Journal of Agricultural Chemistry and Environment, 2020, 9, 314-330
https://www.scirp.org/journal/jacen
ISSN Online: 2325-744X
ISSN Print: 2325-7458

# Effect of Organic and Chemical Fertilizer Application on Growth, Yield, and Soil Biochemical Properties of Landrace *Brassica napus* L. Leaf-and-Stem Vegetable and Landrace (Norabona)

**Takamitsu Kai*, Masahiko Tamaki**

Kurokawa Field Science Center, Meiji University, Kurokawa, Asao-ku, Kawasaki, Kanagawa, Japan
Email: *mtamaki@meiji.ac.jp

**How to cite this paper:** Kai, T. and Tamaki, M. (2020) Effect of Organic and Chemical Fertilizer Application on Growth, Yield, and Soil Biochemical Properties of Landrace *Brassica napus* L. Leaf-and-Stem Vegetable and Landrace (Norabona). *Journal of Agricultural Chemistry and Environment*, **9**, 314-330.
https://doi.org/10.4236/jacen.2020.94023

**Received:** August 31, 2020
**Accepted:** November 20, 2020
**Published:** November 23, 2020



## Abstract

Norabona is generally cultivated in Japan under management systems that use chemical fertilizers and synthetic chemical pesticides. However, the continuous use of these fertilizers and pesticides damages the soil environment and reduces the number of soil microorganisms. There has been little research investigating the effect of organic and chemical fertilizer applications on soil biochemistry and the growth and yield of norabona. In this study, we investigated the effect of organic and chemical fertilizer application on these factors during the norabona growing season from September 2019 to May 2020. Leaf length, shoot height, and shoot width were significantly higher under organic fertilizer management in the early stage of cultivation (in March) than under chemical fertilizer management. However, there was no significant difference between treatments for these growth parameters in later months, nor for any other parameters. Soil TN, and TP contents were significantly higher in the organic fertilizer treatment after harvest than prior to cultivation or after the chemical fertilizer treatment. In addition, soil TC, and volumetric water content were significantly higher in the organic fertilizer treatment than in chemical fertilizer treatment. The higher TC, TN, and C/N ratio in organic fertilizer treated soil appeared to increase the bacterial biomass, leading to enhanced nutrient circulation via N and P circulation activity, producing a rich soil environment with active soil microorganisms.

## Keywords

Organic Fertilizer, Soil Microorganisms, Soil Fertility, Agricultural Environment,

Environmental Conservation

## 1. Introduction

Norabona (*Brassica napus* L.) is a leaf-stalk vegetable grown mainly in the Kanto region in Japan, and is an important traditional vegetable in the spring season. The plants are harvested between February and May [1]. The eating quality of Norabona is relatively sweet due to the high sugar content in the stems and the high fructose and glucose contents [2]. Consumers eat them in boiled or stir-fried, or raw in salads and smoothies [1] [2]. Norabona is cultivated mainly by conventional methods using chemical fertilizers, and synthetic chemical pesticides applied around planting and early spring. However, the continuous use of these fertilizers and pesticides damages the soil environment and reduces the number of soil microorganisms. A recent report showed that only 1% of agricultural fields in the world are cultivated under organic farming systems [3]. Although the yield is relatively stable in conventional farming systems, excessive use of chemical fertilizers and synthetic chemical pesticides can cause severe environmental, socio-economic, and human health problems. As a result, consumer awareness towards organic foods has recently increased. Organic farming methods cause relatively lower environmental damage compared with conventional farming and organic crop products are considered tasty and healthy [4]. However, the yield in organic farming systems is more unstable and/or lower than in conventional farming systems [5] [6] [7] [8]. Therefore, an updated organic farming system is required to ensure high yield and quality of agricultural products. Soil microorganisms play several beneficial roles, such as decomposing organic resides, releasing nutrients to plants, and bioremediation of pesticide polluted soils [9] [10] [11]. Therefore, soil microorganisms are considered key players in maintaining soil fertility. A large and active microbial community is needed for efficient nutrient cycling and steady supply of nutrients to the plants. In a previous study, we developed a soil fertility index, SOFIX, for the evaluation of soil fertility [12]. Analysis of SOFIX data from several agricultural fields clearly showed that the number and activity of microorganisms can be significantly enhanced by controlling total carbon (TC) and total nitrogen (TN) contents. It has been found that excessive levels of TC, TN, total phosphorus (TP), and total potassium (TK) in conventional chemical fertilizer management systems can lead to yield reduction in Japanese orchards [13]. Furthermore, apple orchards are relatively rich in TC, TN, TP, and TK compared with annual croplands such as paddy fields and uplands [14]. Also, organic management of apple cultivation increased bacterial biomass while enhancing N and P circulation activity and high TC [15]. Similarly, in small-sized tomato cultivation, appropriate controls such as TC, TN, and C/N ratio of organic fertilizer increased microbial biomass and enhanced material circulation [16]. Stable and reproducible organic soil was used by base soils and additive materials based on SOFIX database [12], values of TC (≥25,000 mg/kg), TN (≥1,500 mg/kg), TP (≥1,100 mg/kg), TK (2,500 to 10,000 mg/kg), and C/N ratio (8 - 25) in the soil were mainly controlled by wood chips, peat moss, and vermiculite, and additive materials [17]. However, the relationship between microbial activity and plant growth remains unknown. There has been little research investigating the effect of organic and chemical fertilizer applications on soil biochemistry and the growth and yield of norabona. Therefore, the objective of this study is to investigate the effect of organic and chemical fertilizer application on soil biochemical properties and the growth, yield of norabona.

