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Chemical and Biological Properties of Paddy Soil Treated with Herbicides and Pyroligneous Acid

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Abstract

In this study, the effect of different herbicide x pyroligenous acid mixtures on soil chemical and biological properties was evaluated in order to elucidate its potential impacts on nutrient availability and soil quality. The experiment was conducted under greenhouse conditions consisted of 100-fold diluted wood vinegar (100 WV), 50% BCB (Bentazone cyhalofop-butyl), 100% BCB, 50% BCB+100 WV, 50% BCB+250 WV and 50% BCB+500 WV applied against *Echinochloa crusgalli*. Ten days after herbicide application, the chemical properties were altered and the changes were influenced by the amount of WV dilutions. The soil pH was near neutral to slightly alkaline coupled with decreased electrical conductivity (EC). The total carbon, available phosphorous, exchangeable magnesium, exchangeable sodium, exchangeable calcium, exchangeable potassium and cation exchange capacity (CEC) were relatively lower compared to the control. Biolog™ assay showed that different treatments resulted to variable microbial activity, Shannon-Weaver index and richness of carbon utilization potential. Principal component analysis showed segregation of samples based on extracted principal components indicating variable carbon utilization potential due to different treatments combination. Correlation analysis between soil and microbial properties revealed that Principal Component 2 has negative correlation with pH and available P. On the other hand, Principal Component 3 was positively correlated with total C, exch. Ca, Mg, Na, and CEC (0.475, 0.490, 0.555, 0.489, and 0.517, respectively). Application of pyroligneous acid combined with herbicides resulted to changes in soil chemical and biological characteristics which may have unique implications on nutrient availability and overall soil quality.

Keywords: bentazone cyhalofop-butyl, Biolog™, pyroligneous acid, soil chemical and biological properties

1. Introduction

The continuous use of environmentally persistent herbicides and other synthetic agricultural chemicals posed great risks to soil and water contamination. A viable alternative pesticide that is equally effective and less harmful to the environment is in demand. The role of pyroligneous acids (PA), also as plant and soil treatments gained the attention of research enthusiasts. Pyroligneous acid is a dark brown solution obtained as a by-product of wood carbonization. Large number of substances has been found in the pyrolysis liquids from different resources. The detected substances from the acids belong to different classes of organic compounds, namely, aldehydes, ketones, alcohols, organic acids, esters, derivatives of furan and pyran, phenolics, hydrocarbons and nitrogen compounds, in which the major ones are organic acids and phenolics (Souza et al., 2012). These compounds are known to increase herbicide efficacy (Kim et al., 2001).

The acidic nature of PA may have an effect on displacement of ions from soil exchange complex. An advantage of this would be improved nutrient availability. However, increased displacement would possibly result to leaching of substances through the soil profile (Jones, 1998; Andrade, 2003). For that reason, studies on the impact of the use of this kind of product are of great agricultural and environmental importance, and absolutely necessary to support the correct and safe application of chemicals (synthetic or alternative agricultural supplies) on the soil.

Soil microorganisms play an important role in soil-plant-herbicide-fauna-man interactions. They participate in herbicide degradation and activity, they may serve as bioindicators of changes in soil biological activity and, some microbial species may be used as bioherbicides. The microbial biomass can also represent a large pool of nutrients especially on less fertile sites (Giardina et al., 2000). Microorganisms can act as sinks, during immobilization and sources, during mineralization of labile nutrients (Garcia-Oliva et al., 1998; Stenstrom et al., 1998). The soil biota activity can be stimulated as an indirect result of the chemical changes in the soil.

There is no or limited scientific information with regard to the influence of pyroligneous acids coupled with synthetic herbicides on soil chemical and biological properties. Hence, the objective of this study was to evaluate the combined effect of different herbicide and pyroligenous acid mixtures on soil chemical and biological properties in order to elucidate its potential impacts on nutrient availability and soil quality.

2. Methods

2.1 Experimental Set Up

The experiment was conducted under greenhouse conditions at the Agricultural Experiment Station and Research Facility, College of Agriculture and Life Sciences, Kyungpook National University, Daegu, South Korea. The experimental set up was in a Completely Randomized Design (CRD) with three replications.

