Science of the Total Environment 634 (2018) 974–982

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Soil microbial communities and glyphosate decay in soils with different herbicide application history

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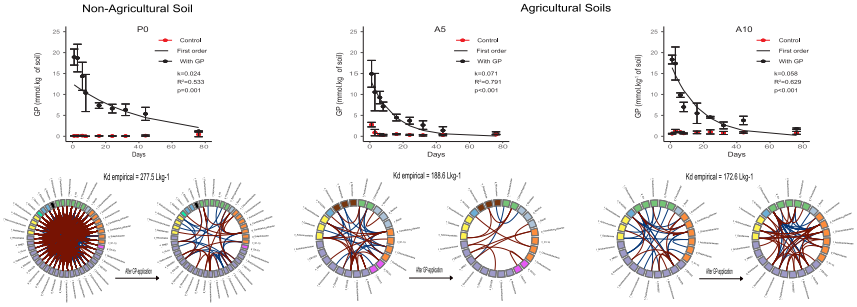
HIGHLIGHTS

- Glyphosate dissipation in soil is evaluated under field conditions.
- Redundant bacterial populations of potential degraders
- Application of glyphosate disrupt bacterial association network.
- Bioavailability is a key factor for the persistence of GP and AMPA.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 January 2018
Received in revised form 29 March 2018
Accepted 31 March 2018
Available online xxxx

Editor: Jay Gan

Keywords:
Soil microbial communities
Next-generation sequencing
Glyphosate
Nonparametric Microbial Interdependence Test

ABSTRACT

This study evaluates the glyphosate dissipation under field conditions in three types of soil, and aims to determine the importance of the following factors in the environmental persistence of herbicide: i) soil bacterial communities, ii) soil physicochemical properties, iii) previous exposure to the herbicide. A soil without previous record of GP application (P0) and two agricultural soils, with 5 and >10 years of GP exposure (A5 and A10) were subjected to the application of glyphosate at doses of 3 mg·kg^{-1}. The concentration of GP and AMPA was determined over time and the dynamics of soil bacterial communities was evaluated using 16S ARN ribosomal gene amplicon-sequencing. The GP exposure history affected the rate but not the extent of GP biodegradation. The herbicide was degraded rapidly, but P0 soil showed a dissipation rate significantly lower than soils with agricultural history. In P0 soil, a significant increase in the relative abundance of *Bacteroidetes* was observed in response to herbicide application. More generally, all soils displayed shifts in bacterial community structure, which nevertheless could not be clearly associated to glyphosate dissipation, suggesting the presence of redundant bacteria populations of potential degraders. Yet the application of the herbicide prompted a partial disruption of the bacterial association network of unexposed soil. On the other hand, higher values of linear (Kd) and nonlinear (Kf) sorption coefficient in P0 point to the relevance of cation exchange capacity (CEC), clay and organic matter to the capacity of soil to adsorb the herbicide, suggesting that bioavailability was a key factor for the persistence of GP and AMPA. These results contribute to understand the relationship between bacterial taxa exposed to the herbicide, and the importance of soil properties as predictors of the possible rate of degradation and persistence of glyphosate in soil.

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https://doi.org/10.1016/j.scitotenv.2018.03.393
0048-9697/

1. Introduction

The herbicide glyphosate [N-(phosphonomethyl)-glycine] is a synthetic phosphonate used extensively in the entire world. The introduction of glyphosate-resistant crops, pre-emergence applications and weed control between crops has broaden its application (Székács and Darvas, 2012), with a concomitant increase in the volume applied per hectare. Environmental concern related to the widespread use of glyphosate has derived in a large number of experimental studies (reviewed by Cerdeira and Duke, 2006; Borggaard and Gimsing, 2008; Mamy et al., 2016) and modelling studies (la Cecilia and Maggi, 2018; Wang et al., 2016) focusing on its fate. The persistence of glyphosate in the environment increases the possibility of freshwater and groundwater contamination, as well as the interception and absorption by weeds and crops (Bento et al., 2017; Doublet et al., 2009).

Degradation of glyphosate (GP) in soils is mainly microbiological (Sprankle et al., 1975), and the role of abiotic factors on its dissipation is negligible (Bento et al., 2016). Therefore, the role of soil microorganisms is critical in minimizing the environmental concentration of the herbicide. GP biodegradation occurs by two alternative pathways (Singh and Walker, 2006). One of the pathways, carried out by microorganisms that utilize the herbicide as a source of phosphorous, involves the conversion to sarcosine, which is subsequently mineralized to carbon dioxide and water. This pathway rarely occurs in natural environment because the enzymes involved are induced when the intracellular P_i is deficient, a situation not typically encountered in agricultural soils (Sviridov et al., 2015). In the other pathway, GP is metabolized to glyoxylate and aminomethylphosphonic acid (AMPA) by microorganisms that use GP as a source of N. The capacity of many soil bacteria to degrade GP, yielding both sarcosine and AMPA, has been demonstrated in the laboratory (Sviridov et al., 2015). The biodegradation of GP via AMPA has been well documented, and this metabolite has been detected at higher concentrations than GP in agricultural fields (Aparicio et al., 2013; Battaglin et al., 2014; Primost et al., 2017; Silva et al., 2018). An important body of literature, mostly performed in microcosms, has revealed that GP exposure affects the structure of soil microbial communities. A wide variety of responses have been described, ranging from transient to permanent changes, affecting members of phylum *Acidobacteria* (Newman et al., 2016a), ammonia-oxidizing bacteria (Allegrini et al., 2017), mycorrhiza (Druille et al., 2013), and others. Either the reduction or the enhancing of the microbial activity and biomass in soil has also been reported (Gómez et al., 2009; Haney et al., 2002).

It has been shown that repeated use of the same pesticide for several years brings about the ability of soil biota to degrade it rapidly (James et al., 2010). However, this process depends on the intervals between successive pesticide applications, and on the stability of the active microbiota (Kaufman et al., 1985). Previous studies on the effect of repeated applications of GP focused on the activity of microbial communities rather than on the kinetics of biodegradation. Araújo et al. (2003) found an increase in respiration and FDA activity in agricultural soils after GP application, compared to soils with no history of GP exposure. On the other hand, Allegrini et al. (2015) did not find differences in the microbial community tolerance to GP from contrasting soils with and without history of exposure to the herbicide.

