted due to the required application of herbicide. The objective of this work was to establish the effect of glyphosate on soil biochemical parameters and how long did these effects last on a sandy clay loam Entisol in SW Spain under different tillages.

To test the hypothesis that glyphosate could lead to an erroneous evaluation of biochemical parameters when different tillages are compared, two experiments were carried out in 2011: (i) the first one was conducted under incubation conditions; no-tillage (NT) and reduced tillage (RT) pots with and without herbicide addition were compared, after sunflower (*Helianthus annus* L.) sowing, during 101 days; (ii) in the second one, samples were directly collected from an experimental farm after wheat (*Triticum aestivum* L.) sowing; NT and RT soil samples where glyphosate was applied were compared for 60 days with plots under traditional tillage in the absence of glyphosate.

Biochemical parameters such as water soluble carbon (WSC), microbial biomass carbon (MBC) and nitrogen (MBN), as well as dehydrogenase (DHA) and β-glucosidase (β-Glu) activities were analyzed in both experiments.

Results showed that glyphosate acted as a source of organic carbon in both experiments, however, stimulation effects on MBC, MBN, DHA and β-Glu were more evident and lasted longer for the incubated samples. In this experiment, the maximum values for MBC, MBN and DHA were registered between 18 and 37 days after application, while higher β-Glu values under reduced tillage were still registered after 101 days. Those effects were less noticeable in the on-field experiment.

Under our experimental conditions (Entisol, Mediterranean condition, rainfed agriculture, wheat–sunflower–fodder pea rotation), an interval of at least 30 days between glyphosate application and soil sampling should be adopted as a standard to avoid data misinterpretation. Differences in glyphosate metabolism are expected for different conditions and crops and should be evaluated case by case.

1. Introduction

Conservation agriculture (CA) constitutes the most important change in soil management in modern agriculture (Lal et al., 2007). FAO define CA as "the agriculture that aims to achieve sustainable and profitable methods that improves livelihoods of farmers through the application of three main principles: minimal soil disturbance, permanent soil cover and crop rotations". Among conservation agriculture, conservation tillage is the collective umbrella term that is commonly given to no-tillage, direct drilling, minimum tillage or ridge-tillage to denote that the specific practice has a conservation goal of some nature.

Normally, the retention of 30% surface cover by residues characterizes the lower limit of classification for conservation tillage but other objectives include time, fuel and water savings, and improvement of soil quality.

Numerous researches have pointed out the positive effects of CA in soil quality under rainfed conditions in the Mediterranean basin (Álvaro-Fuentes et al., 2008; Melero et al., 2011; Mrabet et al., 2001). The CA increases soil organic carbon (SOC) by preventing carbon losses operated by erosion and mineralization (Engel et al., 2009) and by adding fresh carbon sources proceeding from crop residues. Carbon storage has several environmental benefits since agriculture is responsible for 20% of CO_2 emission (Lal, 2004). Soils under CA also showed higher microbial activities and better physical properties compared with corresponding soils

* Corresponding author. Tel.: +34 954624711; fax: +34 954624711.
E-mail addresses: marco.panettieri@csic.es, marcopanettieri@gmail.com (M. Panettieri).

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under traditional tillage (TT) (Franzluebbers, 2004). Furthermore, water infiltration and water loss prevention displayed higher values under CA and these characteristics are particularly relevant under Mediterranean conditions that could turn into semi-arid conditions, like those in the southwest of Spain (Moreno et al., 1997). However, one of the common problems under CA is the absence of a preliminary tillage that eliminates weeds before seeding procedures. In these conditions, farms always need herbicides to prevent weeds proliferation. This practice causes contamination and chemical persistence in soil, and groundwater pollution after percolation. Glyphosate ($C_3H_8NO_5P$, *N*-phosphonomethylglycine) is one of the most important herbicide in the world and the most sold in the USA. Glyphosate mode of action is to inhibit the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which catalyses the reaction of shikimate-3-phosphate and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate. That results in the total inhibition of the synthesis pathway of three fundamental amino acids: phenylalanine, tyrosine and tryptophan. The shikimate pathway is not present in vertebrate metabolism and this makes glyphosate relatively non-toxic for human, reptiles and mammalians.

Many soil enzymes can be used as indicators of soil quality for sustainable management because they are sensitive to ecological stress and land management (Benedetti and Dilly, 2006). Enzymes may react to changes in soil more quickly than other physic-chemical variables and therefore may be useful as early indicators of biochemical changes (Melero et al., 2008; Nannipieri, 1994). Evidence of the stimulation effect of herbicides on soil biochemical properties has been reported (Benitez et al., 2006; García-Ruiz et al., 2008; Reinecke et al., 2002), even if herbicides are not designed to directly interact with soil enzymes (Speir and Ross, 2002). However, other authors defined as negligible the effect of glyphosate on soil microbial communities (Busse et al., 2001). Gianfreda and Rao (2008) reported that this effect is concentration-dependent and herbicide type-dependent and Zabaloy et al. (2008) described glyphosate as the herbicide with the most pronounced stimulation effect on soil biochemical properties if compared with 2,4-dicholorophenoxyacetic acid and metsulfuron-methyl. The main objective of this work was to evaluate the effect of glyphosate on various biochemical indicators of soil quality in soils under conservation tillage in Mediterranean, rainfed conditions. We hypothesize that the herbicide presence could have a time dependent effect on soil biochemical properties. To test the hypothesis two different experiments were carried out: one under controlled conditions and another one directly under field conditions. Parameters like water soluble carbon (WSC), microbial biomass carbon (MBC) and nitrogen (MBN) and the activity of two enzymes (dehydrogenase, DHA, and β-glucosidase, β-Glu) were determined.

