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研究課題名(和文) 持続システム構築のための日本各地における果樹園土壌微生物群集及び化学特性の評価

研究課題名(英文) Evaluation of soil microbial communities and chemical properties in fruit orchards across all of Japan for the establishment of sustainable agriculture

研究代表者

Cruz A・F (Cruz, Andre Freire)

京都府立大学・生命環境科学研究科・講師

研究者番号：80405240

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研究成果の概要(和文)：日本全国の果樹園から採取された土壌は、アンプリコン遺伝子分析によって微生物群集が測定された。酵素活性によって土壌微生物バイオマスおよび活性も測定された。さらに、土壌の化学的性質：pH、全N、全P、可溶化したP、非晶質、およびECを分析した。微生物群集の豊富さ、多様性、および構成は、果実の種類と地域によって異なった。微生物多様性、バイオマスNおよびC、ならびに酵素活性は、南部のものより北部地域の方が一般的に高かった。これらのデータ間の関係を考慮すると、酵素活性は細菌の豊富さと正の相関を示したが、真菌のものとは相関しなかった。また、土壌微生物バイオマスPは、非晶質と相関があった。

研究成果の学術的意義や社会的意義

This research could demonstrate that the soil characteristics (microbial community, biomass, activity and chemical properties) can range according to land using in fruit orchards. Especially the high values of diversity and enzyme activity could confirm the influence of organic matter.

研究成果の概要(英文)：The soils from fruit orchards from all over Japan had their microbial community (bacterial and fungal) determined by amplicon gene analysis (Illumina MiSeq 250bp). The soil microbial biomass (C, N, P and K) and activity, represented by enzymes (Phosphatase, Dehydrogenase, Arylsulphatase, b-glucosidase, protease and Urease) were also determined. Furthermore, soil chemical properties: pH, Total N, Total P, Available P, Amorphous fraction, and EC were analysed. The abundance, diversity, and composition of the microbial communities were specific according to fruit type and region (Location). The estimated microbial richness was generally higher in northern regions than in those from southern. Similar pattern was found for microbial biomass N and C, and enzyme activity. Considering the relationship between these data, the enzyme activities were positively correlated to bacterial richness, but not with fungal ones. Also, the soil microbial biomass P had correlation with amorphous fraction.

研究分野：土壌科学

キーワード：土壌微生物 多様性 酵素活性 土壌微生物バイオマス 土壌化学 果樹園

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## 1 . 研究開始当初の背景

Soil microorganisms, mostly characterized by their diversity and abundance, greatly affect the nutrient dynamics. In particular, a higher diversity, could have more ability to decompose organic matter. The soil activity is also affected by land using type and crops, depending on region and soil type their activities are also affected. Similar pattern can be found for the soil microbial biomass, which is strongly affected by soil management and land using.

The agricultural management probably increases the C and N inputs in soils, which has effect on microbial diversity, biomass, and activity. This improvement of microbial status on soil sometimes function as an indicator of soil quality. Many reports has shown the effect of land use and management on soil microbial activities, but these studies were mostly restricted to a few soil samples, limited to a small area. Soil analysis on a national scale, representing the agricultural areas of a whole country, has scarcely been reported, but most such studies are linked to the chemical characterization of soil. A survey at national scale of fruit soils, including the chemical and biological properties has been not well reported.

Considering these reasons, it is fundamentally to research about the soil microbial status in these fruit orchards and their relationship with soil chemical properties related to fertility. Such information would provide basic approach to recommend the fertilizers in appropriate manner.

## 2 . 研究の目的

The current study aimed to determine at national scale the characteristics of fruit soils in Japan, including their chemical properties related to fertility, soil microbial activity, biomass and microbial community (Diversity and Composition).

## 3 . 研究の方法

### **Soil Samples and chemical analysis**

The samples were obtained from fruit orchards in the 47 prefectures of Japan, where the most important species were considered for each one. 68 samples were taken from 13 fruit species (Apple, Cherry, Chestnut, Grape, Japanese pear, Kiwi, Mandarin, Mango, Japanese apricot, Peach Persimmon, Pineapple and Plum). These soils were separated in three groups of storage method (air-dried, Frozen and Fridge). The chemical properties of the soils were determined: pH, EC, CEC, available phosphorus, amorphous aluminum and iron, Total N and Total C.

### **Microbial analysis**

The microbial community was performed by DNA extraction with buffer and SDS, followed by cleaning with Mag Extractor (Toyobo, Co., Japan). The 16S rRNA and ITS rRNA gene sequences were analyzed by amplicon PCR using a primer pair V3/V4 and ITS1F/ITS2, followed by index PCR to construct their library using the Illumina protocol. The validation until the NGS was conducted at Genome Quebec Co. Montreal– Canada. The gene sequences were processed using the QIIME 1.9.1 pipeline to determine the relative abundance (RA) and diversity (Shannon-Wiener index - SW) of the microbes in

soils.

The microbial growth, here represented by the microbial biomass C, N, P and K, was determined in fridge soils. The same stored soils were used to calculate the enzyme activities, where the final product was used to calculate it. The dehydrogenase, phosphatase, arylsulphatase, b-glucosidase, urease and protease were analyzed in the soil samples. The statistical analysis was performed with all data to calculate the ANOVA and the Pearson correlation was used to compare the data.

