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Full Length Article

Soil Chemical and Microbial Properties and its Relationship with the Root Growth of *Panax ginseng*

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Abstract

In this study, the relationship between soil chemical and microbial properties and ginseng root growth was determined. Rhizosphere soil and ginseng root samples were collected from three different ginseng farms within Gyeongsangbuk-do, South Korea. Our results showed that available phosphorus, magnesium and cation exchange capacity (CEC) in soils have significant correlation with the overall growth of ginseng roots. Soil chemical properties such as pH, percent total C and N, C:N ratio, available P, exchangeable Ca, K and Na were also found to have correlation with soil microbial properties such as PC 1, PC 2, PC 3, AWCD and richness of metabolized carbon based on Biolog EcoPlate™. However, direct correlation between soil microbial properties and ginseng root growth was not observed. Establishment of the relationship between soil chemical and microbial properties with ginseng root growth is important to fully understand the factors affecting nutrient availability in ginseng cultivated soils which could help identify cultural strategies that would enhance the root growth and quality of *P. ginseng*.

Keywords: Ginseng root growth; Soil microbial functional structure; Nutrient availability; Cultivation area

Introduction

Panax ginseng C.A. Meyer is a valuable medicinal plant that has been traditionally used in Oriental medicine. Its root is the main part used for medicinal purposes and its efficacy has been recognized for thousands of years (Keifer and Pantuso, 2003; Helms, 2004). At present, many scientists have already asserted various pharmacological effects on ginseng which includes efficacy in improving cerebral functions, relieving pain, preventing cancers, increasing immune functions, anti-diabetic efficacy, etc (Choi, 2008). The main active constituent in *P. ginseng* that is responsible for its multifunctional properties is known as ginsenosides. Several studies have reported that the qualitative and quantitative characteristics of these ginsenosides from roots may vary depending on its cultivation area (Lee *et al*., 2011; Lee *et al*., 2011; Chung *et al*., 2012) while a study made by Shi *et al*. (2007) reported that the changes in ginsenoside content with age may also be related to the growing area of ginseng roots. Also, the growth of ginseng roots varies depending on its area of cultivation. According to Khrolenko *et al*. (2006), cultivated *P. ginseng* develops much faster than those naturally grown ginseng plants. Choi *et al*. (2007) also stated that mountain-cultivated *P. ginseng* grows very slowly compared to field-cultivated *P. ginseng*. These results were mostly obtained by classification and characterization of various ginseng cultivation areas but only through physiological and chemical analysis. Understanding not only the soil's chemical properties, but also its microbial properties is important because the interaction between plant and soil microorganisms in the rhizosphere is a determinant of plant health and soil fertility (Hayat *et al*., 2010). Soil is a complex ecosystem wherein microorganisms have significant influence on plants as they are involved in various microbial functions such as transformation of soil chemical properties, degradation of organic compounds, transformation of soil physical structure and enhancement of plants' nutrient uptake (Lopes *et al*., 2011). Soil microorganisms play an important role in converting essential nutrients in soil from organic to inorganic forms that are soluble for plant uptake and this decomposition of soil organic matter is affected by soil properties such as pH, moisture, particle size and soil depth (Hayat *et al*., 2010; Hoorman and Islam, 2010; Fontaine *et al*., 2003). Plants may also contribute to the uniqueness of soil quality as the growing roots can promote a large population of particular soil microorganisms depending on the plants' needs (Bais *et al*., 2001; Bertin *et al*., 2003).

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In this study, the chemical and microbial properties in soils which promote growth of *P. ginseng* roots were determined. The relationships between soil chemical and microbial properties with ginseng root growth were investigated to understand the factors affecting nutrient availability in soils which may have an overall effect on the growth of *P. ginseng* roots. Analysis of the microbial functional structure in soils collected from different *P. ginseng* farms partially explained the factors that contribute to the uniqueness of soils cultivated with *P. ginseng*.

Materials and Methods

Sample Collection and Preparation

Ginseng roots and rhizosphere soil samples were collected in triplicate on May 29, August 2 and October 2, 2013 coded as D1, D2 and D3, respectively from Punggi, YeongJu; Sogok, SangJu; and Naeye, Gumi coded as S1, S2 and S3, respectively within Gyeongsangbuk-Do, South Korea. Ginseng roots were rinsed with distilled water and were tap-dried. The lengths, diameters and fresh weight of the ginseng roots were recorded. Soil samples were cleaned by removal of visible root debris and were homogenized by manual mixing. Soil samples for microbial community-level physiological profiling were immediately stored at 4ºC until analysis while samples for chemical analysis were air-dried, ground and passed through 2 mm sieve.

