The Responses of Soil Microbial Community to Glyphosate Stress Studied at Biochemical, Catabolic, and Genetic Levels

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Abstract

Glyphosate is a non-selective and post-emergence organophosphate herbicide that is widely used in agriculture. We report here the *in situ* and *ex situ* effects of glyphosate on the soil microbial communities using culture-independent patterns of microbial biomass, phospholipid fatty acids (PLFAs), 16S rDNA denaturing gradient gel electrophoresis (DGGE), real-time quantitative PCR, and culture-dependent methods of plate enumeration and community level catabolic profiles (CLCPs).

The results showed microbial biomass reduced by 45%, as well the numbers of cultivable bacteria and fungi decreased by 84% and 63%, respectively. However, phosphobacteria were significantly enriched by 39 folds.

PLFAs analysis showed fungal and part of gram-positive (G+) bacterial biomass were restrained remarkably by 29% and 21%, respectively, followed by a significant increase (38%) in the ratio of bacterial to fungal PLFAs in glyphosate input soils.

However, the CLCPs showed high dosage input of glyphosate had a significant boost on the catabolic activity of gram-negative (G-) bacterial community.

Furthermore, DGGE analysis indicated that the genetic diversity of bacterial community decreased in the soil contaminated by high dosage of glyphosate. Among 18 sequenced DGGE bands, 13 bands were related to G- bacteria.

Real-time PCR result indicated that copies of the glyphosate tolerance gene, 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*), increased significantly in high glyphosate input soils.

In conclusion, our results demonstrated comprehensively that fungi and G+ bacteria were inhibited while G- bacteria played an important role in degrading glyphosate under stress of high glyphosate dosage. Soil fungi have been harmed even in the recommended concentration of glyphosate.

Soil description and experimental design

The loam soil was collected from a site without agrochemical use and then loaded into pots. Three treatments were defined:

GLY0: no-glyphosate control;

GLY1: recommended dosage glyphosate input with 50 mg active ingredient kg^{-1}soil;

GLY10: high dosage glyphosate input with 500 mg active ingredient kg^{-1}soil;

CONTROL: soil were treated with distilled water.

Analysis indices

(1) Soil microbial biomass carbon, and soil organic carbon analyses

(2) Phospholipid fatty acids (PLFAs) analysis

(3) CLCPs of soil G- & G+ bacteria

(4) Quantification of cultivable bacteria, phosphobacteria and fungi

(5) 16S rDNA-DGGE analysis

(6) Real-time PCR analysis of *EPSPS* gene

Table 1 C, N and P profiles in the soils [a]

Treatment	C_{mic} (μg.g^{-1})	C_{org} (mg.g^{-1})	N_{tol} (mg.g^{-1})	P_{tol} (mg.g^{-1})	C_{mic} / C_{org}	C_{org} / N_{tol}	C_{org} / P_{tol}
GLY0	150.0 a	15.9 a	1.7 a	0.49 b	0.0094 a	9.24 a	3.25 a
GLY1	163.2 a	14.9 a	1.4 a	0.49 b	0.011 a	10.00 a	2.84 a
GLY10	82.1 b	16.4 a	1.4 a	1.07 a	0.005 b	10.60 a	1.36 b

[a] Data are expressed as "mean (standard deviation) significant difference label". Means between any two soils in a column followed by a same lowercase letter indicate no significant difference using ANOVA LSD test at $p<0.05$, n=4. Soil weight is based on oven-dried soil.