Because soil is a very complex and dynamic environmental matrix, herbicide degradation in soil is not only determined by the microorganisms and the environmental factors, such as land use, soil moisture, temperature and sources of nitrogen and carbon (Girvan et al., 2003; Lauber et al., 2008; Bento et al., 2016; Zabaloy et al., 2016), but it is also critical that the molecule is available for enzymatic attack (Throckmorton et al., 2015). Once GP is sprayed, a part of the herbicide attaches to soil particles due to their high adsorption capacity, and will be more or less bioavailable, depending on the reversibility of adsorption equilibrium. Some mechanisms have been proposed to describe the interaction between GP and soil particles (Cruz et al., 2007; Gimsing and Borggaard, 2002; Ololade et al., 2014). Yet it is not entirely clear what are the main factors that control the adsorption of GP to soil. Weber et al. (2004) proposed a pedotransfer function for predicting the linear sorption coefficient (Kd) of different pesticides, and more recently Dollinger et al. (2015) put forward a specific function for GP, in which Kd is mainly driven by cation exchange capacity (CEC) and clay content.

Although it is known that sorption influences both the immobilization and the microbial degradation of the herbicide, less attention was devoted to studying the interplay between soil properties, microbial community composition and GP biodegradation. Our working hypothesis is that soil characteristics, rather than the dependency on specific microorganisms, determine the glyphosate dissipation in soil. To that aim, the specific objectives of this work are: i) to evaluate the degradation rates in soils with and without previous exposure to GP, ii) to establish the relationship between the dynamics of biodegradation and the changes in soil bacterial communities iii) to elucidate the influence of soil properties on microbial community structure and GP bioavailability. We based our study in three soils located in the southeast of Buenos Aires Province, Argentina, with similar edapho-climatic conditions, but different history of land use and herbicide exposure. These fields have contrasting characteristics in clay, CEC and soil organic matter, which make them appropriate for evaluating, on a field experiment, the relationship between soil parameters and herbicide dissipation, as well as the role of native bacterial communities in response to glyphosate application.

2. Materials and methods

2.1. Experimental design

The field experiments took place between November 2013 and February 2014 at INTA Balcarce Agronomic Experimental Station, Province of Buenos Aires. Soils studied are classified as Luvic Phaeozem (IUSS Working Group WRB, 2007). Three locations were selected: a soil without previous exposure to herbicides (P0: S37°45′47.9″ WO58°18′28.4″) belonging to a football stadium surrounded by a row of trees, and two agricultural soils, with 5 and almost 10 years of GP application history (A5: S37°45′49.7″ WO58°17′33.1″ and A10: S37°45′17.4″ WO58°17′51.8″, respectively). Agricultural soils were managed under conventional tillage with maize-wheat/soybean rotation. The history of GP use and spraying dosage during the last year and the 5 years before this experiment are shown in Table 1 and Supplementary Table S1. A randomized block design with six plots of 10 m^2 was made at each location. Commercial glyphosate (DuPont® Premium HL 48% w/v) was sprayed onto three of the six plots, whereas the other three plots remained as controls (no herbicide added). Considering a depth of 5 cm and a soil bulk density of 1.2 t m^{-3}, the sprayed soils received uniform manual application of approximately 3 mg of active ingredient kg^{-1} of soil.

Soil samples were collected the day before application and on days 1, 3, 5, 8, 16, 24, 32, 44 and 72 after herbicide application. Each sample was a composite of ten sub-samples per plot, collected from the top 0 to 5 cm, using a soil core device, which was cleaned by flashover to avoid cross-contamination between samples. Samples were homogenized, dried at 30 °C and sieved through 2-mm mesh, and stored at −20 °C until DNA extraction.

Soil texture was determined using the pipette method (Gee and Bauder, 1986). Cation-exchange capacity (Chapman, 1965), pH (1:2.5 soil: water ratio), total organic carbon (Nelson and Sommers, 1982) and available phosphorus (Bray and Kurtz, 1945) were determined by standard procedures.

Temperature and rain data during the experiment were collected in the Meteorological Station of INTA Balcarce (http://anterior.inta.gov.ar/balcarce/info/meteorologia/meteoro2.htm) and the information is summarized in Fig. S1.

Table 1
Properties of the studied soils and soil properties dataset analyzed.

Soil properties	A10_Balcarce	A5_Balcarce	P0_Balcarce
Coordinates	S37°45′17.4″ WO58°17′51.8″	S37°45′49.7″ WO58°17′33.1″	S37°45′47.9″ WO58°18′28.4″
Spraying dosage in the last year (mg ia kg soil^{-1})	7.30	8.05	–
SOM (%)	5.0 ± 0.45 b	4.1 ± 0.51 b	10.6 ± 0.92 a
pH	5.70 ± 0.10	5.80 ± 0.06	6.10 ± 0.15
CEC (cmol kg^{-1})	25.00 ± 0.75 b	27.50 ± 5.44 b	40.40 ± 2.59 a
Sand (%)	46.80 ± 3.15 a	40.40 ± 2.07 b	39.50 ± 2.28 b
Silt (%)	29.20 ± 3.37	33.40 ± 1.80	30.50 ± 2.68
Clay (%)	24.60 ± 1.83 b	26.10 ± 1.45 b	29.20 ± 1.47 a
P-Bray (mg kg^{-1})	58.46 ± 9.89 a	24.01 ± 2.28 c	28.27 ± 1.64 b
k constant (days^{-1})	0.058 ± 0.011 a	0.071 ± 0.009 a	0.024 ± 0.006 b
*t*½ (days)	8.56	10.35	16.12
Pedotransfer functions (Dollinger et al., 2015)			
Kd (L kg^{-1})	172.59 ± 7.88	188.63 ± 5.74	277.45 ± 27.35
Kf (L kg^{-1})	199.39 ± 8.88	225.57 ± 6.26	292.47 ± 32.46

OC organic carbon, CEC cation exchange capacity. Different letters indicate significant differences between sites ($p < 0.05$).

