

Synergistic effects of Microplastic and Glyphosate on Soil Microbial Activities in Chinese Loess Soil



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Abstract

In order to improve yields, pesticides and plastic mulching are widely used in the Loess Plateau, China. Effects of microplastic and glyphosate on the soil microbial activity have been studied. The soil was collected in the farmland of Ansai city, Shaanxi province. Nine treatments (CK M1, M2, G1, G2, M1G1, M1G2, M2G1 and M2G2) were prepared for this research. All treatments were incubated in a climatic chamber and collected at the 0, 1st, 3rd, 7th, 14th and 30th incubation day. According to the results, the half-life of glyphosate of treatments without microplastics, M1 and M2 are 37.06d, 36.48d and 34.83d respectively. Soil respiration of M2 was 2.33 times stronger than CK samples in the 30th incubation day, and glyphosate didn't stimulate the soil respiration individually. Soil respiration was significantly stimulated by the synergetic effects of glyphosate and microplastics. Soil respiration of M2G2 was 3.68 times higher than the control group. The SIR was accelerated by microplastic and glyphosate addition separately, and no significant difference was found with both microplastic and glyphosate applications. No remarkable results were found for urease activity with effects of microplastics and glyphosate, while in treatment G1, the results showed a significant inhibiting effect on the β – glucosidase activity in the 7th incubation day. Soil phosphatase activity was significantly enhanced by the microplastics application whereas glyphosate enhanced the soil phosphatase activity slightly. Additionally, the microplastics particles became smaller after 30 day's incubation, a 4.10% increase of small microplastics (diameter between <50 μ m to 100 μ m) was found in treatments M1G2.

Keywords: microplastics; glyphosate; soil enzymes activity; respiration.

Abbreviation

AMPA	Aminomethylphosphonic acid
SIR	Substrate-induced Respiration
ANOVA	Analysis of Variance
LC-MS/MS	Liquid Chromatography tandem Mass Spectrometry
PP	Polypropylene
NaOH	Sodium Hydroxide
CK	Control treatment
M1	Treatment with 7% microplastic application
M2	Treatment with 28% microplastic application
G1	Treatment with 3.6 kg/ha glyphosate application
G2	Treatment with 7.2 kg/ha glyphosate application
M1G1	Treatment with 7% microplastic content and 3.6 kg/ha glyphosate application
M1G2	Treatment with 7% microplastic content and 7.2 kg/ha glyphosate application
M2G1	Treatment with 28% microplastic content and 3.6 kg/ha glyphosate application
M2G2	Treatment with 28% microplastic content and 7.2 kg/ha glyphosate application
ISWC	Institute of Soil and Water Conservation, Yangling, China
OC	Organic matter
TN	Total nitrogen
TP	Total phosphate

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1. Introduction

Loess Plateau is a typical agricultural and pasture interlaced region in China, with the fragile ecological environment, as well as the central area of Chinese Dryland Agriculture (Yong.Li, Jiying.Tian 2014). Loess Plateau is approximately 640,000km² area of land, supporting the life of more than 100 million people in this region (Yiping Chen et al. 2015). Covered with 44% cultivated land, Loess Plateau owns abundant cultivated land resource and solar heat resource, thus, this region plays a key role in achieving food security (Yanlong Chen et al. 2015) (Zilong Zhang, Chenyu.Lu 2014). In order to improve the agricultural production, various agrochemical products had applied in this region. According to the survey of the utilization of pesticides in Loess Plateau of Shaanxi province in 2008, the application of pesticides especially the organophosphorus pesticides is very common among the agricultural products (Zhang Yi, 2008). Furthermore, due to lack of irrigation, the supply of water for some crops like winter wheat is totally depending on the natural precipitation. In this case, in order to capture and maintain soil moisture from the limited natural precipitation, water management practices are essential to the crop yield (Yanlong Chen et al. 2015). Plastic mulching has been regarded as an important measurement in China to increase yields, according to the statistic report, 1.25 million tons of plastic film was used in 2011 (Yan 2014).

Glyphosate (N-(phosphonomethyl) glycine, C₃H₈NO₅P, Structural formula in Figure.1) is a broad-spectrum, non-selective, systemic and post-emergence herbicide, which is widely used in the agricultural cultivation in the world (Bennicelli et al. 2009). The widespread application of the glyphosate causes problems regarding environmental contamination, indicating the glyphosate residue in the soil, food-chain and aquatic environments (Jan et al. 2009). Herbicide may show toxicity to the soil microorganism by changing the soil environment, through the influx of carbon (C), phosphorus (P) and nitrogen (N) from the co-metabolic decay of glyphosate (Zabaloy et al. 2012). There are several studies focus on the effects of glyphosate on the soil microbial activity, and most of the studies reported glyphosate shows a lack of influence or short-term effects on the microbial community (Zabaloy et al. 2012). However, the effects of glyphosate on the loess soil still remain very few. Glyphosate is moderately persistent in soil, which half-life ranging from 1 to 151 days according to the soil types (Veiga et al. 2001)(Yang et al. 2015)(Bergström, Börjesson, and Stenström 2011).

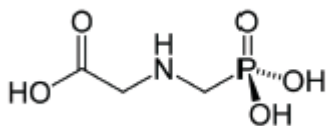


Figure 1. Structural Formula of Glyphosate

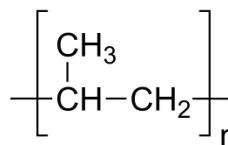


Figure 2. Structural Formula of Polypropylene

The aim of glyphosate application is to protect crops, but it should be environmentally safe as well. Which means during the pesticide decay progress, the pesticides compounds should stay low or non-toxicity (Perry et al. 2014). The metabolism of the glyphosate in the soil mainly depends on the microbial decay through two pathways: one leads to the intermediate formation of sarcosine and glycine, another one leads to the formation of aminomethylphosphonic acid (AMPA) (Borggaard and Gimsing 2008). Biodegradation of glyphosate by soil microorganism is an essential decay procedure of this herbicide (Liphadzi et al. 2005). However, what influential factors mostly in the soil mostly affect the decay of glyphosate still remain to be clarified (Borggaard and Gimsing 2008).

Meanwhile, there is a rising problem that should be aware, the usage of plastics, principle component is polyethylene and polypropylene (C_3H_6)_n (Figure.2). Due to the semi-arid climate

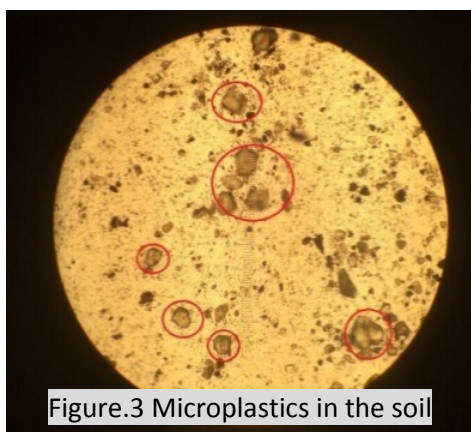


Figure.3 Microplastics in the soil

and lack of precipitation in the Loess Plateau, in order to maintain the soil moisture, plastic mulching is widely used in this area as a water management practice for agricultural cultivation (Yanlong Chen et al. 2015). However, after harvest, the plastic film was left in the field frequently. According to an investigation of a major plastic film use area in China, with long-term plastic film application (more than 10 years), the soil plastic residue level could reach to 50-260 kg/hm² (Yan 2014). Due to high dispersibility of plastic film and these plastics

become debris accumulating in the field, degraded to the microplastics (Figure.3), the diameter of the microplastic is commonly less than 5mm (Rocha-Santos and Duarte 2014). These small plastics lead to side-effects: eaten by biota and accumulated in animal bodies via the food chain. Besides, microplastics could absorb harmful contaminants, pollutants as well as pesticides, enriching these chemical compounds on its' surface, accumulating these compounds in the soil (Rillig 2012). There is a rising awareness of the effects of plastic pollution on the global ecological environment, but most of the studies are focus on the marine environment (Oberbeckmann, Löder, and Labrenz 2015)(Driedger et al. 2015), and the study of the microplastic effects on the soil microbial community remains very little.

In terms of soil, microbial activity plays an important role in the soil ecological system. Soil enzymes are maintaining the soil health, involved in the decomposition of organic matter and nutrient (Cáceres et al. 2009). Soil enzyme reacts more quickly to soil changes than other physicochemical variables (Panettieri et al. 2013). Besides, soil enzymes are considered as one of the bioindicators of soil health and it has been used frequently to estimate biological processes in a number of contaminated soil and their reclamation processes. Besides, soil enzymes could be regarded as a manifestation of microbial activity in the soil as well, they're sensitive to the effects of the climate, chemical compounds such as pesticides and herbicides (Pandey and Singh 2006). Thus, in order to discover the effects of microplastics and glyphosate on the microbial

activity, soil enzyme, such as phosphatase, urease and β -glucosidase are determined in this study.

