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Bio-organic fertilizers from food wastes induce changes in soil microbial community composition and the development of bok-choy (*Brassica rapa*)

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Abstract

Conversion of food wastes into bio-organic fertilizers is a rational solution that both reduces the burden on the environment and creates positive impacts on increasing agricultural productivity and securing world food security. Bio-organic fertilizers are mixtures of organic matter decomposition and living microorganisms. There are still unknown points about how these microorganisms carry out beneficial activities to the development of plants and how they interact with other microbes in a competitive environment in soils. This study examines the effects of two types of bio-organic fertilizers converted from either fruit peels or leafy vegetable wastes on the early development of bok-choy (*Brassica rapa*) and the microbial community in soil. Results indicated that both fertilizers positively impact the germination of bok-choy by increasing the sprouting rates while maintaining the stem development of the plants compatible with plants in the control case with unfertilized soil. The fresh root mass of plants grown in unfertilized soil was statistically higher than that of plants grown in fertilized soils, which correlated with a greater mean root length in this soil condition, although not significantly. A microbial count test pointed out shifts in the microbial populations that grow on Czapek agar (CZA) and De Man, Rogosa, and Sharpe (MRS) agar media. An increase in the lactobacilli population of the soil supplemented with bio-organic fertilizer from fruit peels suggested a connection to the significant increase in soil microbial respiration observed in this environmental condition.

1. Introduction

Food waste disposal is a global problem. It is estimated that about a third of food is disposed of each year as waste (Kang et al., 2021). To reduce the burden of burying waste in landfills or investing in technology for the treatment, composting food waste into organic biofertilizers is a rational solution (Song et al., 2021; Jara-Samaniego et al., 2017). Chinese cabbage has shown an increase in leaf length, root length, and fresh and dry weight when being treated with a mixture of fertilizer and food waste (Kang et al., 2021). An anaerobic digestate from four leafy vegetables used as fertilizer has been shown to be compatible with a commercial fertilizer for vegetable growth and overall bacterial diversity and composition (Song et al., 2021). Co-composting mixtures consisting of food market wastes and pruning of trees and ornament palms showed suitable for being used as organic fertilizers with no phytotoxicity, significant organic matter decomposition, and appropriate agronomic parameters (Jara-Samaniego et al., 2017). Land application of anaerobic digestion of organic fraction of household waste (OFHW) in Denmark would increase soil organic C build-up from 4 to 12% compared to the reference system that uses incineration of all OFHW (Klinglmair and Thomsen, 2020).

Soil health depends greatly on the composition and functions of the microbial community present in the soil (Wilhelm et al., 2023; Hermans et al., 2020). Human activities, however, make tremendous impacts on soil quality. To meet the demand for food, fuel, and fibre, efforts have been made to increase agricultural productivity by inputting fertilizers and pesticides into the soil, improving irrigation systems, and crop and soil management systems (Trivedi et al., 2016). Organic fertilizers transformed from food wastes could contain many fermentative microorganisms that facilitate the fermentation of food wastes into compost. The use of these fertilizers may accelerate the population of thermotolerant and thermophilic microbiomes, thus helping to achieve the highest growth of plants by improving the fertility of the soil (Areeshi, 2022). Microbes in biofertilizers perform the function of plant growth promotion through direct and indirect mechanisms. The direct mechanism involves some modifications in the hormone levels or fulfilling the nutrient requirements while the indirect mechanism involves the inhibitory effects of harmful microorganisms in soil. Employing metagenome analysis, the application of bio-organic fertilizer for pear fruit productivity yield has been demonstrated to be associated with changes in the abundance of ecological clusters of rhizosphere microbiomes dominated by *Mitsuaria* spp. and *Actinoplanes* spp. (Wang et al., 2022). Bio-organic fertilizer applied to cucumber cultivation along with successful control of Fusarium wilt disease can improve soil chemical condition and manipulate the composition of the soil microbial community (Huang et al., 2017).