Table 2 The concentration of PLFAs associated with different components of microbial communities in the soils [a]

Soil sample	Total (mmol.g^{-1}soil)	Bacterial (mmol.g^{-1}soil)	Fungal (mmol.g^{-1}soil)	Fungal:Bacterial
GLY0	16.08 a	8.67 a	0.59 a	0.069 a
GLY1	16.43 a	9.90 a	0.42 b	0.043 b
GLY10	16.39 a	9.64 a	0.41 b	0.043 b

Table 4 CFU enumeration of cultivable microbes in the soils

Soil sample	Bacteria ($\times 10^5 \cdot g^{-1}$)	Fungi ($\times 10^5 \cdot g^{-1}$)	Phosphobacteria ($\times 10^5 \cdot g^{-1}$)
GLY0	186.33 a	99.67 a	0.933 c
GLY1	173.67 a	23.00 b	21.90 b
GLY10	29.00 b	37.33 b	37.17 a

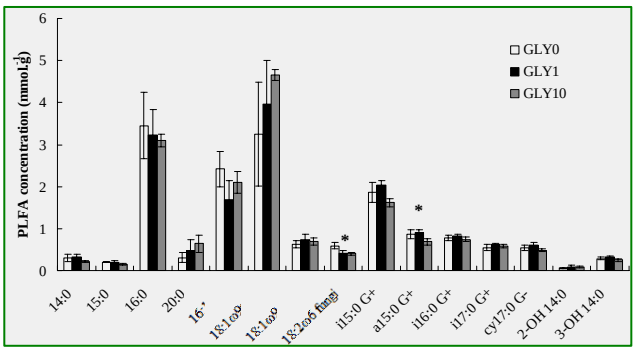

Fig.1 Abundance of phospholipid fatty acids (PLFAs) in the three soil samples, G-: gram negative bacteria, G+: gram positive bacteria

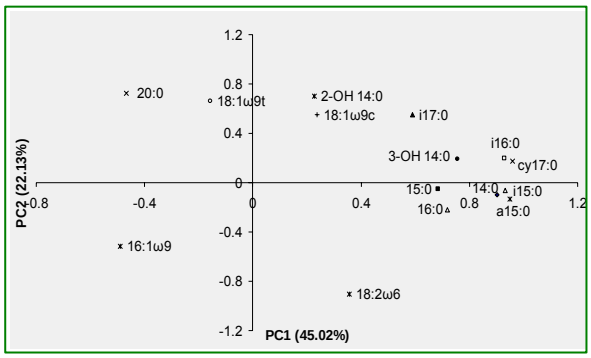

Fig.2 Loadings of individual PLFAs for the first two principal components

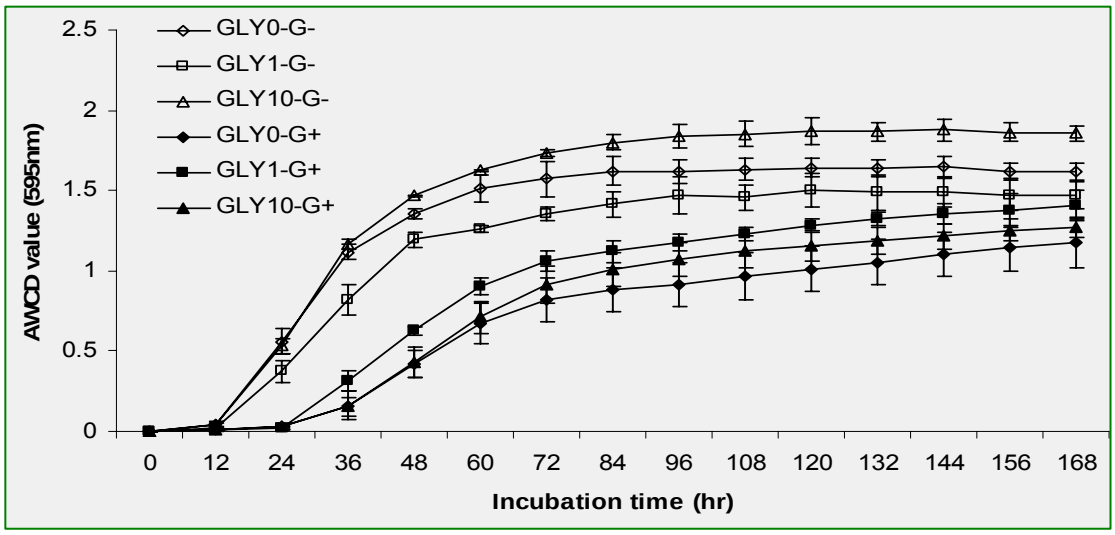

Fig.3 The changes of AWCD value for the three soil microbial communities during whole incubation. Bars indicate standard deviation (SD), n=3. G-: gram negative bacteria, G+: gram positive bacteria

