EVALUATION OF GLYPHOSATE EFFECTS ON ACRISOL SOIL QUALITY - A FIELD SURVEY AT DONG NAI AND CU CHI RUBBER FAMRS

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Received: 4 January 2018; Accepted for publication: 18 May 2018

ABSTRACT

Glyphosate is commonly used in modern agriculture and research due to its effects in prevention of weed growth. Recently, glyphosate becomes more important to unprogressive agricultural countries as Vietnam. To examine glyphosate impacts on soil quality, a number of soil samples were collected before and after glyphosate treatment and qualified via some criteria such as pH, soil moisture, total NPK content, total soil microbes (TSMs) and herbicide degrading microbes (HDMs). Results showed that soil pH was not affected by glyphosate however it decreased soil moisture of dry season and nitrogen content (NiC) in both seasons. Furthermore, glyphosate also increased either phosphorus content (PhC) or potassium content (PoC) in dry season. However, TSMs and HDMs were uninfluenced by glyphosate during 15 days of the survey. Results also indicated some of recommendations as spraying glyphosate in dry and rainy seasons. Nevetheless, it is necessary to have wise insights about glyphosate treatment. Developing non-chemical weed management to minimize its negative impacts on the ecosystem must be considered.

Keywords: Glyphosate, rubber farms, herbicide, soil environment.

1. INTRODUCTION

For the agricultural production, prominences to soil quality must be concerned. Criteria such as pH, soil moisture, N-P-K content and microbes reflect directly soil quality [1]. pH value indicates ion H^+ amount and determines soil is alkaline or acidic. Therefore, pH significantly affects nutrient uptake in soil. Nitrogen (N), phosphorus (P) and potassium (K) are macronutrients tightly involved in the plant growth. Nitrogen demand proportionates largely for development. Phosphorus is important to root, flowering and seed development while potassium maintains disease resistance in plants. Microbes take part in nitrogen fixation, help uptake nutrients or water to root and their activities during metabolising or their symbiosis may help generate new nutrients for soil and plant [1]. Therefore, such criteria determine to crop yield.

However, the weed invasion competes nutrients, space, light and moisture with interested plants, which might lower crop yield. In Mekong basin countries, the crop yield will reduce if herbicide is unemployed [2]. Moreover, cons in conventional weeding (manual handling, air drying, water supplying…) and pros such as quick time and high efficiency, have actuated herbicide demand in agricultural production. Nevertheless, it is undeniable that herbicide application enabled crop yields more profitable but also created negative impacts to soil environment [3].

Glyphosate is widely used in the world for weeding. Many reports examined the effects on glyphosate to soil environment, to soil quality or to plant growth [3]. However, there is no report on rubber farms. In this study, we examined effects of glyphosate, a commonly used

herbicide in Vietnam on rubber farms in Dong Nai and Cu Chi via pH value, soil moisture, macronutrients (N-P-K) content, total soil microbes (TSMs) and herbicide degrading microbes (HDMs) in dry and rainy season. Furthermore, some notes as using glyphosate were also discussed. The study will premise for further evaluations of glyphosate effects on other plants.

