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Impact of organic liquid fertilizer on plant growth of Chinese cabbage and soil bacterial communities

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Organic liquid fertilizers from livestock manure are increasingly recognized as sustainable amendments influencing soil bacterial communities. Yet, their direct impacts on bacterial composition and crop functionality remain unclear. Addressing this gap, we developed a bio-liquid fertilizer (LBF) by culturing *Chlorella fusca* in a purified pig manure-based medium. We compared its effects with chemical (CLF) and fermented (FLM) liquid fertilizers on Chinese cabbage (*Brassica rapa* subsp. *pekinensis*). We aimed to determine how organic bio-liquid fertilizers enhance crop health and soil bacterial balance, contributing to sustainable agricultural practices. Although LBF did not surpass CLF in promoting growth, it significantly increased antioxidant compounds (polyphenols, flavonoids), sugars, and antioxidant activities, including nitrite-scavenging capacity and reducing power. Soil bacterial communities were strongly correlated with key chemical properties (Na, K, NO_3^--N, Ca, pH). Notably, *Litorilinea* decreased under CLF, and *Sphingomonas* and *Nocardioides* declined under FLM, whereas LBF treatment increased all three genera, suggesting improved bacterial conditions. These findings demonstrate that a well-designed organic bio-liquid fertilizer can bridge knowledge gaps by enhancing plant functionality and promoting beneficial soil bacteria. This approach supports more efficient nutrient recycling and may foster greater resilience and sustainability in modern farming systems.

Keywords Bacterial community, Bio-liquid fertilizer, Organic fertilizer, Soil fertility, Chinese cabbage, Sustainable agriculture

The use of agrochemicals is largely responsible for the capacity of global crop production to keep pace with the increasing global food demand owing to the ever-growing human population despite the continuous reduction in cropping area[1,2]. However, the excessive use of chemical fertilizers causes severe environmental pollution. Thus, for example, 50% and 90% of the nitrogen (N) and phosphorus (P) applied as fertilizer in agriculture, respectively, are released into the atmosphere or water resources, contributing to greenhouse gas emissions, soil salinity, and eutrophication[3–5]. Moreover, chemical fertilizers adversely affect the soil nutrient balance in natural ecosystems, thereby affecting all components of water and land food webs[6,7]. Indeed, previous studies have shown that long-term chemical fertilizers can lower the soil pH, reducing microbial diversity[8].

Furthermore, such reduction can reduce the soil's ability to suppress diseases, making it more vulnerable to harmful soil pathogens[9–11]. Therefore, environmentally friendly organic fertilizers can contribute to more sustainable agriculture[12–16]. In particular, applying organic fertilizers with crop residues and other biological wastes reportedly increases crop production and plant nutrition, improving kiwi (Actinidia deliciosa) plant growth and yield[17]. Similarly, organic manure application allegedly improves okra (Abelmoschus esculentus) growth, yield, and mineral content compared with the results obtained by applying NPK 15-15-15 fertilizer[18]. Another previous study found that applying a mixture of Hanwoo (Korean cattle) manure and chemical fertilizer enhanced maize growth compared with results observed upon application of chemical fertilizer alone[19]. Therefore, research on organic fertilizers has decidedly contributed to the development of sustainable agriculture[20].

Numerous studies have revealed that microbial inoculation, which can substitute synthetic farm inputs, improves the soil environment and plant growth[21,22]. Further, plant growth-promoting bacteria directly and

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indirectly affect the solubilization of soil nutrients (P, K, and Fe), concomitant with a marked enhancement of plant resistance to pest and disease attack[23,24]. Thus, for example, rhizobacteria improve water and nutrient uptake and plant abiotic and biotic stress resistance[25]. Furthermore, applying microbial inoculants, including *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum*, reportedly increases maize root and shoot dry weights compared to untreated plants[26]. Moreover, previous studies have attempted to determine the effect of the interaction of two or more microbes on crop growth and yield; however, the underlying mechanisms remain unclear[23,27]. *Chlorella fusca* is a photosynthetic microorganism with a high potential to produce useful metabolites[28]. Specifically, previous studies have revealed that *C. fusca* influences plant metabolism by increasing auxin and cytokinin hormones, which regulate plant growth and development[29,30]. These findings suggest combining bio-liquid fertilizers with microorganisms improves crop production and soil physiochemical properties. Therefore, in this study, we aimed to produce organic bio-liquid fertilizer through *C. fusca* culture using media developed from livestock manure.

Studies on the sustainable application of organic fertilizers for economic benefits are on the rise, clearly showing that organic fertilizers improve soil quality, lodging resistance, and crop production[31–33]. However, the mineralization of organic matter is necessary for a steady supply of nutrients, whereby the effects of organic fertilizers on plant growth occur at a slower rate than those of chemical fertilizers. Greenhouse cultivation ensures stable crop production by significantly overcoming environmental limitations. However, in greenhouse farming, the slurry of liquid organic fertilizers can cause nozzle clogging during irrigation. Therefore, we aimed to develop an organic-liquid fertilizer for use in greenhouse farming to supply plant nutrients sustainably. Specifically, we determined the effects of different organic liquid fertilizers on Chinese cabbage (*Brassica rapa* subsp. *pekinensis* (Lour.) Rupr.) growth and antibiotic activity and soil bacterial communities.

Results

Chemical components subsection

The chemical components of the Chemical Liquid Fertilizer (CLF), Fermented Liquid Manure (FLM), and Liquid Bio-Fertilizer (LBF) treatment used in the experiments were based on an Electrical Conductivity (EC) of 1.5 (Table 1). Among the different liquid fertilizers, pH was significantly ($P \leq 0.05$) higher in CLF (7.53) and FLM (7.63) than that in LBF (7.07). FLM (75.6 mg kg^{-1}) and LBF (80.42 mg kg^{-1}) organic-liquid fertilizers had relatively high amounts of NO_3^--N. The highest amounts of exchangeable cations were observed in the CLF for K (242 mg kg^{-1}) and in the FLM for Na (114 mg kg^{-1}) and Ca (109 mg kg^{-1}). The soil pH was significantly ($P < 0.05$) increased in the FLM (7.17) and LBF (7.03) groups compared with that of the CLF group (6.76). The soil NH_4^+-N content was the highest in the CLF group (200.81 mg kg^{-1}) and lowest in the LBF group (114.79 mg kg^{-1}). The FLM (184.49 mg kg^{-1}) and LBF groups (182.34 mg kg^{-1}) had relatively high amounts of NO_3^--N in the soil and liquid fertilizers. The highest amounts of exchangeable cations were observed in the soils of the CLF group for K (0.15 mg kg^{-1}) and Ca (109 mg kg^{-1}) and the FLM group for Na (0.53 mg kg^{-1}). However, EC, total N and P, and OM were not significantly different among the liquid fertilizers tested. We observed changes in pH and NH_4^+-N considering the quantitative order of the chemical components between the liquid fertilizers and soils after liquid fertilizer treatments. Overall, the CLF group soil had the lowest pH and highest amounts of NH_4^+-N, K, and Ca, whereas the FLM and LBF groups had higher amounts of NO_3^--N and Na than those of the CLF group.