2.2. Extraction and quantification of glyphosate and AMPA

Five grams of soil were spiked with 50 μL of 10 mg L^{-1} isotope-labeled glyphosate (1,2-^{13}C ^{15}N, Sigma-Aldrich) and incubated 30 min at room temperature. Particulate material was extracted using 25 mL of a solution containing (100 mM $Na_2B_4O_7$ $10H_2O$; 100 mM K_2HPO_4; pH = 9, reagent-grade), and the extract was analyzed according to the methodology proposed by Peruzzo et al. (2008). Briefly, samples were sonicated and then centrifuged to separate the suspended material. Supernatants were derivatized with 9-fluorenylmethylchloroformate (FMOC-CL) in acetonitrile (HPLC-grade) and incubated overnight in the dark at room temperature. Subsequently, 5 mL of dichloromethane (CH_2Cl_2, HPLC-grade) were added, and the mixture was vortexed and centrifuged. The aqueous phase was collected and filtered through a 0.22 μm nylon filter for chromatographic analysis.

Standards of GP (Pestanal®, Sigma) and AMPA (Sigma-Aldrich) were prepared in extract solution, in concentrations ranging from 0.5 μg L^{-1} to 200 μg L^{-1} for each analyte. In order to evaluate GP recovery from samples, 4 μL of 10 mg L^{-1} isotope-labeled GP were added to this series of dilutions. The standards were derivatized and processed by the same methodology described above.

Samples and standards were injected into a Waters® ACQUITY® UPLC MS/MS system (Waters), calibrated for positive detection, using a column ACQUITY® UPLC BEH C18 column (1.7 μm, 50 × 2.2 mm) (Waters), and eluted using a methanol/water gradient containing 5 mM ammonium acetate. Methanol and water were HPLC-grade. Calibration curves were adjusted using a weighted least square regression 1/x, considering a satisfactory linearity when $R^2 \geq 0.99$. The recovery of GP in soil was between 70 and 100%.

2.3. Dissipation analysis and constants of glyphosate sorption

GP dissipation was described using a first order kinetic model: $GP_t = GP_o * e^{-kt}$, where GP_t (mg kg^{-1}) is the concentration at time *t* (in days), GP_o is the average initial concentration (n = 3), and *k* is the first-order dissipation constant (days^{-1}). Additionally, the half-life (t½) was calculated as: t½ = ln 2/*k*.

First order kinetics was fitted using nonlinear least square included in the R package *stats* (R Core Team, 2017). Statistical differences among means of GP first order dissipation constants (*k*) were analyzed with the Kruskal-Wallis nonparametric rank test ($p < 0.05$).

We have also used a closed form solution for the measured GP decay as a function of time, to obtain the maximum velocity, V_{max}, and the Michaelis–Menten rate constant, K_M (Schnell and Mendoza, 1997):

$$[GP](t) = K_M W\left(\frac{[GP_0]}{K_M}\ exp\left(\frac{-V_{max}t + [GP_0]}{K_M}\right)\right)$$

where *W* is the omega function, which satisfies the transcendental equation $W(x)\exp.(W(x)) = x$ (Schnell and Mendoza, 1997). Goodness of fit was assessed using the normalized root mean square error (NRMSE).

To relate the properties of soils to their ability to adsorb GP, Kd and Freundlich constant (Kf) were calculated empirically, as proposed by Dollinger et al. (2015). The pedotransfer functions are: Kd (L kg^{-1}) = 7.20 * CEC − 1.31 * Clay + 24.82 and Kf (L kg^{-1} n^{-1}) = 50.904 + 9.246 * CEC − 1.985 * Clay − 11.811 OC (CEC in cmol kg^{-1}, with clay and OC expressed as %: %OC = SOM%/1.724). The Kd and Kf were compare with *k* (dissipation constant) and correlation analyses were performed using Spearman's Rho coefficient.

Additionally, in order to confirm the relationship between properties of soils and their ability to adsorb GP and AMPA, we related our results with a dataset from 16 edaphically characterized soils of the southeast of the Buenos Aires Province, for which, environmental concentrations of both GP and AMPA were reported by Aparicio et al. (2013). The Kd and Kf values were calculated empirically, as described above, and correlation analyses were performed with the sum of molecules (moles of GP and AMPA kg^{-1} of soil) reported for each soil. This analysis does not consider the initial concentration and history of use. The sum of molecules of GP and AMPA for soils P0, A5 and A10 at day 42th was included in the analysis.

2.4. DNA extraction and sequencing

DNA was extracted from 0.25 g of soil using PowerSoil® DNA Extraction kit (MoBio, USA), following the manufacturer's instructions. A fragment of the 16S rRNA gene (~640 bp) spanning V3–V5 hipervariable region was PCR amplified, using primers: 357-forward 5′CACGACGTT GTAAAACGACCCTACGGGAGGCAGCAG 3′ and 926-Reverse 5′CAGGAA ACAGCTATGACCCCGTCAATTCMTTTRAGT 3′. PCR was performed in a final volume of 30 μL, containing 1 U Phusion® High Fidelity polymerase and 1× Buffer (NEB, USA), 0.2 mM dNTPs and 0.03 μM of each primer. Thermocycler program consisted of an initial step at 95 °C for 2 min, followed by 30 cycles of 95 °C for 20 s, annealing at 51 °C for 1 min, and extension at 72 °C for 1 min. The final extension was at 72 °C for 5 min. Three PCR reactions per sample was performed and pooled before sequencing. Amplicons were purified using Nucleospin® DNA, RNA and protein purification" (Macherey-Nagel, Düren, Germany), and used as template for a second PCR. These reactions used primers with ligation adaptors for 454 sequencing and barcodes or MID (multiplex identifiers) to the 5′ends. PCR reactions consisted in an initial step at 95 °C for 5 min, and 20 cycles at 95 °C for 30 s and 72 °C for 1 min, finished with a final extension at 72 °C for 5 min. Amplicons were purified and quantified to adjust its concentrations. DNA was sequenced using a GS FLX Titanium 454 Sequencer (Roche) at INDEAR (Rosario, Argentina).