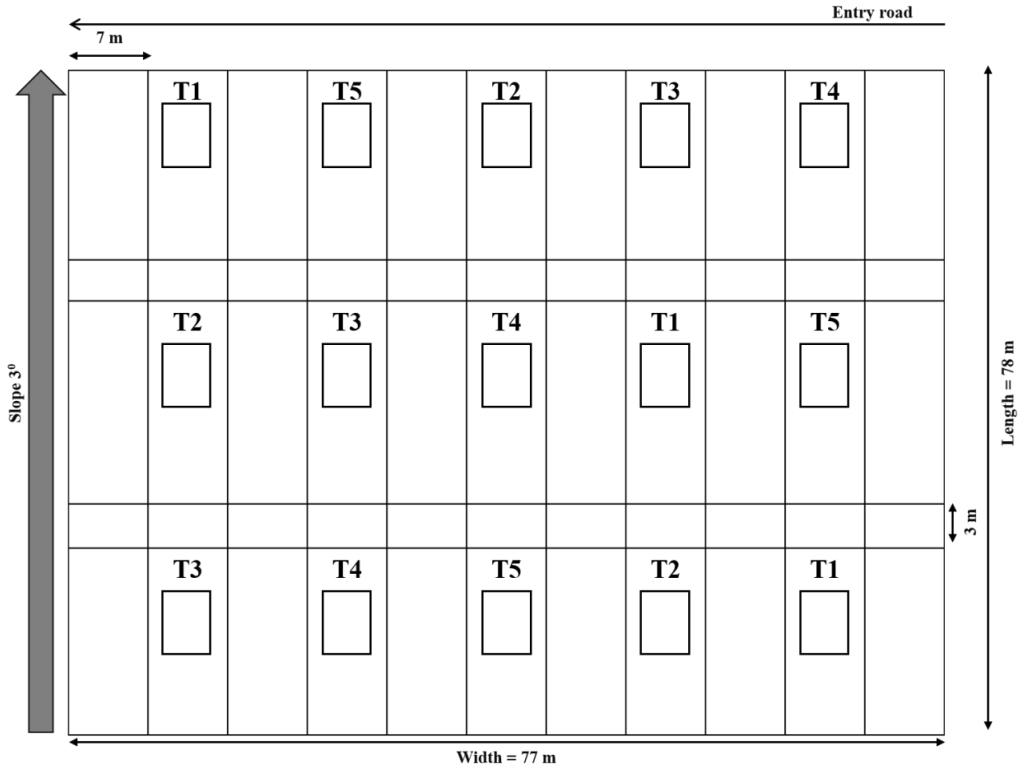
2. MATERIALS AND METHODS

2.1. Experimental design

Experiment was designed as Table 1 below:

Table 1. Treatment groups and their layouts

Groups	Experiments	Note
T1	Mowing grass	Experiments were duplicated. Each of treatment was put a pot 1 m^2 for sampling: soil, water, pH, nutrients and microbes
T2	Removing grass	
T3	Removing grass tree-tree interval and spraying line-line inverval	
T4	Whole spraying	
T5	Let grass grow natually	



Treatments were laid out in completely randomized design

: Representative for 1 m^2 sampling pot

2.2. Description of experiment

The experiment was carried with the following factors: Location: block 4, altitude less than 200 m; an area larger than 22 hectares; type of soil: haplic acrisols; year of planting: 2002; planting density: 476 trees/ha; breed of tree: VM 515; line to line interval space: 7.0 m, tree to tree interval space: 3.0 m; time to experiment: dry and rainy seasons; herbicide: mainly glyphosate, mixtured with low dose of 2, 4-D (20-30 mL/ 8L) to enhance weeding efficiency; dose of spray: herbicide was diluted as recommendation from manufacturer. Usage dose was 4 L/ha, spraying in dry season (February-March) and rainy season (June-August).

2.3. Soil sampling

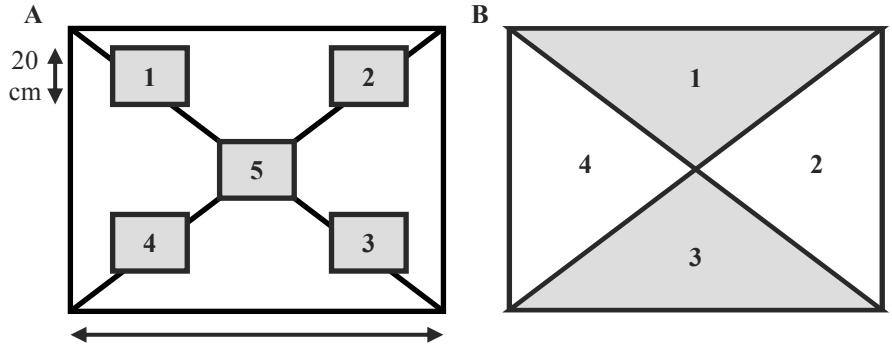


Figure 1. Process of soil sampling

Soil sampling was described in Figure 1. Each of sampling pot in Table 1 was represented by a square in Figure 1A. Soil samples from 5 different places (1-5) in each sampling pot were collected as indicated times. Soil sample in each of place was volumed as 20 x 20 x 20 cm and followed by mixing homogenically with all the rest of places. Soil then was spread out on plastic bag as shown in Figure 1B. Mixture of Part 1 and Part 3 was representive for further analysis in each 1 m^2 sampling pot. Analysis methods were done in accordance with the Vietnam standards including: TCVN 6647, TCVN 7373, TCVN 7374, TCVN 7375.

2.4. Statistical analysis

Data were input into Excel, graphs were drawn and statistically analyzed by Graphpad prism 6.0.1. Data were shown as mean and SD (Standard Deviation). *P value* < 0.05 is considered as significance.

3. RESULTS AND DISCUSSION

3.1. Effects of glyphosate on soil pH

Herbicide efficiency is tightly related to pH in soil and pH helps maintain nutrient uptake [1, 4]. To address whether glyphosate is able to affect soil pH, samples were collected in dry and rainy seasons before and after glyphosate treatment. Results showed that soil pH after glyphosate treatment in both dry and rainy seasons were not significantly different from untreated glyphosate groups (T1, T2, T5) (Fig. 2). However, soil pH in T5 and T1 were increased in dry and rainy seasons, respectively (Fig. 2A-B). The reason is that some of leaves or shoots in T5 were died in dry season. These grass biomasses act as source of ash which might cause soil pH increase [5] (Figure 2A). Increasing evidences showed that

mowing practice can promote the root of plant growing deeper as well as can stimulate some symbiotic fungi in grassland [6-8]. Moreover, higher rainfall would leach water to underground effectively and neutralize soil H^+ in rainy season. These factors might be the causes faciliting soil pH increase in T1 (Fig. 2B). Taken together, glyphosate treatment is effectiveless on soil pH.