## 2. Materials and Methods

### 2.1. Experimental Treatments and Soil Texture

Between September 2019 and May 2020, we investigated the cultivation of Norabona (*Brassica napus* L.) using organic and conventional farming methods in an upland field at Ikuta Campus, Meiji University. Generally, norabona is cultivated with chemical fertilizers and synthetic pesticides, but in our study, no pesticides were used in either the organic or conventional farming treatments. The field has been managed with chemical fertilizers and synthetic pesticide management for about eight years. The norabona seeds were sown on a seedling bed in a greenhouse in September. Then, in October when they had reached the development stage of two to three main leaves, they were potted up in 9 cm pots. Then, in November they were planted in the field in rows, with 60 cm between plants and 70 cm between rows. The soil surface was then covered then with black mulch. In the conventional farming treatment, the chemical fertilizer was applied according to typical rates for high yielding growers in the local area. Specifically, basal fertilizer ($N:P_2O_5:K_2O$ = 150:200:150 kg·ha$^{-1}$) was incorporated into the field soil in September, before planting. Then subsequent fertilizer was applied on two occasions in December and January: ($N:P_2O_5:K_2O$ = 120:0:120 kg·ha$^{-1}$) on each occasion. The organic fertilizer was commercial cattle manure ($N:P_2O_5:K_2O$ = 270:200:270 kg·ha$^{-1}$, Oobari Corporation, Tochigi, Japan), and applied in September.

The soil was a light andosol, and particle size analysis was conducted by sieve analysis to determine the soil texture; sand (2 - 0.02 mm), silt (0.02 - 0.002 mm), and clay (less than 0.002 mm), with particles of less than 75 μm measured by sedimentation [18].

### 2.2. Soil Chemical Properties

Soil samples (top 15 cm layer, excluding the top 2 - 3 cm surface crust) were taken near the base of five selected norabona plants in each treatment, and mixed to provide one composite sample for each treatment. The following chemical properties were analyzed: TC, TN, ammonium-nitrogen ($NH_4^+$-N), nitrate-nitrogen ($NO_3^-$-N), TP, available phosphoric acid (SP), TK, and exchangeable potassium (SK). The TC content was analyzed with a TOC analyzer (Model: SSM-5000A, Shimadzu, Kyoto, Japan). $NH_4^+$-N and $NO_3^-$-N were analyzed by extracting the soil sample with 1 M KCl, followed by the indophenol blue and brucine methods, respectively [19]. SP and SK were analyzed by shaking a soil-water suspension (1:20, w/v) at 100 rpm for 1 h and analyzing the extract with the molybdenum blue method [20] and atomic absorption spectrophotometry, respectively. The TN, TP, and TK contents were analyzed by digesting soils in a Kjeldahl Therm digestion unit (Gerhardt, Königswinter, Germany) with $H_2SO_4$ and $H_2O_2$; $NH_4^+$-N, SP, and SK contents in the digest were determined. The pH of a soil-water suspension (1:2.5, w/v) was analyzed using a pH meter (Model: LAQUA F-72, Horiba Scientific, Kyoto Japan).

## 2.3. Soil Biological Properties

Organic nitrogen substances such as proteins in soil are decomposed by soil microorganisms into ammonia nitrogen ($NH_4^+$) → nitrite nitrogen ($NO_2^-$) → nitrate nitrogen ($NO_3^-$). The $NH_4^+$ oxidation activity ($NH_4^+ \rightarrow NO_2^-$), $NO_2^-$ oxidation activity ($NO_2^- \rightarrow NO_3^-$), and bacterial biomass were determined. Bacterial biomass was determined by eDNA analysis, which establishes an accurate and simple measurement by extracting microbial DNA from soil. $NH_4^+$ oxidation activity, $NO_2^-$ oxidation activity, and the number of microorganisms were quantified with a triangular radar chart, and the ability of the soil to convert organic nitrogen to $NO_3^-$ was evaluated as "N circulation activity." 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 inorganic phosphate (phytic acid-degrading activity) before the plant can absorb phosphate. Therefore, the ability to convert phytic acid into organic phosphate was evaluated as "P circulation activity".