Physicochemical Soil Analysis

Soil pH was measured with Thermo Scientific Orion Star A215 Benchtop pH meter at a ratio of 1:2 with distilled water (Hendershot *et al*., 2006). Total organic C and N were determined using a ThermoFisher Flash 2000 automatic elemental analyzer (Thermo Scientific, USA) in triplicate per sample. Available phosphorus in soil was extracted using Bray and Kurtz P-1 solution based on 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 rpm) for 5 min at room temperature. The extracts were filtered through Whatman No. 42 filter paper and were analyzed for phosphorus by ICP using blank and standards prepared in the Bray P-1 extracting solution. Soil exchangeable bases were extracted with 1 M ammonium acetate solution (pH 7) from the modified procedure described by 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 h. The extracts were filtered through Whatman No. 42 filter paper and were analyzed by ICP using blank and standards. Extracted P and exchangeable bases were analyzed using an ICP-OES (PerkinElmer Optima 7300 DV, USA).

Soil Microbial Community-level Physiological Profile

Soil microbial activity was evaluated based on substrate (sole carbon source) utilization profiles that were established using BIOLOG EcoPlate™ (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 of the microplate. The microplates were incubated at 28ºC and their absorbance was measured at an optical density of 590 nm using a Multiskan™ Go Microplate Spectrometer (ThermoFisher, USA) every 24 h for 7 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 following the procedure described by Ultra *et al*., 2012.

Statistical Analysis

The data on soil properties, AWCD, richness and Shannon-Weaver index were statistically analyzed using the IBM SPSS Statistics ver. 21 for Windows. Two-way analysis of variance (ANOVA) were performed to determine treatments effects and Tukey's honestly significant difference (HSD) was used to determined significant differences between treatments ($P < 0.05$). The optical density (OD) data from the Biolog EcoPlate™ were subjected to principal component analysis using SPSS (Ultra *et al*., 2012).

Results

Soil Nutrient Analysis and Root Measurements

The chemical properties of soils collected from S1, S2 and S3 on D1, D2 and D3 are shown in Table 1. The pH of soils collected from S1 and S3 were slightly acidic and did not vary significantly during D1, D2 and D3 while soils collected from S2 exhibited neutral pH, however slightly fluctuated from pH 7.15 to pH 6.94 and 6.81 on D2 and D3, respectively. The percent total carbon and percent total nitrogen of the soils collected from S3 were significantly higher compared to S1 and S2. In general, the percent total carbon and nitrogen of the soil samples increased from D1 to D2, but declined on D3. However, the percent total carbon of soils from S3 decreased from D1 to D3. Soils collected from S3 generally had the highest available phosphorus and potassium while soils from S2 had the highest exchangeable calcium. Exchangeable sodium in soils collected from S1, S2 and S3 decreased from D1 to D2, however increased on D3. The exchangeable magnesium and CEC of the soils collected from S2 and S3 were higher compared to soils from S1.

Table 1: Chemical properties of rhizosphere soil collected from Punggi, Yeong-Ju (S1); Sogok, Sang-Ju (S2) and Naeye, Gumi (S3) within Gyeongsangbuk-do, South Korea on May 29 (D1), August 2 (D2) and October 2 (D3), 2013[1]

Sampling date	pH	Total C (%)	Total N (%)	C/N ratio	Available P (mg kg^{-1}, Bray P1)	CEC	Ca^{2+}	K^{+}	Mg^{2+}	Na^{+}
						($cmol_c$ kg^{-1})				
Punggi										
May 29	5.93a	0.43b	0.034b	12.76a	13.43b	8.48a	6.12a	0.24b	1.27a	0.86b
August 2	5.62a	0.46b	0.036b	13.04a	9.23a	8.56a	6.77b	0.26b	1.38a	0.14a
October 2	5.64a	0.25a	0.0023a	116.27c	11.10a	9.63b	6.95b	0.24b	1.37a	1.09b
Sogok										
May 29	7.15c	0.43b	0.017b	26.54b	59.05c	13.09c	9.27c	0.17a	2.78b	0.87b
August 2	6.94b	0.57c	0.041c	13.89a	61.29c	12.93c	9.57c	0.20b	3.09b	0.17a
October 2	6.81b	0.34b	0.0020a	190.65c	93.15d	13.40c	9.24c	0.20b	2.94b	1.02b
Naeye										
May 29	5.63a	1.09e	0.11b	10.29a	186.23e	10.47b	5.82a	0.86d	2.71b	1.08b
August 2	5.54a	0.96ed	0.12b	7.97a	174.05e	13.67c	8.74b	0.79d	3.93c	0.21a
October 2	5.83a	0.66d	0.0017a	431.53c	207.35f	9.73b	6.22a	0.43c	2.11b	0.97b