Effects of different liquid fertilizers on plant growth

The effects of CLF, FLM, and LBF on plant development are presented in Fig. 1. The phenotypes of Chinese cabbage after applying different liquid fertilizers were considerably different. CLF and LBF application improved leaf growth and plant width compared with those of FLM application (Fig. 1a). A significant ($P < 0.05$) difference in the length of Chinese cabbages was observed in the FLM group 29 days after transplanting. The FLM group exhibited significantly ($P < 0.05$) lower cabbage length than the other groups. Furthermore, 57 days after transplanting, Chinese cabbage had the most significant increase in length in the CLF group (24.88 cm), followed

	Fertilizers			Soil after treatments		
	CLF	FLM	LBF	CLF	FLM	LBF
pH	7.53 (± 0.06) a	6.63 (± 0.04) b	7.07 (± 0.08) a	6.76 (± 0.09) b	7.17 (± 0.05) a	7.03 (± 0.03) a
EC (dS m^{-1})	1.57 (± 0.08) a	1.59 (± 0.11) a	1.53 (± 0.09) a	0.12 (± 0.02) a	0.11 (± 0.01) a	0.10 (± 0.01) a
NH_4^+-N (mg kg^{-1})	578 (± 25.05) a	590 (± 17.67) a	560 (± 25.13) a	1,279.61 (± 142.77) a	1,266.67 (± 153.93) a	1,158.01 (± 20.17) a
NO_3^--N (mg kg^{-1})	64.96 (± 7.21) a	46.2 (± 9.25) a	74.29 (± 5.53) a	200.81 (± 14.58) a	140.10 (± 18.53) ab	114.79 (± 14.21) b
P (mg kg^{-1})	14.66 (± 5.04) a	19.61 (± 9.17) a	37.26 (± 7.1) a	1,115.74 (± 71.23) a	1,028.84 (± 65.46) a	1,110.33 (± 36.53) a
K (mg kg^{-1})	242 (± 2.57) a	212 (± 3.33) b	177 (± 3.88) c	0.15 (± 0.006) a	0.13 (± 0.007) b	0.14 (± 0.003) b
Na (mg kg^{-1})	47.5 (± 1.23) c	114 (± 1.8) a	78.53 (± 0.75) b	0.22 (± 0.02) b	0.53 (± 0.05) a	0.51 (± 0.04) a
Ca (mg kg^{-1})	37.47 (± 0.34) c	109 (± 1.04) a	90.79 (± 1.27) b	9.62 (± 0.4) a	8.08 (± 0.4) b	8.50 (± 0.27) ab
OM (%)	0.02 (± 0.03) b	0.08 (± 0.06) a	0.11 (± 0.04) a	1.69 (± 0.34) a	1.45 (± 0.24) a	1.63 (± 0.19) a

Table 1. Chemical components of different liquid fertilizers with EC of 1.5 and the chemical components of soil after treatment. As determined by Duncan's test, lowercase letters represent significant differences ($P < 0.05$) between groups. *CLF* chemical liquid fertilizer, *FLM* fermented liquid manure, *LBF* liquid bio-fertilizer.

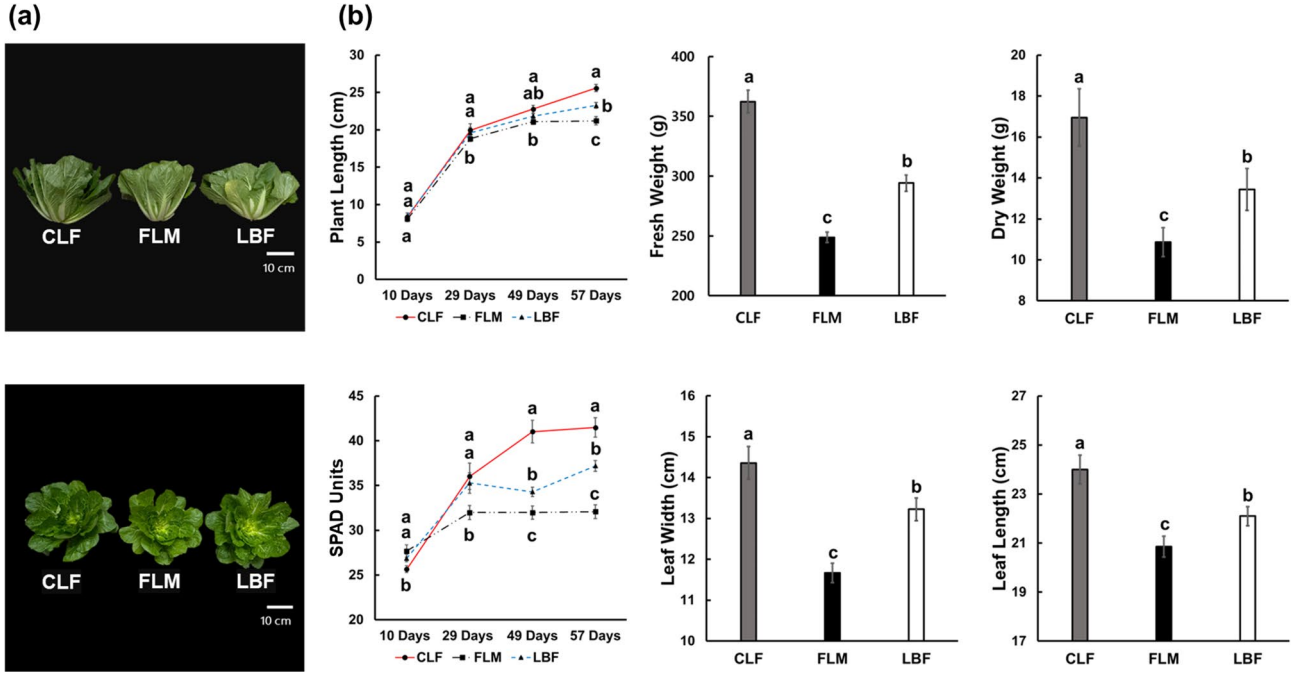


Fig. 1. Growth of Chinese cabbages treated with different fertilizers (**a**) Phenotypes by different fertilizer treatments. (**b**) The plants' plant length, chlorophyll content, leaf length, and width, and fresh and dry weight at 57 days after planting. The line colors represent the different fertilizer treatments (red; CLF, black; FLM, blue; LBF). The values are the mean ± standard deviation ($n = 9$). Lowercase letters indicate significant differences ($P < 0.05$) between groups by the Duncan test. *CLF* chemical liquid fertilizer, *FLM* fermented liquid manure, *LBF* liquid bio-fertilizer.

by the LBF (22.55 cm) and FLM (21.93 cm) groups (Fig. 1b). At 57 days after transplanting, leaf width and length had the highest increase in the CLF group (14.35 and 24 cm, respectively), followed by the LBF group (13.22 and 22.1 cm, respectively) and the FLM group (11.66 and 20.85 cm, respectively). Chlorophyll concentrations significantly ($P < 0.05$) increased in the CLF group (41.48), followed by the LBF (37.2) and FLM groups (32.08) between 49 and 57 days after transplanting. The fresh and dry weights of plant tissues, except roots, were higher in the CLF group (362.33 and 16.95 g, respectively) than those in the LBF (294.22 and 13.43 g, respectively) and FLM (248.77 and 10.86 g, respectively) groups. LBF-treated plants exhibited improved growth responses, while the other fertilizers had moderate effects.

