

Article

Effects of Liquid Organic Fertilizers on Plant Growth and Rhizosphere Soil Characteristics of Chrysanthemum

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Abstract: Organic fertilizers are generally thought to be an effective way to sustain soil fertility and plant growth. To promote the productivity of chrysanthemum, five sources of liquid organic fertilizers (L1–L5), as well as a chemical fertilizer, were applied at an early stage of the growth cycle to investigate their effects on plant growth. In the short-term pot experiment, the liquid organic fertilizers significantly promoted root and aboveground growth by 10.2–77.8% and 10.7–33.3%, respectively, compared with the chemical fertilizer. The order of growth promotion was: L1 (shrimp extracts) > L2 (plant decomposition) > L4 (seaweed extracts)/L5 (fish extracts) > L3 (vermicompost). Morphological and chemical analyses indicated that, compared with other organic fertilizers, the treatment with shrimp extract (L1) produced the greatest increases in root dry weight, total length, surface area, volume, tips, and thick root length, respectively. Furthermore, the shrimp extract treatment significantly increased the nutrient contents and altered the soil's functional microbial community at the rhizospheric level compared with the chemical fertilizer treatment. Thus, the shrimp extract liquid organic fertilizer could be part of an effective alternative to chemical fertilization during the early stage of chrysanthemum growth.

Keywords: liquid organic fertilizer; chrysanthemum; root architecture; nutrient level; microbial community

1. Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.), which originated in Asia and Northeastern Europe, has been cultivated for more than 1600 years [1,2]. It is widely cultivated for ornamental, culinary, and medicinal uses throughout the world. For instance, in Italy, chrysanthemum production covers 1180 ha with 437 million plants and continues to grow [3]. The current trends in the chrysanthemum industry are focused on improving flower quality and creating environmentally-friendly production systems [4,5]. To achieve these goals and accelerate chrysanthemum production, further innovation is needed in improving fertilization regimes and other production techniques [6,7]. In many areas of the world, especially in China, chrysanthemum plants are cultivated under greenhouse conditions, and the over-application of chemical fertilizers has influenced the soil quality and caused serious environmental problems [8,9]. In addition, chrysanthemum plants are sensitive to chemical fertilizers, and their improper use affects the plant's reproductive growth and the secondary metabolism of chrysanthemum plants [10,11]. Moreover, chrysanthemum has a great demand for nutrients, especially at the early stage of the growth cycle, and the nutrient status during the first seven weeks of chrysanthemum growth has a strong effect on flower size and quality [4]. Thus, growth regulation and fertilizer optimization at the early stage play critical roles during the growth cycle of chrysanthemum.

Organic fertilizers are effective in promoting environmental sustainability and plant growth after long-term use, but previous studies have focused primarily on the conventional solid organic fertilizer product, such as straw and manure [12,13]. Specialized horticultural production has fostered the emergence of new liquid organic fertilizers [14], which have usually been derived from natural products and their biological activities occur at limited doses. Compared with conventional organic fertilizer, the abundant organic matter and soluble nutrients in the liquid organic fertilizers could maintain soil sustainability and plant health [15,16]. In addition, the integration of watering and fertilization patterns could improve the nutrient use efficiency and decrease the risk of nutrient loss [17,18]. Moreover, the special compounds in liquid organic fertilizers, such as chitin, humic and fulvic acids, and other biopolymers, can be biostimulants to plants [19–22]. Canfora et al. reported that liquid organic fertilizers containing stillage and vermicompost promoted the root growth of tomato and improved the soil microbial communities Eubacterial and Archaeal diversity, and this was in accordance with the results of liquid residues from lipopeptide production that could promote tomato growth and increase the diversity of the soil's microbial community, as well as the related enzyme activities and nutrient cycles [23,24]. Given the ecological and economic benefits of liquid organic fertilizer, evaluating plant growth under organic versus chemical fertilizer use and studying the possible mechanisms of action are promising steps in developing an effective alternative fertilizer for chrysanthemum production [25,26].

The root systems of terrestrial plants perform two primary functions: acquiring nutrients and water from soil-based resources and recruitment of desired microbial partners for greater mutualistic benefits [27–30]. In a previous study, Xu et al. found that restricted the growth of shoot-borne roots of maize decreased nutrient absorption, leaf formation, and shoot growth [31]. The rhizosphere is the thin layer of soil contacted by the root and is the habitat for soil microbial communities [32]. Ecological processes in the soil are often complex interactions between the plant's roots, soil nutrients, and the rhizosphere's microorganisms [33,34]. Soil microorganisms in the rhizosphere play critical roles in nutrient cycling and soil structure maintenance, which could further promote nutrient cycles and plant growth [34,35]. The Biolog microplate technique is a powerful tool for monitoring the soil bacteria's functional diversity, although it cannot determine the total microbial community but, rather, the active microbes, which can indicate environmental changes [36,37]. Therefore, root growth and rhizosphere microbial community changes appear to be extremely vital to evaluating liquid organic fertilizers.