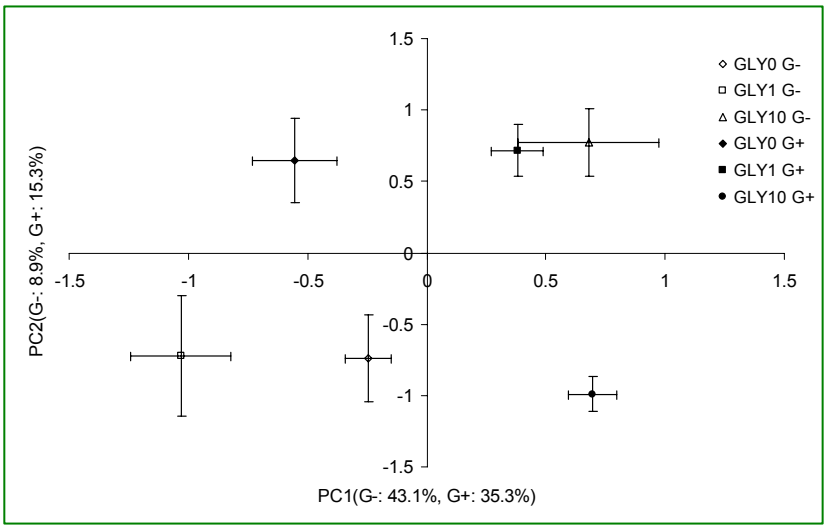

Fig.4 Principal Component Analysis (PCA) of community level catabolic profiles (CLCPs) based on Biolog plates for the three soil samples, Bars indicate standard deviation (SD), n=3. G-: gram negative bacteria, G+: gram positive bacteria.

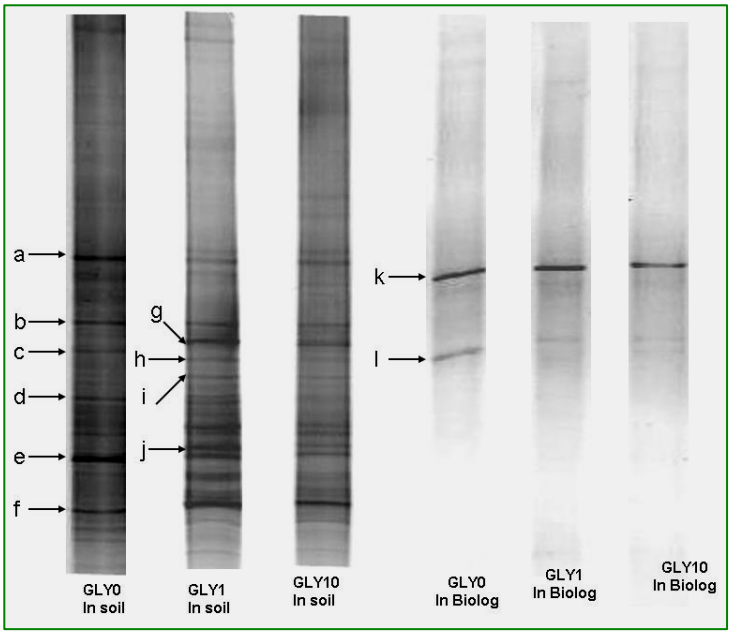

Fig.5 DGGE profiles of amplified 16S rDNA fragments from soil and Biolog samples treated with glyphosate. The letters from a to l indicate the positions of the bands.

