

# A Study on Rice Growth and Soil Environments in Paddy Fields Using Different Organic and Chemical Fertilizers

Takamitsu Kai<sup>1\*</sup>, Motoki Kumano<sup>2</sup>, Masahiko Tamaki<sup>1</sup>

<sup>1</sup>Kurokawa Field Science Center, Meiji University, Kurokawa, Asao-ku, Kawasaki, Kanagawa, Japan

<sup>2</sup>School of Agriculture, Meiji University, Higashimita, Tama-ku, Kawasaki, Kanagawa, Japan

Email: \*mtamaki@meiji.ac.jp

**How to cite this paper:** Kai, T., Kumano, M. and Tamaki, M. (2020) A Study on Rice Growth and Soil Environments in Paddy Fields Using Different Organic and Chemical Fertilizers. *Journal of Agricultural Chemistry and Environment*, 9, 331-342. <https://doi.org/10.4236/jacen.2020.94024>

**Received:** August 5, 2020

**Accepted:** November 23, 2020

**Published:** November 26, 2020

Copyright © 2020 by author(s) and Scientific Research Publishing Inc. This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

## Abstract

Currently, the majority of paddy fields in Japan are grown using chemical fertilizers and synthetic chemical pesticides, since chemical fertilizers can provide the nutrients necessary for plant growth. However, there are concerns regarding the environmental impact of chemical fertilizer and pesticides production, such as reduction of soil microorganisms and water pollution due to the runoff of fertilizer components from the soil caused by excessive fertilizer application. In this study, we investigated the effects of the application of organic and chemical fertilizers on the plant growth of paddy fields, in addition to their effects on the chemical and biological properties of the soil. The panicle numbers of rough and brown rice, the 1000-grain weight of the rough and brown rice, and the percentages of ripened grains were significantly higher in paddy soils grown with organic fertilizers than in those grown with chemical fertilizers. In addition, the total carbon (TC) contents and pH values were significantly higher in the soils of paddy fields grown with organic fertilizers. Furthermore, the soils of paddy fields grown with organic fertilizers exhibited greater bacterial biomasses, N circulation activity, and P circulation activity than the soils of paddy fields grown using chemical fertilizers, although the differences were not significant. In this study, the difference in plant growth was appeared in fertilizer application such as organic and chemical fertilizers. It was indicated that the organic fertilizer and pesticide reduction management increased the soil bacterial biomass and activated the material cycle such as N circulation activity.

## Keywords

Rice, Organic Fertilizer, Soil Microorganism, Soil Fertility, Agricultural Environment, Environmental Conservation

## 1. Introduction

Rice is grown in various parts of the world between latitudes of 50° north and 35° south [1]. At present, Asia accounts for ~90% of worldwide rice production, although outside of Asia, rice is also grown in Brazil and Colombia (South America), and in Egypt, Senegal, and Madagascar (Africa). Agriculture is a production system that utilizes part of the ecosystem (material cycle) and is supported by soil organisms at the base of the production system pyramid. However, these natural environments are considered to be external economies, and the use of large amounts of chemical fertilizers and synthetic pesticides has resulted in soil degradation and stagnation of the material cycle, thereby leaving the ecosystem in a critical state. Furthermore, since chemical fertilizers do not contribute to the biotic and physical properties of the soil, or enhance soil fertility, soil degradation is likely to occur in agricultural practices that use only chemical fertilizers for long periods of time. This in turn can lead to poor crop growth.

A recent report showed that only 1% of agricultural fields are cultivated under organic farming systems [2]. Although yields tend to be relatively stable in conventional farming systems, the excessive use of chemical fertilizers and synthetic chemical pesticides can cause severe environmental and socio-economic problems, in addition to issues associated with human health. As a result, consumer awareness towards organic foods has recently increased.

Compared to conventional farming techniques, organic farming methods cause relatively lower environmental damage, and the resulting crops tend to be flavorful [3]. However, yields tend to be lower and less stable in organic farming systems than in conventional farming systems [4] [5] [6] [7]. Organic farming systems must therefore be modified to ensure high yields and qualities for the resulting agricultural products.

Soil microorganisms play several beneficial roles in terms of plant growth and soil quality, including the decomposition of organic materials, the release of nutrients to plants, and the bioremediation of pesticide-polluted soils [8] [9] [10]. Soil microorganisms are therefore considered key players in maintaining soil fertility, and so a large and active microorganism community is required for efficient nutrient cycling and to ensure a steady supply of nutrients to the plants.

In a previous study, we developed a soil fertility index, SOFIX, for the evaluation of soil fertility [11]. Analysis of the SOFIX data from several agricultural fields clearly showed that the number and activity of microorganisms can be significantly enhanced by controlling the total carbon (TC) and total nitrogen (TN) contents. It has also been found that too high TC, TN, total phosphorus (TP), and total potassium (TK) levels in conventional chemical fertilizer management systems can lead to yield reductions in Japanese orchards [12], although apple orchards are relatively rich in TC, TN, TP, and TK, compared with annual crop-lands such as paddy fields and uplands [13]. However, the relationship between microbial activity and plant growth remains unknown. In recent years, the application of organic farming to paddy fields has received growing attention. As

pointed out above, the excessive use of chemical fertilizers may lead to soil degradation and other problems. In contrast, organic fertilizers have the advantage of a sustained efficacy and a low environmental impact, due to a reduction in the amount of pesticides applied. However, few studies have been reported into the effects of bacterial biomass, material cycling as N circulation activity, and P circulation activity values on soil fertility in paddy fields. Therefore, we newly herein report our investigation into the differences between rice crops grown in paddy fields using organic and chemical fertilizers.