Bacteria strains of *Bacillus* and *Trichoderma* have been shown to have the ability to stimulate the plant immune system by activating plant-induced systemic resistance (ISR), thus promoting growth in several crops (Bahramissharif and Rose, 2019). A combination of oak-bark compost and the two bio-agents, *Bacillus subtilis* subsp. *subtilis* and *Trichoderma harzianum* demonstrated to increase suppression of late blight disease in tomato plants. On lettuce plants, different integrated pest management strategies using Trichoderma-enriched compost, a combination of *T. gamsii*, *T. asperellum*, *B. amyloliquafaciens* and potassium phosphite, and a combination of *T. polysporum* and *T. atroviride* have shown efficiently reduce the severity of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lactucae* (Bellini et al., 2023). Another study demonstrated that mixing organic compounds prepared based on compost heaps of trimmed garden grass, chopped banana stems, and livestock and poultry manure with *Bacillus* spp. and *Trichoderma asperellum* is beneficial to banana seedlings by providing the best morphophysiological quality (Moreira et al., 2021).

Bio-organic fertilizers derived from food and agricultural wastes have been used for years to improve soil conditions and the resilience of plants to stress and diseases. This study was conducted in the laboratory using two types of bio-organic fertilizers converted from two different types of food wastes, fruit peels and leafy vegetables, through aerobic fermentation by a microbial mixture of *Trichoderma* spp. and *Bacillus subtilis*. The effects of these organic fertilizers on the budding and growth of bok-choy (*Brassica rapa*) and their impacts on the soil microbial community composition and soil respiration have been assessed.

2. Materials and methods

2.1. Materials

The soil used in this study was Tribat clean soil (Saigon Xanh Biotechnology Co. Ltd., Saigon, Vietnam), which has the main ingredient of coir peat mixed with red-worm farming soil. Coir peat is biologically pre-treated with *Trichoderma* spp. to remove pathogens before mixing with soil. The Tribat soil is preconditioned with minimal nutrients and minerals necessary for plant growth (total organic compounds 24.91%, humic acid 14.45%, a total nitrogen 0.9%, K_2O 0.73%, P_2O_5 0.3%, cation exchange capacity of 44.69 cmol kg^{-1}, and other necessary medium- and micro-elements in chelated forms). The bio-preparation used in the study was Tricho (Dien Trang Co. Ltd., Saigon, Vietnam), which contains the main ingredient: *Trichoderma* spp. 10^8 CFU g^{-1} and *Bacillus subtilis* 10^8 CFU g^{-1} in starch substrate, pH 6.5 and moisture 30%. The vegetable seeds used in this study were bok-choy (*Brassica rapa*) (Phu Nong Co.Ltd., Saigon, Vietnam).

Fertilizers used in the study are homemade fertilizers made from food by-products. Two types of food waste used in this study were peels of oranges and grapefruits and wastes from leafy vegetables. Wastes are sorted, chopped, and put in different plastic bags. The bags were then sealed and sterilized in an autoclave (MSLPS13, Neuvar, CA, USA). After the pasteurization, the wastes were sprayed with sterilized water to a moisture of 80% before mixing with the Tricho bio-preparation at a ratio of 1% w/w. They were incubated with periodic stirring under aerobic conditions for one week to convert into bio-organic fertilizers: fruit-peel and vegetable fertilizers before being used in the study.

2.2. Microbial counting experiment

The microbial media used in this study were Czapek agar (CZA) medium (saccharose 30 g L^{-1}, sodium nitrate 2 g L^{-1}, dipotassium phosphate 1 g L^{-1}, magnesium sulfate 0.5 g L^{-1}, potassium chloride 0.5 g L^{-1}, ferrous sulfate 0.01 g L^{-1}, and agarose 20 g L^{-1}; final pH 7.3 ±0.2) and De Man, Rogosa, and Sharpe (MRS) agar medium (Merck, Germany). CZA medium is suitable for qualitative procedures for cultivating saprophytic fungi, soil bacteria, and other microorganisms. MRS agar medium is optimized for the isolation and growth of all species of the genus *Lactobacillus*.