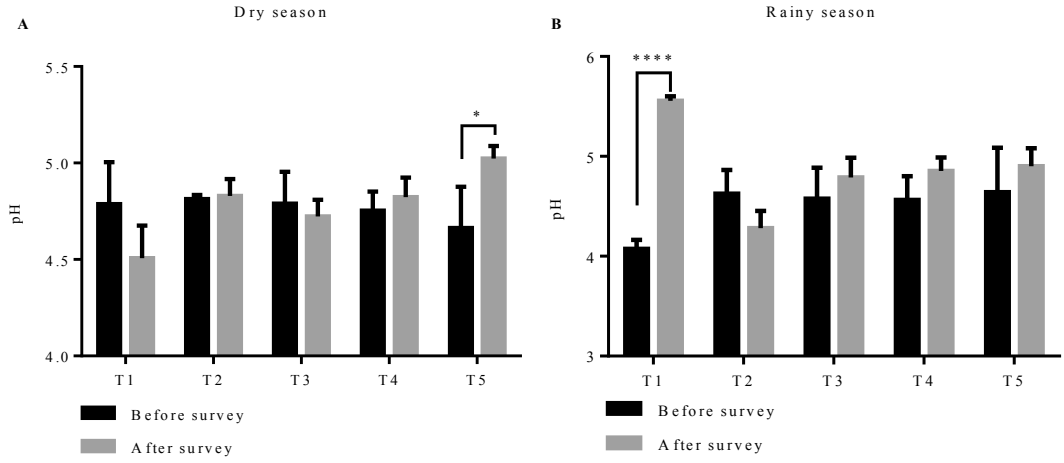


Figure 2. Soil pH state under glyphosate treatment

3.2. Seasonal effects of glyphosate on soil moisture

Moisture is important to assess soil quality [1]. To examine glyphosate effects on the soil moisture, moisture was recorded in dry and rainy seasons before and after glyphosate treatment. After 15 days of the survey, T3 showed the decrease of moisture in dry season while there was no significant difference in all treamtents of rainy season (Figure 3 A-B). This result indicated that the glyphosate treatment at line to line interval/ removing weed tree to tree interval significantly reduced soild moisture (*P value < 0.01*). Therefore, it is necessary to assess the soil moisture, especially in dry season prior to T3 treatment.

Besides, present herbicide in rubber farms also affected soil moisture seasonally. Before the survey, there were almost no difference between treatments in both seasons, except T4 (Figure 3C). This might be high waterflow rate at T4 as compared to other sites, leading to high soil moisture at the time of collecting sample. However, soil moistures were decreased considerably in dry season as compared to rainy season after survey (T3, T4 ; Figure 3D). The reason is that much rain was absorbed into ground, leading to the increase of soil moisture in rainy season versus dry season. Another possiblity is that glyphosate itself reduced soil moisture. Indeed, glyphosate has chemical structure containing carboxyl and phosphate residues, resulting in high dissolution of glyphosate in water [9] and therefore directs to soil moisture decrease in dry season. Taken together, the glyphosate treatment subsides the soil moisture in dry season while there is no change in rainy season. Therefore, it is unadvisable to weed by glyphosate in dry season at low moisture lands.

3.3. Glyphosate effects on soil nitrogen content

Nitrogen content (NiC) plays an important role in plant growth [1]. In soil, there are organic and inorganic nitrogen, however, only inorganic nitrogen can be uptaken by plant. These nitrogen sources can be generated by nitrogen fixation, fertilizer or from death

animals. To certify whether glyphosate can alter soil NiC, we analyzed NiC in soil before and after survey. Results showed that herbicide treated-NiC in dry season was significantly reduced as compared to pre-survey (Figure 4A). In rainy season, NiC in all treatments was statistically decreased (Figure 4B). These evidences are correlative to other studies which claimed that glyophosate has negative impacts on nitrogen fixation and related microorganisms, leading to the reduction of soil NiC [10-11]. Therefore, it is necessary to take a wise step when spraying glyphosate to low soil NiC lands.