Soils in which all phytic acid was converted to phosphoric acid, and there was no mineral chemisorption were assigned a P circulation activity of 0 (zero). A P circulation activity of 100 points would indicate that there is little mineral content. Therefore, soil with a moderate mineral content and abundant microorganisms (due to phosphoric acid being supplied) was assigned a P circulation activity value of 40 - 60. The following biological properties were analyzed: total bacterial biomass, $NH_4^+$ oxidation activity, $NO_2^-$ oxidation activity, N circulation activity, and P circulation activity. Total bacterial biomass was estimated by quantifying environmental DNA (eDNA) using the slow-stirring method following the procedures of Aoshima *et al.* (2006) [21]. The N circulation activity was analyzed by pooling the values of $NH_4^+$, $NO_2^-$ oxidation activities, and total bacterial number. The $NH_4^+$ and $NO_2^-$ oxidation activities were estimated by analyzing the percent reduction in N in soil sample with added ammonium sulfate and sodium nitrate, respectively, that were incubated for 3 days at 25˚C, as described by Matsuno *et al.* (2013) [22] and Adhikari *et al.* (2014) [12]. Similarly, 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 [23].

## 2.4. Main Stem Harvest

While the main stem was still at the immature stage, before stem elongation and having about 15 to 17 main leaves and before stem elongation, the top of the main stem was cut off to leave the main stems on all plants with 10 to 12 leaves. After cutting off the top of the main stem, the flower stalks of all side branches were harvested to leave the primary side branches with 0 to 4 leaves and the other side branches with 0 to 3 leaves, based on management methods reported by Nomura [24] and Odawara *et al.* [25].

The number of leaves was counted on each plant. Also, shoot height, shoot with and the width and length of the four largest leaves on each plant were measured with a ruler. SPAD values of the four leaves were measured by chlorophyll meter (SPAD-502Plus, Konica Minolta Co., Osaka, Japan).

## 2.5. Growth and Yield Measurement

Eight replicate samples of 5 plants each were harvested for each fertilizer treatment. Average values were calculated on a per plant basis. The harvest period started on 4 March 2020 and ended on 26 May 2020. Main stems and primary lateral branches were harvested in March, secondary lateral branches in April, and tertiary lateral branches in May. Flower stems that reached 30 cm or more in length to the leaf tip were harvested on the primary lateral branches, and flower stems that had emerged on the secondary lateral branches and beyond were harvested based on the report by Tsuge *et al.* [26].

The roots and shoots of the 5 plants × 8 replicates (= 40 plants) from each fertilizer treatment were collected. The roots and shoots from the 8 replicates of 5 plants each, were harvested every month, and the roots were washed carefully. The total dry weights of shoots and roots were measured after oven drying at 80˚C for 3 days. The number of lateral branches was defined as the number of flower stems harvested from one plant. The lateral branch saleable yield was defined as the amount of flower stems generated from one plant, and the weight of all harvested flower stems. Based on the reports of Yoshida [27] and Tsuge *et al.* [28], flower stalks exceeding 30 cm length were cut to 30 cm in length and weighed. The saleable weight per lateral branch is a value related to the quality of the flower stalk, with higher values indicating better quality, and was determined by dividing the lateral branch saleable yield by the number of lateral branches.

## 2.6. Sugar Content of Norabona

The sugar content was analyzed using a pocket sugar meter (Model: PAL-S, Atago, Tokyo, Japan). Sugar content was determined by dropping undiluted norabona juice onto the sensor of the pocket sugar meter. Each norabona juice sample was measured three times, and the average was calculated.

### 2.7. Statistical Analysis

Data are presented as the mean ± standard deviation (SD) values and analyzed using BellCurve for Excel 2016 for Windows (Social Survey Research Information Co., Ltd., Tokyo, Japan). All data were analysed using ANOVA followed by Fisher's least significant difference test where appropriate. All statistical analysis was conducted at a significance level of $\alpha = 0.05$ ($p < 0.05$).

## 3. Results

### 3.1. Soil Chemical Properties

The soil grain size of the field site was 53.2% sand, 29.0% silt, and 17.8% clay, meaning the soil had a clay loam texture. Soil chemical properties before norabona cultivation in September 2019, and in the organic and chemical treated soils in May 2020 (after harvest) are shown in Table 1. The soil TC, TN, TP, and TK content values before cultivation were 25862, 2806, 2137, and 2746 mg kg$^{-1}$, respectively. The C/N ratio value was 9.2. In addition, $NO_3^-$-N, $NH_4^+$-N, SP, SK content values were 40, 2, 2, and 245 mg·kg$^{-1}$, respectively. Furthermore, pH, EC, and volumetric water content values were 6.3, 0.66 mS·cm$^{-1}$, and 24.7%, respectively. After harvesting the norabona in May 2020, the soil TC, TN, TP, TK contents in the organic fertilizer were 49,033, 3584, 2721, and 3394 mg·kg$^{-1}$, respectively, giving a C/N ratio of 13.7. The $NO_3^-$-N, $NH_4^+$-N, SP, SK contents were 2, 11, 15, and 48 mg·kg$^{-1}$, respectively, and the pH, EC, and volumetric water contents were 6.9, 0.71 mS·cm$^{-1}$, and 42.5%. In comparison, the chemically fertilized soil had TC, TN, TP, TK contents of 28,733, 1,793, 1,504, and 3,107 mg·kg$^{-1}$, respectively. giving a C/N ratio of 14.4. The $NO_3^-$-N, $NH_4^+$-N, SP, SK contents were 5, 18, 37, and 172 mg·kg$^{-1}$, respectively, and the pH, EC, and volumetric water contents were 5.6, 0.65 mS·cm$^{-1}$, and 33.5%.