The treatments were: T1-Control, T2-100 WV (100-fold diluted wood vinegar), T3-50% BCB (Bentazone cyhalofop-butyl), T4-100% BCB, T5-50% BCB+100 WV, T6-50% BCB+250 WV (250-fold diluted wood vinegar) and T7-50% BCB+500 WV (500-fold diluted wood vinegar). The herbicide BCB and the WV was applied singly or consecutively by spraying. The wood vinegar (Table 1) was obtained from a local distributor. The planting media was composed of 1:1 paddy and sandy soil (ph = 6.69, EC = 72.4 uS/cm). Water depth in pots was maintained at 3cm on the soil surface during the experiment period. Treatment application was done at 2 to 3 leaf growth stage, approximately 6 to 8 days after transplanting of barnyard grass. The experiment was terminated 10 days after treatment application.

Table 1. Physical and chemical components of wood vinegar

Item	Wood vinegar
pH	2.97
Electrical conductivity	2313 uS/cm
Transparency	Transparent
Color	Golden brown
Methanol	0.31%
Phenolic compounds	2.44%
Propanoic acid	0.89%
Acetic acid	9.95%

Typically, wood vinegar is high in low-molecular weight acids (formic and acetic), alcohols (methanol) and aldehydes, which can serve as a carbon and energy resource for prototrophic bacteria occurring in the soil (Focht, 1999 as cited by Hagner, 2013). However, the high acidity, methanol and phenol content have strong bactericidal effect at a high concentration. Therefore, wood vinegar if applied in correct concentration, can enhance the soil biological properties.

2.2 Soil Sample Preparation and Analysis

Immediately after sampling, soil samples for microbial characterization were stored at 4 ºC until analysis while samples for chemical analyses were air-dried, ground, and passed through 2 mm sieve. Soil pH and EC were determined in 1:5 soil-water ratio (Thermo Scientific Orion Star A215 Benchtop pH/EC meter). Total organic C was determined using an elemental analyzer (ThermoFisher Flash 2000). Available P was determined following the method described by Pierzynski (2000). Air-dried soil samples (2.0 g) were added in 20 mL Bray and Kurtz P-1 extracting solution (0.025 M HCl in 0.03 M NH_4F) and were placed in a mechanical shaker (200 or more epm) for 5 minutes at room temperature. The extracts were filtered through Whatman No. 42 filter paper and were analyzed for phosphorus by ICP-MS (PerkinElmer NexION 300X). Soil exchangeable bases were extracted through ammonium acetate method according to Brix (2008). Air-dried soil samples (2.0 g) were added in 20 mL 1 M NH_4OAc and were placed in a mechanical shaker for 2 hours. The extracts were filtered through Whatman

No. 42 filter paper and were also analyzed by ICP-MS (PerkinElmer NexION 300X). Soil microbial activity was evaluated based on substrate utilization profiles that were established using BIOLOG EcoMicroPlate^{TM} (Biolog, Hayward, CA, USA). Soil sample (5 g) was suspended in 0.1 M NaH_2PO_4 solution (pH 6) at a ratio of 1:9 (w/v). The soil suspension was diluted 1,000-fold with 0.15 M NaCl and 100 µL of the diluted suspension was inoculated into each well. The microplates were incubated at 28 ºC and their absorbance was measured at an optical density of 590 nm using a Multiskan^{TM} Go Microplate Spectrometer (ThermoFisher, USA) every 24 h for 6 days. The absorbance of the 31 substrates was used to calculate the average well color development (AWCD) and the values were plotted against the incubation period of the plate (Ultra et al., 2012). The Shannon-Weaver index and richness of bacterial communities were calculated based on the absorbance readings of the microplate wells after 96 h of incubation.

2.3 Statistical Analysis

The data on soil properties were analyzed statistically following the analysis of variance (ANOVA) and the mean differences among treatments were compared by Fisher's Least Significant Difference (LSD) using the statistical computer package program, R-Software. On the other hand, AWCD, richness, and Shannon-Weaver index were statistically analyzed using the IBM SPSS Statistics ver. 21 for Windows. Mean values per treatment were compared using two-way analysis of variance. Tukey's honestly significant difference was performed to determine significant differences ($P < 0.05$). The optical density data from the Biolog EcoMicroPlate^{TM} were subjected to principal component analysis using SPSS.