Herbicide glyphosate have diverse effects on different soil enzymes (Bennicelli et al. 2009). Some of these enzymes are widely used to assess the effects of the contaminations in the soil, such as phosphatase, urease and β – glucosidase (Yu, Zhang, and Zhou 2011). Plant roots, bacteria and fungi produce several of extracellular phosphatase which mineralizes organic P in soil and releasing orthophosphate ions (Godin et al. 2015). This indicates that phosphatase plays an important role in the phosphorus acquisition of plants and microorganism, it determines the P cycle within the soil (Srinivasulu et al. 2012). According to (Godin et al. 2015), the organic P is regarded as an important source of P for plants and microbes. Glyphosate, as organophosphorus herbicides, may promote the production of soil phosphatase due to the import of P source. Moreover, Urease is regard as the most generally assayed enzymes, for it shows great impacts on the transformation rate of urea, and urea is an important fertilizer (Qin, Hu, and Dong 2010). Besides, Urease is the key factor in the nitrogen cycle transforming urea to ammonia, these are important processes involved in nitrogen metabolism(Gianfreda et al. 1994b). In bacteria and fungi, β – glucosidase plays an important role in cellulose saccharification. β -glucosidase is one of the glucosidase family that catalysing the breakdown of complex carbohydrates, as well as shorting cello-oligosaccharide to glucose. (Sinnott, M.; Garner, C.D.; First 1998) This enzyme also indicates the carbon cycle within the soil microorganism (Iizuka et al. 2013). Both urease and β -glucosidase are the common enzymes representing the intensity of microbial transformation process of soil carbon and nitrogen.

Since the carbon dioxide as a biologically processed carbon, it is the most ubiquitous metabolites which generated from respiration (Dilly et al. 2011). Respiration is essentially the microbial litter decomposition and the mineralization of soil organic matter(Fanin et al. 2011), the respiration intensity is used to assess the overall activity of the microorganism, fluctuations of microbial respiration indicates whether the soil microorganism is becoming active or not (Karhu et al. 2014). In addition, the substrate-induced respiration (SIR) is commonly used to analyse the microbial activity, with an access of excess nutrients (usually glucose), SIR indicates the maximum respiration of the soil microorganism. Furthermore, the glyphosate and AMPA residues play an important role in this study. The glyphosate decay is one of the objectives of this study, illustrates how glyphosate is changing in the loess soil. The residue of the glyphosate determines the decay process in the soil. And this process indicates how glyphosate is decomposed by the soil microflora and how microplastic affects the decay of herbicide. The study of glyphosate and AMPA residue including precisely the rate of glyphosate decay and the AMPA formation rate soil in different days, and the residue data would reveal the effects of soil microorganism on the glyphosate.

The aim of this study is to i) study glyphosate decay in Chinese loess soil in lab condition; ii) understand the synergistic effects of microplastics and glyphosate addition on soil microbial activities.

2. Materials and Methods

The methodology part of this research could be divided into three different steps (Figure.4). First step is the collection of the soil samples, including the application of microplastic and glyphosate. Secondly, the samples are collected and analysed in the laboratory. Finally, the data is processed by statistical software (Statistica 13.0 and Sigemaplot 10.0).

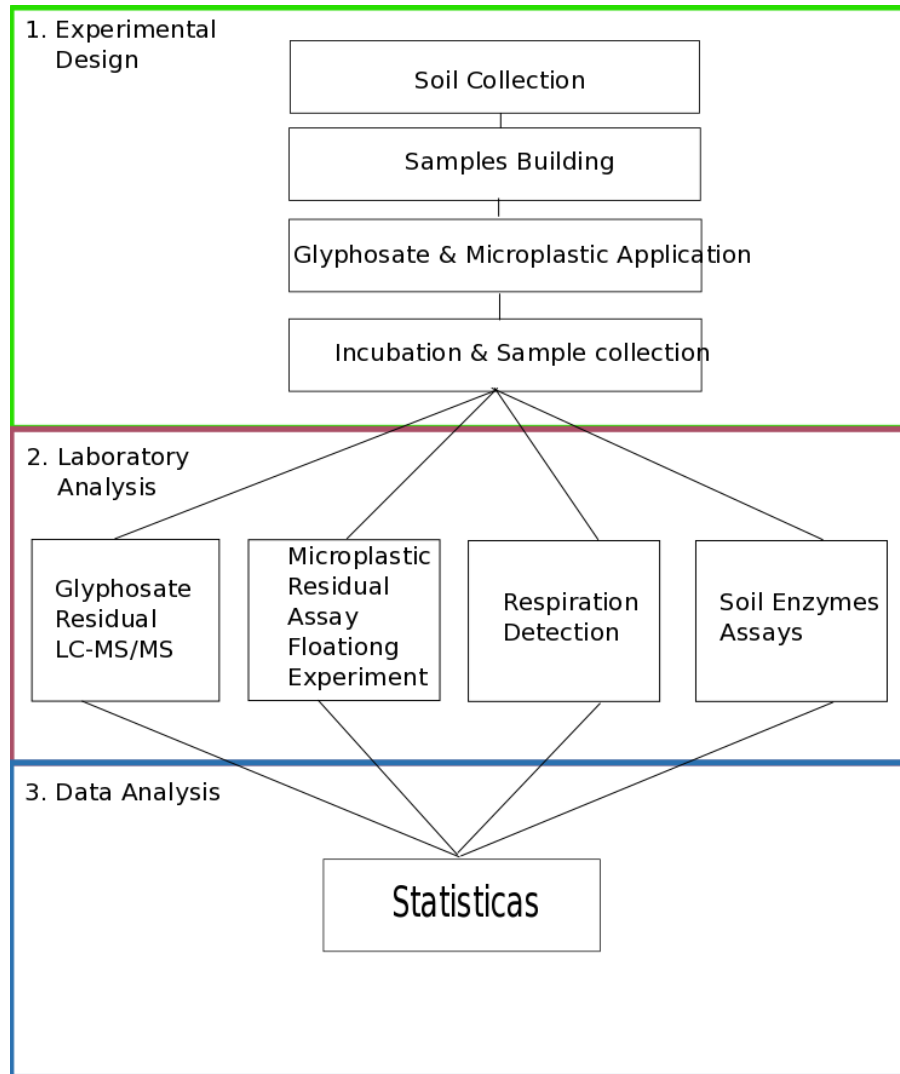


Figure.4 Flow chart of methodology

2.1 Soils and samples

The soil was collected from farmland of Ansai area (109°32' N, 36°87' W) in Loess Plateau, located in the northern part of Shaanxi Province, the middle part of China. The climate of this area belongs to temperate zone, with continental semi-arid monsoon climate. Windy, less

rainfall at spring and winter is cold and dry. The average temperature is 8.8 °C and the annual precipitation is 505.3mm, with 157 days of the frost-free season.

Soil samples were collected from 0.5m depth of surface soil, homogenized, air-dried and sieved through a 2mm sieve. The soil properties data is shown in Table.2. The experiments design was based on 4 groups of treatments: in M treatments, only microplastics would be applied in the samples; glyphosate only would be applied in G treatments; the combination of glyphosate and microplastics would be applied on samples named MG; CK is the control group. And according to different density of glyphosate and microplastics, nine treatments were designed (Table. 3). According to (Huerta Lwanga et al. 2016), 28% of microplastic content would start to be harmful to earthworm in the laboratory condition, and in order to investigate the ultimate effects of microplastic, this destiny was used in this research continuously. The soil was weighed, mixed with the compounds according to the table. The field capacity of the loess soil is 18-24% (L. Chen et al. 2007), in order to represent the normal condition of soil moisture, the moisture content of all samples was controlled at 10%. All samples were incubated at climate chamber with 28°C degree, 80% humidity for 1 week to ensure the soil microorganisms of every sample stays at the similar condition and active (Figure.6). Soil samples were weighed every day and deionized water was added to maintain the soil moisture at 10%.

Table.2 Soil properties

Parameters	
OC (g/kg)	5.13
TN (g/Kg)	0.86
TP (g/kg)	0.57
NO ₃ ⁻ (mg/Kg)	1.95
Available P(mg/Kg)	5.04
pH (H ₂ O)	8.62

Table.3 Treatments

Treatment No.	Glyphosate Density	Microplastic Content
CK	0	0
M ₁	0	7% (14g)
M ₂	0	28% (56g)
G ₁	3.6kg/ha (2.3mg)	0
G ₂	7.2kg/ha(4.6mg)	0
M ₁ G ₁	3.6kg/ha (2.3mg)	7% (14g)
M ₁ G ₂	7.2kg/ha(4.6mg)	7% (14g)
M ₂ G ₁	3.6kg/ha (2.3mg)	28% (56g)
M ₂ G ₂	7.2kg/ha(4.6mg)	28% (56g)

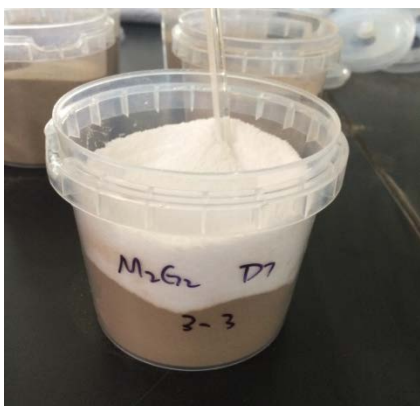


Figure. 5 Example of Soil Sample



Figure. 6 Incubation of soil samples

After 1-week incubation, glyphosate was sprayed into every soil sample according to the content above. Meanwhile, sample collection started at the same day when glyphosate was sprayed. There are 3 replicates prepared for each treatment. Soil samples were collected at day 0th, 1st, 3rd, 7th, 14th, 30th, all the collected samples were placed in hermetic bags and stored in a -80°C freezer to ensure the inactivation of soil enzymes and glyphosate decay.