2.5. Sequence data processing

Data were processed using QIIME 1.9.0, following pipelines for 454 data (Caporaso et al., 2010). Sequences were demultiplexed using FASTA-formatted sequences, their corresponding quality scores data files and the validated mapping file. This step assigned to each read one barcode/sample, quality filtering based on minimum length of reads of 220 bp, 2 errors in barcode as maximum and 2 mismatches to the primer, removing of low quality or ambiguous reads and the accomplishing of reverse primer removal. Additionally, the identification of chimeric sequences was performed by ChimeraSlayer algorithm. The workflow for *pickotus* was carried out picking Operational

Taxonomic Units (OTUs) based on sequence similarity within the reads (method *uclust* and sequence similarity threshold 0.97), selecting representative sequences for each OTU by abundance and their alignment (by PyNAST) and their taxonomy using Bacterial SILVA database as template (available at: https://www.arb-silva.de/no_cache/download/archive/release_123). The filtered data was used to obtain the OTU table, indicating the number of times an OTU appeared in each sample. The sequences were normalized via random sub-sampling at 3173 reads per sample for downstream analyses. OTUs with an occurrence lower than 25% of the total of samples were removed. These rarified OTU tables were used to calculate alpha diversity metrics including: Shannon index (Ludwig and Reynolds, 1988), observed OTUs, Chao 1 (Chao, 1984) and dominance. Beta diversity metrics were also estimated through weighted and unweighted UniFrac (Hamady et al., 2010). Venn diagram was constructed with package gplots in R 2.10.1(R Core Team, 2017). All sequences were submitted to the NCBI Sequence Read Archive (SRA) and are available under the accession number PRJNA393173.

2.6. Statistical analysis of bacterial community structure

To test the existence of differences in the interdependence of microbial species associated to GP application in the three soils over time, data was analyzed using the Nonparametric Microbial Interdependence Test using phyloseq data structure (NMIT-Phyloseq) (Zhang et al., 2017). Taxa with relative abundances higher than 0.3% and present in >20% of samples were included in the pair-wise correlation analysis (Kendall rank correlation) within each experimental plot, separated by treatment: GP-applied and control. Then, the statistical differences between correlated structures associated with the GP-treatment, were determined by permutation MANOVA (Zhang et al., 2017). Additionally, correlations >0.8 (positive and negative), in at least two of the three plots per treatment, were graphed to visualized the group microbial interdependence relationships.

The statistically significant differences of taxa abundances were determined between treatments (GP- applied and control) for each site using permutational multivariate analysis of variance using Adonis, 999 permutations (Vegan package). Alpha diversity metrics were compared using compare_alpha_diversity.py script in the QIIME between control and GP-treated soils for each site. Additionally, the variations of alpha diversity metrics were analyzed over time through mixed linear model using the PROC MIXED procedure (SAS Institute version 9.0, 2002). The differences in the relative abundance over time of different taxa by treatment also were analyzed using this procedure. Treatment and site were considered as fixed and random effects, respectively, and time as a repeated measure. Weighted UniFrac distances between treatments for each site were compared using ANOSIM method, with 999 permutations (Vegan package). Principal coordinate analyses (PCoA) was performance, using the beta_diversity_through_plots.py script in the QIIME pipeline. Mantel test was used to identify the soil properties that significant correlated with the bacterial community compositions.

3. Results

3.1. Dissipation of glyphosate in the field

Fig. 1A shows that GP was degraded under field conditions in the three soils, regardless the previous exposure. Interestingly, no lag phase was observed in the soil that had not previously received GP applications. Heavy rains had not occurred after spraying of the herbicide, making negligible losses due to leaching or runoff. GP dissipation followed in all cases first order kinetics ($p < 0.05$); yet the rates of dissipation, measuring by constant *k*, were significantly different between sites ($p \leq 0.001$; Fig. 1A). When attempting to fit the Michaelis-Menten model, the GP decay curves approached a first-order relationship, with Vmax/Km almost equaling the first-order rate constant *k*, and with similar MRSE values (Table S2). For the P0 soil, the rate of herbicide degradation (k) was significant lower in comparison with the agricultural soils (A5 and A10). Consequently the t½ of P0 almost double (16.1 days) that of A10 (8.6 days). Values for these variables in the two agricultural soils were not significantly different, irrespective of the application history, i.e. number of years exposed to herbicide and application doses (Tables 1, S1). Levels of the main metabolite AMPA increased over time, and remained high throughout the experiment in all soils, when compared to GP (Fig. 1B). The small concentration of both GP and AMPA detected on day 72 in one of the 3 control plots, suggest possible dispersion of GP-containing soil particles from close areas of agricultural production.

The use of empirical Kd and Kf functions as proxies for the degree of GP sorption to soil suggested that the molecule adsorbs more strongly to P0 soil, which had the highest values of organic matter, clay and CEC contents in comparison to the agricultural soils A10 and A5 (Table 1). Additionally, the values of Kd and Kf constants correlated negatively with *k* values (Kd: $r = -0.73$, $p = 0.025$ and Kf: $r = -0.80$, $p = 0.009$), indicating that soils with a lower capacity to sorb GP show faster rates of GP dissipation.

3.2. Glyphosate depletion and soil characteristics

To test the relation between soil properties and the extent of total GP depletion, we sought to confirm the results obtained in this study with data from agricultural soils from the Pampa region reported previously (Table S3). The amount of residual GP and AMPA correlated significantly with both Kd (Spearman $R = 0.4702$; $p = 0.04221$) and Kf ($R = 0.4789$, $p = 0.03802$), regardless the differences in the amount of applied herbicide, as well as the time elapsed after the last application (Aparicio et al., 2013).