#### 4 . 研究成果

The soil chemical properties indicated a high pH level in grape and plum soils. Also, the available P was found very high in mango and pear soils. In terms of relationship between the microbial community and soil chemical properties, the biodiversity indexes were mostly positively correlated with enzyme activities in bacterial communities but not with fungal ones (Table 1). Although the *Proteobacteria* and *Acidobacteria* were very common in all fruit types, the *Chloroflexi*, *Bacterioidetes* and *Verrumicrobia* were very specific for Mango soils. In terms of genus, *Bacillus*, *Brevibacterium* and *Streptomyces* could be found in Mango areas in higher abundance (Table 2).

The fungal community indicated some unique genus in Chestnut (*Inocybe*, *Russula*) and Mango (*Chaetomium*, *Aspergillus* and *Metarhizium*) orchards (Table 3). The Shannon-Wiener index (Biodiversity) had shown lower values in Mango and Pineapple areas for both bacterial and fungal genes. However, the Cherry and chestnut areas had higher diversity than other fruits. Furthermore, the soil microbial biomass (SMB) N and K were higher in tropical fruit areas than in temperate ones. Positive correlation was found from CEC with SMB-C, and Amorphous Fe Al with SMB-P.

Table 1. Correlations between the bacterial and fungal diversities with enzyme activities

	Bacteria			Fungi		
	Chao	Shannon	Simpson	Chao	Shannon	Simpson
<b>Dehydrogenase</b>	0.255*	0.344**	0.197	0.039	0.067	0.079
<b>Phosphatase</b>	0.372**	0.220	0.121	0.121	0.061	0.061
<b>Arylsulphatase</b>	0.063	0.067	0.000	0.086	0.101	0.015
<b>-glucosidase</b>	0.293*	0.257*	0.234	-0.101	-0.027	0.008
<b>Urease</b>	0.288*	0.088	-0.165	0.000	-0.153	-0.098
<b>Protease</b>	0.056	0.167	0.208	-0.239	-0.118	-0.126

Table 2- Relative abundance of major bacterial genus in fruit orchards of Japan.

	Apple	Chestnut	Mandarin	Mango	Jap. Apricot	Pineapple
<i>Bacillus</i>	0.232	0.207	0.479	4.497	0.342	1.312
<i>Brevibacterium</i>	0	0	0.00123	4.176	0	0
<i>Burkholderia</i>	0.298	0.353	0.876	0.0479	0.328	0.0945
<i>Dermaococcus</i>	0.00216	0.00181	0.00950	1.141	0.00196	0
<i>Erwinia</i>	0.0116	0.00903	0.00157	0	0	0.00166
<i>Flavobacterium</i>	0.751	1.106	0.346	0.290	1.014	0.139
<i>Kaistobacter</i>	1.758	0.864	1.569	0.607	1.464	2.416
<i>Mesorhizobium</i>	0.0981	0.0927	0.117	0.133	0.0988	0.0530
<i>Nitrospira</i>	0.792	0.775	0.771	0.523	0.887	0.671
<i>Paenibacillus</i>	0.0304	0.0343	0.0570	0.158	0.0386	0.0696
<i>Pseudomonas</i>	0.319	0.265	0.270	0.268	0.133	0.0795
<i>Streptomyces</i>	0.159	0.140	0.318	2.435	0.105	0.0746

Table 3- Relative abundance of major fungal genus in fruit orchards of Japan.

	Apple	Chestnut	Mandarin	Mango	Jap. Apricot	Pineapple
<i>Acremonium</i>	0.0446	0.0481	0.0763	0.0497	0.0376	1.07
<i>Chaetomium</i>	1.58	0.600	0.502	22.2	0.661	1.72
<i>Cortinarius</i>	0.0668	0.0904	0.00296	0.188	0	0.00187
<i>Glomus</i>	0.00300	0.00409	0.0178	0.0525	0.0204	0
<i>Inocybe</i>	0	0.877	0	0	0	0
<i>Metarhizium</i>	0.821	0.103	1.06	0.00829	0.925	0
<i>Penicillium</i>	0.739	0.188	2.15	2.12	0.346	0.252
<i>Rhodotorula</i>	0.0484	0.0566	0.0312	0.00829	0	1.46
<i>Russula</i>	0.000857	0.152	0.000987	0	0	0
<i>Trichoderma</i>	0.335	0.206	0.144	0.0497	0.0194	1.62
<i>Alternaria</i>	0.0188	0	0.00252	0	0	0
<i>Aspergillus</i>	0.170	0.0157	0.423	8.423	0.602	0.375
<i>Cladosporium</i>	0.0103	0.0239	0.00767	0	0	0
<i>Colletotrichum</i>	0.00257	0.00852	0.0546	0	0.1000	0
<i>Cylindrocarpon</i>	0.0715	0.0358	0.0301	0.227	0.00968	0
<i>Fusarium</i>	7.66	3.03	9.84	0.602	8.55	1.66
<i>Gibberella</i>	0.846	0.336	2.95	0	0.162	0.00187
<i>Rhizoctonia</i>	0.0129	0.00273	0.00548	0	0	0
<i>Humicola</i>	2.58	2.63	2.97	2.14	5.60	3.33

## 5 . 主な発表論文等

〔雑誌論文〕(計 0 件)

〔学会発表〕(計 5 件)

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## 6 . 研究組織

### (1)研究分担者

研究分担者氏名：クルス、アンドレ フレイリ

ローマ字氏名：Cruz, Andre Freire

所属研究機関名：京都府立大学

部局名：大学院生命環境科学研究科

職名：講師

研究者番号(8桁)：80405240

### (2)研究協力者

研究協力者氏名：矢内 純太

ローマ字氏名：Junta Yanai

研究協力者氏名：福田秀美

ローマ字氏名：Fukuda Hidemi

研究協力者氏名：中尾 淳

ローマ字氏名：Nakao Atsushi

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