2.1.1 Chemicals

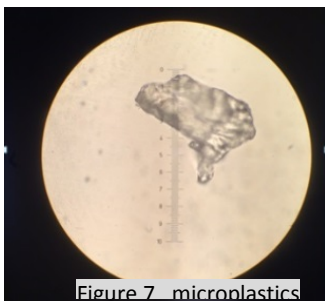


Figure 7 microplastics

Glyphosate (98% purity) was purchased from Dr. Ehrenstorfer, Germany. It is a reference material only for research purpose in a fully equipped chemical laboratory. The glyphosate powder was accurately weighed and mixed with deionized water to ensure the concentration at 0.46g/L (G₁) and 0.92g/L (G₂), 5mL of glyphosate solution was sprayed homogeneously on the soil sample surface and mixed by a plastic stick afterwards.

2.1.2 Microplastics

The microplastic used in this research is polypropylene powder (commonly used in plastic films of China, Figure. 1), which diameter is smaller than 250 micrometres, the majority of the microplastic is between 125-250 μ m. It is crystal irregular cube under the microscope (Figure.7). The microplastics was weighted according to the table below (Table.4) and mixed homogeneously with soil samples by a glass stick.

Table.4 Size distribution of the original microplastics

Plastic Diameter	250 μ m	125 μ m	100 μ m	63 μ m	50 μ m	<50 μ m
Particle size distribution (%)	58.35%	35.86%	2.27%	1.07%	0.42%	0.21%

2.2 Soil microbial activities

2.2.1 Soil respiration

According to the respiration method of West (West and Sparling 1986), the detection of respiratory of microorganism was undertaken immediately after soil collection from the climatic chamber. The respiratory was detected by measuring the generation of CO₂ in a certain period of time.

In order to detect the microbial respiration, 10g soil was extracted from the sample, and incubated in a sealed glass assay bottle (Figure.8) at 25°C for 2 hours. Within this period of time, all carbon dioxide generated by the microorganism would be sealed in the glass bottle, and the generation of carbon dioxide (CO₂) was detected by an infrared CO₂ Detector QGS-08B (Figure.9). Induced respiratory was measured by the same method, the only difference is additional 6mg glucose was added in every 10g soil samples and incubated for 1 hour instead of 2 (West and Sparling 1986).

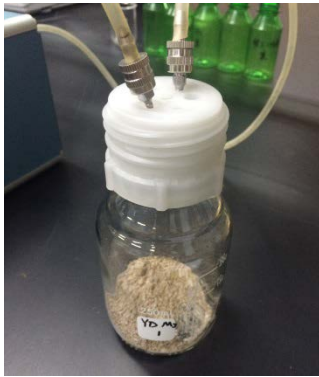


Figure.8 Assay Bottle



Figure.9 Infrared CO₂ Detector QGS-08B

2.2.2 Soil enzymes

The enzymes in this research are all extracellular enzymes, which indicates all the bacteria would be killed by toluene in advance before detection. The detection of urease was on the basis of the spectrophotometric method by Kandeler (Kandeler and Gerber 1988). 3g soil sample was collected for urease detection, by adding 1mL toluene to kill the soil microorganism, after 15min, 10mL 10% urea solution was added. Then the samples were incubated for 24 hours at 37°C, next, 0.5mL filtrate was extracted, 2mL sodium hypochlorite and 1.5mL sodium hypochlorite were added. It took 20mins for coloration, constant volume at 25mL, the production of the blue indophenol was detected by using a spectrophotometer UV2800 (See Figure.10) at 578nm.

The spectrophotometric method by Kind and King was selected to detect phosphatase activity (Kind and King 1954). 5g soil samples were collected, by adding 1.5mL toluene to kill the microflora, then the soil samples were incubated at 37°C in an incubator for 24hrs with the addition of 0.5% disodium phenyl phosphate (dissolved in 20mL critic acid buffer at pH 9.6).

100mL aluminum sulfate was added to the solution, shaking afterwards, the coloration would take place with the addition of 5mL critic buffer and 0.4mL 2, 6 -Dibromoquinone-4-chloroimide. The coloration was examined through spectrophotometer UV2800 at 660nm. The production rate of the phenol represents the soil phosphatase activity.



Figure.10 Spectrophotometer UV2800



Figure 11. SpectraMax M5

According to (Saiya-Cork, Sinsabaugh, and Zak 2002), the fluorescence method is introduced in this research to assay the activity of β -glucosidase. With 4-Methylumbelliferyl β -D-galactopyranoside as a substrate, under the effect of β -glucosidase, the substrate will be broken up for 4-Methylumbelliferone especially, and the 4-Methylumbelliferone has a strong fluorescence characteristic.

Soils were unfrozen for analysis. Homogenate was prepared by dispersing 1.00 g of soil (wet soil, <2 mm, dry weight equivalent) in 125 mL of buffer (50 mM sodium acetate buffer) prepared at the appropriate pH (6.0) for each sample site. After that homogenizing for 2 min, and then 50 μ L (200 μ M substrate) of fluorometric substrate solution would be combined with 50 μ L of soil homogenate in a microplate and incubated for 1 h at 37 $^{\circ}$ C. Each assay microplate also contained two columns of blanks for measuring background fluorescence in the substrate: one column of soil homogenate blanks, and one column of sodium acetate buffer blanks.

After incubation, 10 μ L of 1 M NaOH was added to each well to stop enzyme activity. Following termination of each reaction, after 1 min we used a fluorometer set at 365 nm excitation and 450 nm emission to measure fluorescence by SpectraMax (Figure.11). From these fluorescence values, we calculated enzyme activity by the rate of substrate converted in μ mol g $^{-1}$ dry soil h $^{-1}$ (DeForest 2009).

2.2.3 Microplastics residue analysis

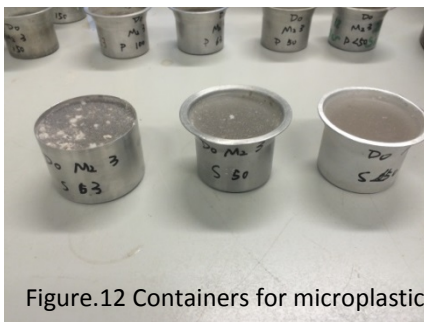


Figure.12 Containers for microplastic

The floating method is the main way to determine the microplastics content in soils, following (Huerta et al. 2016). 10g of soil samples were put into the oven at 40 $^{\circ}$ C, in order to exclude the excess of soil moisture. The dried soil was sieved with 6 different diameter nets: 250 μ m, 125 μ m, 100 μ m, 63 μ m, 50 μ m and smaller than 50 μ m. In this way, Soil and microplastic were separated according

to their size in 6 groups, and 100 mL water was added in each sieved sample. Due to the different density, plastic would float on the water surface, with 2 hours stewing and soil particles would deposit on the bottom of the container. Even though most of the soil particles would precipitate, the area of floating microplastic coverture was considered only as 90% of the area, attributing 10% to very fine floating soil particles (then the plastic weight calculation would be at least multiplied by 0.9, if the floating area is not completely covered by the microplastics, then the calculation will correspond to the surface area of coverture (ie.60, 50, 40%, using a coverture area circle and the estero microscope as help). Then microplastic was collected and deposited in a different container. Soil and microplastic were dried in a 40°C oven for 24-48 hours at in different containers. Once samples were dried, soil and microplastic were weight separately, and the calculations would be done.

2.2.4 Glyphosate and AMPA residue analysis

Glyphosate concentration in different soil samples is determined by High-performance Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). The accurate procedures of LC-MS/MS are displayed on the flow chart below (Figure.13). All steps and selection of reagents of glyphosate residue detection are entirely based on (Yang et al. 2015).