3.3. Analysis of microbial soil communities

The composition of bacterial communities was determined using high-throughput sequencing of 16S rRNA gene amplicons. A total of 399,466 high-quality sequences were obtained after processing. Following the exclusion of singletons, an average of 114,004 sequences per site was obtained.

The analysis of alpha diversity metrics over time revealed transient differences. For all soils, the observed-OTUs, Chao1 richness and Shannon's diversity estimates, increased transiently after GP application at day 16, recovering the initial conditions at the end of experiment. A slightly increase in dominance was also observed (Table S4). However, the observed changes could not be attributed to bacterial communities' exposure to herbicide, because similar changes were also observed for control plots (Table S4).

As expected from the fact that soil samples were taken from closely-spaced locations, all samples shared a large number of OTUs (Fig. 2). On the other hand, OTUs unique to P0 soil or to agricultural soils A5 and A10 represented a heterogeneous group of taxa with low representation within the communities.

Principal coordinate analysis (PCoA) indicates that bacterial communities of agricultural soils were more similar between them, and more distant from those of the non-agricultural soil (Fig. 3). The first principal coordinate of the PCoA analysis explained almost 19% of the total variance. The bacterial community composition was significantly correlated with soil organic matter content, CEC, pH and clay content (Table 2).

The most abundant phyla were *Proteobacteria*, *Actinobacteria*, *Acidobacteria* and *Bacteroidetes* (Fig. 4). The microbial interdependences profiles determined by the NMIT test were not statistically different between control and GP-applied soils for all sites (P0: $p = 0.4$; A5: $p = 0.7$ and A10: $p = 0.3$). However, changes in relative abundance of individual phyla exhibited small, but consistent differences, especially in P0 soil.

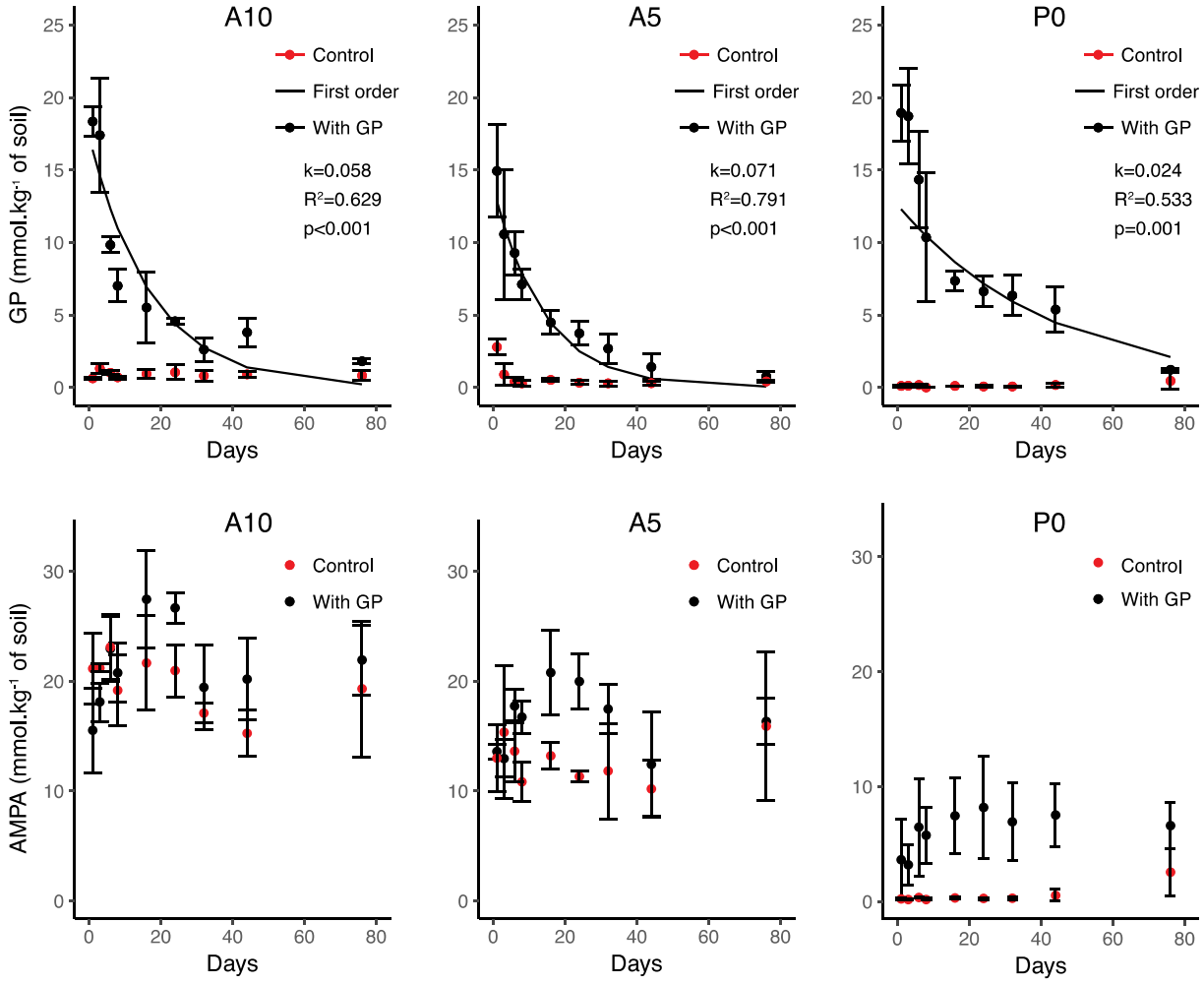

Fig. 1. Dissipation of glyphosate in the field conditions (A) and AMPA formation (B) at 3 soils. Two treatments were plotted (soils with GP application and control without application: P0 (with no history of GP application), A5 and A10 (agricultural soils with 5 and 10 years of glyphosate history, respectively). Error bars represent standard deviation (n = 3).

The decrease observed in the abundance of *Actinobacteria* after GP application ($p = 0.0428$), was accompanied by a concomitant increase in the abundance of *Bacteroidetes* ($p = 0.0261$), which was noticeable from day 16 onwards, and was maintained over time. Within this phylum, families *Flammeovirgaceae* and *Saprospiraceae* (Sphingobacteriales-order) increased following GP treatment from 16% to 20.3%, and 4% to 8.5%, respectively. No significant changes between treatments were observed for other major taxa.