[1] Means within the same column followed by the same letter(s) are not significantly different from each other based on Tukey's test at 5% level of significance

Table 2: Root measurements of ginseng collected Punggi, Yeong-Ju (S1); Sogok, Sang-Ju (S2) and Naeye, Gumi (S3) within Gyeongsangbuk-do, South Korea at two sampling periods - May 29 (D1) and August 2 (D2), 2013[1]

Sampling date/Site	Root Length (cm)		Root Base Diameter (cm)		Roots Fresh Weight (g $plant^{-1}$)	
	May 29 (D1)	August 2 (D2)	May 29 (D1)	August 2 (D2)	May 29 (D1)	August 2 (D2)
Punggi (S1)	10.67 1.15a	18.67 ± 3.21a	7.21 ± 2.85a	12.26 ± 2.32a	1.90 ± 0.55a	7.21 ± 2.23a
Sogok (S2)	18.17 ± 0.58b	18.67 ± 3.06a	9.28 ± 0.32a	17.48 ± 1.27b	4.74 ± 0.45b	10.24 + 1.05b
Naeye (S3)	14.00 ± 2.29a	22.00 ± 3.00b	12.16 ± 0.81b	23.19 ± 8.43c	10.94 ± 0.84c	55.21 ± 6.54c

[1] Means within the same parameter followed by the same letter are not significantly different based on Tukey's test at 5% level of significance. Data are means and standard deviation of triplicate analysis

Measurement of the ginseng roots in terms of length, diameter and fresh weight are shown in Table 2. The ginseng roots collected from S1 and S2 were 4 years old while ginseng roots from S3 were 5 years old. As expected, on D1, the ginseng roots collected from S3 exhibited larger root size than the roots collected from S1 and S2. On the other hand, comparing the size of the ginseng roots collected from S1 and S2, which have the same age of cultivated ginseng, showed that roots collected from S2 were larger. From the comparison of the measurements of roots collected from S1, S2 and S3 on D1 and D2, it was observed that the rate of growth of ginseng roots was highest in S3. There was no significant difference found on the growth rate of ginseng roots collected from S1 and S2, however, roots collected from S2 on D2 are still larger in size than that of S1.

Table 3: Correlations of ginseng root measurements with soil chemical and microbial properties of rhizosphere soils collected from ginseng plantations[1]

Soil Properties	Root length	Root diameter	Root Fresh weight
pH	0.185	0.077	-0.302
Total C (%)	0.249	0.393*	0.529**
Total N (%)	0.157	0.294	0.553**
C/N ratio	0.213	0.121	-0.003
Available P	0.462*	0.548**	0.575**
CEC	0.520**	0.489**	0.418*
Exchangeable Cations			
Ca^{2+}	0.475*	0.372	0.202
K^{+}	0.169	0.326	0.562**
Mg^{2+}	0.595**	0.621**	0.631**
Na^{+}	-0.467*	-0.418*	-0.346
Microbial Properties			
PC1	0.105	0.063	-0.215
PC2	-0.124	-0.177	-0.068
PC3	-0.311	0.002	-0.008
AWCD	-0.169	-0.108	-0.212
Richness	-0.040	-0.085	-0.246
Shannon-Weaver index	0.186	-0.214	0.024

*$P < 0.05$, **$P < 0.01$

PC 1, PC 2, and PC 3 are the principal components extracted from the principal component analysis of the microbial community substrate utilization potential of ginseng cultivated soils

Microbial Community-level Physiologic Profile

The average well color development (AWCD) on the Biolog EcoPlate™ inoculated with soils collected from S3 was higher compared with those from other sampling sites however a large decrease in AWCD was observed on D2 and D3 (Fig. 1-C). Similarly, the AWCD of soils collected from S2 gradually decreased from D1 to D3 (Fig. 1-B). On the other hand, soils collected from S1 had an increase in AWCD from D1 to D2, but had a decrease on D3 (Fig. 1-A). For 7 days incubation, a great increase was observed on the AWCD of soils from S1 and S2 on D1 and D2, but not on D3. For soils collected from S3, great increase on AWCD was observed only on D1.