3. Results

3.1 Soil Chemical Characteristics

The pH observed for all treatments was neutral to slightly alkaline and relatively lower compared to the control. Noticeable decrease in pH compared to the control was seen in soils treated with 50% BCB and 50% BCB + 250 WV. The EC was highest in soils treated with 100% BCB (Table 2). However, the result was comparable to other treatments except that of 50% BCB.

A general trend was seen in total C, available P, exch. Mg, exch. Na, exch. Ca, exch. K and CEC (Table 2) with the highest values obtained in control treatment. In terms of total C content, there were no significant differences observed among treatments. The data on available P, exch. Mg, exch. Na, exch. Ca, exch. K and CEC revealed similar trends. Relatively lower values were obtained compared to the control (Table 2).

Table 2. Effect of herbicide and pyroligneous acid application on the chemical properties of soils under greenhouse conditions

Treatments	ph	EC (*u*S/cm)	Total C (%)	avail. P	exch. Ca	exch. Mg	exch. K	exch. Na	CEC (cmol$_c$kg^{-1}soil)
				--------------------------------ppm-----------------------------					
Control	7.70 a	162.83 ab	0.39 a	10.32 a	4.57 a	1.41 a	0.12 a	0.40 a	6.49 a
100 WV	7.30 ab	141.10 ab	0.32 a	7.87 b	3.69 b	1.02 b	0.10 ab	0.33 b	5.14 b
50% BCB	7.28 b	119.37 b	0.29 a	6.89 b	3.48 b	0.98 b	0.10 ab	0.31 b	4.87 b
100% BCB	7.50 ab	163.10 a	0.29 a	7.80 b	3.90 ab	1.05 b	0.08 b	0.33 b	5.37 ab
50% BCB + 100 WV	7.46 ab	131.50 b	0.29 a	7.72 b	3.68 b	1.02 b	0.10 ab	0.31 b	5.11 b
50% BCB + 250 WV	7.17 b	122.73 ab	0.31 a	6.14 b	3.54 b	0.96 b	0.11 ab	0.31 b	4.93 b
50% BCB + 500 WV	7.30 ab	128.57 ab	0.31 a	7.66 b	3.26 b	0.92 b	0.09 ab	0.30 b	4.57 b

3.2 Soil Biological Characteristics

The highest AWCD on the Biolog EcoMicroplate^{TM} was observed under 50% BCB + 500 WV treatment after 4 days of incubation which indicates high microbial activity (Figure 1). In addition, an increased activity of soil microorganisms was also observed in the control. On the other hand, lower AWCD was observed in the 100% BCB treatment. A noticeable decrease in microbial activity was observed in 50% BCB+250 WV. This suggests that treatment combination synergistically affected the soil microorganisms. In terms of Shannon-Weaver index, there were no noticeable differences among treatments (Figure 2). However, a significant difference was observed in richness which was attributed to the 50% BCB treatment (Figure 3).

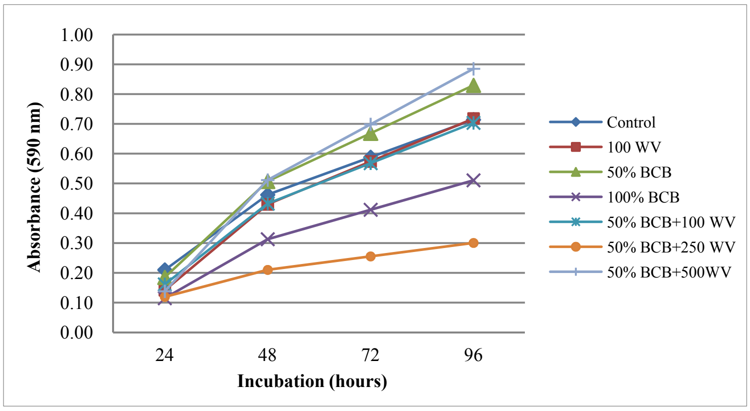

Figure 1. Effect of herbicide x pyroligneous acid application on the Average Well Color Development (AWCD) based on Biolog Ecomicroplate^{TM} absorbance data of soils under greenhouse conditions