Microbial count experiments were conducted using the plate count method. One (1) g of sample is accurately taken and crushed before performing dilution steps in distilled water to a concentration of 10^{-5}. Fifty (50) μl of diluted sample was then spread on each Petri dish containing agar medium and incubated at 35–37°C for two days in an incubator (IF110, Memmert, USA). Finally, microbial colonies that grew on the dishes were counted to calculate the microbial population expressed as CFU g^{-1} (colony-forming unit per gram of sample).

2.3. Soil preparation and evaluation of germination and plant growth

Three types of soil were used in the study: a Tribat clean soil (original soil pH and moisture were 7 and 28%, respectively, measured by a soil tester (MS04; Sonkir, Hanoi, Vietnam)) (original soil, O), a Tribat clean soil mixed with fruit-peel fertilizer (P soil, P) at the ratio of 5% w/w, and a Tribat soil mixed with vegetable fertilizer (V soil, V) at the ratio of 5% w/w. Each prepared soil was then moisturized to 80%, and equal amounts (150 g) were placed into 500-mL PET cups. Bok-choy seeds were carefully selected at the same size. Five (5) seeds were sown on each cup with equal spacing.

The experimental cups were placed in a well-ventilated place at the conditions: ambient temperature ranged from 22°C to 27°C, 80–90% relative humidity, and 1500–1600 lx natural sunlight. The soil moisture during the experiment was maintained at approximately 80%. Plant development in response to each experimental condition was observed. Each treatment was conducted in triplicates. The sprouting rate and the lengths of stem and root after 11 days of sowing were recorded.

2.4. Measure of the soil microbial respiration

The measure of soil microbial respiration followed a procedure described in a previous study by Dang et al. (2022a). In brief, soil samples were pre-dried naturally to constant weight before re-moisturization with sterilized water to 80%, measured with the soil tester. Twenty (20) g of soil sample was put in and evenly coated on the bottom of a sterilized PET jar. A plastic tripod was then placed at the centre of the jar. The tripod is about 1 cm higher than the topsoil layer. Next, a glass beaker containing 10 ml of 0.5 M KOH solution, an initial pH of 13.35 (CAS 1310-58-3; Sigma-Aldrich, Germany), is placed on the tripod. The jar's mouth is then covered with a lid and sealed with Vaseline to ensure no influx or efflux of gas available. Finally, the sealed jar is placed in a well-ventilated area with natural light.

Along with microbial activities, CO_2 is a product of microbial respiration that escapes from the soil and is partially absorbed by the KOH solution to form K_2CO_3, thereby gradually reducing the pH of the solution. The solution is considered saturated when all the KOH is converted into K_2CO_3, i.e., 10 ml of 0.5 M KOH is converted into 10 ml of 0.25 M K_2CO_3. The pH of the saturated solution is calculated at 11.83 according to the calculation described in a previous study (Dang et al., 2022a). At different time points, 1, 2, and 4 days, the pH of the KOH solutions in the beakers is measured using a pH meter (Hanna pH-211, Sigma-Aldrich, Germany). After each measurement, opened jars are withdrawn from the experiment.

The relative pH variation indicates the respiration rate of soil microorganisms, which highly depends on the structure and composition of the soil microbial population. It can be determined using the following formula:

$$R_{pH}\ (\%) = ((pH_{KOH} - pH_t) \times 100)/(pH_{KOH} - pH_{K2CO3})$$

$$= ((pH_{KOH} - pH_t) \times 100)/(pH_{KOH} - 11.83) \quad (1)$$

Where R_{pH} is the relative change in the pH of the solution due to the conversion of KOH to K_2CO_3; pH_{KOH} is the pH of the original KOH solution; pH_{K2CO3} is the pH of the saturated solution; pH_t is the pH of the solution obtained at the day of measure. Each measurement condition was repeated at least thrice.

2.5. Statistical analysis

A statistical analysis was performed using one-way ANOVA. Fisher's LSD test was used to compare the means of the treatments. All statistical analyses were conducted using the statistical package StatPlus LE Build 7.7.0.0 for Windows (AnalystSoft Inc., Walnut, CA, USA). The significance level was set at $p < 0.05$.