## 2. Materials and Methods

### 2.1. Study Site

We investigated the production of rice (*Oryza sativa* “Milky Queen”) and the soil properties in the paddy fields differing in the use of fertilizers, pesticides, and tillage management systems in Iizuna Town, Nagano Prefecture, Japan, in 2019. The climate in Nagano Prefecture is humid temperate; July is the warmest month, while January is the coolest. Chemical fertilization of the study site is carried out to the standards recommended in Nagano Prefecture (*i.e.*, N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 110:120:100 kg·ha<sup>-1</sup>), where solid fertilizer was added to the conventional paddy field according to the standard basal dressing [14] every April over a period of 10 years. Synthetic pesticides are employed approximately 12 times per year in paddy fields grown with chemical fertilizers. In contrast, paddy fields grown with organic fertilizers were fertilized each April over 10 years with compost mainly composed of poultry manure (N:P<sub>2</sub>O<sub>5</sub>:K<sub>2</sub>O = 120:150:100 kg·ha<sup>-1</sup>). In terms of pesticides, one herbicide was applied in May prior to planting the rice. In combination with organic fertilizers, synthetic pesticides were sprayed on the crops each July. Although growers wish to reduce the application of synthetic pesticides to zero, this has not been possible due to their inability to reduce the incidence of pests and diseases, and so Organic-JAS certification for pesticides would be desirable. The mowing of paddy fields grown with organic and chemical fertilizers was carried out twice a year.

Clay loam soils were employed in the paddy fields, and the fields grown with organic and chemical fertilizers were located across the road from one another. Each is a standard plot of 100 m in length × 30 m in width (3,000 m<sup>2</sup>). Both paddy fields gave a yield of approximately 5 t·ha<sup>-1</sup> (the average yield of rice crops in the Nagano Prefecture is ~6 t·ha<sup>-1</sup>). The rice was planted on 17 May 2019. Seedlings were planted in both paddy fields with a distance of 15 cm between plants and 30 cm between rows. The paddy fields were dried for ~10 days in mid-to-late July, and the rice was harvested on 28 September 2019.

### 2.2. Rice Cultivations and Plants

The rice yields were measured by harvesting 30 plants in each of the paddy fields grown with organic and chemical fertilizers. After measuring the panicle number per hill and the number of rough rice per panicle, the weight of rough rice

was recorded. The number of brown rice that passed through a grain thickness of  $\geq 1.8$  mm (Fuji Metal Industry Co., Ltd., Ibaraki, Japan) was measured, and the weight of brown rice was recorded. From these values, the 1000-grain weight of rough rice, the 1000-grain weight of brown rice, and the percentage of ripened grains were calculated.

### 2.3. Soil Chemical Properties

Composite soil samples (top 15 cm layer, excluding the top 2 - 3 cm surface crust) were collected near the base of five randomly selected trees in each orchard. Soil sampling was performed in July when the soil microorganisms were active [12]. The following chemical properties of the composite soil samples were analyzed: TC, TN,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , TP, SP, TK, and exchangeable potassium (SK). The TC content was analyzed with a TOC analyzer (SSM-5000A; Shimadzu, Kyoto, Japan). The  $\text{NH}_4^+$  and  $\text{NO}_3^-$  levels were analyzed by extracting the soil sample with 1 M KCl, followed by the indophenol blue and brucine methods [15]. A soil-water suspension (1:20, w/v) was reciprocally shaken at 100 rpm for 1 h, and the extracts were analyzed using the molybdenum blue method [16] and atomic absorption spectrophotometry for quantitative determination of the SP and SK levels, respectively. The TN, TP, and TK contents were analyzed by digesting the soil samples in a Kjeldahl Therm digestion unit (Gerhardt, Königswinter, Germany) with  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$ ;  $\text{NH}_4^+$ -N, SP, and SK contents in the digest were determined. The pH of the soil-water suspension (1:2.5, w/v) was analyzed using a pH meter (Model: LAQUA F-72; Horiba Scientific, Kyoto, Japan).

### 2.4. Soil Biological Properties

Total bacterial biomass was determined by environmental DNA (eDNA) analysis using the slow-stirring method [17], which allows accurate and simple measurement through the extraction of microbial DNA from the soil. The following biological properties were analyzed: total bacterial biomass,  $\text{NH}_4^+$  oxidation activity,  $\text{NO}_2^-$  oxidation activity, N circulation activity, and P circulation activity. The N circulation activity was analyzed using the  $\text{NH}_4^+$  and  $\text{NO}_2^-$  oxidation activity values and the total bacterial number, as described by Matsuno *et al.* [18] and Adhikari *et al.* [11]. The P circulation activity was determined by analyzing the rate at which soluble P was released from phytic acid (a dominant form of organic P in soil) over a three-day incubation period [12] [13] [19].

Nitrogenous organic substances, such as proteins, are decomposed in soil as follows: protein  $\rightarrow$  peptide  $\rightarrow$  amino acid followed by  $\text{NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$ , and low molecular weight molecules derived from this amino acid decomposition are rendered by the soil microorganisms. During these processes, the  $\text{NH}_4^+$  oxidation activity ( $\text{NH}_4^+ \rightarrow \text{NO}_2^-$ ), the  $\text{NO}_2^-$  oxidation activity ( $\text{NO}_2^- \rightarrow \text{NO}_3^-$ ), and the bacterial biomass were determined. The  $\text{NH}_4^+$  oxidation activity, the  $\text{NO}_2^-$  oxidation activity, and the number of microorganisms were quantified using a triangular radar chart, and the ability of the soil to convert the N present in or-

ganic matter to  $\text{NO}_3^-$  was evaluated as the “N circulation activity” [11]. The larger the area of the triangle, the more active the nitrogen circulation in the soil, and vice versa. In addition, phytic acid (organic phosphate) must be broken down into phosphate before the plant can absorb phosphate (phytic acid-degrading activity). Therefore, the ability to convert phytic acid into organic phosphate was evaluated as the “P circulation activity” [11].