After harvesting, we measured the colors (lightness [L], redness [a], and yellowness [b]) of the Chinese cabbage leaves with each sample measured from an independent individual (Fig. 2). Low Hunter L* values indicating reduced brightness were lower in the CLF group (40.57 L*) than those in the LBF (42.13 L*) and FLM (43.77 L*) groups. Thus, Chinese cabbage leaves were darker in the CLF group and lighter in the LBF and FLM groups. Hunter a* values, indicating the chromatic value from positive (red) to negative (green), were − 13.28 a*, -14.86 a*, and − 14.05 a* in CLF, FLM, and LBF, respectively, indicating a relatively low leaf content in the FLM group. The Hunter b* value indicates yellowness (positive number) or blueness (negative number). The highest and lowest Hunter b* values were observed in the FLM group (20.06 b*) and CLF groups (16.37 b*), respectively.

Antioxidant activities

The results of the analysis of the sugar and ascorbic acid contents and antioxidant activities in the leaves of the CLF-, FLM-, and LBF-treated plants are presented in Fig. 3. The LBF group had a significantly higher sugar content (6.33 mg mL^{-1}) than those of the CLF (5.2 mg mL^{-1}) and FLM (5.47 mg mL^{-1}) groups (Fig. 3a). Across all groups, the ascorbic acid content was higher in the leaves than that in the stems. Ascorbic acid content was significantly higher in the leaves of the FLM and LBF groups than in the leaves of the CLF group. Furthermore, ascorbic acid content was significantly higher in the stems of the CLF and FLM groups than in the stems of the LBF group. The effects of organic fertilizers on the antioxidant content of Chinese cabbage leaves are presented in Fig. 3b. The LBF group had relatively higher levels of antioxidants than the other groups. Total polyphenol content, flavonoid content, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, nitrite scavenging activity, reducing power, and ferric reducing antioxidant power (FRAP) were 2.89, 4.56, and 5.77 mg GAE mL^{-1}; 17.11, 19.43, and 22.55 mg QE mL^{-1}; 35.7, 54.74, and 63.96%; 65.36, 63.31, and 73.87%; 1.08, 1.7, and 2.21; 15.3, 23.61, and 28.61 μMFe2 + mL^{-1} for CLF, FLM, and LBF, respectively. Among these antioxidant activities, the nitrite scavenging activity did not show a significant difference ($P < 0.05$) among the fertilizer groups. The CLF group exhibited significantly ($P < 0.05$) lower antioxidant activities than the other groups, except for the nitrite scavenging activity, indicating that LBF can be utilized as a liquid bio-fertilizer to increase plant biomass in agriculture.

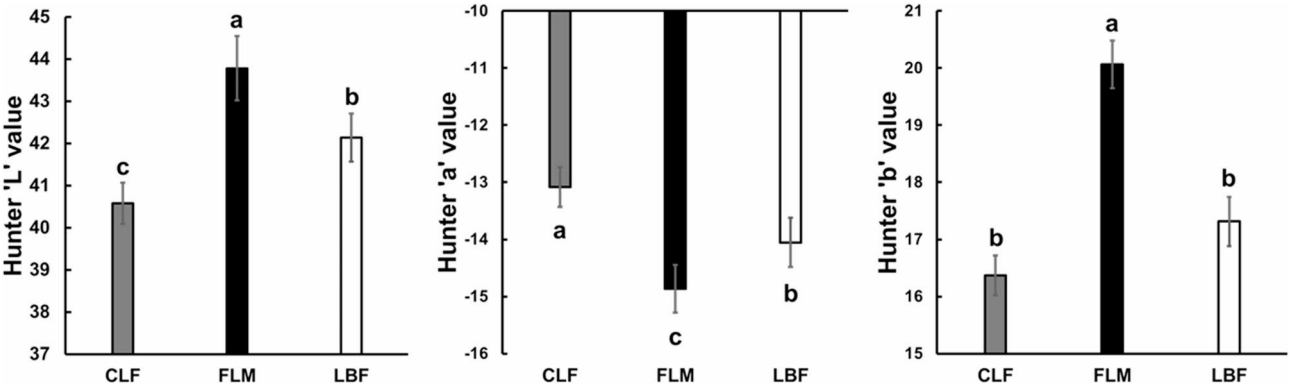


Fig. 2. Color analysis in Chinese cabbage leaves after harvest using the Hunter (L, a, b) system. The values are the mean ± standard deviation ($n = 9$). Lowercase letters indicate significant differences ($P < 0.05$) between groups by the Duncan test. *CLF* chemical liquid fertilizer, *FLM* fermented liquid manure, *LBF* liquid bio-fertilizer.

Soil bacterial composition

The effects of different liquid fertilizers on soil bacteria were determined by analyzing the 16 S rRNA sequences of the bacteria in each group. The results are presented in Fig. 4. The amplicon sequence variants (ASVs) in the untreated soil (77) were lower than those in the other groups (147.83, 146, and 157 in CLF, FLM, and LBF [Fig. 4a]). The LBF group exhibited relatively high alpha diversity in untreated and treated soils. However, no significant ($P < 0.05$) differences were among the liquid fertilizer-treated soils. The ASVs of the soil bacterial communities were clustered into 27 phyla of bacteria (Fig. 4b). Ten of the phyla (*Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Firmicutes*, *Gemmatimonadetes*, *Nitrospirae*, *Planctomycetes*, *Proteobacteria*, and *Verrucomicrobia*) comprised over 1,000 of the ASVs detected from the soil bacteria in each sample. The ASVs of *Actinobacteria* and *Proteobacteria* comprised a larger percentage of soil bacterial DNA (Fig. 4b and S3). A relatively small number of ASVs from *Acidobacteria* and *Planctomycetes* were found in the CLF group, and from *Bacteroidetes*, *Gemmatimonadetes*, and *Nitrospirae* were found in the non-treated group (Fig. S3). Large amounts of bacterial DNA from the untreated and FLM and LBF groups were from phyla *Firmicutes* and *Verrucomicrobia*, respectively.