The present study was conducted to investigate the short-term effects [24,38] of five liquid organic fertilizers from different sources (shrimp extracts (L1), plant decomposition (L2), vermicompost (L3), seaweed extracts (L4), and fish extracts (L5)), on chrysanthemum plant growth at the seedling stage using the root architecture and plant growth parameters. To understand the effects of various liquid fertilizers on the soil quality, the soil nutrient level and functional bacterial diversity, which sustains microbes at the rhizosphere level, were also studied. The chief objectives of this study were: (1) to identify the effects of liquid organic fertilizers and a chemical fertilizer on chrysanthemum growth at the seedling stage; (2) to find a suitable source of liquid organic fertilizer to be applied at the early stage of the growth cycle during chrysanthemum production; and (3) to study the effects of liquid organic fertilizers on the soil characteristics at the rhizosphere level.

2. Materials and Methods

2.1. Materials, Experimental Design, and Sampling

The chrysanthemum cultivar Hangju 'No. 2 of Jinju' was obtained from the Amway Botanical Research and Development Center, Wuxi, Jiangsu, China. The cutting seedlings of chrysanthemum were cultivated on a sterilized substrate of vermiculite and perlite (1:1, *v:v*) without fertilization. After rooting for 15 days, seedlings of a similar height and diameter were transplanted into plots filled with 500 g of a peat and paddy soil (2:1, *v:v*) substrate and grown for 60 days, with one plant per pot.

At transplanting and at one additional time (30 days after transplanting), the CK, NPK, and five liquid organic fertilizers (L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; L5: fish extracts) were applied to the substrate of the chrysanthemum. The major characteristics of these fertilizers are presented in Table 1. All products were applied as a diluted solution according to the instructions provided by the manufacturers. The total amount of the macro-elements applied with the mineral and organic fertilizers was as follows:

NPK: 60.0 mg N pot⁻¹; 13.1 mg P pot⁻¹; 41.2 mg K pot⁻¹;
 L1: 36.8 mg N pot⁻¹; 0.5 mg P pot⁻¹; 9.1 mg K pot⁻¹;
 L2: 35.0 mg N pot⁻¹; 2.9 mg P pot⁻¹; 40.4 mg K pot⁻¹;
 L3: 18.4 mg N pot⁻¹; 0.3 mg P pot⁻¹; 24.6 mg K pot⁻¹;
 L4: 15.1 mg N pot⁻¹; 3.3 mg P pot⁻¹; 12.5 mg K pot⁻¹; and
 L5: 27.4 mg N pot⁻¹; 4.1 mg P pot⁻¹; 14.6 mg K pot⁻¹.

Table 1. Nutrient elements content of the liquid organic fertilizers applied in the experiment.

Product	Source	Biostimulants	pH	N ¹ (g/L)	P (g/L)	K (g/L)	Using Instructions	Providers	Production Organic Certification
CK	/	/	/	/	/	/	/	/	/
NPK	chemical reagent	/	8.9	267.7	58.2	184.4	0.20%	/	/
L1	shrimp extracts	chitosan	7.2	98.0	1.3	23.2	0.25%	Shenbotai Biotechnology and Chemical Co., Ltd., Zhanjiang, Guangdong, China	China OFDC ² certified organic
L2	plant decomposition	humic acid	10.4	140.1	11.4	169.5	0.17%	Tiancibao Agrtcultural and Technology Co., Ltd., Changsha, Hunan, China	/
L3	vermicompost	amino acids	4.0	49.0	0.7	65.5	0.25%	Wenxing Biotech Co., Ltd., Shanghai, China	China OFDC certified organic
L4	seaweed extracts	alginate	7.1	60.4	13.0	51.0	0.17%	Qingdao Seawin Biotech Group Co., Ltd., Qingdao, Shandong, China	EU ³ certified organic
L5	fish extracts	fish emulsion	3.6	91.3	13.6	48.5	0.20%	Yirong Bio-engineering Co., Ltd., Ningde, Fujian, China	China OFDC certified organic

Notes: ¹ The concentration of N, P, and K in the mineral and organic fertilizers was determined by the chemical analysis methods in Section 2.3; ² OFDC, Organic Food Development Center; ³ EU, European Union.

To exclude the influence of chrysanthemum roots, one more treatment, Non-R (without chrysanthemum plant), was also included in our experiment to assess the non-rhizospheric effects. Each treatment consisted of three pots placed in a completely randomized design. At the end of the trial (60 days after transplanting), plant growth was monitored and the rhizospheric soil of the chrysanthemum root was gathered by removing the loose soil and collecting the remaining soil that was tightly adhered to the roots. The soil was divided into two parts: one air-dried for the soil properties analyses, and the other stored at 4 °C for further microbial analysis.

2.2. Root Morphology and Aboveground Growth Parameters

Root morphological parameters, including total root length, root surface area, root volume, and root tip number, were analyzed using the root analysis instrument WinRhizo-LA1600 (Regent Instruments Inc., Quebec, QC, Canada) [39]. Thick root lengths were calculated from root diameters >0.5 mm. Root weight was measured after determining of the root morphological parameters.