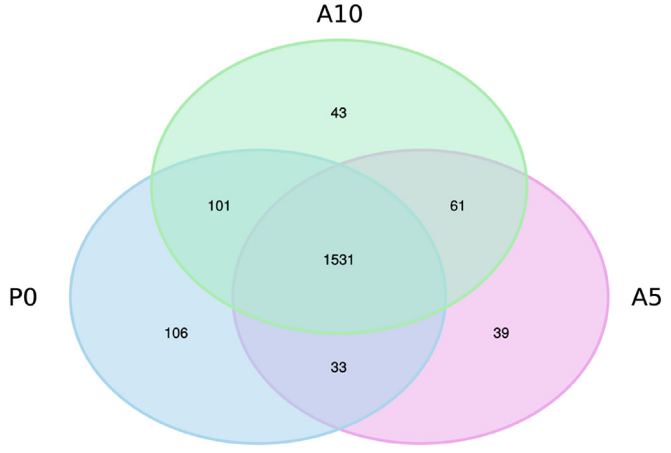

Fig. 2. Venn diagram representation of OTUs that were found at the three soils: P0, A5 and A10.The numbers of overlapping tag sequences are indicated in the graph.

Fig. 5 shows that control groups had higher interdependent structure than GP-treated soils, which indicates that GP treatment disturbs the bacterial association network. Changes were more striking in the P0 site, where the complex network of interactions visualized at the control soil decreased strongly in the presence of GP. Similar loss of interactions was observed in A5, whereas in A10 the initially weak association is not greatly affected by the use of the herbicide.

4. Discussion

4.1. Dissipation of glyphosate in the field

In this study, we show that glyphosate was degraded in soil without major accompanying changes in bacterial community structure. This might not come as a complete surprise, given that many soil microorganisms are able to use GP as a source of nitrogen, phosphorous and carbon (Ermakova et al., 2010; Liu et al., 1991; Sviridov et al., 2015; Zboinska et al., 1992), and that tolerance to GP (Allegrini et al., 2015), as well as the capability of converting GP to AMPA, might not necessitate from previous exposure of bacteria to the herbicide (Sviridov et al., 2015). However, it should be noted that despite the fact that our control soil has never been directly exposed to herbicides, low amounts of GP may have reached the site from the air due to its close proximity to an agricultural area.

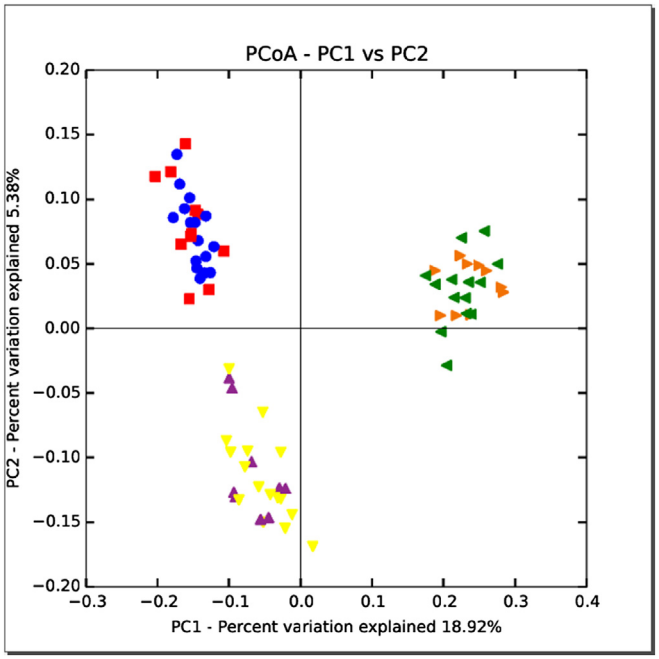

Fig. 3. PCoA plot show the bacterial communities clustered by localities and treatments, based on the weighted UniFrac distance matrix generated. The localities are the Balcarce soils P0, A5 and A10 and the treatment are GP-applicated (A) and control (C) soils. Each point corresponds to a sample: red squares: A5_A; blue circles: A5_C; purple triangles: A10_A; yellow triangles: A10_C; orange triangles: P0_A; green triangles: P0_C. The percentages of variation explained by the plotted principal coordinates are indicated on the axes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

It has been suggested that physico-chemical properties may prove to be more important on degradation rates than the composition of soil bacterial communities (Baker et al., 2010). Our observation that the Michaelis-Menten model became essentially first order implies that Km ≫ GP, i.e. a low overall affinity to GP, which points to the complexity of the biodegradation under field conditions. Parameters such as organic matter and clay had a major influence on GP mineralization potential and sorption in the soil (Vinther et al., 2008). Soils with high sorption and low desorption of GP had shown less biological degradation, suggesting limited GP bioavailability (Sørensen et al., 2006; Okada et al., 2017). The high GP affinity in soils of the Pampa region has been associated to high content of clay, CEC and Al^{3+} Fe and low pH and phosphorus content (Gómez Ortiz et al., 2017; Okada et al., 2016). Since previous soil exposure to GP had a small effect on biodegradation kinetics and on microbial dynamics, we hypothesized that bioavailability was a major factor affecting the extent of total GP depletion. Correspondingly, we show that empirical Kd and Kf pedotransfer functions, which are a priori estimators of the degree of GP sorption to soil, were associated with the amount of GP and AMPA found in Pampa soils, regardless the initial doses of herbicide sprayed or the time elapsed after the last application.

Table 2
Correlations (r) and significances (P) between the composition of bacterial communities and physicochemical soil properties, determined by Mantel test.