The results for determining the bacterial changes induced by liquid fertilizers at the genus level are presented in Fig. 4c. The results revealed that 17 genera from 7 bacterial phyla were observed in the CLF group, which was higher than those in the control group. Many CLF-induced genera were included in the phyla *Actinobacteria* (nine) and *Proteobacteria* (eight). *Arthrobacter* and *Streptomyces* of the phylum *Actinobacteria*, *Neobacilus* of the phylum *Firmicutes*, and *Litorilinea* of the phylum *Chloroflexi* were lower in the CLF group than those in the FLM and LBF groups. Furthermore, 23 genera (FLM application increased and decreased 14 and 9 genera, respectively) from 6 phyla were differentially distributed in the FLM group compared with those in the control group. Two genera (*Luteolibacter* in the phylum *Verrucomicrobia* and *Actinoplanes* in the phylum *Actinobacteria*) exhibited significant differences in bacterial composition between the FLM and control groups. FLM application increased the abundance of many genera of the phylum *Proteobacteria*, whereas *Priestia* and *Litchfieldia* of the phylum *Firmicutes*; *Nocardia*, *Nocardioides*, *Actinophytocola*, *Conexibacter*, *Pseudarthrobacter*, and *Aeromicrobium* of the phylum *Actinobacteria*; *Sphingomonas* of the phylum *Proteobacteria* were decreased. The bacterial composition of *Luteolibacter* (phylum *Verrucomicrobia*) and *Actinoplanes* (phylum *Actinobacteria*) had relatively large changes in the LBF group and significant differences ($P < 0.05$) in distribution between the FLM and control groups. Many bacterial genera from the phyla *Actinobacteria* (four genera) and *Proteobacteria* (five genera) were increased due to LBF application, and *Hydrogenispora* of the phylum *Firmicutes* and *Trichocoleus* of the phylum *Cyanobacteria* were decreased after LBF application when compared with those in control.

Bacterial composition significantly correlated with chemical compositions

NO_3^--N, K, Na, and Ca from fertilizers, pH, and NO_3^--N and exchangeable Na from soil were significantly ($P < 0.05$) correlated with the community of differentially distributed bacteria in the soils (Fig. 5a). We hypothesized that the seven fertilizer or soil chemical compositions influenced soil bacterial changes. Therefore, we conducted a Canonical Correspondence Variant (CCA) among the seven chemical compositions and the differentially distributed bacteria (Fig. 5b) and evaluated the significance of the correlation. The results are presented in Table 2. Finally, we identified the significantly correlated genera among the five phyla. We found that six genera (*Terracoccus*, *Virgibacillus*, *Lysobacter*, *Mesorhizobium*, *Nitrospira*, and *Rhizobium*) were significantly correlated with the fertilizer K content. However, except for K, the six K-related genera had significant positive or negative correlations with one of the six chemical compositions. The CCA plot was divided into four quadrants (I–IV). The FLM group was close to Na (soil) in quadrant I, whereas the LBF group was close to NO_3^--N (fertilizer), Na (fertilizer), Ca (fertilizer), pH (soil), and NO_3^--N (soil) in quadrant II (Fig. 5b). K (fertilizer) was correlated with the CLF group in quadrant IV, suggesting that the chemical composition of the fertilizers led to distinct bacterial communities. The results for determining the specific distribution of bacteria with each fertilizer treatment and