The calculation of the glyphosate residue was based on the equations below. In all samples with glyphosate application, the decay rate of the glyphosate could be calculated by equation (Eq. 2). C_t represents for the glyphosate residues in different incubation day, C_0 stands for the original glyphosate content and k is the incubation day.

$$\frac{C_t}{C_0} = e^{-kt} \quad (2)$$

At the same time, according to Eq.2, the half-life of the glyphosate could be expressed as the equation (Eq.3) below. The result of this equation is explained as after DT_{50} days of incubation, half of the glyphosate would be degraded by the loess soil.

$$DT_{50} = \frac{\ln 2}{k} \quad (3)$$

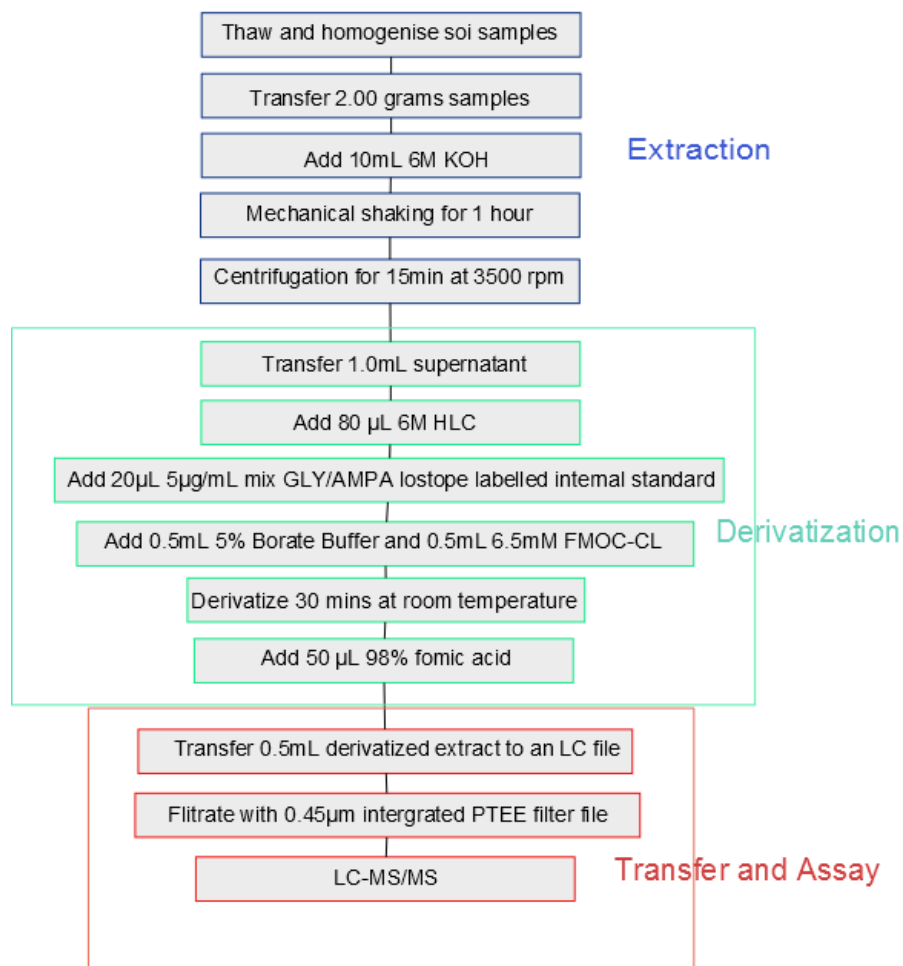


Figure.13 Flow steps of measuring the glyphosate residue in soil samples

2.3 Statistical Analysis

Results were performed on the pure soil basis, and the influence of soil moisture was excluded. Standard-deviation was calculated basing on the three replicates in each treatment. One-way Anovas were performed in order to identify significant differences among the treatments. Poshoc Ducan analysis were also developed with Statistica software Statistica 13.0 and sigmaplot 10.0 (Megharaj, Pearson, and Venkateswarlu 1991).

3. Results

3.1 Glyphosate and AMPA decay

3.1.1 Glyphosate residue

The residue of glyphosate and AMPA was assayed in all samples with glyphosate application (G1, M1G1, M2G1, G2, M1G2 and M2G2). The results showed that, glyphosate decayed significantly during these 30 days (Table. 5). In treatments with 3.6kg/ha or 7.2kg/ha glyphosate application, there are significant differences between treatments with 28% microplastic content and other samples, and the glyphosate residue of M2G1 and M2G2 is the lowest value among other treatments. Meanwhile, if simply compare the M1G1 to G1 and M1G2 to G2, there is no significant difference between them. In 30th incubation day, M1G1 decayed 1.1 µg/g more glyphosate than G1, and M2G2 decayed 0.9 µg/g more glyphosate.

Table.5 Glyphosate residues of different treatments at different incubation-days

Treatment	Glyphosate residue at the incubation day (µg/g)					
	D0	D1	D3	D7	D14	D30
G1	11.4±0.56a	10.9±0.58a	10.4±0.16a	8.7±0.14a	7.6±0.27a	6.4±0.38a
M1G1	10.6±0.225b	9.3±0.42b	8.8±1.1b	7.5±0.54b	6.7±0.94b	5.3±1.85b
M2G1	10.9±0.90b	9.7±0.60b	9.4±0.05a	8.6±0.26a	7.9±0.89a	5.8±0.32c
G2	21.9±1.00a	20.1±0.17a	19.7±0.20a	16.6 0.39a	15.4±0.62a	12.3±0.98a
M1G2	21.8±0.81a	19.4±0.47a	17.2±0.27b	15.0±0.36b	14±0.62b	12.2±0.38a
M2G2	21.4±0.90b	21±0.64b	17.4±0.48b	15.9±0.85a	14.1±0.63b	11.4±0.92b

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$). G1(3.6kg/ha glyphosate); M1G1(3.6kg/ha glyphosate, 7% microplastic); M2G1(3.6kg/ha glyphosate, 28% microplastic); G2(7.2kg/ha glyphosate); M1G2(7.2kg/ha glyphosate, 7% microplastic); M2G2(7.2kg/ha glyphosate, 28% microplastic).

The decay curves of different treatments were showed in Figure. 14. Generally, in the two figures below (a and b), the glyphosate decay of treatments without microplastics application is slower than those with the microplastics application (M1G1, M1G2, M2G1 and M2G2). It is identical to the results of decay rate (Figure. 15). In figure a, M2G1 decays glyphosate the fastest among M1G1 and G1. In the 30th incubation day, glyphosate residues of treatment M1G1 and G1 are in the same level whereas the glyphosate content of M2G1 is much lower. Meanwhile, in figure b, the glyphosate decay curves of M1G2 and M2G2 are very close to each other.

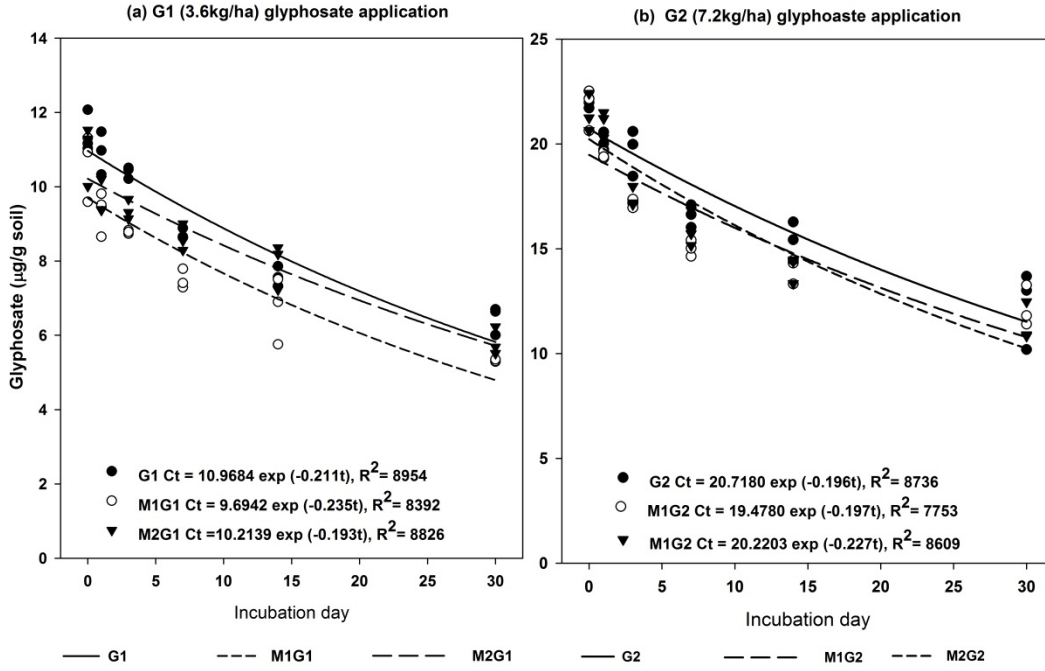


Figure.14 Glyphosate residue of G1 and G2 in different incubation day

Furthermore, according to the equations (Eq.3), the half-life of the glyphosate in M_0 , M_1 and M_2 are 37.06d, 36.48d and 34.83d respectively. Theoretically, according to the result of glyphosate half-life, none of the samples decayed half of the glyphosate during 30 days of incubation. Meanwhile, the equation of glyphosate decay could be expressed as the equations below (Eq.4, Eq.5, and Eq.6).

$$M_0: \frac{C_t}{C_0} = 0.9319e^{-0.0187t} (R^2 = 0.841, P < 0.01) \quad (4)$$

$$M_1: \frac{C_t}{C_0} = 0.8852e^{-0.0190t} (R^2 = 0.724, P < 0.01) \quad (5)$$

$$M_2: \frac{C_t}{C_0} = 0.9072e^{-0.0199t} (R^2 = 0.808, P < 0.01) \quad (6)$$

In Figure.15, Glyphosate decayed gently during 30 days incubation, no significant was observed among all treatments. Meanwhile, it is observed that the decay curves of treatments M1 and M2 is similar.