Aboveground growth was monitored by shoot height, diameter, and dry weight, as well as leaf width, length, area, and dry weight. The SPAD values of leaves were quantified using the hand-held Minolta SPAD-502 (Minolta corporation, Ltd., Osaka, Japan).

2.3. Chemical Analyses of Fertilizer and Soil

The content of N, P, and K of the mineral fertilizer and L1–L5 organic fertilizers was determined with an ICP (inductively-coupled plasma) spectrometer (Thermo Electron Corporation, Ltd., Waltham, MA, USA) [24]. Soil NH_4^+ -N and NO_3^- -N were extracted from the fresh soil samples with 1 M KCl (1:10 soil:solution ratio) for 1 h, and their levels were determined using a continuous flow analyzer (Skalar Analytical B.V., Breda, The Netherlands). The content of soil mineral N was calculated as the sum of the soil NH_4^+ -N and NO_3^- -N contents [40]. Available P was extracted using a 0.5 M NaHCO_3 solution (1:10 soil: solution ratio) and was measured using the colorimetric method with molybdenum. Available K was extracted with 1 M NH_4OAc solution (1:10 soil:solution ratio) and was determined using flame photometry [38]. The EC of air-dried soil was determined by means of an EC meter (Bante, Ltd., Shanghai, China) with a soil: water ratio of 1:5 (*w/v*). The soil pH was measured using the pH meter (Agilent technologies, Ltd., Palo Alto, CA, USA) with a soil: water ratio of 1:2.5 (*w/v*) [41].

2.4. Community Level Physiological Profile Analyses

Biolog EcoPlates (Biolog Inc., Hayward, CA, USA) and a BIOLOG_EmaxTM reading (Biolog Inc.) were used to determine the community level physiological profiles of the chrysanthemum rhizospheric soil. Each plate contains three sets of 31 carbon substrates and one control, and uses tetrazolium dye as the substrate utilization indicator (Janice Young, Biolog, Inc., personal communication). The substrates were classified into six substrate sources, namely, carbohydrates, carboxylic acids, phenolic compounds, amino acids, polymers, and amines. Briefly, 5 g of chrysanthemum rhizospheric soil was mixed in 45 mL of a sterile NaCl solution (0.85%, *m/v*) and then oscillated at 180 rpm for 30 min. Using a sterile NaCl solution (0.85%, *m/v*), a serial dilution was performed until a final 1:1000 dilution was reached. Then, 150 μL of the supernatant was added to each well. Microplates were incubated at 25 °C for 192 h, and the influence of turbidity on the OD values at 590 nm and 750 nm were recorded every 12 h and calculated by subtracting the absorbance values of the two wavelengths. The OD values at the 96 h and 192 h of incubation were used for subsequent statistical analyses. All of the treatments had three replications. The well absorbance values were adjusted by subtracting the absorbance of the control well (water only) before the data analyses. Negative readings ($\text{OD} < 0$) were excluded from all subsequent analyses. The microbial activity in each microplate, expressed as the average well color development (AWCD), was determined as follows: $\text{AWCD} = \sum \text{OD}_i / 31$, where OD_i is the optical density value from each well. The Shannon diversity index was calculated as follows: $H = -\sum P_i (\ln P_i)$, where P_i is the ratio of the activity on each substrate (OD_i) to the sum of activities on all substrates ($\sum \text{OD}_i$). The Shannon evenness index was calculated as $E = H / \ln(\text{richness})$, where richness refers to the number of substrates utilized [36].

2.5. Statistical Analyses

Statistical analyses of data were conducted with the SPSS software program (ver. 20.0 for Windows, Chicago, IL, USA). Variations among chrysanthemum root morphological and aboveground growth parameters, the chemical analyses of rhizospheric soil properties, carbon utilization, and diversity index were analyzed using a two-way analysis of variance. Duncan's test was used to determine the different treatment levels. The PCA was conducted to analyze the substrate utilization pattern based on the Biolog EcoPlates data at 96-h after incubation and was performed to visualize the carbon utilization characteristics using CANOCO for Windows 4.5. All of the graphs were created with OriginPro 8.5 (OriginLab Corporation, Northampton, MA, USA).

3. Results

3.1. Effects of Organic Fertilizers on the Root Architecture and Aboveground Growth of Chrysanthemum

The root architecture of chrysanthemum is of great importance in nutrient uptake and transport. The statistical results of the effects on the root architecture of the various fertilizers are shown in Figure 1. Each liquid organic fertilizer had a positive effect on root growth. Compared with the CK and NPK treatments, the application of liquid organic fertilizers significantly promoted the root growth by 76.2–179.6% and 10.2–77.8%, respectively. L1 showed the highest promotional effect on the root growth of chrysanthemum than the other liquid organic fertilizer treatments. The root dry weight, root total length, root surface area, root volume, root tips, and thick root length of chrysanthemum under the L1 treatment was higher than the NPK treatment by 63.4%, 63.9%, 65.6%, 67.8%, 115.4%, and 90.5%, respectively. The L2 treatment had the second highest promotional effect and the root indices were enhanced by 35.1%, 44.2%, 41.9%, 40.1%, 75.3%, and 43.6%, respectively, compared with the NPK treatment. All of the root indices under L3, L4, and L5 treatments were similar to each other and slightly higher than those of the NPK treatment even at a limited mineral nutrient input rate. Analyses of chrysanthemum root growth showed that the application of L1 resulted in the greatest growth-promoting effects among the various liquid organic fertilizers.