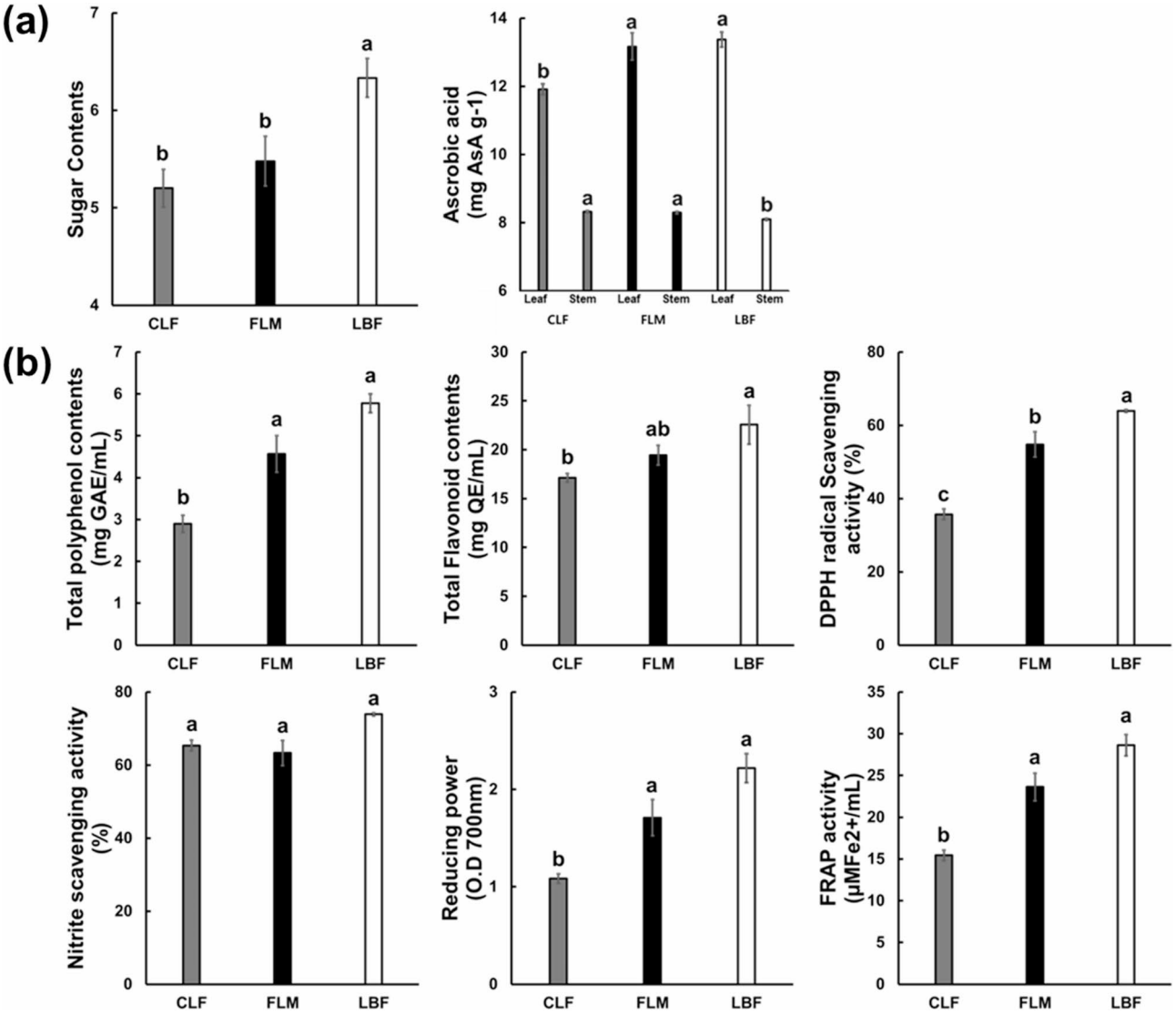


Fig. 3. Differences of functional plant materials with different fertilizer supplies (**a**) The contents of sugar and ascorbic acid. (**b**) The contents and activities of antioxidants. The analysis used Chinese cabbage leaves. The values are the mean ± standard deviation ($n = 3$). Lowercase letters indicate significant differences ($P < 0.05$) between groups by the Duncan test. *CLF* chemical liquid fertilizer, *FLM* fermented liquid manure, *LBF* liquid bio-fertilizer.

comparing their relative ASV abundance to that of the control are presented in Fig. 5c. *Litorilinea* decreased in the CLF group but not in the FLM and LBF groups. The FLM group exhibited a decrease in *Sphingomonas* and *Nocardioides*. LBF exhibited increases in *Sphingomonas* and *Nocardioides*, similar to CLF, and an increase in *Litorilinea*, similar to FLM, suggesting that the bacterial communities were in the middle of CLF and FLM.

Discussion

This study aimed to investigate the effects of different liquid fertilizers on crop growth, soil chemical properties, and bacterial communities. Our results emphasize the significance of carefully analyzing the chemical reactions of crops and the chemical interactions within the soil before introducing liquid fertilizers to determine the appropriate fertilization method. Moreover, the composition of organic liquid fertilizers varies depending on the source of livestock manure[34], requiring various research outcomes to determine the application method. This study demonstrates significant insights into liquid fertilizers utilizing pig manure. Our results showed that NO_3^--N and Na from the fertilizer and soil correlated with the FLM and LBF groups. The chemical fertilizers' NH_4^+-N or NO_3^--N ratio significantly affected plant growth. Low NH_4^+-N (25%) and high NO_3^--N (75%) increase the growth of flowering Chinese cabbage cultivars (cv. Lvbao70, cv. Youlv80, and cv. Chixin No.2), while high NH_4^+-N (75%) and low NO_3^--N (25%) had no significant effect[35].

Furthermore, a previous study revealed that supplying NO3$^-$-N increased the water content and leaf area of spinach, sunflower, and pea plants by 15% and 30%, respectively, compared to an NH4$^+$-N supply[36]. Although