Different soils were assigned a score: soils in which all phytic acid was converted to phosphoric acid without mineral chemisorption were given a score of 100 points, while soils in which phosphoric acid was not produced at all were assigned a score of 0 points. However, a P circulation activity score of 100 points indicated a low mineral content. Therefore, soils with a moderate mineral content and abundant microorganisms (due to phosphoric acid being supplied) were assigned a score of 40 - 60 points.

## 2.5. Statistical Analysis

Data are presented as the mean  $\pm$  standard deviation (SD) values and are analyzed using BellCurve for Excel 2016 for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan). All data were analyzed using Tukey's t-test, where appropriate. Different marks (\*) within a plot are significantly different at  $p < 0.05$ , while double marks (\*\*) within a plot are different at  $p < 0.01$ . The abbreviation n.s. indicates no significant difference, according to the t-test method. All statistical analyses were conducted with a significance level of  $\alpha = 0.05$  ( $p < 0.05$ ).

## 3. Results

### 3.1. Panicle Number, Number of Rough Rice, Number of Brown Rice, 1000-Grain Weight of Rough Rice, 1000-Grain Weight of Brown Rice, and Percentage of Ripened Grains of Rice

The panicle number, number of rough rice, number of brown rice, 1000-grain weight of rough rice, 1000-grain weight of brown rice, and percentage of ripened grains of rice are shown in **Table 1**. For the rice grown using organic fertilizers, these values were 15.4 (hill), 92.7 (panicle), 53.1 (panicle), 23.8 (g), 21.6 (g), and 58.1 (%), respectively, while for the crop grown with chemical fertilizer, the corresponding values were 16.5 (hill), 85.2 (panicle), 44.1 (panicle), 22.6 (g), 20.8 (g), and 52.8 (%), respectively. These results indicate that for the number of rough rice, number of brown rice, 1000-grain weight of rough rice, 1000-grain weight of brown rice, and percentage of ripened grains of rice, significantly higher values were obtained when an organic fertilizer was employed; no significant difference was found for the panicle number.

### 3.2. Comparison of the Soil Chemical Properties

The soil chemical properties of the paddy fields grown under the organic and chemical fertilizer systems are outlined in **Table 2**. As shown, the TC content

**Table 1.** Growth status of rice.

Experimental treatment	Panicle number (hill)	Number of rough rice (panicle)	Number of brown rice (panicle)	1000-grain weight of rough rice (g)	1000-grain weight of brown rice (g)	Percentage of ripened grains (%)
Organic	15.4 ± 1.88 <sup>z</sup>	92.7 ± 30.4	53.1 ± 20.8	23.8 ± 3.12	21.6 ± 1.57	58.1 ± 17.1
Chemical	16.5 ± 3.50	85.2 ± 27.6	44.1 ± 14.4	22.6 ± 2.32	20.8 ± 0.82	52.8 ± 11.0
t-test	n.s. <sup>y</sup>	**	**	**	**	**

<sup>z</sup>Mean ± standard deviation of a sample (panicle number, number of rough rice, number of brown rice, 1000-grain weight of rough rice, 1000-grain weight of brown rice, and percentage of ripened grains: n = 463 - 496). <sup>y</sup>Different marks different marks (\*\*) within a pot are different at p < 0.01. In addition, n.s. indicates no significant difference, according to the t-test method.