3. Results

3.1. Bio-organic fertilizers from food wastes induce changes in the early development of bok-choy (*Brassica rapa*)

This experiment examined the sprouting rate of bok-choy in three different soil environments: a Tribat clean soil, a clean soil with bio-organic fertilizer made from fruit peels, and a clean soil with bio-organic fertilizer made from leafy vegetable wastes. The ratio of fertilizer to soil was 5% w/w, as explained in the Materials and Methods section. Analysis of the sprouting rate of bok-choy showed that the highest rate was observed in the soil supplemented with fertilizer from vegetable wastes, followed by the soil supplemented with fertilizer from fruit peels and that without fertilizer supplement (Fig. 1).

Statistical analysis of growth indices for plant biomass in different soil environments showed that samples in all soil environments had no significant difference in root length, although the root length of plants on soil without fertilizer supplement

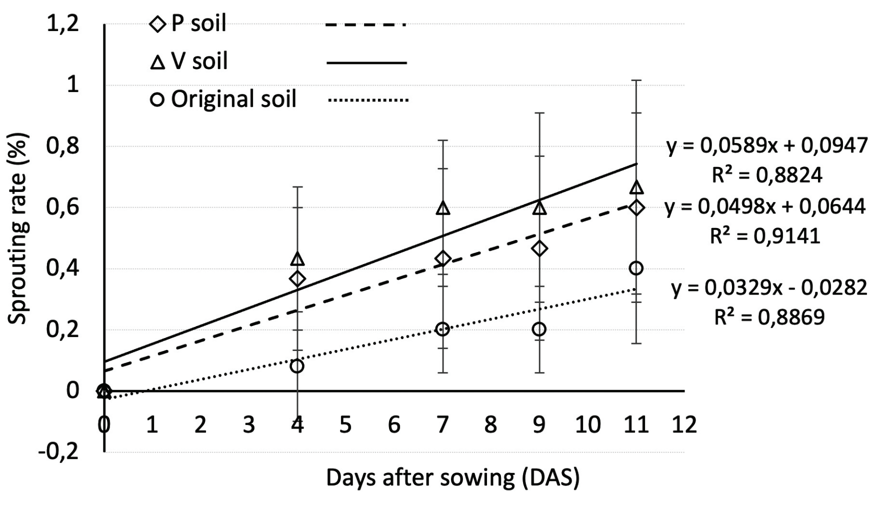


Fig. 1. Sprouting rate of bok-choy in different soil environments. Original soil, P soil, and V soil indicate clean soil without fertilizer supplement, soil supplemented with bio-organic fertilizer from fruit peels, and soil supplemented with bio-organic fertilizer from vegetable wastes, respectively.