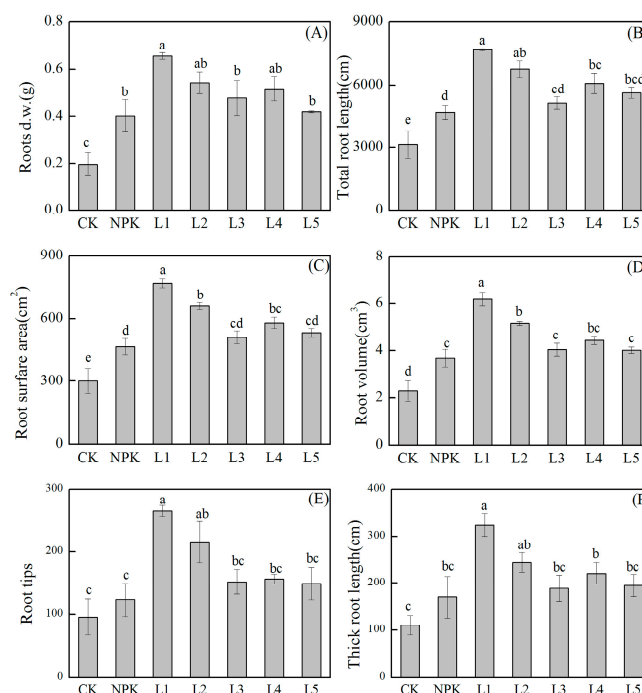


Figure 1. Effects of CK, NPK, and organic liquid fertilizers (L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; L5: fish extracts) treatments on the root architecture of chrysanthemum at 60 days after transplanting. (A) Root dry weight; (B) total root length; (C) root surface area; (D) root volume; (E) root tips; and (F) thick root length. Every value is expressed as the mean \pm standard deviation. Different letters indicate significantly statistical differences at the $p < 0.05$ level as determined by Duncan's multiple range test.

The statistical results of the aboveground growth indices affected by different fertilization regimes are shown in Figure 2. The shoot and leaf growth of chrysanthemum was significantly improved by the application of liquid organic fertilizers. Each type of liquid organic fertilizer produced greater seedling growth than the NPK treatment, especially in terms of shoot height and weight, leaf length, width, area, and weight, which were enhanced by 28.9%, 30.8%, 15.9%, 18.9%, 36.2%, and 28.2%, respectively.

The shoot heights of all the plants under the liquid organic fertilizer treatments were higher than under the NPK treatment, and the tallest plants were exposed to the L2 treatment, having an increase of 35.7%, but there were no significant differences among the liquid organic fertilizers treatments. Treatments with L1, L2, and L4 showed significant promotional effects that led to the cultivation of strong seedlings, and the shoot diameters of L1-treated plants were 1.2 times those of the NPK-treated plants. Plants treated with L1 achieved the greatest shoot and leaf biomasses, which were greater than the CK treatment by 70.0% and 66.7%, respectively. All of the liquid organic fertilizer treatments significantly promoted the leaf growth of chrysanthemum, and the leaf length, width, and area under the L5 treatment were 44.7%, 29.7%, and 90.7% greater than those under the CK treatment, respectively. The SPAD measurements of leaves were elevated after the application of liquid organic fertilizers and greater than under the NPK treatment (except L3). The SPAD value of the L1 treatment was 29.3% greater than the CK treatment. Analyses of the aboveground indices of chrysanthemum showed that the L1, L2, and L5 treatments had the greatest promotional effects on chrysanthemum shoot and leaf growth.