The TN and TP contents were significantly higher after the organic fertilizer soil than in the chemical fertilizer soil or before cultivation, as was soil pH. The TC content and volumetric water content were significantly higher after the organic fertilizer treated soil than before cultivation, but SK content was significantly lower. On the other hand, the C/N ratio, $NH_4^+$-N, and SP contents were significantly higher after the chemical fertilizer treated soil than before cultivation. However, $NO_3^-$-N content was significantly in the organic and chemical fertilizers treated soils than before cultivation, but there was no significant difference for TK or EC.

### 3.2. Soil Biological Properties

Soil biological properties before norabona cultivation in September 2019, and in the organic and chemical fertilizer treated soils in May 2020 (after harvest) are shown in Table 2. Bacterial biomass number, $NH_4^+$ oxidation activity, $NO_2^-$ oxidation activity, N circulation activity, and P circulation activity values before cultivation were 0.3 × 10$^8$ cells·g$^{-1}$, 51.3, 66.0, 13.3, and 0.7 points, respectively. After harvest in the organic fertilizer treated soil, the bacterial biomass number,

**Table 1.** Soil chemical properties. [z]Mean ± standard deviation of a sample (TC, TN, TP, TK, C/N ratio, $NO_3^-$-N, $NH_4^+$-N, SP, SK, pH, EC, and volumetric water content: n = 8). [y]Different letters within columns are significantly different at $P < 0.05$ level, according to the Tukey-Kramer method.

| Period | Experimental treatments | Total carbon (TC) ($mg \cdot kg^{-1}$) | Total nitrogen (TN) ($mg \cdot kg^{-1}$) | Total phosphorus (TP) ($mg \cdot kg^{-1}$) | Total potassium (TK) ($mg \cdot kg^{-1}$) | C/N ratio | Nitrate nitrogen ($NO_3^-$-N) ($mg \cdot kg^{-1}$) | Ammonia nitrogen ($NH_4^+$-N) ($mg \cdot kg^{-1}$) | Available phosphoric acid (SP) ($mg \cdot kg^{-1}$) | Exchangeable potassium (SK) ($mg \cdot kg^{-1}$) | pH | EC ($mS \cdot cm^{-1}$) | Volumetric water content (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| September 2019 | Before cultivation | 25,862 ± 2,590[z]b[y] | 2,806 ± 146b | 2,137 ± 150b | 2,746 ± 499a | 9.2 ± 1.2b | 40 ± 28a | 2 ± 0b | 2 ± 1b | 245 ± 82a | 6.3 ± 0.7ab | 0.66 ± 0.05a | 24.7 ± 5.9b |
| May 2020 | Organic | 49,033 ± 1,449a | 3,584 ± 69a | 2,721 ± 50a | 3,394 ± 106a | 13.7 ± 0.5ab | 2 ± 2b | 11 ± 8ab | 15 ± 3ab | 48 ± 4b | 6.9 ± 0.7a | 0.71 ± 0.02a | 42.5 ± 2.1a |
| | Chemical | 28,733 ± 3,482ab | 1,793 ± 113c | 1,504 ± 100c | 3,107 ± 145a | 14.4 ± 2.0a | 5 ± 3b | 18 ± 2a | 37 ± 8a | 172 ± 9ab | 5.6 ± 0.2b | 0.65 ± 0.05a | 33.5 ± 4.2ab |

**Table 2.** Soil biological properties. [z]Mean ± standard deviation of a sample (bacterial biomass, $NH_4^+$-N oxidation activity, $NO_2^-$-N oxidation activity, N circulation activity, and P circulation activity: n = 8). [y]Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Period | Experimental treatments | Bacterial biomass ($\times 10^8$ cells·g$^{-1}$) | $NH_4^+$ oxidation activity (point) | $NO_2^-$ oxidation activity (point) | N circulation activity (point) | P circulation activity (point) |
|---|---|---|---|---|---|---|
| September 2019 | Before cultivation | 0.3 ± 0.2[z]b[y] | 51.3 ± 12.5b | 66.0 ± 6.5b | 13.3 ± 5.4b | 0.7 ± 0.9b |
| May 2020 | Organic | 16.1 ± 4.1a | 72.0 ± 2.1a | 83.3 ± 5.1a | 67.8 ± 4.7a | 4.5 ± 0.9a |
| | Chemical | n.d.c | 58.0 ± 4.5ab | 55.0 ± 4.8b | 10.6 ± 0.5b | 0.0 ± 0.0c |

$NH_4^+$ oxidation activity, $NO_2^-$ oxidation activity, N circulation activity, and P circulation activity values were 16.1 × $10^8$ cells·g$^{-1}$, 72.0, 83.3, 67.8, and 4.5 points, respectively. In comparison, the respective values after harvest in the chemical fertilizer treated soil were not detectable (n.d.) cells·g$^{-1}$, 58.0, 55.0, 10.6, and 0 points, respectively.