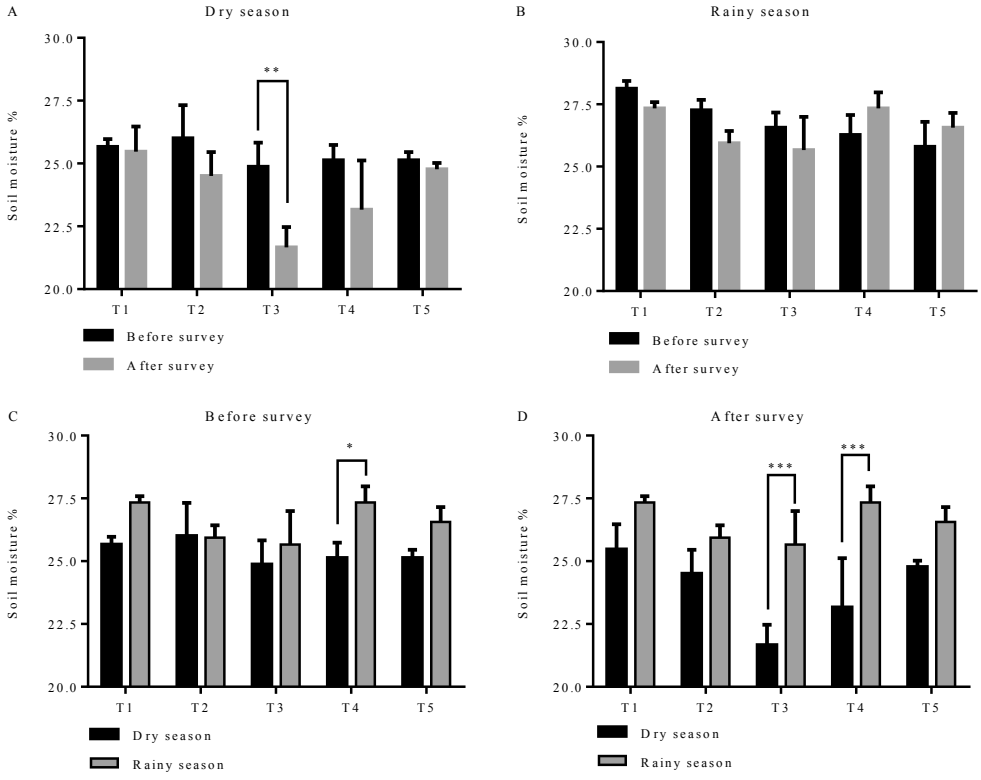


Figure 3. Negative impacts of glyphosate on soil moisture

3.4. Glyphosate effects on soil phosphorus content

Phosphorus content (PhC) is the second large proportion in macronutrients [1]. To assess glyphosate effects on PhC in soil, samples were collected in dry and rainy seasons before and after survey. Figure 5A showed that T3 and T4 increased soil PhC as compared to other treatments in dry season while soil PhC was not statistically differed from pre-survey in rainy season (Figure 5B). It is probable that glyphosate has phosphate residue and directly increases PhC in soil (Figure 5A). Furthermore, a vast of grass suddenly died under glyphosate treatment may act as phosphorus source and subsequently increased soil PhC. Besides, the glyphosate dose in T3 is lesser than in T4, but the efficiency in soil PhC deposition was not different (Figure 5A). This evidence suggested that T3 but not T4 can be applied in rubber farms in an attempt to limit glyphosate dose, which benefits economic and environmental profits. In rainy season, although glyphosate treatment can increase soil PhC, however, washout restrained soil PhC deposition, leading to no difference between before and after survey in T3-T4 (Figure 5B). Therefore, there is no benefit regarding PhC deposition as supplementing glyphosate in rainy season. Furthermore, surface vegetations

loss in T1 and T2 might facilitate washout PhC attenuation (Figure 5B). Thus, T1 and T2 are not recommended for rainy season. In term of seasonal effects, there was no statistical change between treatments before survey (Figure 5C), excepting for T1. However, after the survey, there was soil PhC loss in all treatments in rainy season as compared to dry season (Figure 5D). This evidence demonstrated the soil PhC sensitivity to washout and surface vegetation is necessary in this case. Taken together, weeding by glyphosate might be an alternative phosphorus fertilizer and glyphosate based-T3 weeding might be a considerable option in dry season. Surface vegetation has a crucial role in preventing soil PhC loss induced by the washout.