**Table 2.** Soil chemical properties.

Experiments	soil adoption day	Total C (mg·kg <sup>-1</sup> )	Total N (mg·kg <sup>-1</sup> )	Total P (mg·kg <sup>-1</sup> )	Total K (mg·kg <sup>-1</sup> )	C/N ratio	NO <sub>3</sub> <sup>-</sup> -N (mg·kg <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N (mg·kg <sup>-1</sup> )	Available phosphoric acid (mg·kg <sup>-1</sup> )	Exchangeable potassium (mg·kg <sup>-1</sup> )	pH	EC (mS·cm <sup>-1</sup> )
Organic	February	22,135 ± 81.6 <sup>z</sup>	1,922 ± 40.8	1,056 ± 8.16	2,300 ± 163	11.5 ± 0.08	3 ± 0.00	0 ± 0.00	0 ± 0.00	26 ± 2.45	6.6 ± 0.00	0.07 ± 0.01
	May	22,973 ± 8.16	1,289 ± 24.5	1,032 ± 4.08	2,633 ± 16.3	20.0 ± 0.22	1 ± 0.82	1 ± 0.00	92 ± 3.27	315 ± 8.16	6.9 ± 0.20	0.11 ± 0.01
	August	23,960 ± 16.3	1,059 ± 32.7	1,159 ± 3.27	2,391 ± 25.3	23.0 ± 0.86	0 ± 0.00	1 ± 0.82	162 ± 4.08	348 ± 12.2	6.7 ± 0.10	0.18 ± 0.02
	October	24,310 ± 24.5	1,645 ± 40.8	1,179 ± 89.8	2,363 ± 18.8	15.0 ± 0.21	1 ± 0.82	0 ± 0.00	162 ± 2.45	368 ± 5.72	6.5 ± 0.10	0.23 ± 0.01
	<b>Average</b>	<b>23,345 ± 853</b>	<b>1,479 ± 330</b>	<b>1,107 ± 63.5</b>	<b>2,422 ± 126</b>	<b>17.4 ± 4.44</b>	<b>1 ± 1.09</b>	<b>1 ± 0.50</b>	<b>104 ± 66.5</b>	<b>264 ± 139</b>	<b>6.7 ± 0.15</b>	<b>0.15 ± 0.06</b>
Chemical	February	22,000 ± 408	1,800 ± 8.16	1,000 ± 8.06	2,108 ± 81.6	12.2 ± 0.16	6 ± 0.82	6 ± 0.00	4 ± 0.82	39 ± 0.82	5.9 ± 0.10	0.91 ± 0.01
	May	20,275 ± 32.7	1,227 ± 81.6	1,022 ± 24.5	2,340 ± 19.6	19.0 ± 1.08	2 ± 0.82	4 ± 0.82	84 ± 4.90	365 ± 4.90	6.4 ± 0.20	0.36 ± 0.02
	August	19,280 ± 16.3	811 ± 8.16	923 ± 0.82	2,341 ± 24.5	23.0 ± 0.22	0 ± 0.00	2 ± 0.00	146 ± 5.72	355 ± 6.53	6.5 ± 0.20	0.12 ± 0.01
	October	21,703 ± 4.08	1,541 ± 49.0	1,290 ± 1.63	2,257 ± 49.0	15.0 ± 0.45	1 ± 0.82	0 ± 0.00	129 ± 4.90	379 ± 1.63	6.1 ± 0.00	0.25 ± 0.01
	<b>Average</b>	<b>20,815 ± 1100</b>	<b>1345 ± 369</b>	<b>1,059 ± 138</b>	<b>2,262 ± 95.0</b>	<b>17.3 ± 4.08</b>	<b>2 ± 2.28</b>	<b>3 ± 2.24</b>	<b>91 ± 55.0</b>	<b>285 ± 142</b>	<b>6.2 ± 0.24</b>	<b>0.41 ± 0.30</b>
<b>t-test<sup>y</sup></b>	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	

<sup>z</sup>Mean ± standard deviation of a sample (total C, total N, total P, total K, C/N ratio, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, available phosphoric acid, exchangeable potassium, pH, and EC: n = 3). <sup>y</sup>Different marks (\*) within a pot are significantly different at p < 0.05. In addition, n.s. indicates no significant difference, according to the t-test method.

ranges for the organic and chemical fertilizers were 22,135 - 24,310 and 19,280 - 22,000 mg·kg<sup>-1</sup>, respectively. while the average TC values were 23,345 and 20,815 mg·kg<sup>-1</sup>. In addition, the TN content ranges were 1,059 - 1,922 and 811 - 1,800 mg kg<sup>-1</sup>, for the organic and chemical fertilizer groups, respectively, while the average TN values were 1,479 and 1,345 mg·kg<sup>-1</sup>. Furthermore, the TP content ranges were 1,032 - 1,179 and 923 - 1,290 mg·kg<sup>-1</sup>, respectively, while the average values were 1,107 and 1,059 mg·kg<sup>-1</sup>. The TK content ranges obtained when organic, and chemical fertilizers were employed were 2,300 - 2,633 and 2,108 - 2,341 mg·kg<sup>-1</sup>, respectively, while the average TK values were 2,422 and 2,262 mg·kg<sup>-1</sup>. For the C/N ratio ranges, the corresponding values were 11.5 - 23.0 and 12.2 - 23.0, respectively, while the average C/N ratio values were 17.4 and 17.3. In addition, the NO<sub>3</sub><sup>-</sup>-N content ranges were 0 - 3 and 0 - 6 mg·kg<sup>-1</sup>, respectively (average values = 1 and 2 mg·kg<sup>-1</sup>), while the NH<sub>4</sub><sup>+</sup>-N content ranges

were 0 - 1 and 0 - 6 mg·kg<sup>-1</sup>, respectively (average values = 1 and 3 mg·kg<sup>-1</sup>). The available phosphoric acid (SP) content ranges were 0 - 162 and 4 - 146 mg·kg<sup>-1</sup>, (average values = 104 and 91 mg·kg<sup>-1</sup>), while the exchangeable potassium (SK) content ranges were 26 - 368 and 39 - 379 mg·kg<sup>-1</sup>, respectively (average values = 264 and 285 mg·kg<sup>-1</sup>). Furthermore, the pH ranges for the samples obtained using the organic and chemical fertilizers were 6.5 - 6.9, and 5.9 - 6.5, respectively (average values = 6.7 and 6.2), while the EC ranges were 0.07 - 0.23 and 0.12 - 0.91 mS·cm<sup>-1</sup>, respectively (average values = 0.15 and 0.41 mS·cm<sup>-1</sup>).

Overall, the average TC contents and the pH were significantly higher under organic fertilizer conditions, although no significant differences were found for the average TN, TP, TK, C/N ratio, NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N, SP, SK, and EC values.