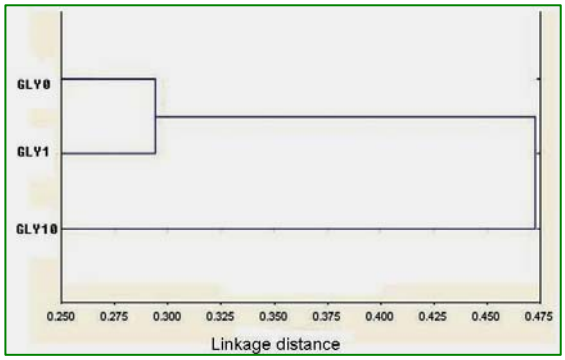

Fig.6 Dendrogram based on presence /absence of DGGE bands.

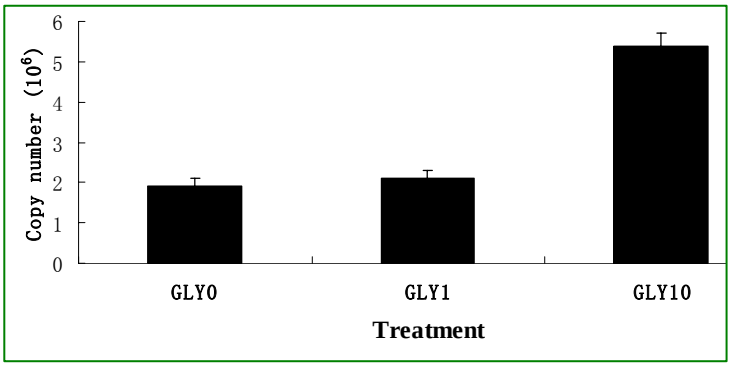

Fig.7 Gene copies in different soil samples determined by real-time quantitative PCR, EPSPS: 5-enolpyruvyl shikimate-3-phosphate synthase gene.

Table 4 Sequence analyses of bands excised from DGGE gels derived from bacterial 16S rDNAs extracted from the soils and Biolog microplates

Genbank accession No.	Bacterium with related bacterial sequence	Treatment	Related to G-/G+ bacteria
EF452410	*Bacillus clausii strain*	GLY0 (soil)	G- bacterium
EF452411	*Flavobacterium sp.*	GLY0 (soil)	G- bacterium
EF452412	*Uncultured deltaproteobacterium*	GLY0 (soil)	G- bacterium
EF452413	*Uncultured Flavobacteria bacterium*	GLY0 (soil)	G- bacterium
EF452414	*Uncultured Acidobacteria bacterium*	GLY0 (soil)	G- bacterium
EF452415	Uncultured bacterium	GLY0 (soil)	Unknown
EF452416	Uncultured bacterium	GLY0 (soil)	Unknown
EF452417	Uncultured bacterium	GLY0 (soil)	Unknown
EU255829	*Burkholderia cenocepacia*	GLY0 (Biolog)	G- bacterium
EU255817	*Pseudomonas sp.*	GLY0 (Biolog)	G- bacterium
EF452371	Uncultured soil bacterium	GLY1 (soil)	G- bacterium
EF452372	Uncultured bacterium	GLY1 (soil)	G- bacterium
EF452373	*Ralstonia sp.*	GLY1 (soil)	G- bacterium
EU255854	*Gamma proteobacterium*	GLY1 (Biolog)	G- bacterium
EU255852	*Burkholderia sp.*	GLY1 (Biolog)	G- bacterium
EF452374	Uncultured bacterium	GLY10 (soil)	Unknown
EF452375	*Uncultured hydrocarbon seep bacterium*	GLY10 (soil)	Unknown
EU255862	*Devosia sp.*	GLY10(Biolog)	G- bacterium

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Thanks for your attention!