Soil properties	r	P
SOM %	0.8486	0.001
pH	0.4155	0.001
CEC.	0.6859	0.001
P-Bray	0.0914	0.014
Sand	0.1137	0.008
Silt	0.0341	0.244
Clay	0.3979	0.001

4.2. Effects on the bacterial community structure

The diversity analysis of soil bacterial communities at the three sites with different history of GP application revealed a separation according to type of land use. Bacterial populations present in agricultural soils were more similar to each other and less similar to populations present in the non-agricultural soil. Differences in bacterial community structure were strongly correlated with soil SOM, CEC, pH and clay content. The three soils belong to the same series, although the edaphic characteristics had been modified as a result of the type of use. Agricultural management practices, such as fertilization, crop rotation and pesticide applications, influence the structural and functional diversity of microbial communities (Berg and Smalla, 2009; Girvan et al., 2003; Lauber et al., 2008). Besides, the type of land use provokes long-lasting effects on physicochemical characteristics of the soils, especially a decrease in the organic matter content and pH changes (Galantini and Rosell, 2006; Sainz Rozas et al., 2011), which are important in shaping microbial community composition (Zhao et al., 2016).

A recent study found that bacterial communities were more influenced by location, cropping history and year of sample than by GP treatment (Schlatter et al., 2017). In general, changes in the relative abundance of specific taxa in this study could not be unequivocally associated to GP dissipation. However, changes observed in *Bacteroidetes* matched the late field dissipation of the herbicide, suggesting a differential response of this group to herbicide treatment, whether related to the degradation of the molecule or its effect on the soil nutrients uptake (Newman et al., 2016b). The increase in the relative abundance of *Bacteroidetes*, in particular within the families *Flammeovirgaceae* and *Saprospiracea*, was observed following GP treatment in the soil that had no previous exposure to GP. Members of *Bacteroidetes* are generally associated with the degradation of complex organic materials, and have been reported as copiotrophs that thrive under conditions of high substrate availability (Fierer et al., 2012). Since commercial GP-based product containing 48% (w/v) of GP were applied, changes in bacterial community structure due to the effect of other ingredients cannot be ruled out. The increase of copiotrophs had been observed when organic compounds are added to soil as fertilization strategy for improving soil organic carbon (Fernandez et al., 2016; Li et al., 2017). Substantial variations in *Proteobacteria* and *Bacteroidetes* relative abundances were similarly reported in wheat rhizosphere upon repeated GP application on soils with and without history of GP use, an effect that was also associated with an increase in biomass of dying roots in soils (Schlatter et al., 2017).

Using a recently described method to detect microbial temporal correlations between bacterial taxa (Zhang et al., 2017), we found that addition of GP disturbed bacterial interdependence, reducing the number of interactions at the family level. The partial disruption of the bacterial association network prompted by the application of GP appears to be especially noticeable in P0 soil. Remarkably, bacterial associations in soils with several years of exposure to GP were already low, and thus less affected by a new addition of the herbicide. Microbial communities' interactions through competitive and cooperative relations contribute with the maintenance of ecosystem function (Hibbing et al., 2010). Thus, what are the functional implications of the observed disturbances on microbial interactions caused by short-term and long-term application of glyphosate have yet to be established.

5. Conclusions

In summary, the results of this study suggest that GP degradation are performed by a variety of taxa and that the bioavailability of the herbicide to the bacterial communities involved in this process is influenced by soil properties. The correlation between pedotransfer functions and constant