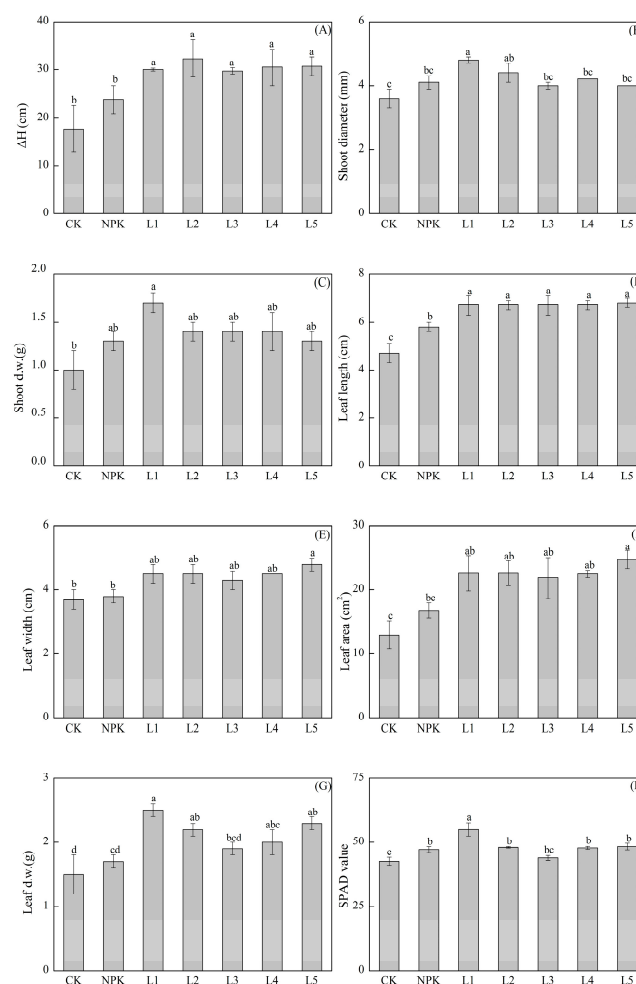


Figure 2. Effects of CK, NPK, and organic liquid fertilizers (L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; L5: fish extracts) treatments on the aboveground growth of chrysanthemum at 60 days after transplanting. (A) ΔH ; (B) shoot diameter; (C) shoot dry weight; (D) leaf length; (E) leaf width; (F) leaf area; (G) leaf dry weight; and (H) SPAD value. ΔH indicates the shoot height change during the growth stage; leaf length and width were measured using the largest leaf of the plant. Leaf area was estimated as follows: leaf length \times width \times 0.75. Different letters indicate significant differences among treatments as determined by Duncan's multiple range test at the $p < 0.05$ level.

3.2. Effects of Liquid Organic Fertilizers on the Nutrient Contents of Chrysanthemum Rhizospheric Soil

To investigate the soil's chemical properties influenced by the application of liquid organic fertilizers, several main nutritional parameters of rhizospheric soil were measured and the results are shown in Table 2. The nutrient content in the non-rhizosphere (Non-R) treatment was significantly different from the rhizospheric soil. The contents of mineral nitrogen and available potassium were much higher in the Non-R treatment. In the rhizospheric soil, the mineral N content was significantly increased with the addition of L1, whereas the content was the lowest under the NPK treatment, with a gap of 12.46 mg/kg. The available P and K contents under the L4 treatment were greater than under other treatments, and were enhanced by 49.0% and 13.4%, respectively, compared with the CK treatment. The EC measurements of rhizospheric soil significantly increased with the application of liquid organic fertilizers, which was consistent with the change in the mineral N content. The L1 treatment showed the highest EC value, followed by the L3 treatment, and no significant differences were observed among the other fertilizer treatments. The pH value of rhizospheric soil changed under different fertilizer treatments, and this was partly due to the different pH values of the fertilizers themselves, and because of different influences on the root exudates of chrysanthemum. Compared with that of the NPK, the pH values of the liquid organic fertilizer treatments were closer to that of the CK.

Table 2. The mineral nitrogen (N), available phosphorus (Avail-P), available potassium (Avail-K), electric conductivity (EC), and pH of chrysanthemum rhizospheric soil at the end of the trial *.

Treatment	Mineral N (mg/kg)	Avail-P (mg/kg)	Avail-K (mg/kg)	EC (us/cm)	pH
Non-R	29.9 ± 2.7 a	15.6 ± 0.3 d	48.3 ± 1.9 a	228.0 ± 17.1 b	5.1 ± 0.1 e
CK	11.9 ± 0.6 d	17.4 ± 0.7 cd	18.3 ± 0.3 b	139.6 ± 5.1 c	5.3 ± 0.1 b
NPK	7.4 ± 0.7 e	18.7 ± 0.5 bcd	17.4 ± 1.3 b	186.8 ± 15.9 bc	5.5 ± 0.1 a
L1	19.8 ± 1.2 b	19.4 ± 1.1 bcd	17.3 ± 0.5 b	340.3 ± 39.6 a	5.2 ± 0.1 cd
L2	12.5 ± 1.1 cd	21.0 ± 0.9 bc	18.5 ± 1.1 b	194.6 ± 9.8 bc	5.4 ± 0.1 a
L3	11.4 ± 0.4 d	17.0 ± 0.7 d	18.7 ± 1.1 b	294.0 ± 10.1 bc	5.1 ± 0.1 de
L4	13.1 ± 1.3 cd	25.9 ± 2.5 a	20.8 ± 1.3 b	171.4 ± 28.9 c	5.3 ± 0.1 bc
L5	16.3 ± 1.0 bc	22.4 ± 1.3 ab	19.0 ± 0.8 b	209.3 ± 21.5 bc	5.2 ± 0.1 bcd

Notes: * Treatments included: Non-R: non-rhizosphere; CK: control; NPK: chemical fertilizer; L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; and L5: fish extracts. Different letters indicate significant differences among treatments as determined by Duncan's multiple range test at the $p < 0.05$ level.