2. Materials and methods

2.1. Experimental area

The selected plots were situated on "La Hampa" experimental farm of the "Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS-CSIC)" (37°17′ N, 6°3′ W), located 13 km southwest of the city of Seville (Spain). Since 2008, 9 plots on the farm were managed under different tillage practices, in order to evaluate the changes originated by tillage on soil quality. The soil is an Entisol (Xerofluvent, Soil Survey Staff, 1999) with a sandy clay loam texture (clay content of about 250 g kg^{-1}, 210 g kg^{-1} silt and 540 g kg^{-1} sand), pH of around 7.8 (calcareous), Kjeldahl nitrogen concentration of 950 mg kg^{-1}, Olsen phosphorus concentration of 18.8 mg kg^{-1} and alkaline-earth carbonates concentration of 280 g kg^{-1}. The climate of the zone is typically Mediterranean, with mild rainy winters (484 mm mean annual rainfall) and very hot and dry summers. The mean annual temperature at the experimental site is around 17 °C, the maximum and minimum mean monthly temperatures were 33.5 °C and 5.2 °C registered in July and January, respectively.

For the on-field experiment, samples collected from three tillage treatments were directly compared: traditional tillage (TT), reduced tillage (RT) and no-tillage (NT). The experiment was carried out in a completely randomized block design with three replicates per treatment. For the incubation experiment, soil cores proceeding from the same plots were used to fill the incubation pots.

The TT consisted of a pass of mouldboard plough (to a depth of 25–30 cm) and two or three cultivator passes at a depth of 15–20 cm and a disc harrow pass at a depth of 15 cm. RT was characterized by the lack of mouldboard ploughing and by a reduction in the number of tillage operations (only chisel plough at a depth of 20–25 cm, every two years and a yearly disc harrowing at a depth of 5–7 cm were retained) as well as by leaving the crop residue on the soil surface. NT was characterized by the absence of tillage (direct drilling) in which the residue is left on the soil surface until it decays, except sunflower stalks, which were broken into smaller pieces before the next crop was sown. The percentage of the soil surface covered by residue in both the RT and NT treatments was determined by stretching a 10 m cord (marked every 10 cm) diagonally across several rows of each conservation tillage (CT) sub-plot as reported by Plaster (1992). The number of marks touching a piece of crop residue gave the percentage of coverage. In our case, the percentage of residues covering the soil surface was greater than 60% in both CT treatments, and this confirmed that conservation tillage was established correctly in both cases.

A wheat (*Triticum aestivum* L.)–sunflower (*Helianthus annus* L.)–fodder pea (*Pisum arvense* L.) crop rotation was established. The sunflower and fodder pea crops were not fertilized (as it is traditional in this zone), while wheat received 100 kg ha^{-1} (fertilizer complex) with no top dressing fertilizer. Weeds were controlled by tillage in TT and by the application of pre-emergence herbicides in CT, at a rate of 4 L ha^{-1} glyphosate (36%).

2.2. Incubation experiment

For the incubation experiment, a week after fodder pea harvest on May 2011, two different areas of 1 m^2 were delimited at random in each one of the 6 plots corresponding to conservation tillage systems (3 plots under NT and 3 under RT), for a total of 12 selected areas. In one of this area for each plot, the herbicide was applied (GLYFOS Ultra, Agrodan, which contains 36% glyphosate) at a dose equivalent to 10 L ha^{-1} that corresponds to the maximum recommended by the manufacturer's label. To ensure the homogeneity of the application a sprayer was used. The second area for each plot was used as a control, for a total of 6 areas with glyphosate application and 6 control areas, equally distributed between NT and RT plots.

After the application of the herbicide, undisturbed soil samples coming from each one of the 12 areas were taken using PVC cylinders. These cylinders contained approximately the top 15 cm of the soil profile and were immediately transferred to 12 pots of similar diameters; a total amount of 600 cm^3 (about 750 g) was placed in each pot. The soil profile (0–15 cm depth) remained unaltered after this operation.

The pots with soil samples were brought to the laboratory, and they were placed at random in a chamber under controlled conditions (between 22 and 24 °C, photoperiod of 18 h light and 6 of dark and light intensity: 111 μE m^{-2} s^{-1}) where they remained throughout the time that the experiment was developed.

In order to prepare the sowing, distilled water was added to bring the water content of soil to 70% of its total water holding