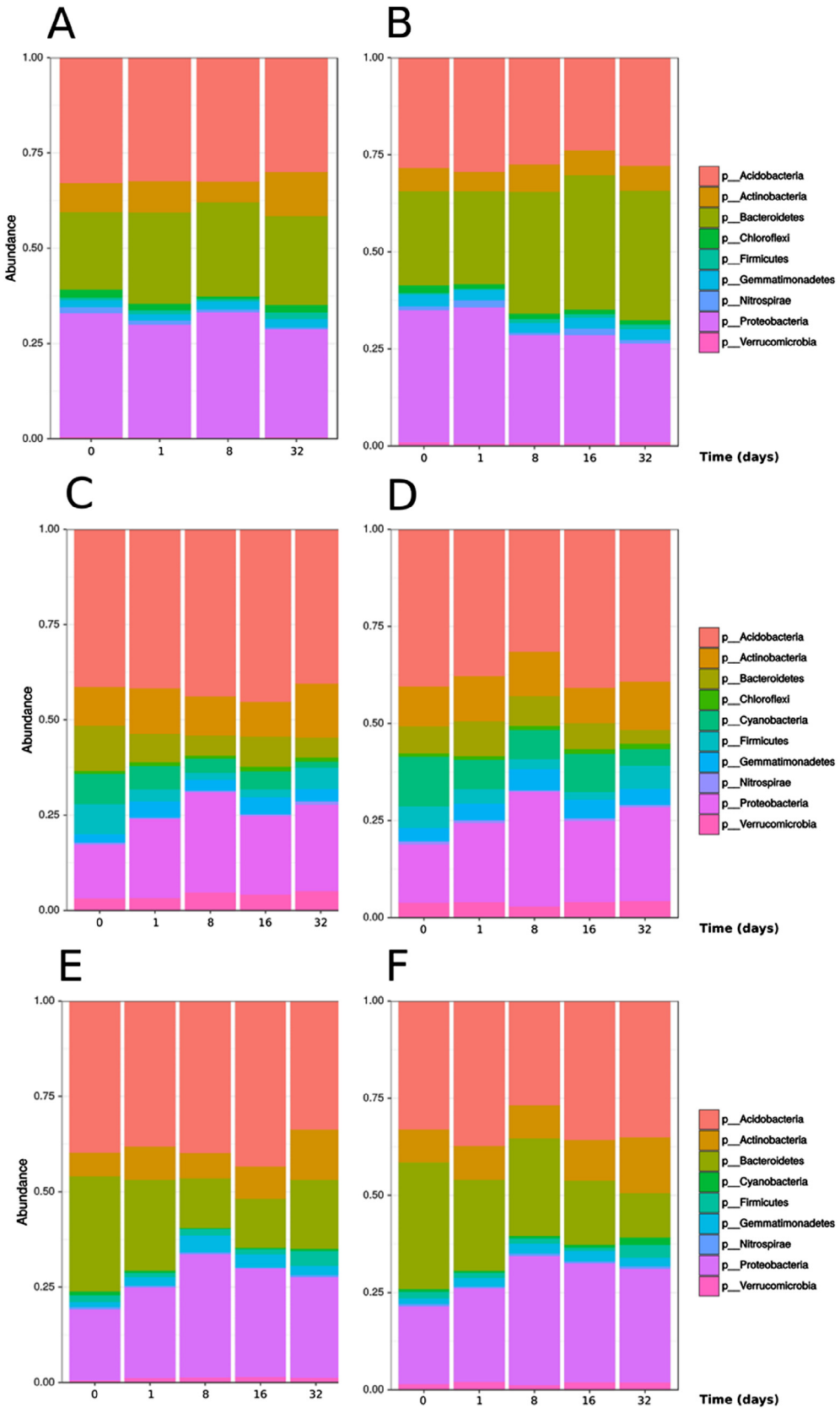

Fig. 4. Relative abundance of major phyla over time, present in soils control (left: A, C and E) and with GP-application (right: B, D and F) at the tree studied sites. Soil PO (A and B), soil A5 (fig. C and D) and soil A10 (fig. E and F). The x axis shows the sample time and the y axis shows the mean of abundances over time per treatment ($n = 3$).

of dissipation point to the role of physico-chemical properties, such as cation exchange capacity, clay and organic matter content as predictors of the rate of degradation and possible persistence of glyphosate in soil. Further research is required to identify a precise mechanism by which addition of glyphosate disturbs bacterial association networks, and to establish whether this impairs soil ecosystem function.

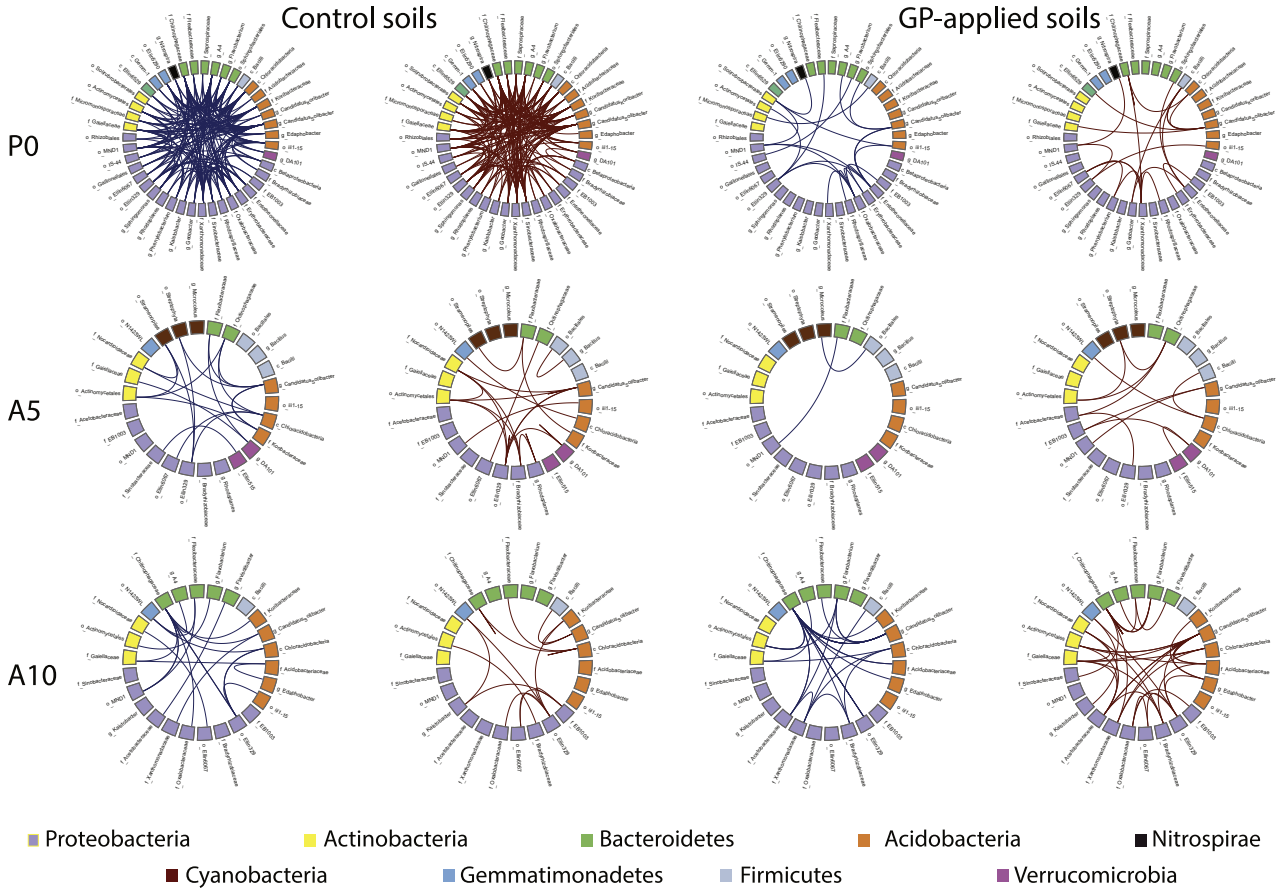

Fig. 5. Microbial interdependence networks for major taxa. Graphics are separated by treatments: control soils (left panels) and GP-treated soils (right panels). Nodes are colored according to their phylum-level taxonomic identification. Edges indicate that the correlation between the connected taxa is >0.8 in at least 2 of the 3 plots assayed for each treatment. Red lines represent positive correlations and blue lines, negative correlations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Acknowledgments

We thank Luis Alonso and Maximiliano Dosanto for their support in the maintenance of the assays and sample collection and processing; Hernán Echeverria and members of the group "Soil-Culture relationship" (EEA Balcarce) for their support in soil analysis. This work was financed by INTA through the Program of Postgraduation and Training (Res. 743/12) and P-N Suelo 1134044.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.393.

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