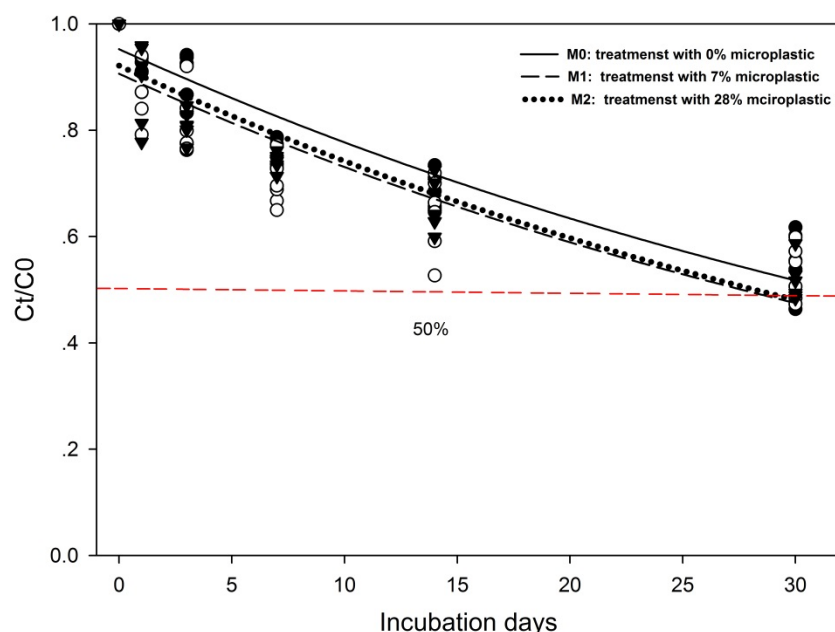


Figure.15 The decay rate of glyphosate (C_t/C_0) at different incubation days

3.1.2 AMPA residue

AMPA was through each soil sample and the result showed an increasing tendency in all treatments during the 30 days' incubation (Table. 6). AMPA was detected in all the samples during the observation days and its contents obviously depend on the initial glyphosate applied. It is observed that G1 formatted more AMPA than M1G1 and M2G1 from 0 to 3rd incubation day. The application of microplastics didn't show any significant effects on the AMPA generation.

Table.6 AMPA residues of different treatments in 30 incubation days

Treatment	AMPA residue from incubation day ($\mu\text{g/g}$)					
	D0	D1	D3	D7	D14	D30
G1	0.39±0.045a	0.43±0.029a	0.87±0.078a	0.96±0.171a	1.85±0.195a	2.26±0.078a
M1G1	0.30±0.046b	0.37±0.011b	0.69±0.034b	1.24±0.099b	1.83±0.049a	2.16±0.008b
M2G1	0.28±0.029b	0.37±0.019b	0.73±0.070c	1.14±0.083c	1.94±0.088b	2.10±0.029b
G2	0.80±0.022a	1.04±0.027a	1.20±0.103a	2.10±0.087a	3.14±0.306a	4.46±0.260a
M1G2	0.77±0.051a	0.82±0.028b	1.33±0.077b	2.21±0.092b	3.10±0.187a	4.74±0.276b
M2G2	0.86±0.055b	1.03±0.021a	1.36±0.059b	2.25±0.117b	3.23±0.103b	4.69±0.369c

Different letters stands for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$). G1 (3.6kg/ha glyphosate); M1G1 (3.6kg/ha glyphosate, 7% microplastic); M2G1 (3.6kg/ha glyphosate, 28% microplastic); G2 (7.2kg/ha glyphosate); M1G2 (7.2kg/ha glyphosate, 7% microplastic); M2G2 (7.2kg/ha glyphosate, 28% microplastic).

The results of AMPA are displayed in the exponential curve below (Figure.16), according to the figure (a) and (b), the AMPA residue in each treatment showed a stable increase from 0 to 30th incubation day. Besides, there was a dramatically AMPA rise in G1 samples from 1st to 3rd

incubation day, increased 0.44 $\mu\text{g/mL}$ from 0.43 $\mu\text{g/mL}$ to 0.87 $\mu\text{g/mL}$ whereas the increase of G2 is only 0.16 $\mu\text{g/mL}$. A contrary result is found in figure b, more AMPA was found in treatments M1G2 and M2G2 than G2. Microplastics demonstrated an inhabitation impacts on AMPA production with 3.6 kg/ha glyphosate application, and a promotion effects was found with 7.2 kg/ha glyphosate application. Besides, no significant impact was found by variation of microplastics amount.

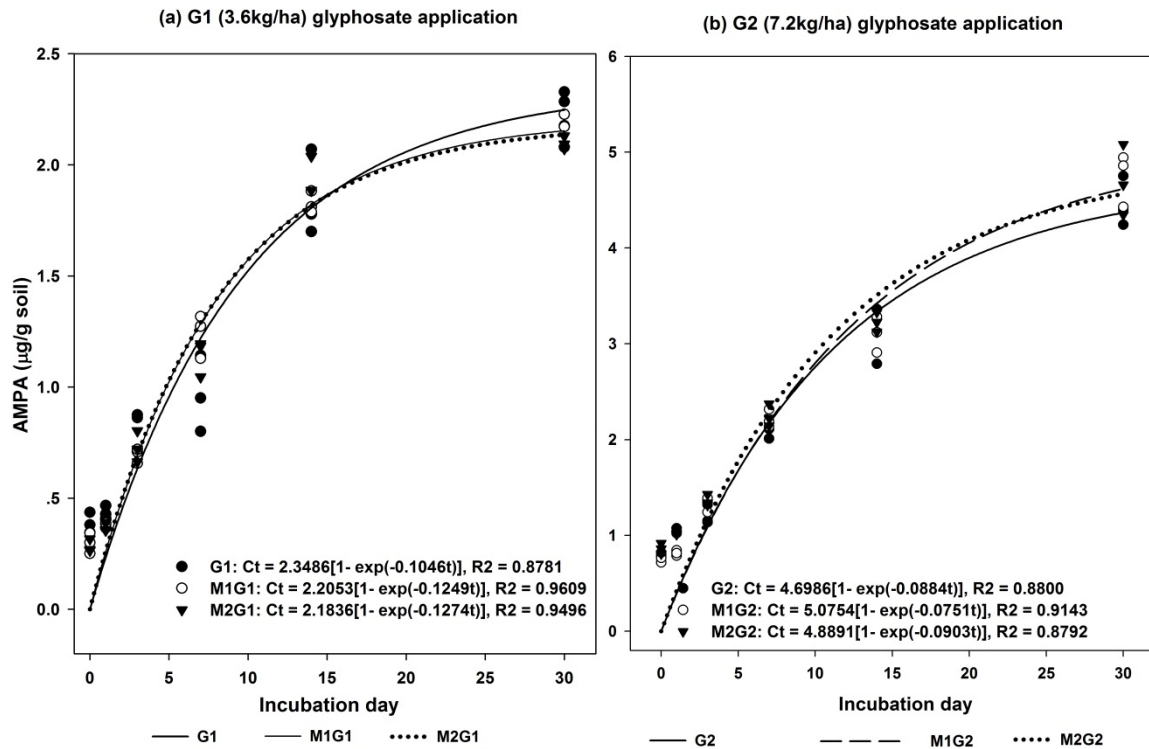


Figure.16 AMPA residue of G1 (3.6kg/ha) and G2 (7.2kg/ha) in different incubation day

3.2 Synergetic effects of glyphosate and microplastic on soil microbial activities

3.2.1 Soil Respiration

In the table below (Table.7) presents the results of soil respiration. According to the table, a significant difference was observed in treatment M2, CK and M1. Especially in day 30th, the respiration of M2 is almost 4 times higher (8.17 ppm $\text{CO}_2/\text{g soil/h}$) than the other 2 treatments. Furthermore, there is a significant difference between treatment CK and M1, soil respiration of treatment M1 has exceeded CK to 1.5 ppm $\text{CO}_2/\text{g soil/h}$.

Table.7 Effects of different treatments on soil respiration in 30 incubation days

Unit: ppm co ₂ /g soil/h	D0	D1	D3	D7	D14	D30
CK	3.57±0.92a	0.75±0.19a	2.00±0.16a	1.93±0.13a	2.78±0.10a	1.77±0.43a
M1	3.57±0.98a	1.02±0.09a	2.92±0.13b	2.47±0.76b	2.75±0.53a	3.27±1.51b
M2	7.15±0.90b	3.81±0.69b	6.34±0.09c	4.14±0.26c	5.41±0.78b	8.17±1.17c
G1	2.71±0.41a	1.15±0.02a	3.06±1.77b	2.55±0.17b	2.91±0.12a	1.80±1.35a
G2	4.06±0.61a	1.35±0.12a	3.12±0.36b	2.65±0.011b	3.12±0.07a	2.58±0.61d
M1G1	2.02±0.18c	2.04±0.49c	3.40±0.18d	3.87±0.48c	3.49±0.28c	4.05±0.15e
M1G2	3.35±0.20a	1.83±0.13c	3.19±0.04d	3.25±0.27d	3.73±0.12c	5.63±0.41f
M2G1	4.00±1.71a	2.69±0.06d	4.61±0.30e	5.09±0.03e	6.02±0.27d	7.85±0.62c
M2G2	6.74±0.90b	4.44±0.36e	5.12±0.31f	7.85±0.71f	7.20±0.43e	11.48±0.93g

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$).