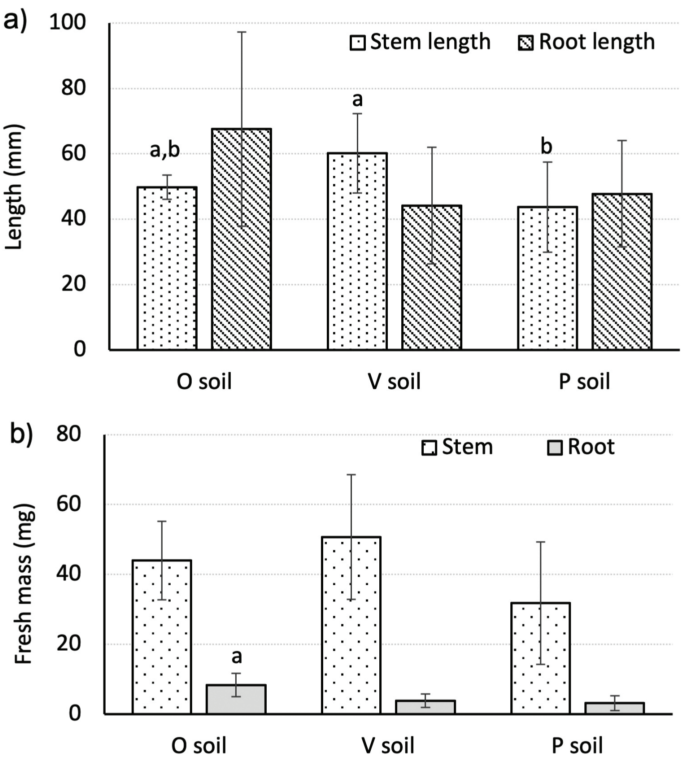
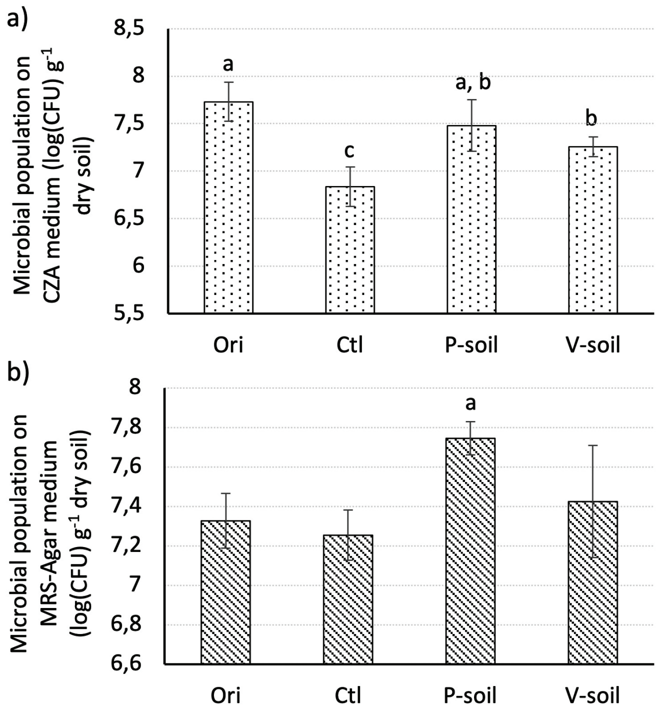


Fig. 2. Development of bok-choy in different soil environments. O soil, P soil, and V soil indicate clean soil without fertilizer supplement, soil supplemented with bio-organic fertilizer from fruit peels, and soil supplemented with bio-organic fertilizer from vegetable wastes, respectively. Letters a and b indicate statistically significant differences (Fisher's LSD, P < 0.05).



was a bit higher on average (57.25 ± 21.6 mm). Plants grown on the soil supplemented with bio-organic fertilizer from vegetable wastes showed significantly higher in the stem height compared with the plants on soil supplemented with bio-organic fertilizer from fruit peels. However, comparing the stem length of the plants grown on these two media with those grown on the soil without fertilizer supplement, there was no significant difference (Fig. 2a).

Fig. 2b shows the fresh masses of stems and roots 11 days after sowing. Although statistical analysis showed no significant difference in the stem mass of plants in different soil environments, the root masses indicated statistical differences between those of plants in unfertilized soil and soils with fertilizer supplements. The root mass of plants in unfertilized soil (8.4 ± 3.4 mg) was higher than the root masses of plants in the other two soil environments. The root weights of plants in the two soil environments supplemented with the two different bio-organic fertilizers did not show a statistically significant difference.

3.2. Bio-organic fertilizers from food wastes induce changes in soil microbial populations that grow on CZA and MRS media

This experiment used the traditional plate counting method to assess the total microbial density in the three types of soil environments described in the previous part after a sowing period. Two types of microbial culture media were used: CZA medium suitable for propagating fungi and aerobic cultures of soil microorganisms and MRS agar optimized for the culture of *Lactobacillus* strains. Statistical analysis indicates the changes in microbial populations on CZA and MRS culture media and in different soil environments (Fig. 3). The microbial populations on CZA medium of the soil without fertilizer supplement and soil supplemented with bio-organic fertilizer from vegetable wastes showed significant decreases after 11 days of sowing. However, the microbial population on CZA medium of the soil supplemented with bio-organic fertilizer from fruit peels remained equivalent to the initial population on CZA medium of a clean soil environment before starting the trial (Fig. 3a).