### 3.3. Comparison of the Soil Biological Properties

The soil biological properties of the paddy fields grown under the organic and chemical fertilizer systems are listed in **Table 3**. More specifically, the bacterial biomass ranges obtained using the organic and chemical fertilizers were 14 - 35 × 10<sup>8</sup> and 10 - 12 × 10<sup>8</sup> cells·g<sup>-1</sup>, respectively (average values = 22 × 10<sup>8</sup> and 11 × 10<sup>8</sup> cells·g<sup>-1</sup>). In addition, the NH<sub>4</sub><sup>+</sup> oxidation activity ranges were 32 - 65 and 21 - 38 point, respectively (average values = 45 and 30 point), while the NO<sub>2</sub><sup>-</sup> oxidation activity ranges were 47 - 60 and 21 - 55 point, respectively (average values = 53 and 41 point). Furthermore, the N circulation activity ranges were 31.0 - 50.0 and 18.0 - 36.0 point, respectively (average values = 40.0 and 27.5 point), while the P circulation activity ranges were 2 - 8 and 0 - 6 point, respectively (average values = 5 and 3 point).

**Table 3.** Soil biological properties.

Experiments	Soil adoption day	Bacterial biomass (×10 <sup>8</sup> cells g <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> oxidation activity (point)	NO <sub>2</sub> <sup>-</sup> oxidation activity (point)	N circulation activity (point)	P circulation activity (point)
Organic	February	35 ± 0.82 <sup>z</sup>	65 ± 2.45	52 ± 1.63	50.0 ± 2.45	8 ± 0.82
	May	20 ± 0.82	46 ± 2.45	60 ± 3.27	44.0 ± 1.63	4 ± 0.00
	August	14 ± 1.63	36 ± 3.27	51 ± 2.45	35.0 ± 0.82	2 ± 0.00
	October	18 ± 2.45	32 ± 0.82	47 ± 0.82	31.0 ± 1.63	4 ± 0.41
	<b>Average</b>	<b>22 ± 0.67</b>	<b>45 ± 0.89</b>	<b>53 ± 0.91</b>	<b>40.0 ± 0.58</b>	<b>5 ± 0.34</b>
Chemical	February	12 ± 0.00	38 ± 4.08	50 ± 0.82	36.0 ± 0.41	6 ± 0.00
	May	11 ± 0.82	33 ± 1.63	55 ± 2.45	35.0 ± 3.27	3 ± 0.82
	August	10 ± 0.82	21 ± 0.82	36 ± 1.63	21.0 ± 0.82	0 ± 0.00
	October	11 ± 1.63	29 ± 1.63	21 ± 0.82	18.0 ± 0.82	3 ± 0.00
	<b>Average</b>	<b>11 ± 0.58</b>	<b>30 ± 1.22</b>	<b>41 ± 0.68</b>	<b>27.5 ± 1.13</b>	<b>3 ± 0.36</b>
	t-test <sup>y</sup>	n.s.	n.s.	n.s.	n.s.	n.s.

<sup>z</sup>Mean ± standard deviation of a sample (bacterial biomass, NH<sub>4</sub><sup>+</sup> oxidation activity, NO<sub>2</sub><sup>-</sup> oxidation activity, N circulation activity, and P circulation activity: n = 3). <sup>y</sup>n.s. indicates no significant difference, according to the t-test method.



Overall, the bacterial biomass,  $\text{NH}_4^+$  oxidation activity,  $\text{NO}_2^-$  oxidation activity, N circulation activity, and P circulation activity were higher under organic fertilizer conditions; however, no significant differences were found for any of these properties.

#### 4. Discussion

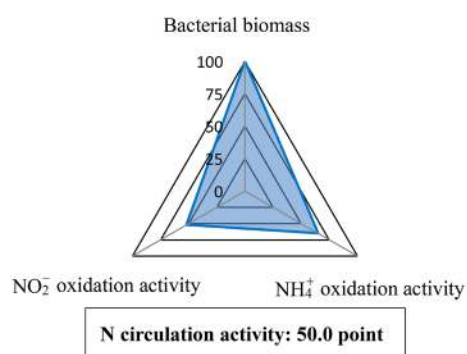
The radar charts of the N circulation activities of the paddy field soils obtained in February, May, August, and November are shown in **Figure 1**, while the P circulation activities are shown in **Figure 2**. As indicated, the paddy soils cultivated with organic fertilizers have higher N and P circulation activities in February, May, August, and November than when chemical fertilizers were employed, thereby indicating active material cycles.

It was previously reported that the recommended carbon and nitrogen contents and the C/N ratio in paddy fields are  $\geq 20,000 \text{ mg}\cdot\text{kg}^{-1}$ ,  $\geq 1,000 \text{ mg}\cdot\text{kg}^{-1}$ , and 10 - 25, respectively [20]. In our study, these recommended values were achieved in all months under organic fertilizer methods. In contrast, when a chemical fertilizer was employed, the soils had lower TC and TN values in August.