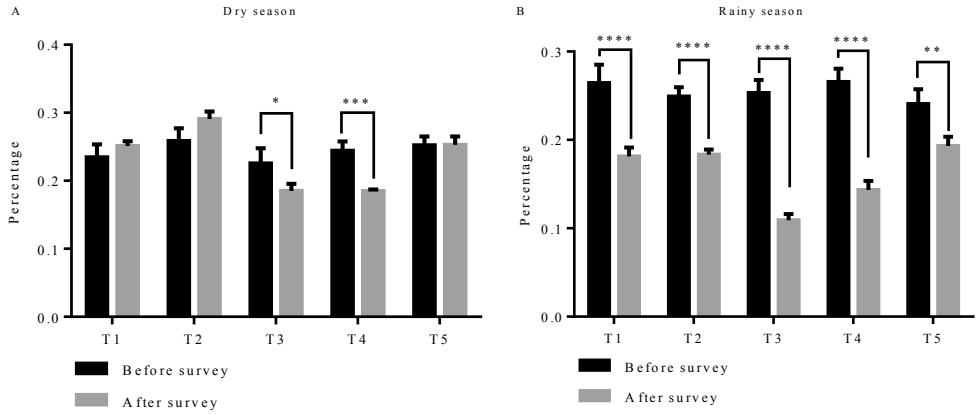


Figure 4. Reduction of soil NiC under glyphosate effects

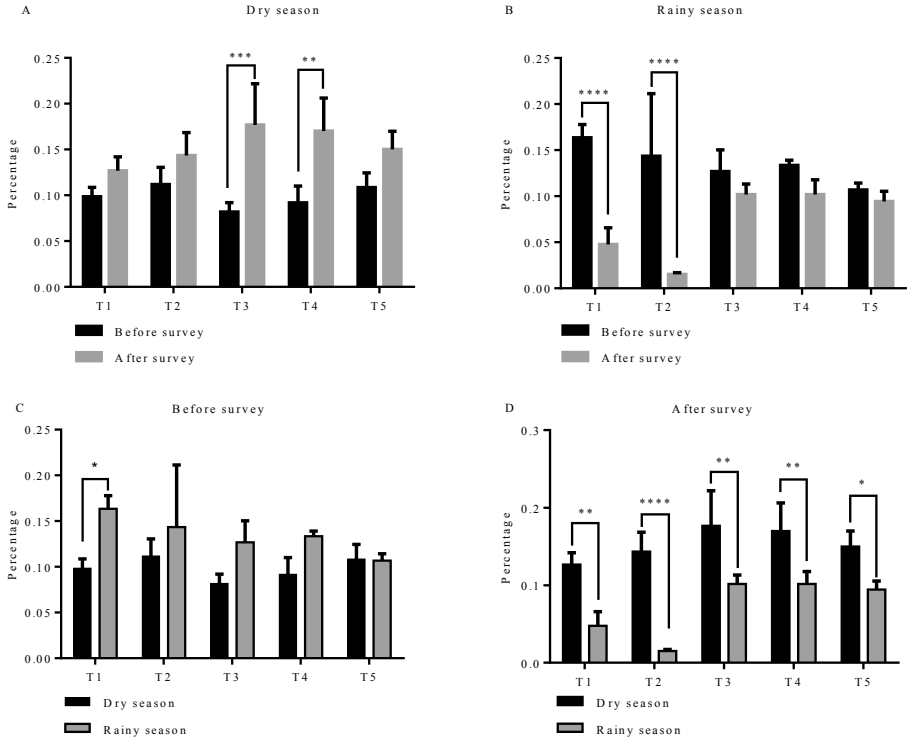


Figure 5. Positive impacts of glyphosate on soil PhC

3.5. Glyphosate effects on soil potassium content

Potassium is important to the disease resistance of plant [1]. Soil samples were collected in dry and rainy season before and after survey. Results showed that T3 and T4 in dry season significantly increased soil PoC between before and after survey (Fig. 6A). The reason was formulation of this commerical herbicide is potassium glyphosate and consequently increases soil PoC after spraying. Therefore, glyphosate supplement might indirectly intensify the disease resistance in plant. Besides, T2 also accumulated soil PoC (Figure 6A). Surface vegetations also use potassium for their growth and removing surface vegetation in T2 would reduce potassium demand and percentage of PoC in T2. This data suggested that T2 can temporarily increase soil PoC. Moreover, there was no significant difference among groups, except T4 in rainy season (Figure 6B). Somehow, glyphosate treatment in T4 reduced soil PoC signifantly compared with T3. Taken together, glyphosate increases soil PoC in dry season and it is necessary to supplement K-fertilizer to replace the washout PoC-induced loss in rainy season and be aware of glyphosate whole spraying in rainy season.