The respiration of all treatments was decreased after the application in 1st day and increased dramatically between 1st and 3rd day. Soil respiration of treatment G1 and G2 were very close to each other and there was no significant difference between them according to Duncan's test. There was no significant different between M1G1 and M1G2 in 1st, 3rd and 14th incubation day. Treatments M2G2 had stronger respiration than the M2. The respiration value of treatment M2G2 at 30th incubation was 11.48 ppm CO₂ /g soil/h whereas the result of M2 was 8.17 ppm CO₂ /g soil/h, additionally from the table above, glyphosate itself showed very small simulation effects on the soil respiration individually. Respiration of treatment M2G2 was the highest value among other treatments and it's significant different from others.

3.2.2 Induced soil respiration

In the table (Table. 8) below the results of SIR is displayed. The SIR value of CK was at the lowest point. In 30th incubation day, the SIR of all treatments was lower than 0th day. Indicated a reduction of SIR after 30 days incubation. At the 3rd day, the induced respiration of M₂ reached it highest peak, with 32.03 ppmCO₂/g soil/h, this value was the highest value among any other samples. Meanwhile, it was observed that the soil samples with higher microplastic content M2 have a higher value of induced respiration than M1. It is observed that there was a significant difference between CK and M1,M2, M1 and M2 had a higher value than CK.

Table.8 Effects of different treatments on SIR in 30 incubation days

Unit: ppm CO ₂ /g soil/h	D0	D1	D3	D7	D14	D30
CK	4.83±0.08a	6.5±0.57a	5.27±2.60a	6.66±0.50a	6.50±0.43a	4.80±1.30a
M1	9.31±1.30b	6.98±0.93a	14.37±2.33b	8.99±1.17b	11.03±0.59b	5.12±2.24a
M2	18.33±3.70c	21.06±4.78b	32.03±3.40c	16.43±1.92c	17.45±1.01c	9.35±0.94b
G1	9.43±0.41b	7.06±0.24a	15.26±1.13b	8.86±0.77b	11.56±0.78b	2.16±1.16c
G2	12.16±2.98d	8.67±0.40c	15.57±0.12b	9.93±0.97d	12.07±0.74b	1.89±1.36c
M1G1	10.09±0.14b	10.00±2.91d	15.63±0.37b	10.04±0.37d	13.26±0.54d	5.41±0.31a
M1G2	11.29±0.95e	10.07±0.28d	16.92±1.27b	11.08±0.94e	14.73±0.35e	6.45±0.28d
M2G1	14.40±3.40f	12.69±1.07e	22.64±0.82d	16.30±0.99c	18.52±0.99f	9.91±0.68e
M2G2	24.84±4.49g	23.70±1.65f	28.66±5.22e	31.02±2.44f	29.49±1.76g	10.86±2.48e

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$).

As for the SIR results of treatments with glyphosate application, SIR of both G1 and G2 showed extremely increase from 1st day to 3rd comparing to CK. G1 and G2 reached the second peak at 14th day, and decreased dramatically from 14th to 30th day. The SIR value of G1 and G2 were smaller than CK at 30th day. And there is no significant difference between G1 and G2 in 3rd, 14th and 30th incubation day. Meanwhile, there's no significant difference between M1 and M1G1. Overall, treatment M2G2 had the strongest induced respiratory value among other samples, and a significant difference was found between M2G2 and M2G1. Besides, a reduction of SIR of all the samples was observed at the last two incubation weeks. treatments M2G2 and M2G1 showed a significant higher SIR value than treatments M1G1 and M1G2.

3.2.3 β -glucosidase data

Refer to the result of the β -glucosidase data (Table. 9). According to the Duncan's test, it was observed that only treatment M2 had a significant difference ($p > 0.05$) from the other 2 treatments from 1st incubation day. The β -glucosidase value of M2 was higher than M1 and CK. The activity of β -glucosidase of M1, M2 appeared to decrease at 7th incubation day. In 1st and 30th incubation day, the difference of M1 and CK is not significant. Meanwhile, it was detected that M1 didn't show a significant difference from the CK, small fluctuation of β -glucosidase activity is observed in these samples, but the value almost stayed the same level during 30 days' incubation. According to the Table, the activity of β -glucosidase of G1, G2 had no significant difference in 3rd, 14th and 30th incubation day. Besides, it is observed that there was a decrease in the 7th incubation day of treatment G1, treatment G2 showed a small decrease as well. However, the value of G1 and G2 increased to the same level as CK at 14th day of incubation. Treatment M1G1 and M1G2 almost stayed at the same level in 30 incubation days. M2G2 had the highest value of β -glucosidase among other 8 treatments, and this is significant different from M2G1. The β -glucosidase value of CK stayed at the lowest point in all time.

Table.9 Effects of treatments on β -glucosidase in 30 incubation days

Unit: $\mu\text{mol/g}$ soil/h	D0	D1	D3	D7	D14	D30
CK	5.37 \pm 0.09a	7.22 \pm 0.63a	5.85 \pm 2.88a	7.40 \pm 0.40a	7.22 \pm 0.48a	5.33 \pm 1.44a
M1	10.35 \pm 1.50b	7.76 \pm 0.70a	15.96 \pm 2.59b	9.99 \pm 1.30b	12.26 \pm 0.65b	5.69 \pm 2.48a
M2	20.36 \pm 4.11c	23.40 \pm 5.31b	35.59 \pm 3.81c	18.26 \pm 2.14c	19.39 \pm 1.12c	10.39 \pm 1.04b
G1	10.48 \pm 0.45b	7.85 \pm 0.27a	16.96 \pm 1.25b	9.85 \pm 0.85b	12.85 \pm 0.87b	2.40 \pm 1.29c
G2	13.51 \pm 3.13d	9.63 \pm 0.45c	17.30 \pm 0.14b	11.04 \pm 1.08d	13.41 \pm 0.82b	2.10 \pm 1.51c
M1G1	12.11 \pm 0.15e	11.11 \pm 3.24d	17.36 \pm 0.41b	11.15 \pm 0.41d	14.74 \pm 0.60d	6.01 \pm 0.34a
M1G2	12.54 \pm 1.05e	11.19 \pm 0.31d	18.80 \pm 1.41d	12.31 \pm 10.4d	16.37 \pm 0.39e	7.17 \pm 0.31d
M2G1	16.00 \pm 3.78f	14.09 \pm 1.19e	25.15 \pm 0.91e	18.11 \pm 1.10c	20.58 \pm 1.10f	11.01 \pm 0.76e
M2G2	27.57 \pm 4.99g	26.34 \pm 1.84f	31.84 \pm 5.80c	34.47 \pm 2.72e	32.77 \pm 1.96g	12.04 \pm 2.75e

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$).

3.2.4 Urease activity

The Urease data of is displayed below (Table. 10). According to the Duncan's test, no significant difference was found among CK, M1 and M2. Only at the 1st incubation day, treatments M1 and M2 showed an increase of urease activity.

Table.10 Effects of treatments on urease activity in 30 days' incubation

Unit: $\mu\text{mol/g}$ soil/h	D0	D1	D3	D7	D14	D30
CK	1.83 \pm 0.52a	3.15 \pm 0.93a	1.50 \pm 2.30a	1.40 \pm 0.29a	1.82 \pm 0.36a	1.04 \pm 0.22a
M1	1.60 \pm 0.27a	2.86 \pm 0.83a	1.01 \pm 0.19b	1.23 \pm 0.18b	1.70 \pm 0.27a	1.27 \pm 0.56a
M2	2.00 \pm 0.78b	1.90 \pm 0.54b	0.88 \pm 0.27b	1.62 \pm 0.11a	2.37 \pm 0.43b	1.16 \pm 0.19a
G1	2.66 \pm 0.58c	0.64 \pm 0.42c	1.21 \pm 0.42c	2.44 \pm 0.67c	1.11 \pm 0.05c	1.60 \pm 1.50b
G2	2.52 \pm 0.53c	1.27 \pm 0.40d	1.20 \pm 0.29c	1.16 \pm 0.17b	1.14 \pm 0.18c	0.92 \pm 0.16a
M1G1	2.76 \pm 1.16c	1.97 \pm 0.46b	1.19 \pm 0.44c	1.40 \pm 0.15a	1.14 \pm 0.53c	2.44 \pm 1.71c
M1G2	3.63 \pm 1.22d	2.67 \pm 0.28a	1.44 \pm 0.28a	1.41 \pm 0.15a	1.14 \pm 0.24c	1.54 \pm 0.40b
M2G1	3.37 \pm 0.16d	1.37 \pm 0.41d	0.81 \pm 0.17b	1.67 \pm 0.19a	1.36 \pm 0.22c	1.84 \pm 0.68d
M2G2	4.38 \pm 1.06e	1.50 \pm 0.31d	1.07 \pm 0.24c	1.46 \pm 0.25a	1.66 \pm 0.12a	1.66 \pm 0.13b

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($P < 0.05$).