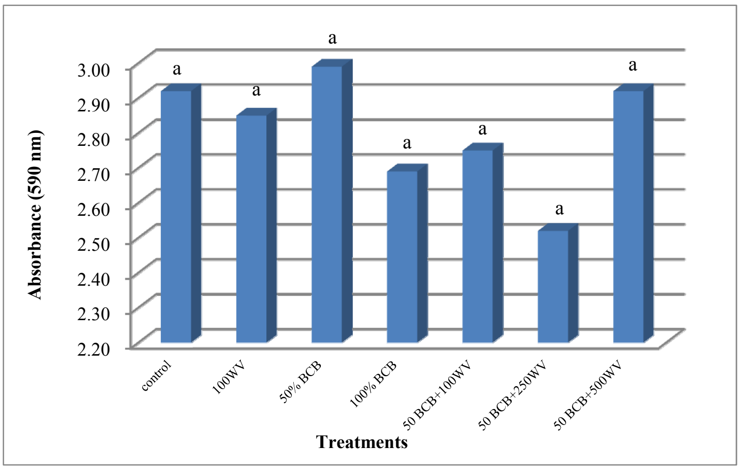

Figure 2. Influence of herbicide x pyroligneous acid application on the Shannon-Weaver index of soils based on Biolog EcoMicroplate^{TM} absorbance data at 96 h of incubation. Same letters indicate non significant differences according to Tukey's test ($P \leq 0.05$)

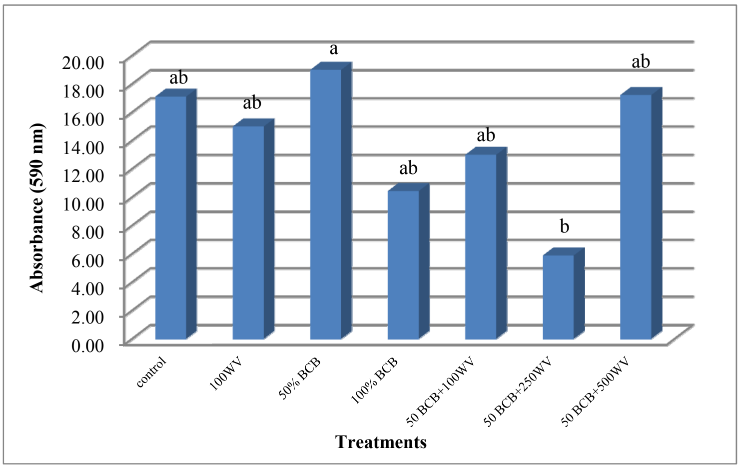

Figure 3. Richness of metabolized carbon of soils as affected by herbicide x pyroligneous acid application based on Biolog EcoMicroplate^{TM} absorbance data at 96 h of incubation. Same letters indicate non significant differences according to Tukey's test ($P \leq 0.05$)

The Biolog EcoMicroplate^{TM} readings at 96 hours were subjected to principal component analysis (PCA) to determine the extent of differentiation of microbial functional structure based on the carbon source utilization among soils from different treatments. This resulted in the extraction of 3 principal components (PC) contributing about 25%, 17%, and 10% to the data variance, corresponding to PC1, PC2, and PC3, respectively (Figure 4). The plots between PC1 and PC2 scores were able to differentiate the soil treated with 50% BCB+250 WV from other treatments (Figure 4a). On the other hand, the plots of PC1 and PC3 scores were able to differentiate the soils from the control and 50% BCB from other treatments (Figure 4b).