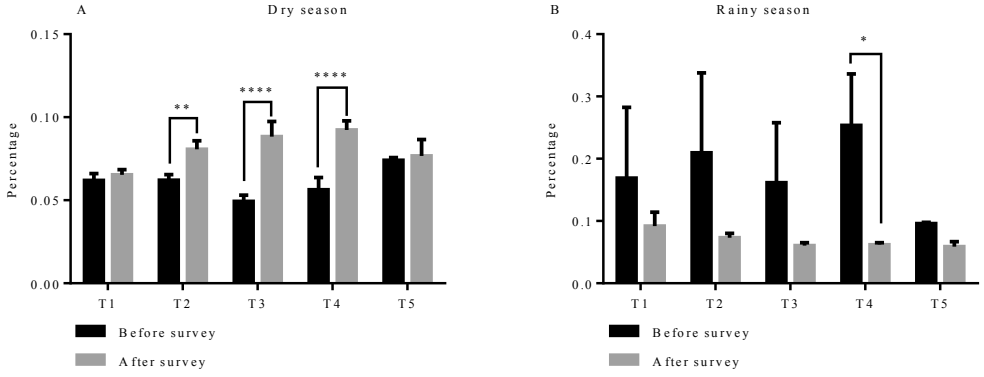


Figure 6. Seasonal impacts of glyphosate on soil PoC

3.6. Total soil microbes and herbicide degrading microbes under glyphosate treatment

Total soil microbes (TSMs) maintain nutrient homeostasis in soil [1]. Herbicide degrading microbes (HDMs) are activated their degrading capacity in presence of herbicide and use herbicide as an energy source for their metabolisms [12]. Soil samples in all treatments were collected and evaluated for TSMs and HDMs to examine 2 possibilities: 1) whether herbicide affects on TSMs; 2) herbicide in soil might increase HDMs. Both TSMs and HDMs before and after survey were not significantly different in both dry and rainy season (Figure 7A-B-C-D). However, results in Appendix showed that there was a correlation between TSMs and HDMs when herbicide existing in soil (Appendix). It's explained that herbicide promoted HDMs growth while inhibiting other microbes, leading to the shuffle in TSMs constituent. Nevertheless, these changes were temporary and rapidly recovered after 15 days of the survey. Consequently, there was no significant difference between before and after survey (Figure 7B and Figure 7D).

In general, glyphosate effects in this study is comparatively friendly to soil microbes. These results are consistent to previous reports [13-14]. However, it is necessary to take a wise step during long term glyphosate consumption. Indeed, glyphosate is believed to negatively influence the reproductive process and activities of earthworms and symbiotic microbes in plant roots [15, 16]. Although there is no clear evidence about glyphosate leaching to groundwater, but some of evidences showed concerns about glyphosate existence or its residue derivative in drainage water or castle and poultry [11, 14]. Glyphosate-based

herbicides degraded or undegraded are reported to have certain harms to animal and human health [17]. It is indispensable to overview glyphosate effects on the general environment while regulating dose of treatment, locations (close to groundwater, urban area, etc…). Moreover, it is urgent to explore new efficent weeding practices or develop non-chemical herbicides to minimize negative impacts on the ecosystem and human health [18-19].