The lactobacilli populations that grow on MRS medium, however, showed the opposite when these populations in all soil environments did not reduce during the experiment (Fig. 3b). In particular, the lactobacilli population in the soil environment supplemented with bio-organic fertilizer from fruit peels indicated a significant increase after 11 days of sowing. These populations in the soil environment without fertilizer supple-

Fig. 3. The soil microbial populations in different soil environments. Ori indicates clean soil without fertilizer supplement at the starting point of the experiment. Ctl, P-soil, and V-soil indicate clean soil without fertilizer supplement, soil supplemented with bio-organic fertilizer from fruit peels, and soil supplemented with bio-organic fertilizer from leafy vegetable wastes 11 days after sowing, respectively. Letters a, b, and c indicate statistically significant differences (Fisher's LSD, P < 0.05).

ment and soil supplemented with bio-organic fertilizer from leafy vegetables showed no statistical difference compared to the initial soil environment.

3.3. Bio-organic fertilizers from food wastes induce changes in soil microbial respiration

Total soil respiration, mainly contributed by soil microbial respiration, is among the substantial factors in assessing soil health and the soil microbial communities. In this experiment, soil respiration rates of three types of soil media were evaluated using a simple method described in the Materials and Methods section. The evaluation results revealed that the highest respiration rate was observed on soil supplemented with bio-organic fertilizer from fruit peels (Fig. 4). This result is also significantly higher than the results of soil respiration rate for the other two soil samples. Meanwhile, the respiration rates of soil supplemented with bio-organic fertilizer from vegetable wastes and soil without fertilizer supplement showed no significant statistical difference.

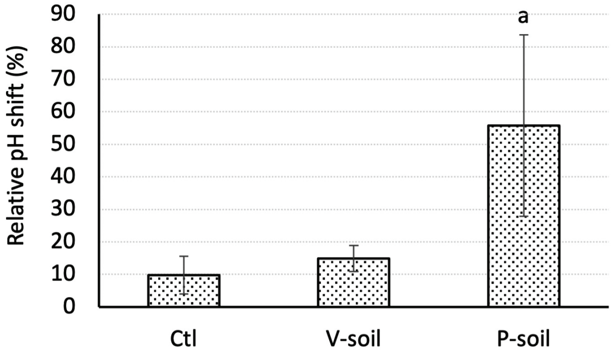


Fig. 4. Respiration rates of the three different soil environments. Ctl, P-soil, and V-soil indicate clean soil without fertilizer supplement, soil supplemented with bio-organic fertilizer from fruit peels, and soil supplemented with bio-organic fertilizer from vegetable wastes 11 days after sowing, respectively. Letter a indicates a statistically significant difference (Fisher's LSD, $P < 0.05$).

4. Discussion

The role of lactic acid bacteria (LAB) in promoting plant growth has been demonstrated in some other studies (Lamont et al., 2017; Raman et al., 2022; Younas et al., 2022). LAB have been used as biofertilizers for the remediation of soil, effective control of bacterial and fungal phytopathogens, improving plant stress tolerance, and possibly acting as bio-stimulants to promote the growth of plants. *Lactobacillus acidophilus* demonstrated an ability to carry out some activities to promote plant growth, thus increasing the potato dry mass (Panetto et al., 2023). Yunaira et al. (2020) proved that the probiotic strain *Lactobacillus fermentum* InaCC B1295 has positive effects on the growth of mustard greens with the increases observed in the height and the number of leaves of the plant. In this study, adding bio-organic fertilizer to the soil, besides inducing an increase in lactobacilli populations (Fig. 3), also accelerates the budding rate of bok-choy (Fig. 1). These two effects could relate to each other and in agreement with the results in previous studies about the effects of LAB on plant growth.

There was no difference in the length and fresh mass of stems in the plants grown on both fertilized and unfertilized soil conditions (Fig. 2). Although the root length of plants grown on different soil environmental conditions did not indicate any difference, the fresh mass of roots in plants grown on soil without fertilizer supplement was significantly higher than that of plants in the two other cases. This suggests possible difference in root structure under these different soil conditions. Therefore, observations of plant growth parameters over a longer period of cultivation time along with changes in the structure of root are necessary to have a better understanding of this difference.