The carbon substrates were most heavily loaded based on the principal component analysis (Table 3). Twelve carbon substrates contributed to the separation of treatments along PC1. The highest utilization of these substrates was observed in 50% BCB+500 WV treatment. The carbohydrate D-mannitol was highly utilized by the microorganisms in all treatments except for 50% BCB+250 WV treatment. The carbon substrate distinct to PC2 was D-cellobiose which was highly utilized under the treatment combination 50% BCB+250 WV. The substrates unique to PC3 were 4-hydroxy benzoic acid, glycyl-L-glutamic acid, α-cyclodextrin, and glycogen, which in general, were less utilized as seen in all treatments.

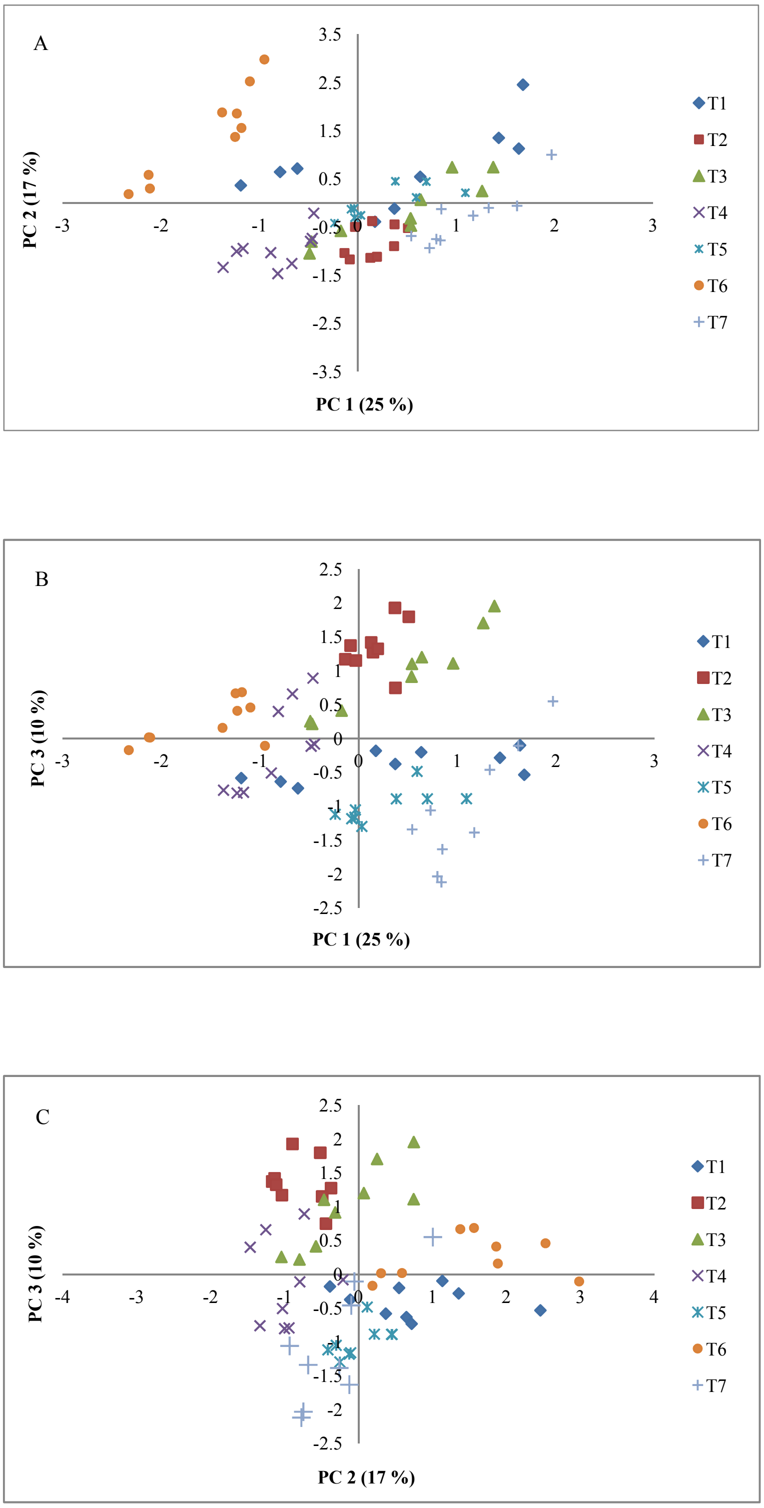

Figure 4. Principal component analysis based on the carbon source utilization among soils from different treatments (T1 – 100 WV, T2 – Control, T3 – 50% BCB, T4 –100% BCB, T5 – 50% BCB+100 WV, T6 – 50% BCB+250 WV and T7 – 50% BCB+500 WV)