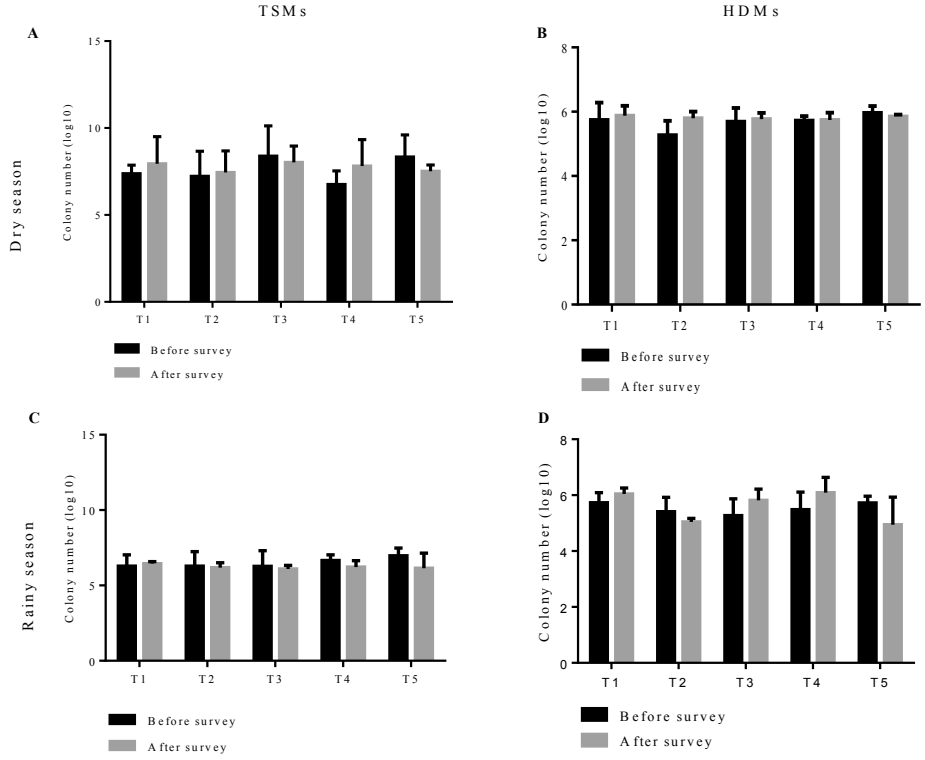


Figure 7. TSMs and HDMs are unchangeable under glyphosate treatment

3.7. Summary of glyphosate effects on soil quality and notices

Glyphosate has some effects on soil quality as decribed in Table 2. In brief, soil pH has no change under glyphosate treatment. In term of soil moisture, glyphosate treatment decreases water content in dry season and therefore, it is unadvisable to spray glyphosate on lands which lack water. To soil NiC, glyphosate treatment decreases nitrogen accumulation in both seasons and therefore the supplement of N-fertilizer should be considered during the glyphosate treatment (Table 2).

Glyphosate has positive impact on soil PhC in dry season (Table 2). Glyphosate serves as phosphorus source, resulting in soil PhC accumulation. Any form of vegetation loss such as mowing or removing grass affects the soil PhC deposition in rainy season. To soil PoC, glyphosate treatment increases PoC in dry season. Whole spraying glyphosate might decrease soil PoC in rainy and as a result, it is necessary to supply K-fertilizer at this stage.

Besides, glyphosate treatment seems to be correlated to soil HDMs (Table 2 & Appendix). The herbicide promotes HDMs growth, rapidly changing soil microbial composition. This change was disappeared after 15 days of the survey.

Table 2. Summary of glyphosate seasonal effects on soil quality and notices

Glyphosate (4 L/ha)	Dry season	Rainy season	Glyphosate effects on soil	Dry season vs rainy season			Notice
				Groups	Before survey	After survey	
pH	ns	ns	Non-affected	T1 T2 T3 T4	Dry > rainy (**) ns ns ns	Dry < rainy (****) Dry > rainy (***) ns ns	Mowing grass increases soil pH in rainy vs dry season Removing grass decreases soil pH in rainy vs dry season. Glyphosate treatment is unharmful to soil pH
Soil moisture	T3 ↓ (**)	ns	Decreases soil moisture in dry season as removing grass in tree to tree interval and spraying at line interval	T3 T4	ns Dry < rainy (*)	Dry < rainy (***) Dry < rainy (***)	On low moisture soil, unadvisable to weed by glyphosate in dry season.
NiC	T3↓ (*) T4↓ (***)	T3 ↓ (****) T4↓ (****)	Decreases soil NiC in all seasons	All groups	ns	ns	NiC in all groups were decreased when weeding by glyphosate. Nitrogen fertilizer should be added in both seasons when weeding by glyphosate
PhC	T3 ↑ (***) T4↑ (**)	T3: ns T4: ns	Increases Phosphorus content in dry season	T1 T2 T3 T4 T5	Dry < rainy (*) ns ns ns ns	Dry > rainy (**) Dry > rainy (****) Dry > rainy (**) Dry > rainy (**) Dry > rainy (*)	For lands with low soil PhC, grass should not be mowed or removed in rainy season. Glyphosate decreases PhC in rainy season vs dry season. Due to washout, soil should be supplied with P-fertilizer in rainy season T3 is suitable for weeding, to save money and to protect soil environment
PoC	T3↑ (****) T4↑ (****)	T3: ns T4↓ (*)	Increases soil PoC in dry season. glyphosate whole spraying decreases PoC in rainy season.	T3 T4	ns Dry < rainy (**)	Dry > rainy (**) Dry > rainy (**)	Be aware of whole spraying glyphosate in rainy season. Should supply K-fertilizer in rainy season.
TSMs	ns	ns	Non-affected	All groups	No correlation	Has correlation (short-term)	Glyphosate increases soil HDMs to adapt to new stress and to degrade herbicide. After 15 days of survey, this correlation is disappeared
HDMs	ns	ns	Non-affected	All groups			

ns: no significant ↑↓ : increase or decrease
NiC: nitrogen content PhC: phosphorus content
PoC: potassium content TSMs: Total soil microbes
HDMs: Herbicide degrading microbes
**P value* < 0.05; ***P value* < 0.01; ****P value* < 0.001; *****P value* < 0.0001

4. CONCLUSION

In this study, glyphosate effects of a dose 4 L/ha on the soil quality in rubber farms within 15 days were surveyed. After herbicide treatment, soil pH, TSMs and HDMs were unchanged while soil NiC was impaired in both seasons. Moreover, soil moisture was decreased and either PhC or PoC were increased in dry season. In rainy season, glyphosate

did not affect soil moisture and PhC, however soil PoC was reduced. This study is a premise for further research on glyphosate-based weeding in other plants and could be an agricultural reference regarding seasonal effects of glyphosate on haplic acrisols. Nevertheless, it is necessary not to misuse glyphosate during crops and to develop non-chemical weedings which are more friendly to the environment.