3.3. Effects of the Organic Fertilizers on Microbial Community Functions in Chrysanthemum Rhizospheric Soil

The AWCD was used as an indicator of the microbial activity in the soil. As presented in Figure 3, the AWCD of the rhizospheric soil was almost zero over the first 50 h of incubation, and it experienced a rapidly increasing stage, subsequently. The highest AWCD values were achieved under L1 and L2 treatments, while the lowest AWCD values were exhibited under the Non-R and CK treatments. The addition of liquid organic fertilizers significantly increased the AWCD values after incubation, and the AWCD values of rhizospheric soil treated with L1–L5 were 2.46, 2.43, 1.70, 1.35, and 1.83 times greater, respectively, than those of the CK soil. Thus, the addition of liquid organic fertilizers generally improved the functions of chrysanthemum rhizospheric soil's microbial community. Additionally, treatments with L1 and L2 affected the increase in the AWCD. The AWCD under treatment L5 showed no significant variation compared with the NPK treatment, whereas the AWCD values of the L3 and L4 treatments at the preliminary stage of incubation were lower than the NPK treatment, but the AWCD of the L3 treatment exceeded that of the NPK treatment at the end of the incubation.

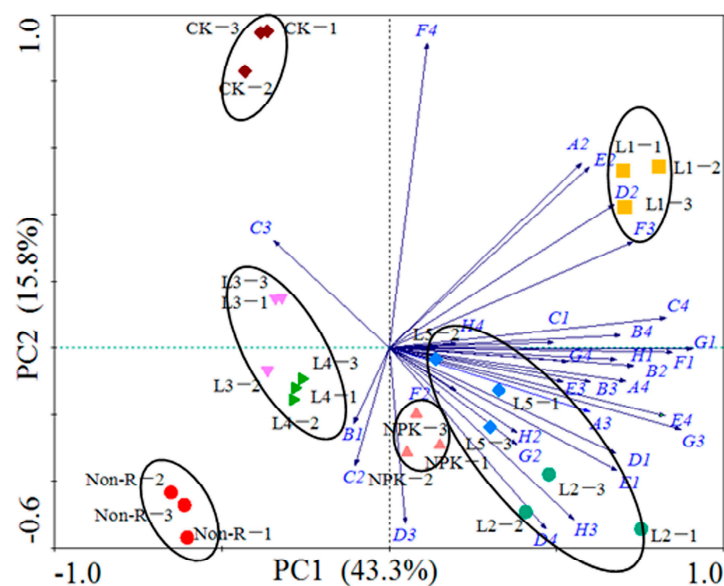


Figure 4. PCA of diverse uses of carbon sources after the 96-h incubation of the chrysanthemum non-rhizospheric (Non-R) soil and rhizospheric soil treated with CK: control; NPK: chemical fertilizer treatment; L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; and L5: fish extracts. Carbon substrates of the Biolog™ Plate include: A2: β -methyl-D-glucoside; A3: D-galactonic acid g-lactone; A4: L-arginine; B1: pyruvic acid methylester; B2: D-xylose; B3: galacturonic acid; B4: L-asparagine; C1: Tween 40; C2: i-erythritol; C3: 2-hydroxybenzoic acid; C4: L-serine; D1: Tween 80; D2: D-mannitol; D3: 4-hydroxybenzoic acid; D4: L-phenylalanine; E1: α -cyclodextrine; E2: N-acetyl-D-glucosamine; E3: γ -hydroxybutyric acid; E4: L-threonine; F1: glycogen; F2: D-glucosaminic acid; F3: itaconic acid; F4: glycyl-L-glutamic acid; G1: D-cellobiose; G2: glucose-1-phosphate; G3: α -ketobutyric acid; G4: phenylethylamine; H1: α -lactose; H2: D,L- α -glycerol phosphate; H3: D-malic acid; and H4: putrescine.