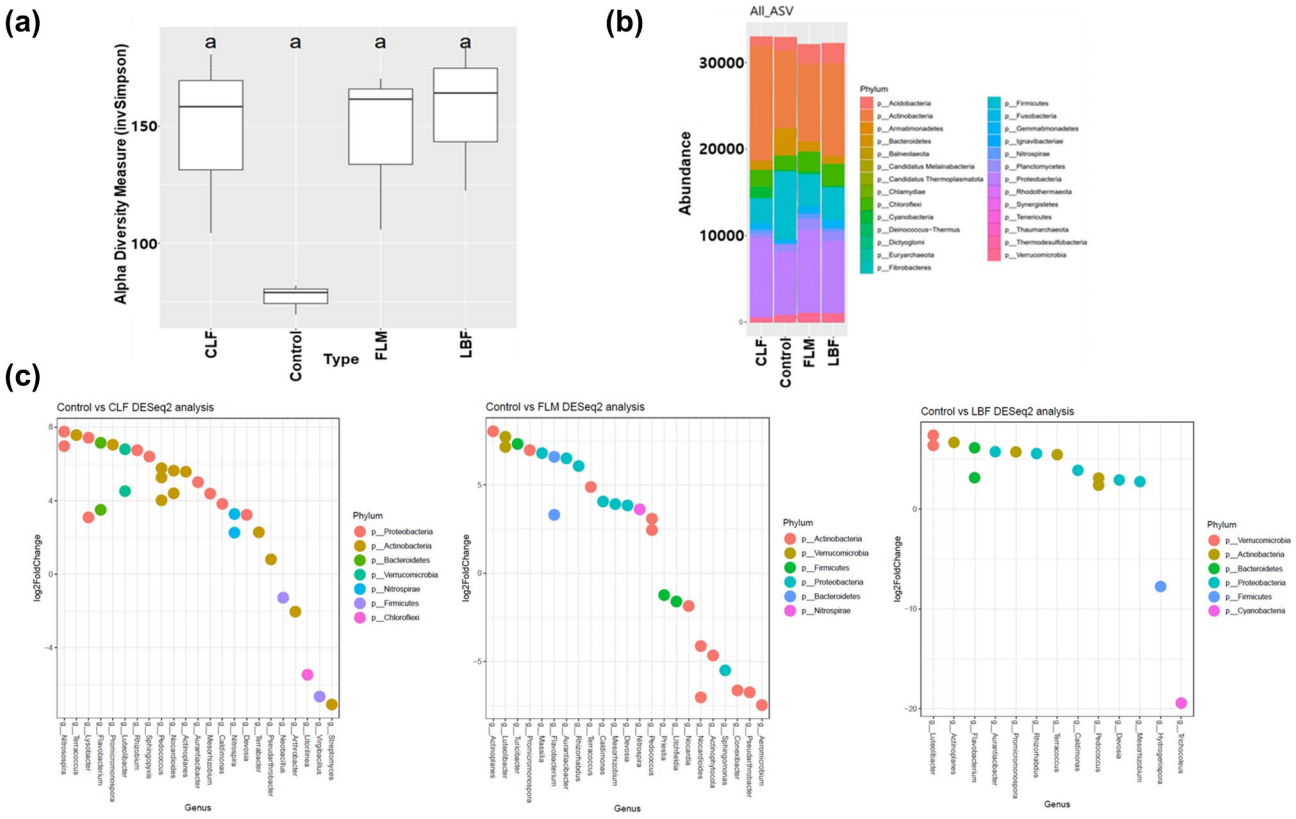


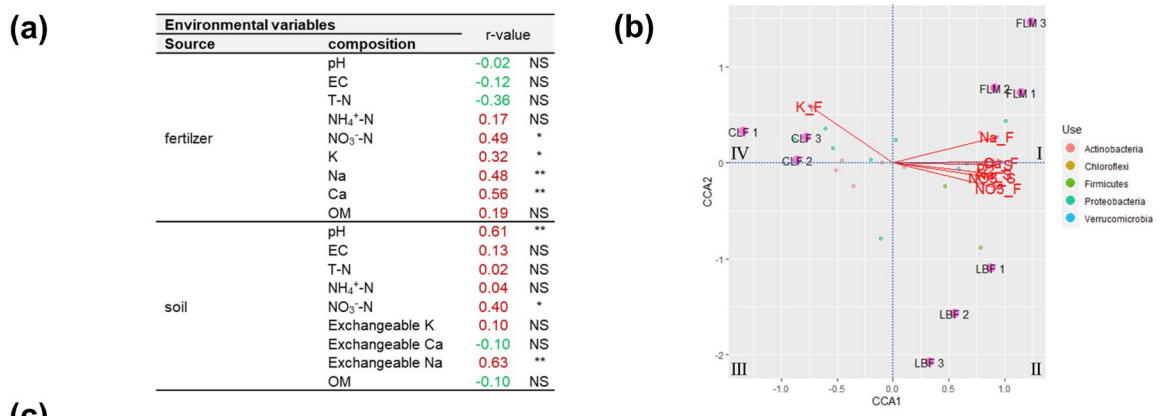
Fig. 4. Amplicon sequence variant (ASV) analysis of the bacteria in the soils treated with different fertilizers (**a**) Alpha diversity of the bacterial population in each sample using the InvSimposn method. Lowercase letters represent the significant differences ($P < 0.05$) between groups by Tukey's HSD test. (**b**) Bacterial communities of each sample at the phylum level. Box colors represent the phylum of bacteria. (**c**) The variations in bacterial DNA quantity in each sample were compared to those in the control. The dot colors indicate the phylum of the bacterial species.

the FLM and LBF treatments resulted in higher NO_3^--N contents in this study, the CLF treatment led to the largest increase in plant growth. Additionally, we measured larger amounts of Na in FLM and LBF and the treated soils than in the control groups. Moreover, exchangeable Na was higher in FLM than in LBF. Excessive Na is well-known to compete with vital nutrients such as Ca and K, leading to deficiencies that ultimately hamper plant growth and development. Indeed, high concentrations of Na can displace Ca and Mg on the soil exchange complex due to its abundance and low flocculating power, as Na has a relative flocculating power of 1 compared to Ca (Ca has a relative flocculating power of 43). This displacement causes dispersion of soil particles, degrading soil structure by reducing porosity and hydraulic conductivity. As a result, water infiltration and absorption are hindered, affecting plants' ability to efficiently uptake water. Indeed, Na-sensitive plants may suffer from toxicity symptoms, impaired growth, and reduced productivity due to nutrient imbalance, poor soil physical conditions, and direct ionic toxicity[37]. The amount of exchangeable Na increased in the soil during a subsequent cropping season following the application of liquid fertilizer developed from pig slurry, as opposed to after using chemical fertilizer[38], suggesting that the high amount of exchangeable Na^+ in FLM and LBF could have inhibited the development of Chinese cabbage.

Our results showed that the organic fertilizers FLM and LBF increased sugars, ascorbic acid, and antioxidants in Chinese cabbage. A previous study revealed that liquid organic fertilizer application increased the ascorbic acid and crude protein content in bell peppers (cv. Red Wonder F1) compared with those observed after mineral fertilizer application[39]. Furthermore, the total contents of broccoli phenolics, flavonoids, and glucosinolates increased after applying organic fertilizers[40]. *Chlorella* extracts promote plant growth in pepper (*Capsicum annuum*)[41]. Applying *Chlorella* extracts for 21 days increased plant height, leaf area, and fruit and shoot weights of pepper plants. The antioxidant enzymes superoxide dismutase, peroxidase, and catalase were increased with *Chlorella* extract treatment in pepper plants, suggesting its role in promoting plant growth. Similarly, *Chlorella* culture treatments improved the height, leaf width, fresh weight, yield of Chinese chives, leaf thickness, leaf number, fresh weight, yield, and mineral content of spinach[42]. *Chlorella* is economically significant as it promotes plant growth and functional food production, including plant growth-promoting bacteria, microalgae, and mycorrhiza[43,44]. Applying *Chlorella minutissima* extracts resulted in significantly ($P < 0.05$) higher DPPH Free Radical Activity, FRAP, and iron chelating activity values than those of the other microalgal species (*Dunaliella salina*, *Isochrysis galbana*, *Nannochloropsis oculate*, and *Tisochrysis lutea*)[45]. However, the effects of a *Chlorella*

(a)

Environmental variables		r-value	
Source	composition		
fertilzer	pH	-0.02	NS
	EC	-0.12	NS
	T-N	-0.36	NS
	NH_4^+-N	0.17	NS
	NO_3^--N	0.49	*
	K	0.32	*
	Na	0.48	**
	Ca	0.56	**
	OM	0.19	NS
soil	pH	0.61	**
	EC	0.13	NS
	T-N	0.02	NS
	NH_4^+-N	0.04	NS
	NO_3^--N	0.40	*
	Exchangeable K	0.10	NS
	Exchangeable Ca	-0.10	NS
	Exchangeable Na	0.63	**
	OM	-0.10	NS



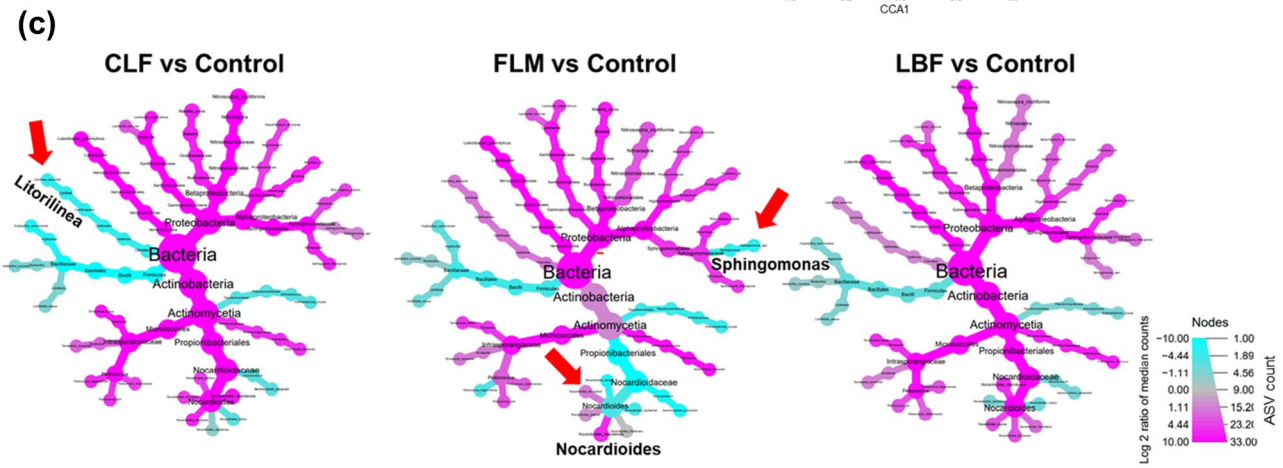


Fig. 5. Correlation of soil properties with bacterial community composition (**a**) Mantel test between soil properties on bacterial community composition. Significant results are indicated by $^*P<0.05$, $^{**}P<0.01$, NS = no significance (**b**) Canonical correspondence analysis (CCA) relating soil bacteria genus group (**c**) The relative abundances of significantly correlated bacteria in CLF, FLM, and LBF-treated soils compared to control. Circle size represents the number of bacteria for ASV. The color indicates the log 2-fold change of ASV with *p*-adjust < 0.05. Arrows represent the specific bacteria in each treatment. *CLF* chemical liquid fertilizer, *FLM* fermented liquid manure, *LBF* liquid bio-fertilizer.