Table 3. Carbon substrates most heavily loaded based on the principal component analysis

Carbon substrates	PC 1*	PC 2*	PC 3*
Carboxylic acids			
γ-hydroxy butyric acid	.580		
D-galactonic acid γ-lactone		.616	
2-hydroxy benzoic acid		.710	
α-keto butyric acid		.727	
4-hydroxy benzoic acid			.752
Carbohydrates			
β-methyl-D-glucoside	.738		
i-erythritol	.527		
D-mannitol	.707		
N-acethl-D-glucosamine	.846		
α-D-lactose	.597	.527	
D-xylose		.798	
D-cellobiose		.505	
Amino acids			
L-arginine	.516		
L-asparagine	.776		
L-serine	.777		
L-phenylalanine		.659	
Glycyl-L-glutamic acid			.720
Polymers			
Tween 40	.687		
α-cyclodextrin			.512
Glycogen			.761
Amines			
Phenylethyl-amine	.625		
Putrescine		-.578	
Phosphorylated chemical	.707		

Note. *PC 1, PC 2 and PC 3 are the principal components extracted from the principal component analysis (PCA) of the microbial community substrate utilization potential of herbicide-wood vinegar treated soils.

Table 4. Correlation analysis between the chemical and biological properties of soils applied with herbicide and wood vinegar

Properties	PC1	PC2	PC3	Richness	Shannon-Weaver Index
EC	-.003	-.372	.226	.056	.086
pH	.091	-.547*	.246	.242	.255
Total C	.352	.157	.475*	.320	.404
P	.136	-.519*	.231	.276	.175
Ca	.090	-.073	.490*	.125	.236
Mg	.147	-.178	.555**	.234	.309
Na	.122	-.077	.489*	.171	.282
K	.085	.388	.243	.048	.119
CEC	.107	-.089	.517*	.155	.260

Note. *$P < 0.05$; **$P < 0.01$.

4. Discussion

4.1 Soil Chemical Characteristics

The experimental set up mimicked a paddy soil condition wherein the soil was under waterlogged conditions. The soil system was generally quite buffered to the extent that waterlogging was not effective in changing the pH, though it has been known that in waterlogged soil, anaerobic conditions take place and as a result fermentation of carbohydrates occur releasing acids which shift the pH to the acidic side (Taha et al., 1967). In addition, the decrease in pH can be attributed to the acidic nature of wood vinegar and herbicides.

The lower values obtained in total C, available P, exch. Mg, exch. Na, exch. Ca, exch. K and C can be attributed to wood vinegar. Wood vinegar has the ability to reduce the cluster value of water. This means that the water is activated and can be easily absorbed by the plants because water with a low cluster value is in a very small mass. Each of these masses will hold one or few mineral elements which can be easily taken up by plants (http://www.agrowingculture.org).

Lower P contents observed on the treatments could be related to fixation in the soil, since it was alkaline in reaction (Taha et al., 1967). Phosphorous can be fixed in the form of calcium, magnesium, iron or aluminum phosphates and clay-adsorbed phosphorous (Mahdi et al., 2012). When alkaline soils were waterlogged, calcium phosphates and aluminum phosphates increased while iron phosphates decreased (IRRI, 1978). The best availability of phosphorous is in the range of pH 6.0 to 7.0. According to Foth (1978), calcium phosphates begin to precipitate at about pH 6.0. On the contrary, above pH 7.0, there is a reduction in phosphorus solubility or availability. Increases in pH above 7.0 create sufficient OH^- to react with $H_2PO_4^-$ to form HPO_4^{2-} and water to cause the latter form of phosphorus to become the most abundant. Since HPO_4^{2-} is less readily taken up by plants than $H_2PO_4^-$, one can conclude that part of the reduced availability of phosphorus in alkaline soils is due to the presence of hydroxyl ions and formation of HPO_4^{2-}. The chemical components of the wood vinegar may have contributed to the production of OH^- reacting with $H_2PO_4^-$ resulting to unavailable forms of phosphorus (Foth, 1978). In addition, soluble $H_2PO_4^-$ quickly reacts with calcium to form a sequence of products of decreasing solubility (Mahdi et al., 2012).