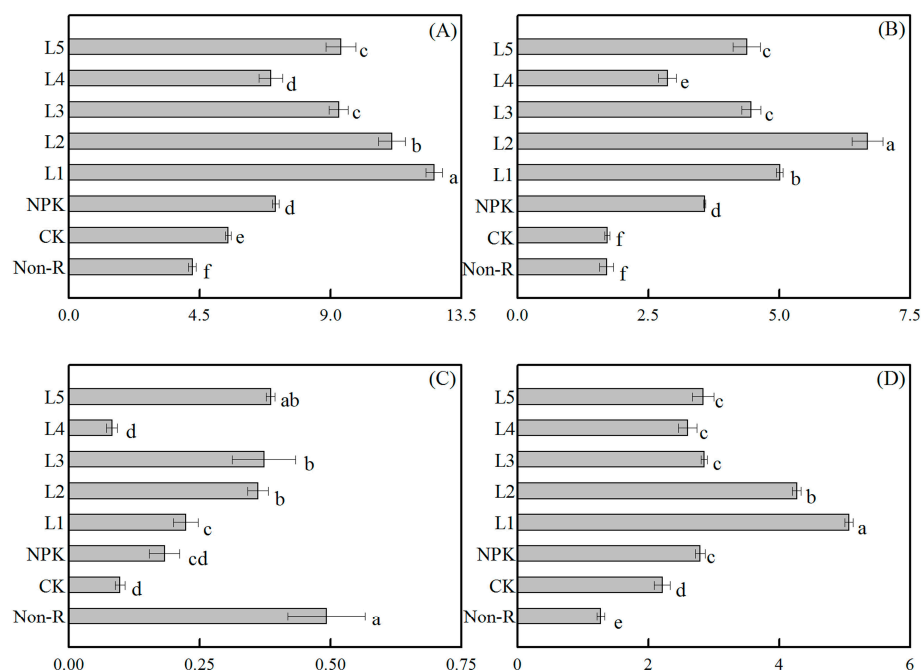


Figure 5. Cont.

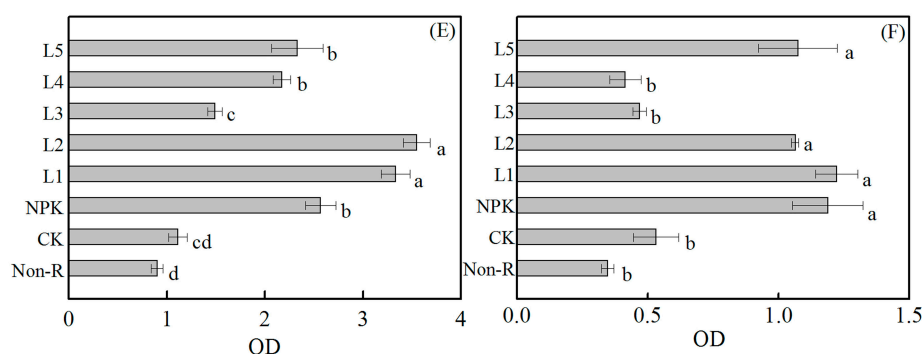


Figure 5. The OD values of diverse carbon substrates after the incubation of chrysanthemum rhizospheric soil treated with CK: control; NPK: chemical fertilizer treatment; L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; and L5: fish extracts. Non-R: non-rhizosphere soil. The main carbon sources were as follows: (A) carbohydrate; (B) carboxylic acid; (C) phenolic compounds; (D) amino acid; (E) polymer; and (F) amine. Different letters indicate significant differences among treatments as determined by Duncan's multiple range test at the $p < 0.05$ level.

The Shannon diversity and evenness indices of the soil microbial community after different fertilization treatments were calculated from the 96-h Biolog data and are shown in Figure 6. The Shannon diversity index for the liquid organic fertilizer treatments (except L3 and L4) was significantly higher than for the Non-R, CK, and NPK treatments. The Shannon diversity index ranged from 2.58 to 3.04, and the averaged index value of L1, L2, and L5 was 11.36% and 7.7% higher than those of the CK and NPK treatments, respectively. There was no significant difference between the Shannon diversity index under the L1, L2, and L5 treatments. The Shannon evenness index under L1, L2, and L5 was significantly higher than that found under the other fertilized and non-fertilized treatments. The averaged Shannon evenness index of L1, L2, and L5 was 10.9% and 6.5% higher than those of the CK and NPK treatments, respectively. Thus, the application of liquid organic fertilizers (especially L1, L2, and L5) had a significantly positive effect on the diversity and evenness of the soil microbial community.

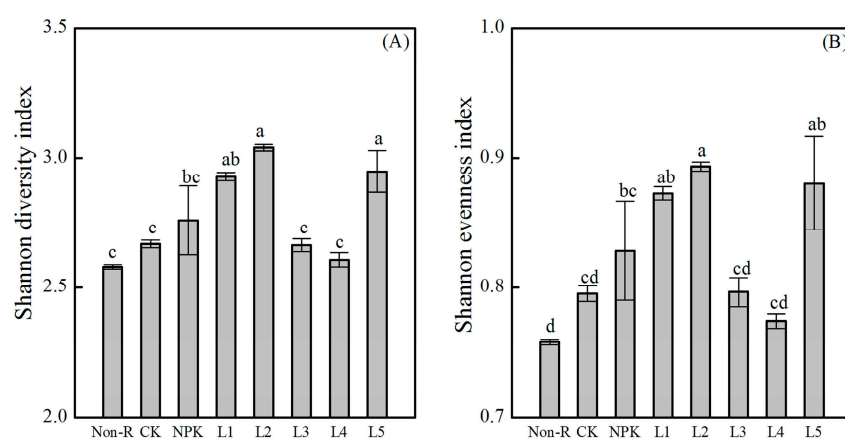


Figure 6. Effects of the various fertilization treatments on the Shannon diversity index (A); and Shannon evenness index (B) of the rhizospheric soil of chrysanthemum. Treatments were as follows: CK: control; NPK: chemical fertilizer treatment; L1: shrimp extracts; L2: plant decomposition; L3: vermicompost; L4: seaweed extracts; and L5: fish extracts. Non-R: non-rhizospheric soil. Every value shown in the bar is expressed as the mean \pm standard deviation. Different letters indicate significant statistical differences at the $p < 0.05$ level as determined by Duncan's multiple range test.