Different trends were observed on the richness of metabolized carbon in soils collected from S1, S2 and S3 at three different sampling periods (Fig. 2). On D1, soils

Table 4: Correlations of soil chemical properties with soil microbial properties

Soil chemical properties	PC1	PC2	PC3	AWCD	Richness	Shannon-Weaver index
pH	0.521**	-0.304	-0.381	0.057	0.100	0.029
Total C (%)	0.184	0.418*	0.475*	0.539**	0.507**	0.236
Total N (%)	0.145	0.414*	0.282	0.439*	0.410*	0.212
C/N ratio	-0.389*	-0.178	0.074	-0.352	-0.385*	-0.051
Available P	0.020	0.287	0.382*	0.291	0.196	0.212
Exchangeable cations						
Ca^{2+}	0.297	-0.351	-0.537**	-0.208	-0.157	0.051
K^{+}	-0.015	0.510**	0.467*	0.426*	0.322	0.234
Mg^{2+}	0.263	0.036	-0.086	0.165	0.132	0.277
Na^{+}	-0.068	0.149	0.425*	0.181	-0.065	0.145
CEC	0.309	-0.136	-0.271	0.016	-0.024	0.216

**P < 0.05, **P < 0.01*

PC 1, PC 2, and PC 3 are the principal components extracted from the principal component analysis of the microbial community substrate utilization potential of ginseng cultivated soils

collected from S3 had the highest richness of metabolized carbon among the three sampling sites, however, a large decrease was observed on D2 and a slight increase on D3. The richness of metabolized carbon of soils collected from S1 increased on D2 however decreased on D3, while the richness of metabolized carbon of soils collected from S2 decreased on D3.

There was no significant difference found on the Shannon-Weaver index of the soils collected from S1 and S2 at three sampling periods (Fig. 3). On D1, the soils collected from S3 had slightly higher Shannon-Weaver index but decreased on D2.

The Biolog EcoPlate™ readings at 96 h 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 sampling sites and periods. The first and second principal component (PC 1 and PC 2) explained 65% and 11% of the data variance, respectively; while PC 3 explained 4% of the data variance. Biplots between PC 1 and PC 2 scores showed that the soils collected from S1 clustered to the negative axis of PC 1 throughout the three sampling periods; however, soils collected from S2 and S3 clustered to the positive axis of PC 1 on the first sampling period (Fig. 4). Soils collected from S2 on D2 still clustered on the positive axis of PC 1, while soils collected from S3 on the same date clustered on the negative axis of PC 1. Soils collected from S2 and S3 on D3, together with the soils from S1, clustered on the negative axis of PC 1. Biplots between PC 1 and PC 3 scores also showed the same results (Fig. 5). However, in biplots between PC 2 and PC 3 scores, only soils collected from S3 on D1 were located on the positive axis of PC 2 (Fig. 6).

The carbon substrates utilized by the microbial community that contributed to the clustering of the PC scores of soil samples that were unique to PC 1 were D-galactonic acid γ-lactone, D-galacturonic acid, 4-hydroxy benzoic acid, D-glucosaminic acid, itaconic acid, L-arginine, L-asparagine, L-serine, tween 80, putrescine, pyruvic acid methyl ester. The carbon substrates 2-hydroxy benzoic acid, α-D-lactose, L-phenylalanine, L-threonine, α-cyclodextrin glycogen and glycogen were unique to PC 2, while β-methyl-D-glucoside was unique to PC 3.

Correlations of Root Growth with Soil Nutrient and Microbial Properties

Correlations of root growth with soil nutrient parameters and soil microbial properties as determined by Biolog EcoPlate™ are presented in Table 3. Some soil nutrient properties were found to have correlation with the growth of ginseng roots; however, there was no direct correlation found with root growth and soil microbial properties. Ginseng root diameter and weight positively correlated with percent total carbon; root weight positively correlated with percent total nitrogen; exchangeable calcium and potassium positively correlated with root length and root weight, respectively; and available phosphorus, magnesium, total CEC positively correlated with root length, diameter and weight. Exchangeable sodium was found to have negative correlation with root length and diameter. Correlations with soil chemical properties and microbial properties were also determined (Table 4).