Bacterial biomass and P circulation activity values were higher in the organic fertilizer soil than before cultivation or in the chemical fertilizer soil. In addition, $NO_2^-$ oxidation activity and N circulation activity were significantly higher in the organic fertilizer treated soil than the chemical fertilizer treated soil or before cultivation. Furthermore, $NH_4^+$ oxidation activity was significantly higher in the organic fertilizer treated soil than before cultivation.

### 3.3. Main Stem Yield

Average per plant leaf number, leaf length, leaf width, SPAD, shoot height, shoot width, and main stem + primary lateral branch yield of the organic and chemical fertilizer treatments in March are shown in Table 3. In the organic fertilizer treatment, leaf number, leaf length, leaf width, SPAD, shoot height, width, and yield were 20, 46.1 cm, 15.2 cm, 51.2, 37.0 cm, 74.0 cm, and 153 g, respectively. In comparison, the respective values in the chemical fertilizer treatment were 19, 41.0 cm, 14.6 cm, 56.0, 32.8 cm, 68.8 cm, and 158 g, respectively. Leaf length, shoot height, and shoot width were significantly higher in the organic fertilizer treatment, but SPAD was significantly lower. There was no significant difference between the treatments for leaf number, leaf width or yield.

The root diameters of the organic and chemical fertilizer treatments were 53.7, and 52.0 mm, respectively in March, 73.4, and 75.2 mm, in April and 71.5, and 71.4 mm, respectively in ay (Table 4). There was no significant difference between treatments in any month. Root fresh weights and dry weights are shown in Table 5 and Table 6, respectively. There were no significant differences between treatments in any month. Shoot fresh and dry weights are shown in Table 7 and Table 8, respectively. There were no significant differences between treatments in any month.

### 3.4. Lateral Branch Yield

The total lateral branch yields of the organic and chemical fertilizer treatments

Table 3. Main stem yield. zMean ± standard deviation of a sample (leaf number, leaf length, leaf width, SPAD, shoot height, shoot width, and yield: n = 40). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Leaf number (number) | Leaf length (cm) | Leaf width (cm) | SPAD | Shoot height (cm) | Shoot width (cm) | Yield (g) |
|---|---|---|---|---|---|---|---|
| Organic farming | 20 ± 3$^{z}$a$^{y}$ | 46.1 ± 6.9a | 15.2 ± 2.8a | 51.2 ± 7.6b | 37.0 ± 6.9a | 74.0 ± 5.9a | 153 ± 47a |
| Conventional farming | 19 ± 3a | 41.0 ± 5.7b | 14.6 ± 2.2a | 56.0 ± 9.5a | 32.8 ± 5.8b | 68.8 ± 6.3b | 158 ± 56a |

Table 4. Root diameter. zMean ± standard deviation of a sample (March, April, May: n = 8). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Root diameter (mm) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 53.7 ± 4.2$^{z}$a$^{y}$ | 73.4 ± 9.3a | 71.5 ± 6.5a |
| Conventional farming | 52.0 ± 6.9a | 75.2 ± 6.1a | 71.4 ± 9.4a |

Table 5. Root fresh weight. zMean ± standard deviation of a sample (March, April, May: n = 8). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Root fresh weight (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 572 ± 139$^{z}$a$^{y}$ | 1,315 ± 422a | 913 ± 260a |
| Conventional farming | 487 ± 172a | 1,321 ± 597a | 1,028 ± 557a |

Table 6. Root dry weight. zMean ± standard deviation of a sample (March, April, May: n = 8). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Root dry weight (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 145 ± 23$^{z}$a$^{y}$ | 499 ± 208a | 346 ± 128a |
| Conventional farming | 117 ± 38a | 488 ± 240a | 380 ± 224a |

Table 7. Shoot fresh weight. zMean ± standard deviation of a sample (March, April, May: n = 8). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Shoot fresh weight (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 1,725 ± 253$^{z}$a$^{y}$ | 3,931 ± 1,103a | 3,348 ± 493a |
| Conventional farming | 1,639 ± 465a | 4,529 ± 914a | 3,553 ± 1,071a |

**Table 8.** Shoot dry weight. zMean ± standard deviation of a sample (March, April, May: n = 8). yDifferent letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Shoot dry weight (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 150 ± 19zay | 288 ± 99a | 245 ± 44a |
| Conventional farming | 148 ± 43a | 359 ± 40a | 282 ± 47a |

in March were 593, and 595 g, respectively (**Table 9**). In April and May, the respective yields were 1,580, and 1,360 g, and 1,518, and 1,583 g (**Table 10**). There were no significant differences between treatments in any month. Lateral branch saleable yields of the two treatments were 385, and 405 g, respectively in March, 1048, and 987 g, respectively in April and 933, and 996 g, respectively in May. No significant difference was found between treatments in any month. The number of lateral branches for each treatment in March, April and May are shown in **Table 11**. There was no significant difference between treatments in any month. The saleable weights per lateral branch are shown in **Table 12**. Saleable weight per lateral branch for the organic and chemical fertilizer treatments in March were 29 g, and 35 g, respectively. In April, the saleable weights were 17 g, and 17 g, respectively, and in May they were 9 g, and 8 g, respectively. However, there was no significant difference between treatments for any month.