Observing the image of plant roots under different soil environmental conditions shows that roots of plants grown in soil supplemented with fertilizers tend to develop more secondary root branches than those grown in soil without supplementation fertilizer (Fig. 5). A study has indicated that adding iron oxide materials to soil can impact the growth and structure of plant roots (Dang et al., 2021). Another study demonstrated that the supplementation of iron oxide materials to soil induces changes in the composition of the soil microbial community, including a significant increase in the microbial population on the MRS medium (Dang et al., 2022b). This may suggest a link between differences in the root structure of plants in different soil environments and changes in the soil microbial community composition in this study. Both soils supplemented with bio-organic fertilizers showed increases in the microbial populations on MRS and CZA media compared to the case without fertilizer supplement, especially in the soil supplemented with bio-organic fertilizer from fruit peels where significant increases can be observed with microbial populations on both MRS and CZA media (Fig. 3). Besides, soil supplemented with fruit-peel fertilizer showed a drastic increase in soil respiration compared to the two other soil conditions (Fig. 4). This agrees with the results of a previous study (Dang et al., 2022a) and suggests a significant role of the lactobacilli community in soil respiration activities.

Fig. 5. Plant roots on day 11 after sowing. From left to right, plants grown on soil without fertilizer supplement, soil supplemented with bio-organic fertilizer from fruit peels, and soil supplemented with bio-organic fertilizer from vegetable wastes, respectively.

Another study has demonstrated that when dissolved organic matter is added to soil, it stimulates an opportunistic subset of the soil bacterial community, particularly members of the Gamma-proteobacteria and Firmicutes groups, thus driving high rates of soil respiration (Cleveland et al., 2007). Du and colleagues (2020) demonstrated that the amendment of soil with organic material reduces the population of heterotrophic microorganisms, while effective microbial supplementation increases the heterotrophic count of soil bacteria both before planting and after harvest.

Lactic acid-producing microorganisms are known to make significant contributions to soil health, providing services such as amendments, nutrient regulation, sterilization, and provision of growth stimulants to the soil (Lamont et al., 2017; Jaffar et al., 2023). LAB, when introduced into the soil as fertilizers, will promote the decomposition of organic materials along with increasing soil respiration activities (Anjum and Khan, 2021), induce changes in the relative abundance of bacterial groups, such as *Delftia*, *Halomonas*, *Lactobacillus*, and *Stenotrophomonas* (Afanador-Barajas et al., 2021). A cocktail of probiotic microorganisms includes *Lactobacillus* spp. added to soil has been demonstrated to positively affect soil quality by increasing the soil microbial activities and microbial biomass (Rajper et al., 2016). Effective microorganisms (EM) consist of a mixture of beneficial, naturally occurring microorganisms such as photosynthetic bacteria, lactobacilli, etc. have been shown to have great potential in application to increase crop productivity through protecting plants from pathogens, enhancing soil microbial activity, and converting humus for the soil (Olle and Williams, 2013). Although LAB are known to have many benefits to soil and plants, so far, the mechanisms behind their abilities to produce stimulants for plant growth and create an abundance in a complicated competitive microbial community in soil still need answers.

5. Conclusions

The study tested two types of bio-organic fertilizers converted from either fruit peels or leafy vegetable wastes on the germination of bok-choy and the soil microbial community. Results indicated that both soils supplemented with either fruit-peel or vegetable fertilizer could increase the sprouting rate of bok-choy. Other developmental indices, however, showed no significant difference except for the root fresh mass. The root mass of plants on soil without fertilizer supplement was significantly higher than that of plants on fertilized soils. A microbial count test demonstrated increases in microbial populations on both CZA and MRS media of fertilizer-supplemented soils. The significant increase in the lactobacilli population in soil supplemented with organic fruit-peel fertilizer could link to a statistically significant increase in total soil respiration. Although lactic acid bacteria have been shown to have many positive effects on soils and plants, the mechanism behind their abundant growth in a competitive environment by soil microorganisms remains a topic that needs an answer.

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