PC 1 positively correlated with soil pH; PC 2, PC 3, AWCD and richness positively correlated with percent total carbon; PC 2, AWCD and richness positively correlated with percent total nitrogen, while PC 1 and richness negatively correlated with carbon to nitrogen ratio; PC 3 positively correlated with available phosphorus but negatively correlated with exchangeable calcium; PC 2, PC 3 and AWCD positively correlated with exchangeable potassium; and PC 3 had positive correlation with exchangeable sodium.

Discussion

Ginseng roots are the main part used for medicinal purposes and several studies have attributed its quality to its ginsenoside content. Previous study showed that the changes in ginsenoside content with age may be related to the growing area of ginseng roots (Shi *et al*., 2007) and may also vary depending on the condition and type of soil (Lee *et*

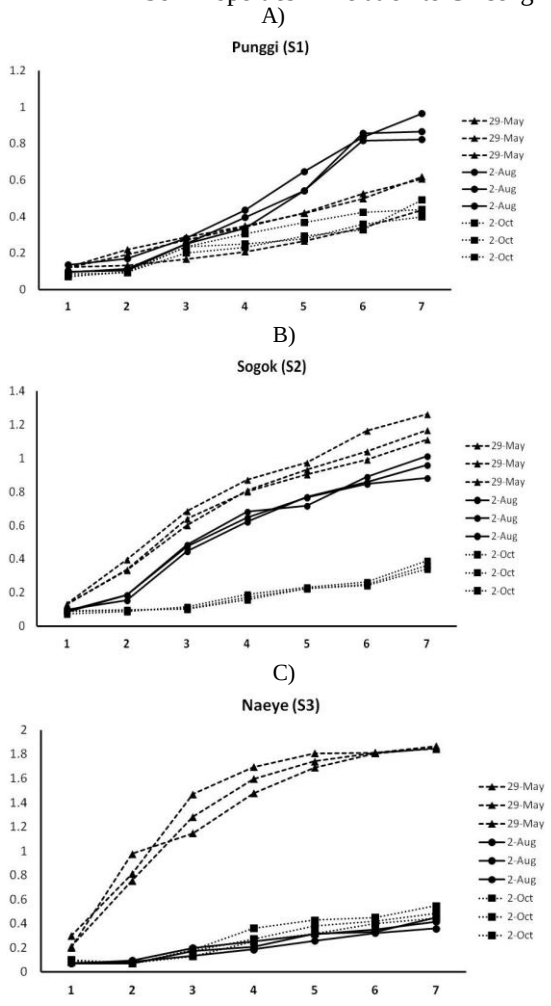

Fig. 1: Average well color development (AWCD) based on Biolog EcoPlate™ absorbance data of soils collected from A) Punggi B) Sogok and C) Naeye in three replicates at three sampling periods (May 29, August 2, October 2, 2013)

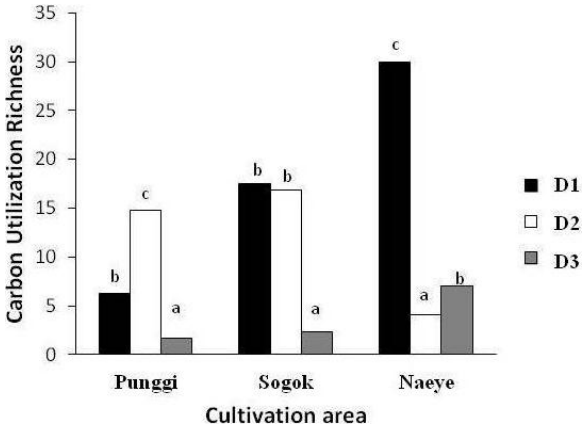

Fig. 2: Richness of metabolized carbon of soils collected from Punggi, Sogok, and Naeye on May 29 (S1), August 2 (S2), and October 2, 2013 (S3) based on Biolog EcoPlate™ absorbance data at 96 h of incubation. Same letters indicate non significant differences according to Tukey's test ($P \leq 0.05$)