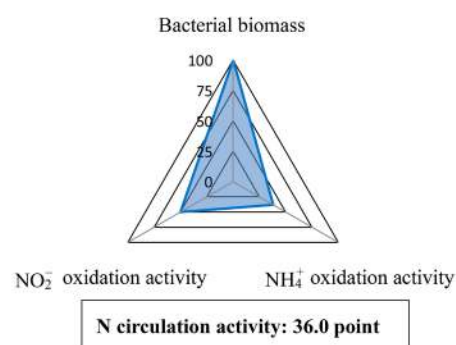
We then moved on to investigate the carbon, nitrogen, phosphorus, and potassium contents. Of these, carbon is supplied only by organic fertilizers, and so the amount of carbon is low when chemical fertilizers are employed. Since many soil microorganisms utilize carbon as a nutrient source, the number of microorganisms and their activity were low in farmland where only chemical fertilizers were used. Therefore, the relationship between carbon (*i.e.*, TC) and the total number of bacteria (*i.e.*, bacterial biomass) was investigated for the two fertilizer groups, as shown in **Figure 3**. To better understand the characteristics of the paddy soil, the average value (bacterial biomass:  $12.1 \times 10^8 \text{ cells g}^{-1}$ , TC:  $15,300 \text{ mg kg}^{-1}$ ) was divided into four groups for analysis. Paddy soil cultivated with organic fertilizer was classified as Group 1 in March, May, August, and November, while paddy soil cultivated with chemical fertilizers belonged to Group 3 in March, May, August, and November. Group 1 soil has a high total bacteria and high carbon content, thereby indicating that the use of organic fertilizer without a pesticide is beneficial to microorganisms. In contrast, Group 3 soil has a high carbon content but a low total bacterial count, thereby suggesting that the use of pesticides was detrimental to microorganism survival. In addition, Group 2 soil has a high total number of bacteria and a low carbon content; this soil was fertilized using mainly chemical fertilizers, and the amount of agricultural chemicals used was small. In addition, newly developed paddy fields and well-prepared paddy fields that do not require much fertilization were included. Finally, Group 4 soil has a low total microbial count and a low carbon content. In this group of paddy fields, mainly chemical fertilizers were used, with pesticides and other chemicals that directly affect microorganisms also being employed. It therefore appeared that a lack of microorganisms correlated with the long-term continuous use of chemical fertilizers and pesticides. In our study, we found that