4.2 Soil Biological Characteristics

The high microbial activity observed under 50% BCB+500 WV treatment could be attributed to low levels of herbicide and wood vinegar. The low concentrations of alcohols, acids, and aldehydes in the wood vinegar served as carbon and energy substrates for soil microorganisms (Steiner et al., 2008). The increased microbial activity under the control treatment was probably due to root exudates released by barnyard grass which provide nourishment to the soil microflora. In contrast, application of 100% BCB decreased the activity of soil microorganisms. This reduction in microbial activity, coinciding with withering plant biomass, could have resulted from drastically reduced root exudates that serve as a resource of the microorganisms in the soil. A shortage in root exudates can lead the rhizosphere microbes to enter a dormant, inactive stage (Hagner, 2013). Therefore, a decline in microbial activity can be observed. This also indicates that 100% application of herbicides is detrimental to soil microorganisms. The Shannon-Weaver index and richness were obtained after 96 hours of incubation. The Shannon-Weaver index is an indicator of the microbial functional diversity in soil. On the other hand, richness refers to the carbon utilization by microorganisms. Based on the results, all treatments did not significantly affect the microbial functional diversity in soil. However, a significant difference was observed in richness which was attributed to 50% BCB treatment. This can be a reflection of the availability of nutrients in the soil that were utilized by microorganisms. In general, herbicides affect soil microbes indirectly. They can be a source of nutrition for microbes or when applied in high doses, may kill microorganisms. The lower rate of BCB application and also its combination with 500 WV favored microbial activity in the soil. Related studies showed that microbes propagate well when higher dilution of wood vinegar was applied. An experiment conducted by Steiner et al. (2008) revealed significant increase in microbial biomass and population growth rate when PA was applied in highly weathered soils. This is mainly due to the effect on the metabolism by acetic acid. Acetyl co-enzyme is produced by plants and microbes from acetic acid. Through the TCA cycle, acetyl co-enzyme is converted into citridic acid, malic acid, fumaric acid, succiric acid and other elements that are necessary for the plants and microbes (Nelson & Cox, 2012). This is the main reason behind the propagation of microbes. The herbicide and wood vinegar may have worked synergistically for microbial growth and multiplication (http://www.agrowingculture.org).

The variability in PC1 can be explained by the high utilization of five carbohydrates and three amino acids. The carbohydrates include β-methyl-D-glucoside, i-erythritol, D-mannitol, N-acetyl-D-glucosamine and α-D-lactose. On the other hand, L-arginine, L-asparagine and L-serine are the amino acids responsible for the variation in

PC1. Variability in the second PC can be attributed by responses to carboxylic acids, carbohydrates and amine (Table 3).

Correlation analysis between soil and microbial properties were also evaluated (Table 4). Results revealed that PC2 had negative correlation with pH and available P. This implies that the set of particular microbial communities that utilized the carbon substrates in the Biolog Microecoplate^{TM} that contributed to PC 2 are pH-dependent and can be influenced by the availability of P. As cited by Bunemann et al. (2011) microorganisms require or preferentially use inorganic forms of P tying up the nutrient in microbial biomass, a process called immobilization. Microbial P immobilization can affect P availability by removing inorganic P from the soil solution, especially when soluble carbon is available for microbial growth. On the other hand, PC3 was positively correlated with total carbon, exch. Ca, Mg, Na, and CEC. The microbial communities that utilized the substrates that were distinct to PC 3 could be related to the availability of total carbon, exch. Ca, Mg, Na, and CEC. This implies the role of mineral nutrition on microbial growth. The alteration of substrate quality due to the addition of wood vinegar triggered the shift of soil microbial functional structure.

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