al., 2011). As expected, roots collected from S3 on the first sampling period (D1) were larger than the roots collected from S1 and S2 due to its age. To determine the effect of soil nutrient status and some chemical properties on the growth of ginseng in their natural growing conditions, we compared the soil attributes between S1 and S2 because the ginseng grown in this area have relatively the same age and the same variety. Based on soil analysis, S2 have more desirable soil chemical properties than soils from S1 resulting to better growth of ginseng. Soils from S2 have near neutral pH throughout the growing period (May to October) and have relatively higher nutrient concentration compared to S1 as indicated by higher exchangeable cations, CEC and P content. Root growth was positively correlated with available P, Mg and total CEC while exchangeable Ca was significantly correlated with ginseng root length. These data showed that the growth of ginseng roots was affected by these nutrients in soil. High available P in soils promotes good root development of ginseng. This can be associated with a study which reported that phosphorus content in soils is a major limiting factor in ginseng growth as low levels of essential mineral phosphorus retards the growth of ginseng in mountainous soils (Rao and Terry, 1989; Kirschbaum and Tompkins, 1990; Jacob and Lawlor, 1991; Choi *et al*., 2007). Aside from available P, exchangeable Ca, Mg, and total CEC were also higher in soils collected from S2 than in soils collected from S1. Correlations of available Mg in soil with the overall root growth of ginseng together with available P can be related to the use of magnesium ions by plants for phosphorus metabolism (British Columbia Ministry of Agriculture, 2003). Cation exchange capacity (CEC) is a useful indicator of fertility in soils as it indicates the capacity of the soil to supply magnesium, calcium and potassium, which are relevant to ginseng root growth. Exchangeable sodium in soils however was found to have negative correlations with root length and diameter which is consistent to the negative effects of salinity on root growth (Kopyra and Gwóźdź, 2003). Percent total C, N and exchangeable K in soil were also found to be essential in the growth of ginseng roots as indicated by their significant correlation with its fresh weight (Table 3). Higher nutrient availability in S2 could be partly explained by its more neutral soil pH compared to soils from S1. Soil pH near neutral is considered to be the most optimum pH for crop growth because at this pH, most of the nutrients are at optimum availability (Marschner, 2012).

Several studies have demonstrated the significance of soil microbial functions and its role to nutrient availability and overall soil health that is necessary for sustainable productivity (Torsvik and Øvreås, 2002; Bowles *et al*., 2014; Hunter *et al*., 2014). Since ginseng production entails longer

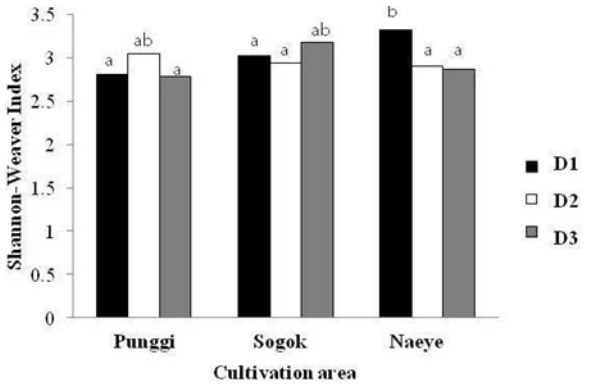

Fig. 3: Shannon-Weaver index of soils collected from Punggi, Sogok, and Naeye on May 29 (S1), August 2 (S2), and October 2, 2013 (S3) based on Biolog EcoPlate™ absorbance data at 96 h of incubation. Same letters indicate non significant differences according to Tukey's test ($P \leq 0.05$)

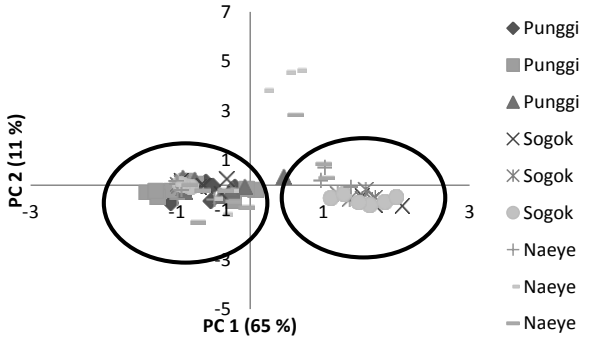

Fig. 4: Biplots of PC 1 and PC 2 extracted from the principal component analysis (PCA) of the carbon utilization profile at 96 h of incubation of Biolog EcoPlate™. Points within a group have comparable microbial structures