culture solution as a liquid bio-fertilizer on plant antioxidant activity remain elusive. Therefore, we hypothesized that *Chlorella* culture liquid fertilizer increases antioxidant levels in Chinese cabbage. The LBF group increased antioxidant levels compared to the CLF and FLM groups.

We found that *Litorilinea* aerobic gram-negative bacterium[46] was significantly decreased in the CLF group and increased in the FLM and LBF groups. The increase in *Litorilinea* could be attributed to the anaerobic fermentation during the composting of the livestock manure to produce liquid fertilizer. *Nocardioides* and *Sphingomonas* genus are gram-positive aerobic bacteria belonging to *Nocardioidaceae* and *Sphingomonadaceae*, respectively[47,48]. Anaerobic fermentation during FLM production decreased *Nocardioides* and *Sphingomonas* bacteria involved in nutrient cycling while decomposing complex biomasses and nitrogen fixation, promoting plant growth and yield[49,50]. *Sphingomonas* can promote plant growth under normal and drought stress conditions owing to its contribution to root development and plasticity[51–53]. A lack of *Nocardioides* and *Sphingomonas* in the soil observed in this study may have decreased the growth rate of the FLM-treated plants compared with those of the CLF- and LBF-treated plants. Both bacteria are commonly negatively correlated with sodium (Na) levels in the fertilizers, suggesting that the high sodium content in FLM may have influenced their abundance (Table 2). The newly developed LBF was produced by culturing *Chlorella* in livestock manure to create an organic liquid fertilizer without a chemical medium. Utilizing recycled organic liquid fertilizers instead of chemical cultures presents a cost-effective method for producing *Chlorella* biostimulants.

While FLM and LBF produced lower yields than CLF, their functional advantages were evident. Furthermore, LBF, enhanced by Chlorella cultivation, outperformed FLM in promoting crop growth and demonstrated potential for further improvement. These results underscore the promise of biotechnological advancements in enhancing the functionality of organic liquid fertilizers and crop productivity, offering a viable alternative to chemical fertilizers. Moreover, this approach efficiently recycles organic waste by transforming livestock manure into value-added organic bio-liquid fertilizers. It aligns with sustainable agriculture practices by reducing environmental impact and reliance on synthetic inputs.

Phylum	Genus	Fertilizer				Soil		
		Na	K	NO_3^--*N*	Ca	pH	NO_3^--*N*	Na
Actinobacteria	*Actinoplanes*	0.77*	-0.21	0.51	0.65	0.63	0.57	0.49
	Nocardioides	-0.77*	0.45	-0.7*	-0.77*	-0.95***	-0.61	-0.72*
	Pedococcus	-0.7*	0.66	-0.84**	-0.81**	-0.94***	-0.7*	-0.78*
	Pseudarthrobacter	-0.45	0.56	-0.59	-0.53	-0.81**	-0.53	-0.62
	Terrabacter	-0.58	0.67	-0.71*	-0.7*	-0.76*	-0.65	-0.78*
	Terracoccus	-0.85**	0.75*	-0.9**	-0.93***	-0.81**	-0.89**	-0.93***
Chloroflexi	*Litorilinea*	0.86**	-0.5	0.75*	0.88**	0.66	0.8**	0.94***
Firmicutes	*Neobacillus*	0.66	-0.9***	0.91***	0.82**	0.7*	0.92***	0.88**
	Virgibacillus	0.27	-0.81**	0.64	0.45	0.48	0.56	0.3
Proteobacteria	*Lysobacter*	-0.78*	0.83**	-0.94***	-0.92***	-0.73*	-0.94***	-0.97***
	Massilia	0.81**	-0.24	0.59	0.78*	0.59	0.52	0.64
	Mesorhizobium	-0.22	0.74*	-0.69*	-0.49	-0.58	-0.47	-0.55
	Nitrosospira	-0.72*	0.74*	-0.9**	-0.85**	-0.89**	-0.79*	-0.87**
	Rhizobium	-0.72*	0.81**	-0.89**	-0.86**	-0.57	-0.92***	-0.9***
	Rhizorhabdus	0.79*	-0.5	0.63	0.78*	0.83**	0.66	0.71*
	Sphingomonas	-0.74*	-0.23	-0.26	-0.51	-0.48	-0.36	-0.4
	Sphingopyxis	-0.47	0.65	-0.72*	-0.6	-0.79*	-0.58	-0.69*
Verrucomicrobia	*Luteolibacter*	0.76*	-0.43	0.78*	0.74*	0.75*	0.75*	0.59

Table 2. Soil bacteria significantly correlated with chemical compositions determined by the mantel test. The fold change value was calculated between treatments and control (log 2 [treatments/control]) by DESeq2 (NS, non-significance). The significance of Pearson's correlation coefficient (PCC) was determined by n-2 degrees of freedom (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

Additionally, high-throughput sequencing provided valuable insights into soil bacterial communities; however, understanding their functional roles remains challenging due to the difficulty of culturing many soil bacteria under standard laboratory conditions[54]. Consequently, this limitation restricts the use of quantitative methods, such as colony-forming unit (CFU)-based assessments, which are essential for accurately evaluating bacteria contributions. Therefore, overcoming these constraints and translating these findings into practical, field-level applications may be possible by effectively correlating bacterial data with environmental factors and integrating advanced approaches such as functional genomics and metabolomics.