### 3.5. Sugar Content of Norabona

The sugar content of the harvested norabona is shown in **Table 13**. The average sugar content in the organic fertilizer treatment in March was 12.2 ˚Brix, compared with 12.1 ˚Brix in the chemical fertilizer treatment. The average sugar contents in two treatments in April was 0.0 ˚Brix and with 9.9 ˚Brix, respectively, and in May was 8.1 ˚Brix and 7.3 ˚Brix. There were no significant differences between treatments in any month. Sugar content of both experimental treatments tended to decrease as the harvesting season got later in the year. This was in agreement with previous studies [29].

## 4. Discussion

The radar charts of average N circulation activity before norabona cultivation, and after harvest in May 2020 in the organic and chemical fertilizer treated soils are shown in **Figure 1**. Average N circulation activity values before cultivation, in the organic and chemical fertilizer treated soils were 13.3, 67.8, and 10.6, respectively. The application of organic fertilizer increased the bacterial biomass and soil N circulation activity compared to before cultivation and in the chemical fertilizer treatment. Average P circulation activity before cultivation, and in the organic and chemical fertilizer treated soils are shown in **Figure 2**. Average P circulation activity values before cultivation, in the organic and chemical fertilizer treated soils were 0.7, 4.5, and 0.0, respectively. The application of organic

**Table 9.** Total lateral branch yield. $^z$Mean ± standard deviation of a sample (March, April, May: n = 8). $^y$Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Total lateral branch yield (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 593 ± 281$^z$a$^y$ | 1,580 ± 342a | 1,518 ± 372a |
| Conventional farming | 595 ± 215a | 1,360 ± 296a | 1,583 ± 515a |

**Table 10.** Lateral branch saleable yield. $^z$Mean ± standard deviation of a sample (March, April, May: n = 8). $^y$Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Lateral branch saleable yield (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 385 ± 122$^z$a$^y$ | 1,048 ± 230a | 933 ± 196a |
| Conventional farming | 405 ± 93a | 987 ± 200a | 996 ± 279a |

**Table 11.** Number of lateral branches. $^z$Mean ± standard deviation of a sample (March, April, May: n = 8). $^y$Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Number of lateral branches (number) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 13 ± 3$^z$a$^y$ | 62 ± 17a | 110 ± 37a |
| Conventional farming | 12 ± 3a | 60 ± 12a | 130 ± 35a |

**Table 12.** Saleable weight per lateral branch. $^z$Mean ± standard deviation of a sample (March, April, May: n = 8). $^y$Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Saleable weight per lateral branch (g) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 29 ± 6$^z$a$^y$ | 17 ± 3a | 9 ± 2a |
| Conventional farming | 35 ± 7a | 17 ± 3a | 8 ± 3a |

**Table 13.** Sugar content. $^z$Mean ± standard deviation of a sample (March, April, May: n = 8). $^y$Different letters within columns are significantly different at P < 0.05 level, according to the Tukey-Kramer method.

| Experimental treatments | Sugar (˚Brix) | | |
|---|---|---|---|
| | March | April | May |
| Organic farming | 12.2 ± 0.8$^z$a$^y$ | 10.0 ± 1.1a | 8.1 ± 0.7a |
| Conventional farming | 12.1 ± 1.2a | 9.9 ± 1.3a | 7.3 ± 1.0a |