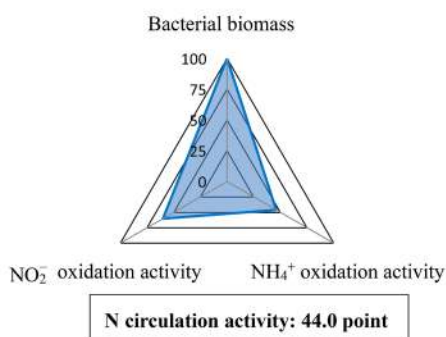
(A) Organic, in February



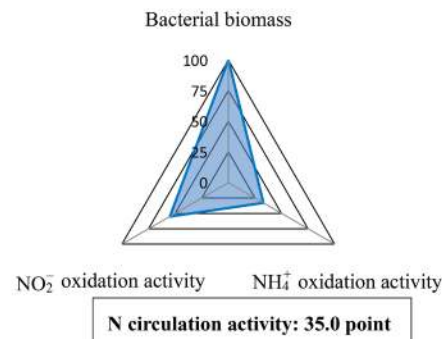
(E) Chemical, in February



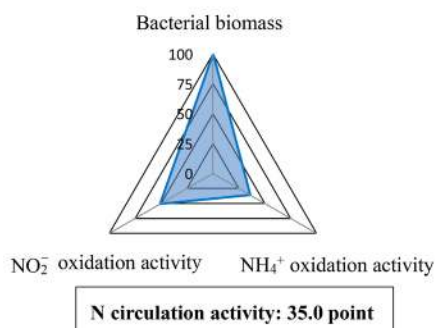
(B) Organic, in May



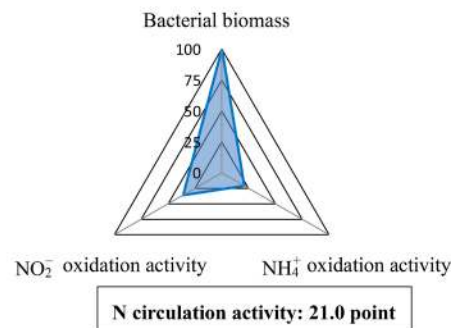
(F) Chemical, in May



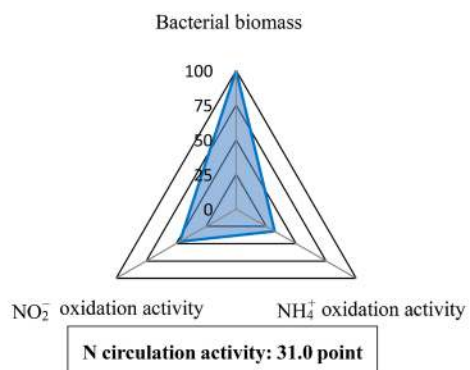
(C) Organic, in August



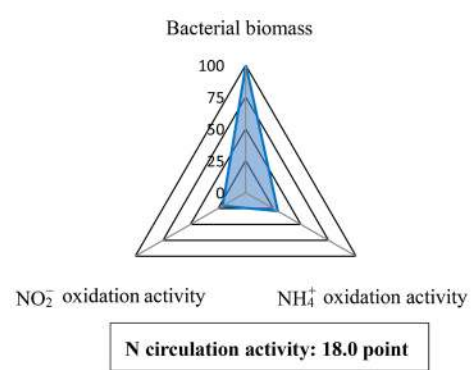
(G) Chemical, in August



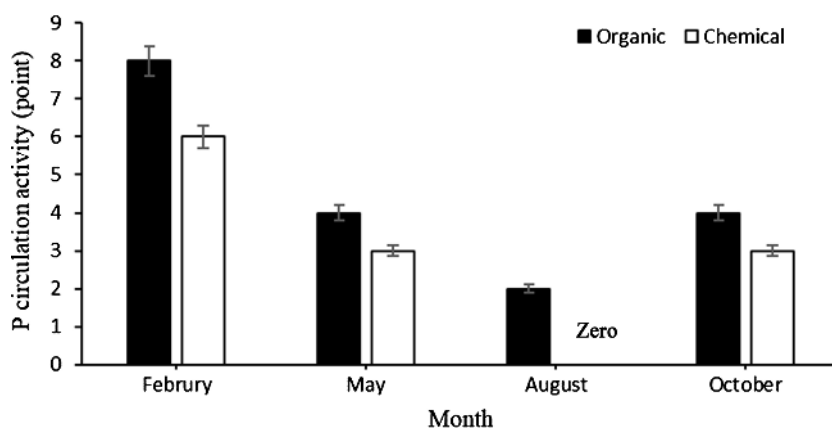
(D) Organic, in October



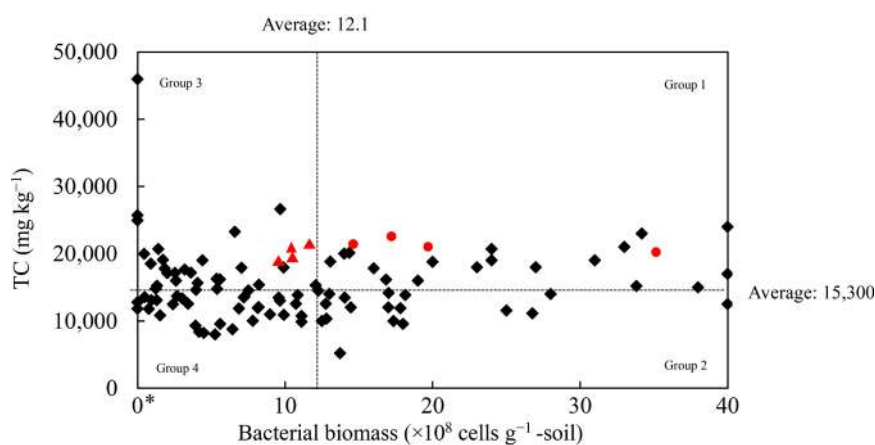
(H) Chemical, in October



**Figure 1.** Radar charts of the average N circulation activity using (A), (C), (E), (G) organic, and (B), (D), (F), (H) chemical fertilizer managements in February, May, August, and November.



**Figure 2.** Average P circulation activities for soil samples taken throughout the year; comparison for soils fertilized using organic and chemical fertilizers.



**Figure 3.** Relationship between the TC and the bacterial biomass in paddy fields using (●) organic, and (▲) chemical fertilizers. Solid lines show suitable conditions and dashed lines show the average values of the bacterial biomass and TC.

approximately 19% of the paddy fields in Japan belong to Group 1, 18% to Group 2, 25% to Group 3, and 38% to Group 4.

In this study, the difference in plant growth was appeared in fertilizer application such as organic and chemical fertilizers. It was indicated that the organic fertilizer and pesticide reduction management increased the soil bacterial biomass and activated the material cycle such as N circulation activity.

In Japan, rice is grown and harvested once or twice in the same paddy field using water. In paddy fields, the chemical composition of the waterlogged soil (carbon, nitrogen, phosphorus, and potassium) results in a large number of microorganisms that fix airborne nitrogen, and this is thought to account for the constant supply of nitrogen, which is taken into account when applying fertilizer. The total number of microorganisms ranges from a small number to a large number, and evaluation of the nitrogen circulation activity, which is an index of the action of microorganisms, tends to indicate low values. This is due to the fact that rice absorbs ammonia nitrogen as a nutrient source (many plants utilize nitrate nitrogen), and so the content of ammonia nitrogen tends to be reduced in

paddy soil, and even in an ideal environment, ammonia oxidation occurs due to a low nitrogen circulation activity.

It can be seen from these results that the total number of microorganisms in the paddy field does not tend to concentrate at low points, with the points being evenly scattered. In addition, the TC is essentially within 10,000 - 30,000 mg·ka<sup>-1</sup>, indicating that the fertilization of paddy fields in Japan is uniform, and that the carbon content is lower than that in upland fields. This can be accounted for by considering that the paddy field's main source of carbon is rice straw after cutting.

Therefore, the differences of the panicle number, number of rough and brown rice, 1000-grain weight of rough and brown rice, percentage of ripened grains, and yield for rice grown in paddy fields in Japan was accounted for by differences in fertilization regime employed, *i.e.*, organic or chemical fertilizers. In addition, the total microbial populations of paddy fields cultivated with organic fertilizers are higher and the material cycles, including N and P circulation, are more active than when chemical fertilizers are employed.

## Acknowledgements

This work was supported by JSPS KAKENHI Grant Number JP19K15937.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

- [1] Sasaki, T. (1988) East Asian Agricultural Theory: Shakehata and Rice Farming. *Kobundo*, 359-361. (In Japanese)
- [2] FiBL and IFOAM (2017) The World of Organic Agriculture Statistics and Emerging Trends, 2017. Research Institute of Organic Agriculture FiBL and IFOAM-Organics International. <http://www.organic-world.net/yearbook/yearbook-2017.html>
- [3] Woese, K., Lange, D., Boess, C. and Bogl, K.W. (1997) A Comparison of Organically and Conventionally Grown Foods—Results of a Review of the Relevant Literature. *Journal of the Science of Food and Agriculture*, **74**, 281-293. [https://doi.org/10.1002/\(SICI\)1097-0010\(199707\)74:3<281::AID-JSFA794>3.0.CO;2-Z](https://doi.org/10.1002/(SICI)1097-0010(199707)74:3<281::AID-JSFA794>3.0.CO;2-Z)
- [4] Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P. and Niggli, U. (2002) Soil Fertility and Biodiversity in Organic Farming. *Science*, **296**, 1694-1697. <https://doi.org/10.1126/science.1071148>
- [5] Mitchell, A.E., Hong, Y.J., Koh, E., Barrett, D.M., Bryant, D.E., Denison, R.F. and Kaffka, S. (2007) Ten-Year Comparison of the Influence of Organic and Conventional Crop Management Practices on the Content of Flavonoids in Tomatoes. *Journal of Agricultural and Food Chemistry*, **55**, 6154-6159. <https://doi.org/10.1021/jf070344+>
- [6] de Ponti, T., Rijk, B. and van Ittersum, M.K. (2012) The Crop Yield Gap between Organic and Conventional Agriculture. *Agricultural Systems*, **108**, 1-9. <https://doi.org/10.1016/j.agsy.2011.12.004>
- [7] Seufert, V., Ramankutty, N. and Foley, J.A. (2012) Comparing the Yields of Organic