There was an obvious reduction of urease activity after 0th day when glyphosate was added in treatment G1 and G2. And there was no significant difference between G1 and G2. However, the urease activity of G1 and G2 was raised after 1st day, and the value of G1 and G2 decreased to the same level as CK after the 3rd day. Besides, treatment G1 showed an increase at the 7th incubation day. Regardless of the reduction at 1st day, no significant difference was found among M1G1, M1G2, M2G1 and M2G2.

3.2.5 Phosphatase data

Phosphatase-mediated phosphorus mineralization is one of the essential processes of the biogeochemical cycling of P, this enzyme also determines the soil P availability in the soil system (Hou et al. 2015). The difference between M1 and CK is not significant according to the Duncan's test. And the phosphatase activity of M2 is higher than CK all the time. Especially for treatment M2, average exceeds the value of pure soil more than 100mmol/g soil, with peak value 3.39 $\mu\text{mol/g soil}$ at 1st incubation day. After day 1, phosphatase activity of all treatments showed a smooth decrease, reached its' lowest point in the 30th day.

Table.11 Effects of treatments on soil phosphatase at 30 incubation days

Unit: $\mu\text{mol/g soil/h}$	D0	D1	D3	D7	D14	D30
CK	1.97 \pm 0.15a	2.40 \pm 0.39a	2.26 \pm 0.11a	2.01 \pm 0.12a	2.04 \pm 0.09a	1.65 \pm 0.05a
M1	2.04 \pm 0.07a	2.94 \pm 0.34b	2.51 \pm 0.14b	2.24 \pm 0.09a	2.11 \pm 0.07a	1.85 \pm 0.80b
M2	2.49 \pm 0.11b	3.39 \pm 0.05c	3.16 \pm 0.19c	2.83 \pm 0.20b	2.55 \pm 0.07b	2.36 \pm 0.02c
G1	2.16 \pm 0.09c	2.62 \pm 0.17d	2.43 \pm 0.15b	2.16 \pm 0.04a	2.07 \pm 0.07a	1.70 \pm 0.73b
G2	2.29 \pm 0.05c	2.50 \pm 0.05d	2.38 \pm 0.16a	2.25 \pm 0.09a	1.88 \pm 0.01c	1.61 \pm 0.02a
M1G1	2.52 \pm 0.08b	2.59 \pm 0.15a	2.70 \pm 0.06b	2.18 \pm 0.13a	1.76 \pm 0.03c	1.84 \pm 0.09b
M1G2	2.37 \pm 0.16c	2.44 \pm 0.01a	3.09 \pm 0.17c	2.38 \pm 0.15a	1.84 \pm 0.07c	1.67 \pm 0.03a
M2G1	2.66 \pm 0.19b	3.16 \pm 0.13c	3.59 \pm 0.13d	2.60 \pm 0.12a	2.26 \pm 0.19c	2.30 \pm 0.05c
M2G2	2.49 \pm 0.07b	3.25 \pm 0.07c	3.58 \pm 0.11d	2.46 \pm 0.03a	2.18 \pm 0.11a	2.48 \pm 0.10c

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($p < 0.05$).

Meanwhile, it is observed that the addition of glyphosate showed no significant effects on the soil phosphatase activity. Although the value of G1, G2 is higher than treatment CK, but the gap is smaller than 20mmol/g soil in general. Meanwhile, there was no significant difference of phosphatase activity between G1 and G2. M2G1 and M2G2 had the highest phosphatase value with 3.60 $\mu\text{mol/g soil/h}$ at 3rd incubation day. Overall, the phosphatase activities between different treatments is not significant, and the phosphatase showed a reduce tendency in all treatments.

3.3 Microplastic residues

The figure.17 shows that the microplastic content decreased in deeper soil layer, and the land use types could be found in Table.1. The majority of microplastics was accumulated in 0-50cm soil layer. The microplastic content of sample B (horticulture land) was concentrated on the upper layer whereas the majority of microplastics in sample C (horticulture land) were between 30-70cm layers. Overall, microplastic content is very low (average weight content: 0.16g microplastic/ kg soil) among these 5 samples even if long-term plastic film had applied.

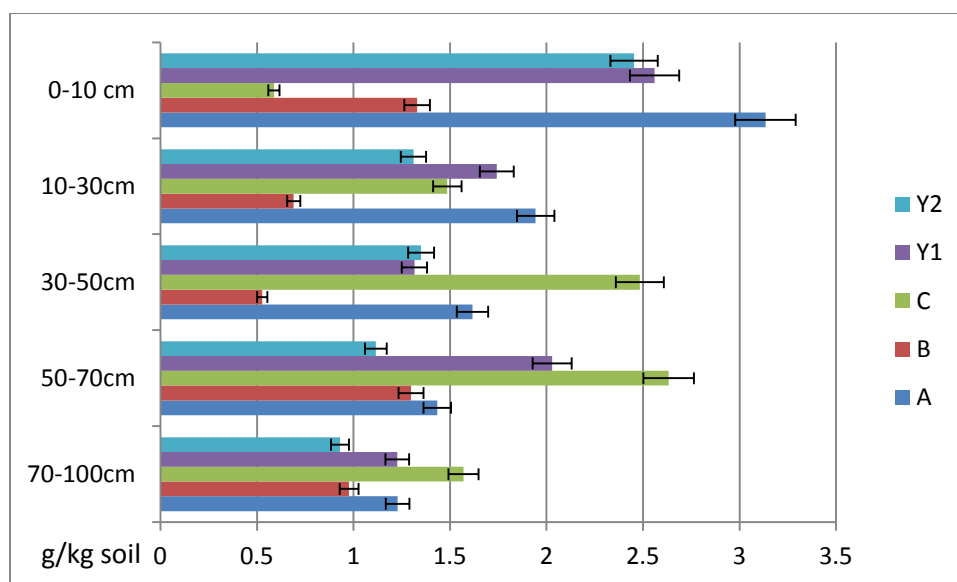


Figure.17 Results of microplastic content in different soil layers in 5 fields

3.3.1 Microplastic residues in the laboratory condition

According to the results (Table.12) of the microplastics residue, it is generally observed that the microplastics diameters of all treatments were becoming smaller than before after 30 days' incubation in the loess soil. In 30th incubation day, microplastics content of M1G2 at diameter <50 μm had increased 4.10% whereas a 27.64% decrease at diameter 125-250 μm was found. Meanwhile, microplastics content of M2G2 with diameter <50 μm increased 0.47% and decreased 10.34% at diameter 125-250 μm .

Table.12 Microplastics weight distribution content of different diameters in 30th incubation day

D30	250 μm	125 μm	100 μm	63 μm	50 μm	<50 μm
Original Microplastic	58.35%	35.86%	2.27%	1.07%	0.42%	0.21%
M1	35.58 \pm 1.78% a	47.93 \pm 5.00% a	4.30 \pm 1.65% a	5.3 \pm 2.09% a	3.61 \pm 1.68% a	3.21 \pm 1.35% a
M2	41.96 \pm 1.46% b	50.73 \pm 1.12% b	2.67 \pm 1.20% b	2.79 \pm 1.03% b	0.95 \pm 0.17% b	0.89 \pm 0.17% b
M1G1	34.46 \pm 0.43% a	50.65 \pm 5.62% b	3.85 \pm 1.03% a	4.62 \pm 1.30% c	3.42 \pm 1.51% a	3.00 \pm 1.34% a
M1G2	30.71 \pm 1.35% c	45.42 \pm 1.04% c	8.56 \pm 1.88% c	5.87 \pm 1.59% a	5.12 \pm 0.30% c	4.31 \pm 2.37% c
M2G1	45.82 \pm 0.48% b	47.30 \pm 0.84% a	2.11 \pm 0.07% d	2.60 \pm 0.08% b	1.30 \pm 0.12% d	0.87 \pm 0.22% b
M2G2	48.01 \pm 1.60% d	40.52 \pm 7.88% d	2.01 \pm 0.26% d	2.93 \pm 1.91% b	0.75 \pm 0.02% b	0.67 \pm 0.02% b

Different letters stand for the significant difference between various microplastic treatments according to Duncan's test ($P < 0.05$).

Furthermore, it is easy to find out that after 30 days of incubation, the microplastic diameter of M1, M1G1 and M1G2 were becoming smaller when comparing to M2, M2G1 and M2G2. Especially for sample M1G2, the percentage of microplastic between 100-125 μm use to be 2.27% before incubation, after applying the glyphosate and incubating in the loess soil for 30 days, this

number increase to 8.56%. It is found that in the 6 treatments, microplastics weights at diameter ranging from <50 to 125 μ m were increased whereas there were fewer microplastics concentrated on 125-250 μ m diameter. Meanwhile, in M1 there was more microplastics were accumulated between <50 μ m to 100 μ m when comparing treatment M1 to M2, microplastics content of M1 at diameter <50 μ m is 3.21% whereas the value of M2 is 0.89%.