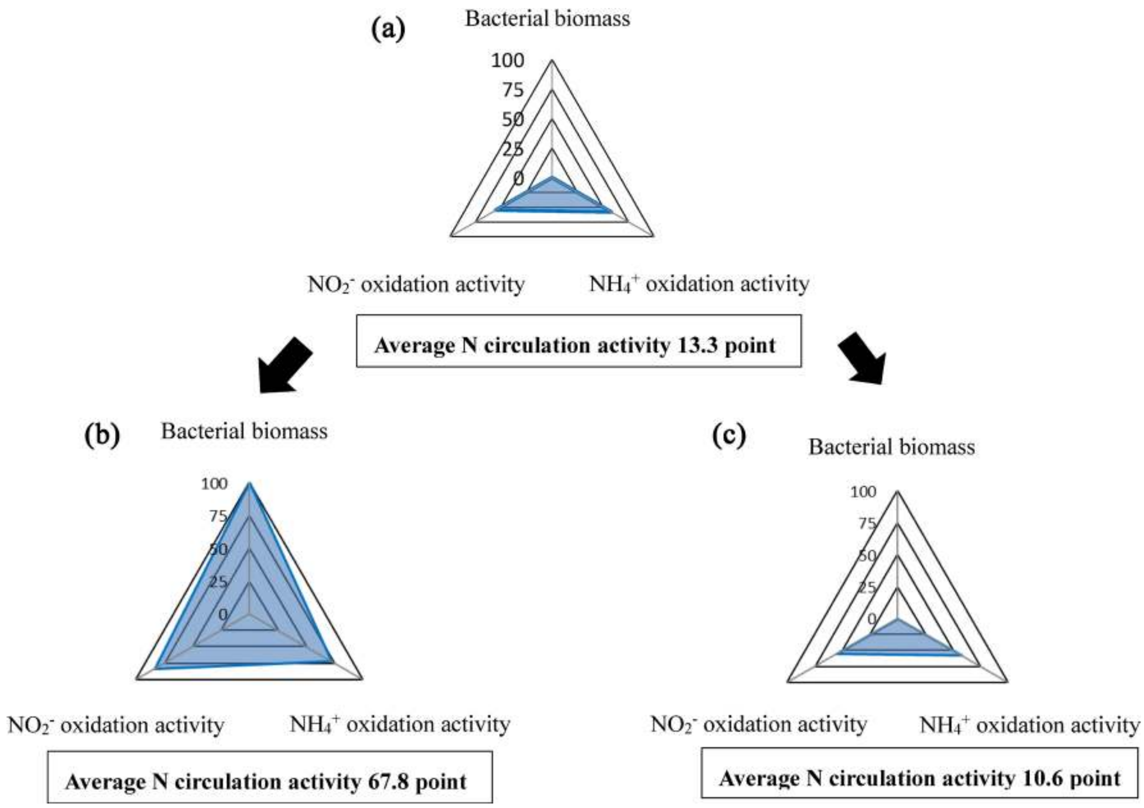


**Figure 1.** Radar charts of N circulation activity (a) before Norabona cultivation in September 2019, (b) under organic farming method in May 2020, and (c) under chemical farming method in May 2020.

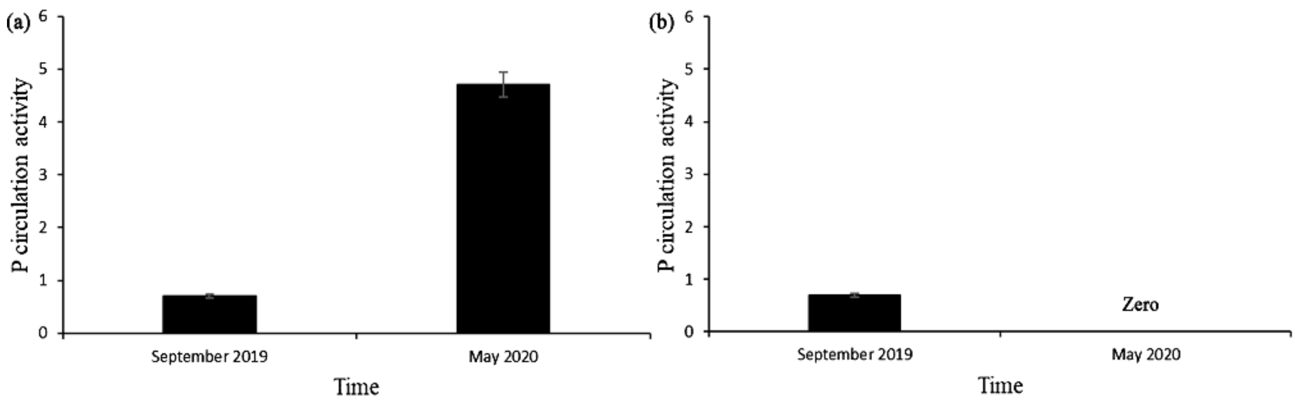


**Figure 2.** Average P circulation activity (a) Organic farming method, (b) Conventional farming method (n = 8).

fertilizer increased P circulation activity, just like for N circulation activity, compared to before cultivation and after chemical fertilizer treatment.

The correlation between total carbon and total nitrogen is shown in **Figure 3**. Kubo *et al.* (2017) [30] reported that the recommended carbon and nitrogen contents and C/N ratio in upland field are 25,000 mg·kg$^{-1}$ or higher, 1,500 mg·kg$^{-1}$ or higher, and 10 - 25, respectively. In our study, these recommended values were not reached before norabona cultivation, because the total carbon and total nitrogen contents were unbalanced. However, these recommended values were achieved by harvest time in the organic and chemical fertilizer treated soils. The balance of nitrogen and carbon were improved by the application of appropriate organic or chemical fertilizers. Furthermore, the relationship between soil TC and soil bacterial biomass is shown in Figure 4. Pitchayapa *et al.* [31] reported that the average bacterial biomass and TC content of the upland fields in different parts of Japan is 8.0 × $10^8$ cells·$g^{-1}$, and 33,120 mg·$kg^{-1}$, respectively. We designated four categories for soils based on these average values: bacteria biomass of above or below 8.0 × $10^8$ cells·$g^{-1}$, and total carbon content of above or below 33,210 mg·$kg^{-1}$. We categorized the individual field data from Pitchayapa *et al.* [31] and found that upland soils had mainly low soil bacterial biomass, but that the total carbon contents ranged from low to high. Thus, even if we only look at the bacterial biomass and the total carbon content, there are