Materials and methods

Plant growth and fertilizers

Chinese cabbage seeds were obtained from the Danong Company, Gyeonggi-do, Korea. The seeds were grown in soil-filled pots (18 × 18 × 30 cm length × width × height) in a greenhouse at the Agricultural Technology Center in Hoengseong County, Gangwon-do, Republic of Korea, with an average temperature of 25℃ and humidity of 65%. The plant treatments for each experimental unit were replicated three times. NPK fertilizer (21-17-17) was obtained from the Namhae Chemical Corporation, Jeollanam-do, Korea, and used as the Chemical Liquid Fertilizer (CLF). The Fermented Liquid Manure (FLM) was produced using livestock manure decomposed for a year obtained from a hog farm (37°30'33.1948211615924" N 128°04' 56.5629151080066" E). The organic medium was produced from livestock manure using a method described previously[55]. Briefly, anaerobic digestate and liquid pig manure were mixed at a ratio of 1:9 and subjected to a 3-day treatment in a field-scale thermophilic aerobic oxidation (TAO) system, maintained at temperatures between 50 and 60 °C. Subsequently, the treated manure underwent a 180-min treatment in an electrocoagulation reactor powered by a 30 V electric supply through two sets of iron and aluminum hybrid electrodes. The *C. fusca* strain was obtained from the National Institute of Agricultural Sciences, Seoul, Republic of Korea. For the initial inoculation, the culture was grown under 28 °C conditions until it reached a concentration of 10^7 cells mL^{-1}. Subsequently, the culture was cultivated to achieve a final concentration of 107 mL^{-1} to prepare for crop application. The incubator (Fig. S1) utilized LED modules (FNB-240LED; F&B Nature, Chungju, Korea) emitting red and blue light in a 16-hour day/8-hour night cycle, with an air supply of 0.1 m^3 air/m^3·min[56]. The incubated medium was filtered using a tubular continuous centrifuge (J-1050 A; Hanil Sci-Med, Chungcheongbuk-do, Korea) at 12,000 × g to obtain the Liquid Bio-Fertilizer (LBF). The liquid fertilizers were treated with 1.5 mS cm^{-1} Electrical Conductivity (EC). After sowing the seeds, 50 mL liquid fertilizers were applied twice daily at 9:00 a.m. and 6:00 p.m. using a dropper.

Analysis of plant growth and chemical components

Plant growth (plant length, fresh and dry weight, leaf color and length, and chlorophyll content) and chemical components of the soil and liquid fertilizer (pH, EC, total N, NH_4^+-N, NO_3^--N, P, K, Na, Ca, and OM) were analyzed as described previously[19]. Briefly, the growth parameters were analyzed four times, 57 days post-transplanting. The soil plant analysis development (SPAD) was measured using a SPAD meter (SPAD-502plus,

Minolta, Japan). Chinese cabbage leaves at 56 days were used for antioxidant analysis and color measurement. Colors (lightness [L], redness [a], and yellowness [b]) were measured using a Hunter Lab Colorimeter (ND-300 A; Nippon Denshoku, Tokyo, Japan). Further, a multiparameter analyzer used 10 g soil and liquid fertilizers for pH and EC analysis (Edge Hl2020, HANNA instruments, Woonsocket, RI, USA). NH_4^+-N and NO_3^--N concentrations were measured using the Kjeldahl method[57], and the Lancaster soil testing method[58] was used to measure P concentration. K, Na, and Ca were detected using the inductively coupled plasma atomic emission spectrometer (ICP-OES; SPECTROBLUE, SPECTRO analytical, Odiham Hampshire, UK). The OM was measured using the Tyurin method[59].

Antioxidant measurements

Antioxidants (total polyphenol and flavonoid contents, 2,2-Diphenyl-1-picrylhydrazyl [DPPH] radical scavenging activity, nitrite scavenging activity, reducing power, and Ferric Reducing Antioxidant Power [FRAP]) were analyzed using previously described methods[19]. Briefly, the plant substances were extracted from 0.5 g dried plant samples in a shaking incubator (ED-SI300R, HYSC, Seoul, Korea) using 25 mL methanol (Daejung, Seoul, Korea). The plant above-ground parts were dried using the drying machine (Henan Baixin Machinery Equipment Co., Ltd, Henan, China) at 60 °C for 24 h. Total polyphenol was measured using the Folin-Ciocalteu method[60,61]. The flavonoid contents[62], DPPH radical scavenging activity[63], nitrite scavenging activity[64], reducing power[65], and FRAP[66] were evaluated using previously described methods. The standard substances used in this study were quercetin for total flavonoid and gallic acid for total phenol, DPPH radical scavenging activity, and FRAP assay, and the actual standard curves were estimated (Fig. S2). The light absorbance was measured using a spectrometer (OPTIZEN POP, KLAB, Seoul, Korea) at 760, 510, 515, 520, 590, and 700 nm for total polyphenol, total flavonoid, DPPH radical scavenging activity, nitrite scavenging activity, FRAP, and reducing power, respectively.

Soil bacterial analysis

Rhizosphere soil samples were collected from the root zone of plants grown for 56 days at depths ranging from 5 to 15 cm. All experimental soil samples were kept in the deep freezer at -80 °C until use. The bacterial DNA was extracted using a Dneasy power soil kit (Qiagen, Hilden, Germany). The polymerase chain reaction (PCR) was performed using a Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, CA, USA) with specific primers for the V3-V4 (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′) region of bacterial 16 S rRNA following a previously described method[19]. The bacterial DNA PCR products were sequenced using a NovaSeq 6000 system (Illumina, San Diego, CA, USA) after constructing a library using the Illumina 16 S Metagenomic Sequencing Library (Illumina)[67]. The adapter sequences were trimmed using the Cutadapt program (Cutadapt, Dortmund, Germany)[68]. The trimmed sequences were used for amplicon sequence variant (ASV)-based sequence analysis using the R packages DADA2 (Rstudio, Boston, MA, USA)[69], and the alpha diversity was evaluated using vegan (Rstudio)[70]. The taxonomic units and phylogenetics of ASVs were analyzed using taxa[71], pyloseq[72], ape[73], and QIME[74] and visualized using dplyr[75], ggrepel[76], ggsignif[77], and ggplot2[78]. The differentially abundant ASVs among experimental samples were detected using DESeq2 (Illumina)[79]. The ASV sequence was identified using the BLAST + program (National Center for Biotechnology Information, Bethesda, MD, USA) 16 S bacterial database[80].

Statistical analysis

Statistical analysis was conducted using the R package Agricolae (RStudio), and significant differences were determined using Duncan's test, with significance set at $P \leq 0.05$. Canonical correspondence was evaluated using the R package canonical correspondence analysis (CCA) (https://cran.r-project.org/web/packages/CCA/index.html).

Data availability

Raw reads from isolates sequenced in this study are available at the NCBI Short Read Archive (SRA) under Bio-Project accession no. PRJNA1013211 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1013211).

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Author contributions

J.L. and S.-G.H. conceived of and designed the study. J.L., N.-Y.J., T.Y.L.L., W.Y.J., K.W.K., H.S.C., B.-O.L., S.-R.K., and M.-G.L. performed the field sampling. J.L., N.-Y.J., S.-Y.S., S.-R.K., M.-G.L., and S.-G.H. collected and analyzed the data. J.L. and S.-G.H. wrote the manuscript. J.L. and N.-Y.J. provided reagents and materials. All authors contributed critically to the article, drafts, and revisions and gave final approval for publication.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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