4. Discussion

4.1 Glyphosate decay

The results showed glyphosate decayed rapidly in the treatment with microplastic addition, comparing to the treatments without microplastics application (Figure. 15). This indicates that microplastic addition would stimulate glyphosate decay. In this study, the result of glyphosate residue indicates the half-life of the glyphosate in loess soil is 37.06d. However, it is reported that the half-life the glyphosate in the soil from Loess Plateau is 3.5d in the upper 2cm soil layer (Yang et al. 2015). Additionally, it is declared that the half-life of the glyphosate is between 110d and 151d in the clay soil (Bergström, Börjesson, and Stenström 2011). In this glyphosate residue experiment, regardless of the thickness of soil layer and difference of soil type, the homogenized soil was used to assay the glyphosate residue and the thickness of the soil layer was 8cm. The variation of the half-life of glyphosate in the same soil could be explained as due to the differences of soil collection process, soil incubation condition and glyphosate application method.

However, according to the results, the half-life of the glyphosate soil samples with 28% microplastic content is shorter (34.83d) compared with the pure soil samples. This indicates that under the same condition, the microplastics application would accelerate the glyphosate decay process by loess soil microorganisms. According to the result of AMPA, only increase tendency was found a basis on the exponential curve, but the AMPA couldn't increase eternally as the glyphosate was decreasing in the loess soil.

4.2 Soil Respiration and SIR

The results indicate that when the plastic weight content was lower than 7%, the effect of microplastic on the respiration of the microorganism was very limited. However, when the concentration of the microplastic is 28% in the soil, the respiratory of the microflora would increase. The results (Table.7) indicate that the microorganism is becoming more active with the abundant microplastic environment. Besides, the results of soil respiration indicates that small density of glyphosate shows no significant effects on the microbial respiration, this finding is in agreement with Carlisle's report (Carlisle and Trevors 1986). According to the results, soil respiration value of treatment M2G1 is higher than M1G1, and curve M2G2 is on the top of M1G2. This indicates that the increase of microplastics amount would enhance soil respiration as a result. Thus, the loess soil respiration is greatly stimulated by the synergistic effects of both glyphosate and microplastic, and this acceleration is stronger than the effects of microplastic and glyphosate separately.

Generally, respiration of soil microorganisms would be stimulated by the herbicide application (Carlisle and Trevors 1986)(Kara, Arli, and Uygur 2004). The similar result was found in this research that a higher value of CO₂ generation was detected in the glyphosate treatments. Meanwhile, with the microplastics application, respiration rate in M2 samples was much higher than the M1 and M0 samples. The microplastic application would increase the soil porosity. And the increased soil porosity would promote more air-contact of soil microorganism, which may

lead to the higher amount of CO₂ generation as a result. Even though all samples were compacted at the same way during the incubation process, but the samples could not be compacted when they are transferred into the assay glass bottle.

The results suggests that in the loess soil, with higher microplastic content, the potential respiratory of the microorganism would be higher. Furthermore, it is observed that the application of glyphosate on the soil samples stimulated SIR of soil from the starting 14 days and inhibited SIR after 14th day (Table.8). However, the tendency differences between G1 and G2 is very small, which indicates the impact of glyphosate dose on SIR is limited. The results (Table.8) illustrate the addition of the glyphosate would enhance the soil microbial potential respiration in a short term period. Besides, only soil moisture index was controled during the incubation period, lack of nutrient could lead to the decrease of the SIR. Comparing CK, SIR was larger in other treatments especially in the treatment with microplastic and glyphosate addition.

Furthermore, the result of substrate-induced respiration (SIR) corroborate that microplastic stimulate the soil respiration, for the SIR stands for the potential respiration of entire soil sample (Aira and Domínguez 2010), which is not related to the soil porosity. In the result of SIR, there's a dramatically increase of the induced respiration at the 3rd incubation day of M2 treatments, and the overall value of M2 is obviously higher than M1 and M0, which confirm the data from the soil respiration is reliable.

4.3 Soil enzymes

The results of β -glucosidase indicate that only high amount of microplastic exists in the soil would stimulate β -glucosidase activity. Low microplastics (7%) content in the soil affected soil B-glucosidase activity. Soil β -glucosidase activity was only restrained by glyphosate in the 7th incubation day. The results showed the activity of this enzyme was stimulated by 28% microplastic application. Compare to the treatments with glyphosate application, the results showed an opposite curve that glyphosate restrains the activity of soil β -glucosidase. On the basis of Gianfreda, glyphosate would enhance the soil urease activity (Gianfreda et al. 1994a). Additionally, Sannino also declared that glyphosate would activate the soil urease to different extent, from +4% to +204% (Sannino and Gianfreda 2001).

The results of urease illustrated that microplastic shows no obvious impacts on the urease activity of loess soil microorganisms. And the results of G1 and G2 indicate that the urease activity would be restrained with the introduction of glyphosate, this phenomenon may disappear after a short period, and the soil urease activity became close to value of CK within 3 days (Table.10). Furthermore, it is determined that within the range of microplastic weight and glyphosate density addition, both of the compounds show fewer effects on the loess soil urease activity for 30 days, and there is no obvious synergistic effect between the glyphosate and microplastic according to the results. According to the results of Duncan's test, after 30 days of incubation, microplastics and glyphosate showed no significant activation or inhabitation on the loess soil urease activity. Glyphosate represented different impacts on the soil urease may due to the difference of soil types. Furthermore, there is less researches focusing on the impacts of

microplastics on soil urease activity. According to my study, the impact of microplastics on the urease soil activity is not significant.

According to the results of phosphatase, it's obvious to find that the phosphatase activity appears a decrease tendency in general. Microplastic showed a positive effect on the soil phosphatase, and the microplastic would enhance the activity of phosphatase to a higher extent comparing to the effects of glyphosate. According to the reports from (Yu, Zhang, and Zhou 2011), impacts of glyphosate application on the soil phosphatase activity is indefinite, differs from the soil types, incubation day and glyphosate density. Additionally, Pandey declares that glyphosate would depress the soil phosphatase activity (Pandey and Singh 2006). On the basis of Table.11 from this study, the activity of loess soil phosphatase was slightly activated by the application of glyphosate on the 3rd incubation day, enhanced by 7.59% and this number decreased to 2.99% in the 30th incubation day. It seems verified that the impacts of glyphosate on the soil phosphatase activity various from the incubation day according to the literature. Due to the soil type in this study is singly the loess soil, the impacts of glyphosate and microplastics on different soil type remains undiscovered. Judging by the Duncan's test of Table.11, various glyphosate density showed no significant differences in the soil phosphatase activity within 7.2kg/ha. However, refer to the impacts of microplastic on the soil phosphatase, and the effects are dramatic and obvious. In Table.11, phosphatase activity was enhanced 41.07% by treatment M2 in the 1st incubation day. Besides, there was a significant difference between M1 and M2, it seems that samples with higher microplastic content possessed higher phosphatase activity according to Table 11.

4.4 Microplastic residues

According to the results of microplastic residue, the distribution of the microplastic in the natural environment is related to the soil depths, the majority of the microplastics were accumulated on the soil surface. The result of treatment M1 determines that microorganism in the loess soil showed promotion effects on the decay of microplastic, for there was an increase of low diameter microplastic content and meanwhile a decrease of high diameter microplastic content (diameter bigger than 125 μ m).

Furthermore, the complete laboratory condition and artificial incubation may not the response the effects of glyphosate and microplastic in the loess soil realistically. Without natural precipitation, plant growth, animal activities and soil dynamic process in the artificial condition with constant temperature, humidity and soil moisture, the results of the study may not be representative and realistic. However, the diversity of effects from the different density of glyphosate and microplastic application on the loess soil microorganisms is reliable.

5. Conclusions and recommendations

5.1 Conclusions

According to the results and discussion, conclusions below could be drawn: First of all, microplastics addition increased glyphosate decay slightly during the observation days. The microplastic particles size decreased after 30 day's incubation in all treatments. And soil respiration is greatly stimulated by the synergetic effects of both glyphosate and microplastic, but glyphosate doesn't stimulate the soil respiration individually. The SIR is accelerated by microplastic and glyphosate addition respectively, the synergetic effects of both glyphosate and microplastic is not significant. Furthermore, glyphosate and microplastics showed no significant effects on the urease activity. Phosphatase activity of was extraordinary enhanced by the microplastic application, and the effects of glyphosate is not significant;

5.2 Recommendations

In the floating experiment, longer and repeated incubation are suggested in order to deliberate on the long term effects of microplastic and glyphosate. Not only water but nutrient is supposed to be applied in the soil samples in order to maintain the activity of the microorganisms, and long term effects could be tackled. Otherwise, the soil microorganisms would lose its activity due to lack of nutrient. According to the microplastic residues results, the microplastic content in the loess soil is far lower in the natural environment, even plastic film had applied in the field for a long term period. The microplastic content is supposed to be lower if further research is carrying on, and a filed-based research is recommended in order to reflect the effects of microplastic on the soil microorganisms in a more realistic condition.

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