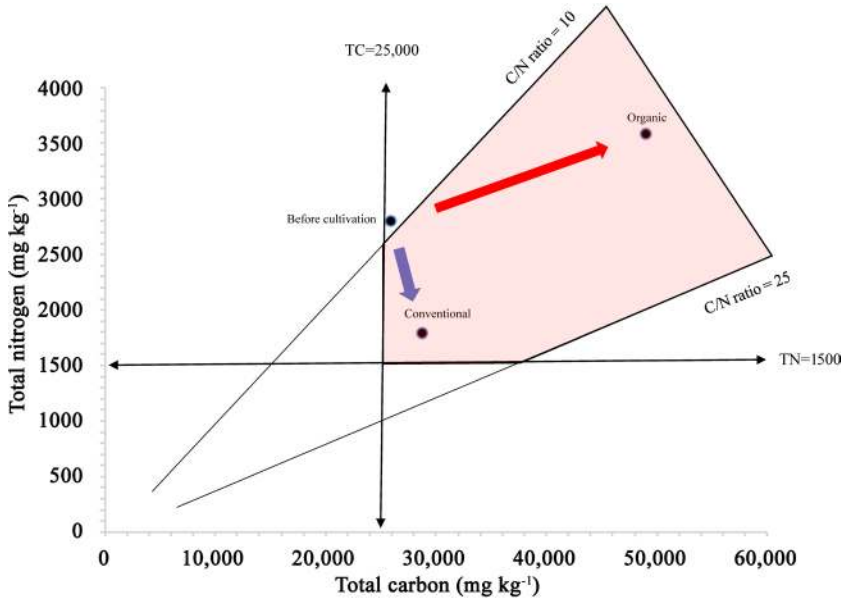


Figure 3. Correlation between total carbon and total nitrogen in upland field soils (n = 8).

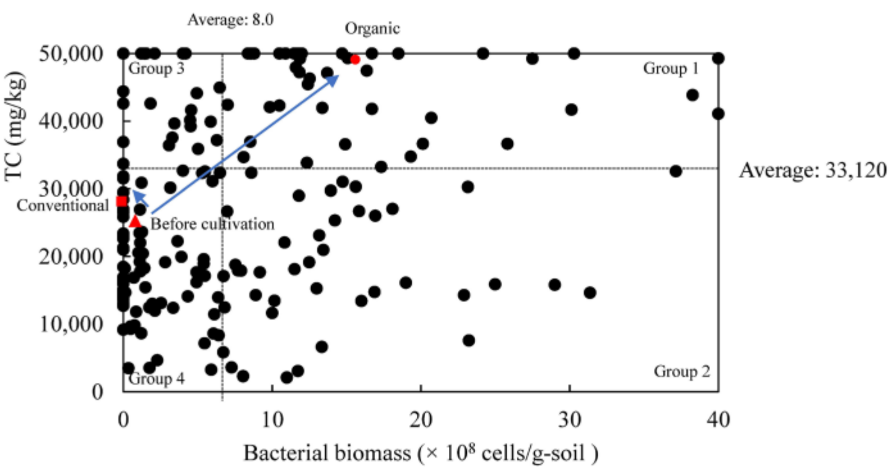


Figure 4. Relationship between soil TC and soil bacterial biomass: (▲) before Norabona cultivation in September 2019, (●) organic farming method in May 2020, and (▪) conventional farming method in May 2020 (n = 8).

various conditions in the soils of upland fields. In this study, the average bacterial biomass and total carbon content of the soil before norabona cultivation and in the chemical fertilizer treated soil were below average. Prior to the start of the norabona cultivation in September 2019, the field probably had received many chemical fertilizer and synthetic pesticide applications that would have affected the soil microbial population. In fields with long-term continuous use of chemical or synthetic fertilizers, there is a high possibility of reduced soil microbial populations. About 43% of the Japanese upland field soils in the Pitchayapa *et al.* (2020) [31] study had lower than average bacterial biomass and total soil carbon. The study fields had been using chemical fertilizers on a long-term basis. However, the application of organic fertilizer to the soil in our study resulted in above-average bacterial counts and total carbon levels. Therefore, the application of organic fertilizers may have increased microbial biomass while enhancing N and P circulation activity and TC.

In terms of the growth norabona, leaf length, shoot height, and shoot width in March were significantly higher in the organic fertilizer treatment than in the chemical fertilizer treatment. However, no other differences in norabona growth or yield were found between the two fertilizer treatments. In future, we intend to study the effects of several years of consecutive cropping of norabona in organic and chemical fertilizer treated soils, and the effects of varying the amount of applied fertilizer.

## 5. Conclusion

Soil conditions were adjusted for TC, TN contents and C/N ratio by applying appropriate amounts of organic fertilizer in an upland field where chemical fertilizers and synthetic chemical pesticides had been applied for about eight years. The cultivation of *Brassica napus* L. leaf-and-stem vegetable and landrace (Nonabona) with organic fertilizer treatment increased TC, TN and C/N ratio. It also increased soil bacterial biomass, N and P circulation activity and led to some improvement in leaf and shoot growth. We will continue to measure bacterial biomass, carbon and nitrogen contents while confirming the results of our soil development efforts. The goal is to create a highly reproducible organic agriculture that anyone can do.

## Acknowledgements

This research was funded by the Meiji University Research Grant for Individuals. We would like to thank them for their support.

## Conflicts of Interest

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

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