duration compared to commonly grown perennial crops, we hypothesized that there exist a unique microbial community in soils devoted for ginseng plantations. Based on our analysis, the soils devoted for ginseng production have unique soil microbial properties as reflected from the Biolog EcoPlate® microbial physiologic profile and activity. The microbial properties significantly differed between sampling sites and within sampling site at different sampling period which correspond to differences brought about by inherent soil properties as well as the influence of plant growth to microbial communities and vise versa (feedback mechanisms). Significantly higher microbial activity in soils collected from S3 on the first sampling period (D1) may indicate that microbial activity in soils increase in direct relation to the age of ginseng plants due to the increasing root biomass. One possible reason is the accumulation of the supply of root deposition in time that may enhance microbial activity (Xiao *et al*., 2014). This can also be

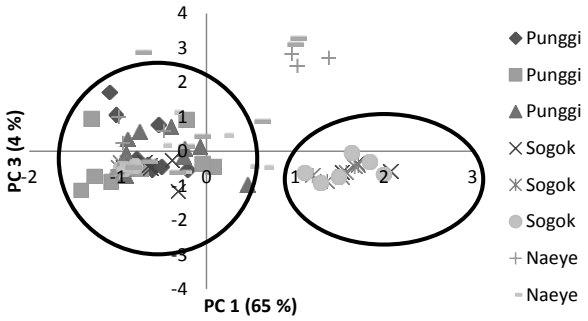

Fig. 5: Biplots of PC 1 and PC 3 extracted from the principal component analysis (PCA) of the carbon utilization profile at 96 h of incubation of Biolog EcoPlate™. Points within a group have comparable microbial structures

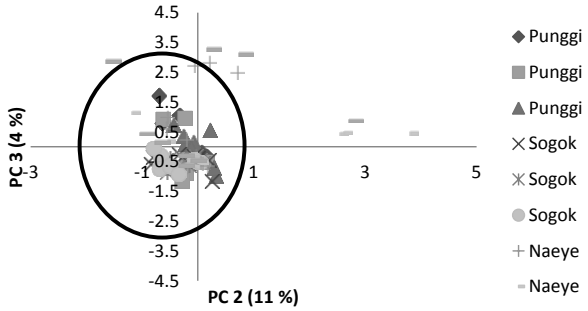

Fig. 6: Biplots of PC 2 and PC 3 extracted from the principal component analysis (PCA) of the carbon utilization profile at 96 h of incubation of Biolog EcoPlate™. Points within a group have comparable microbial structures

supported by higher percent total carbon in soils collected from S3 (Table 1). Soil microorganisms are carbon limited, and growing roots are a significant source of carbon for microbial biomass (Grayston and Jones, 1996). In addition, the availability of plant nutrients is regulated by these microorganisms through mineralization of soil organic matter and solubilization of soil minerals (Hunter *et al*., 2014; Pii *et al*., 2015). Therefore, increasing age of ginseng plants may enhance microbial activity in soils due to its root depositions which in turn may regulate the availability of soil nutrients that may result to higher growth rate of ginseng roots over the years (Thuille *et al*., 2015). This can explain the higher growth rate of ginseng roots collected from S3. Comparing the ginseng roots collected from S1 and S2, which have the same age of ginseng plant, there was no significant difference on the growth rate of ginseng roots collected from the two cultivation areas, however, roots collected from S2 are larger compared to the roots collected from S1.

Observations under field conditions showed general reduction of growth rate of ginseng from D1 to D3 in all site especially at early autumn (October) where almost all