- and Conventional Agriculture. *Nature*, **485**, 229-232.  
<https://doi.org/10.1038/nature11069>
- [8] Singh, J.S., Pandey, V.C. and Singh, D.P. (2011) Efficient Soil Microorganisms: A New Dimension for Sustainable Agriculture and Environmental Development. *Agriculture, Ecosystems and Environment*, **140**, 339-353.  
<https://doi.org/10.1016/j.agee.2011.01.017>
- [9] Chen, M., Xu, P., Zeng, G., Yang, C., Huang, D. and Zhang, J. (2015) Bioremediation of Soils Contaminated with Polycyclic Aromatic Hydrocarbons, Petroleum, Pesticides, Chlorophenols and Heavy Metals by Composting: Applications, Microbes and Future Research Needs. *Biotechnology Advances*, **33**, 745-755.  
<https://doi.org/10.1016/j.biotechadv.2015.05.003>
- [10] Adhikari, D., Perwira, I.Y., Araki, K.S. and Kubo, M. (2016) Stimulation of Soil Microorganisms in Pesticide-Contaminated Soil Using Organic Materials. *AIMS Bioengineering*, **3**, 379-388. <https://doi.org/10.3934/bioeng.2016.3.379>
- [11] Adhikari, D., Kai, T., Mukai, M., Araki, K.S. and Kubo, M. (2014) A New Proposal for a Soil Fertility Index (SOFIX) for Organic Agriculture and Development of a SOFIX Database for Agricultural Fields. *Current Topics in Biotechnology*, **8**, 81-91.
- [12] Kai, T., Mukai, M., Araki, K.S., Adhikari, D. and Kubo, M. (2015) Physical and Biological Properties of Apple Orchard Soils of Different Productivities. *Open Journal of Soil Science*, **5**, 149-156. <https://doi.org/10.4236/ojss.2015.57015>
- [13] Kai, T., Mukai, M., Araki, K.S., Adhikari, D. and Kubo, M. (2016) Analysis of Chemical and Biological Soil Properties in Organically and Conventionally Fertilized Apple Orchards. *Journal of Agricultural Chemistry and Environment*, **5**, 92-99. <https://doi.org/10.4236/jacen.2016.52010>
- [14] Ministry of Agriculture, Forestry and Fisheries (2018) Nagano Prefecture Fertilizer Application Standards.  
[https://www.maff.go.jp/j/seisan/kankyo/hozen\\_type/h\\_sehi\\_kizyun](https://www.maff.go.jp/j/seisan/kankyo/hozen_type/h_sehi_kizyun)
- [15] Nicholas, D.J.D. and Nason, A. (1957) Determination of Nitrate and Nitrite. *Methods in Enzymology*, **3**, 981-984. [https://doi.org/10.1016/S0076-6879\(57\)03489-8](https://doi.org/10.1016/S0076-6879(57)03489-8)
- [16] Murphy, J. and Riley, J.P. (1962) A Modified Single Solution Method for the Determination of Phosphate in Natural Waters. *Analytica Chimica Acta*, **27**, 31-36.  
[https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/10.1016/S0003-2670(00)88444-5)
- [17] Aoshima, H., Kimura, A., Shibutani, A., Okada, C., Matsumiya, Y. and Kubo, M. (2006) Evaluation of Soil Bacterial Biomass Using Environmental DNA Extracted by Slow-Stirring Method. *Applied Microbiology and Biotechnology*, **71**, 875-880.  
<https://doi.org/10.1007/s00253-005-0245-x>
- [18] Matsuno, T., Horii, S., Sato, T., Matsumiya, Y. and Kubo, M. (2013) Analysis of Nitrification in Agricultural Soil and Improvement of Nitrogen Circulation with Autotrophic Ammonia-Oxidizing Bacteria. *Applied Biochemistry and Biotechnology*, **169**, 795-809. <https://doi.org/10.1007/s12010-012-0029-6>
- [19] Horii, S., Matsuno, T., Tagomori, J., Mukai, M., Adhikari, D. and Kubo, M. (2013) Isolation and Identification of Phytate-Degrading Bacteria and Their Contribution to Phytate Mineralization in Soil. *The Journal of General and Applied Microbiology*, **59**, 353-360. <https://doi.org/10.2323/jgam.59.353>
- [20] Kubo, M., Adhikari, D., Araki, S.K., Kubota, K., Shinozaki, A., Matsuda, F., Mitsukoshi, K., Mukai, M. and Watarai, H. (2017) Science of Soil Making. Seibundo Shinkosha Co. Ltd., Tokyo, 2-185.