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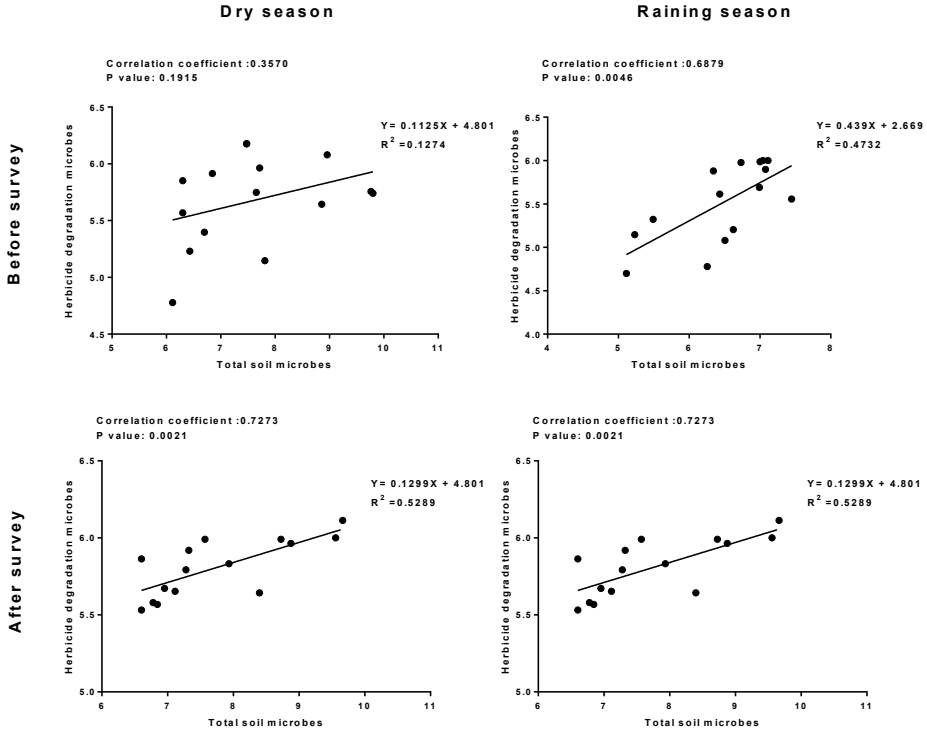
APPENDIX: The relation between total soil microbes (TSMs) and herbicide degrading microbes (HDMs)



TÓM TẮT

ĐÁNH GIÁ ẢNH HƯỞNG CỦA THUỐC DIỆT CỎ GLYPHOSATE ĐẾN MÔI TRƯỜNG ĐẤT TRÊN NÔNG TRƯỜNG CAO SU TẠI ĐỒNG NAI VÀ CỦ CHI

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Glyphosate được sử dụng phổ biến hiện nay trong nông nghiệp và nghiên cứu do có tác dụng ngăn ngừa cỏ dại phát triển. Những năm gần đây, vai trò của glyphosate dần trở nên quan trọng đối với các quốc gia có nền kinh tế nông nghiệp như Việt Nam. Nhằm đánh giá tác động của glyphosate đến môi trường đất trong nông trường cao su, nhóm tác giả đã khảo sát các chỉ số của đất trước và sau khi xử lý với glyphosate; các chỉ số bao gồm: pH, độ ẩm, N-P-K tổng số, vi sinh vật tổng số và vi sinh vật phân huỷ thuốc diệt cỏ trong các mùa khô và mùa mưa tại 2 nông trường cao su Đồng Nai và Củ Chi. Kết quả cho thấy, glyphosate không ảnh hưởng đến pH của đất, làm giảm độ ẩm của đất vào mùa khô, làm giảm nitơ tổng số trong đất, giúp tăng phospho tổng số và kali tổng số vào mùa khô. Glyphosate không ảnh hưởng đến vi sinh vật tổng số và sinh vật phân huỷ thuốc diệt cỏ trong đất. Kết quả của nghiên cứu này bước đầu cho thấy tính khả dụng của glyphosate trong nông nghiệp trồng cao su. Nghiên cứu cũng đã chỉ ra một vài khuyến cáo áp dụng khi phun glyphosate trong mùa khô và mùa mưa. Tuy nhiên, cần có cái nhìn đúng đắn hơn về liều lượng sử dụng đối với loại thuốc diệt cỏ này. Vì vậy, cần ưu tiên sử dụng và phát triển các biện pháp trừ cỏ không hoá chất nhằm giảm thiểu tác động tiêu cực đến hệ sinh thái.

Từ khoá: Glyphosate, nông trường cao su, thuốc diệt cỏ, môi trường đất.