ginseng plants have withered. This naturally occurs to ginseng plants as they are known to be deciduous and the roots and buds are expected to remain dormant throughout winter. The decline of the microbial activity in the soil samples observed from D1 to D3 as indicated by AWCD, wherein the microbial activity for the three locations was lowest on D3, may be due to the very low plant and root activity during its dormancy period. Low root activity during dormancy may result to the decline in root exudation that may decrease the availability of substrates for microbial growth (Lynch and Whipps, 1991). According to Badri and Vivanco (2009), root exudates, which are a major source of soil organic carbon released by plant roots, regulate soil microbial community in their immediate vicinity (Bais *et al*., 2006). The decline of the microbial activity in soils could be related to the decline of the percent total C and N in soils observed on D3. Also, significant correlation was found between soil percent total C and N with AWCD and richness of metabolized carbon. Carbon and nitrogen are two of the major components of soil organic matter (SOM) which provides energy for microorganisms through decomposition (Hoorman and Islam, 2010). During the summer season with hot and humid temperature (D2), increased microbial activity had probably used up most SOM which caused the decrease of the percent total C and N on D3. Aside from percent total C and N, exchangeable K also had positive correlation with AWCD while CN ratio had negative correlation with richness of metabolized carbon. Richness of metabolized carbon indicates the number of microorganisms in soil. High CN ratio could result to slow decomposition of SOM by soil microorganisms thus the negative correlation between the two (Janssen, 1996).

PCA of the normalized Biolog EcoPlate™ data was able to distinguish the differences between the microbial functional structures of the soils collected from S1, S2 and S3. Biplots between PC 1 and PC 2 and PC 1 and PC 3 showed the changes in the microbial functional structure in soils collected on D1 to D3. During the first sampling period (D1), comparable carbon source utilization by the microbial community in soils was found between S2 and S3. However, the microbial structure changed in time as the PC scores of the soils collected from S2 and S3 on D3 clustered towards the negative axis of PC 1. Comparable carbon source utilization by the microbial community in soils was found between S1, S2 and S3 soils collected on D3. In the biplots between PC 2 and PC 3 scores, only soils collected from S3 on D1 were found on the positive axis of PC 2. The results may indicate that PC 1 is more responsible for the differences in the microbial functional structure of ginseng cultivated soils. The highly utilized carbon substrates that contributed to PC 1 can serve as a unique fingerprint for a particular microbial community that contributes to the uniqueness of ginseng cultivated soils.

PC 1, PC 2 and PC 3 extracted from the PCA of the soil had no direct correlation with the growth of ginseng roots; however, correlations between the extracted PCs and soil chemical properties were found. Analysis of the correlation between the extracted PCs and soil chemical properties can partially elucidate the factors that may contribute to the uniqueness of the soil microbial community that can promote the growth of ginseng roots. Percent total C and percent total N in soils, which are significantly correlated with the growth of ginseng root, were found to be correlated with PC 2 and PC 3 and PC 2, respectively. PC 3 was found to have positive correlation with available phosphorus but negative correlation with exchangeable calcium. This result can be associated to the study made by Choi *et al*. (2007) which reported high calcium content but very low amount of phosphorus in ginseng cultivated mountain soils. In relation, the substrate that was highly utilized which contributed to PC 3 was β-methyl-D-glucoside while the substrates that were highly utilized which contributed to PC 2 were 2-hydroxy benzoic acid, α-D-lactose, L-phenylalanine, L-threonine, α-cyclodextrin glycogen and glycogen. These particular substrates may serve as a unique fingerprint for a particular microbial community which is sensitive to changes in total carbon, nitrogen, phosphorus and calcium content in ginseng cultivated soils, which are important factor in promoting larger size ginseng roots.

Conclusion

Overall, establishment of the relationship between soil chemical properties and ginseng root growth has determined that available P, exchangeable Mg and Ca and CEC in soils are the factors that positively affect the growth of ginseng roots while increase in Na concentration retards root growth. In addition, significant correlations between soil nutrient properties – pH, percent total C, N, available P, exchangeable Ca, K and Na with soil microbial properties – PC 1, PC 2, PC 3, AWCD and richness of metabolized carbon in ginseng cultivated soils had established the relationships of soil microbial functions with soil chemical properties that directly determines growth of ginseng. However, based on the PCA of Biolog EcoPlate™ data, we were not able to establish a clearly influence of growth stages of ginseng and the inherent site characteristics on microbial functional communities but rather because of high homogeneity of different samples in PCA bi-plots, it is apparent that ginseng cultivations result to a unique microbial community functional structures regardless of the growth stage of ginseng and site of production. These would indicate the strong influence of ginseng cultivation on soil biology. Whether these soil properties influenced the overall quality of ginseng roots needs further evaluation. On the other hand, further studies on the highly utilized carbon substrates that contributed to PC 1 and PC 3 may elucidate what particular microbial communities contribute to the uniqueness of ginseng cultivated soils and could possibly enhance the root growth of ginseng roots, respectively.

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