4. Discussion

4.1. Effects of Liquid Organic Fertilizers on the Growth of Chrysanthemum

The root systems of plants perform important roles in plant growth [42]. Here, the various liquid organic fertilizer treatments significantly promoted root growth by 76.2–179.6% and 10.2–77.8% compared with the CK and NPK treatments, respectively (Figure 1). The improved root growth of chrysanthemum could be due to the liquid organic fertilizers' abilities to supply soluble organic nutrients and biostimulants more quickly to the plant, which supported its growth [43]. Among the five liquid organic fertilizers, plants treated with L1 achieved greater root dry weights, root total lengths, root surface areas, root volumes, root tips, and thicker root lengths than with the other organic fertilizer treatments by 35.4%, 31.6%, 36.3%, 39.4%, 57.5%, and 34.5%, respectively (Figure 1), followed by the L2 and then the L4 treatments. The L3 and L5 treatments only slightly increased the root growth of chrysanthemum. The shoot and leaf growth of chrysanthemum showed the same trend, and the liquid organic fertilizer treatments significantly promoted the aboveground growth by 30.2–56.5% and 10.7–33.3% compared with the CK and NPK treatments, respectively (Figure 2). These results are consistent with those of Martínez-Alcántara et al., in which either an animal or plant-based liquid organic fertilizer produced a higher total biomass of citrus trees than mineral fertilizers because of the more profuse development of new organs under the organic treatments [26]. Additionally, the shoot diameter, SPAD value and aboveground biomass of chrysanthemum significantly increased with the application of L1 (Figure 2), suggesting that the L1 had a positive effect on seedling growth, leaf chlorophyll concentrations, photosynthetic activities, and nutrient uptake efficiency [44]. In addition, the L2 treatment had a positive effect on shoot height, which may be due to the modified availability of resources [45], whereas the application of L5 significantly enhanced the leaf growth of chrysanthemum, which contributed toward an advanced photosynthetic efficiency [46]. Thus, compared with NPK, the addition of L1 significantly improved the root and plant growth of chrysanthemum under the experimental conditions. The L2 treatment had the next greatest effect, followed by the L4 and L5 treatments. The addition of L3 resulted in only a slight increase in seedling growth.

L1 was derived from shrimp extract, which is the most abundant natural resource on earth, with an estimated chitin yield of 10^{10} – 10^{11} tons per year [22,47]. Additionally, seafood processing wastes do not contain known toxic or carcinogenic materials like liquid wastes from other industries [48]. Therefore, the shrimp extract is a low-cost and environmentally-friendly resource. The chitosan compounds deacetylated from chitin that are extracted from shrimp are effective in promoting seed germination, and root and shoot growth, and can induce resistance to abiotic stresses, as well as acting as biopesticides [14,22,49–51]. Therefore, the positive effect of L1 may be partly due to the presence of chitosan. In the conventional monoculture production system, chrysanthemum is generally affected by *Fusarium* wilt and other diseases [6]. The addition of L1 from this experiment may promote root growth and ecological adaptability and reduce the occurrence of plant diseases and insect pests, thereby improving chrysanthemum growth, and additional experiments are required to verify the hypothesis [52,53].

4.2. Effects of Liquid Organic Fertilizers on Chrysanthemum Rhizospheric Soil's Characteristics

Organic amendments are frequently used to improve the soil structure, microbial diversity, and plant nutrient status [12,54]. In our study, the applications of various liquid organic fertilizers significantly improved the nutrient level (mineral N, available P, and available K contents) by 2.9–28.3% and 8.1–134.2% compared with the CK and NPK treatments, respectively. Increases in the available nutrient content of rhizospheric soil after the application of liquid organic fertilizers have been attributed to the improved diversity of the microbial community, which enhances the nutrient cycle in the soil, increasing the soil nutrients available for plant growth [23,55]. The addition of L1 significantly stimulated the N mineralization process (Table 2), which was different from the findings of Gutser et al. in which the input of organic fertilizers on arable land significantly reduced the mineral-N

content and increased the stability of the organic matter [56]. This was mainly because the organic fertilizer form used by Gutser et al. was compost, whereas the liquid organic fertilizer used in our experiment contained a large amount of soluble nutrients and tended to be more quickly available to plants compared with the traditional substrate-incorporated fertilizers [57]. In our experiment, the L1 treatment produced the highest EC values, which were 15.8–143.8% higher than those of the other treatments (Table 2). The increase in the soil EC value could be correlated with the mineralization of organic matter after the application of liquid organic fertilizers [58], which was in accordance with the change in the mineral N content (Table 2).

The carbon substrate utilization presented by the Biolog-Eco plates was sensitive enough to detect short-term changes in the soil environment, and the appropriate fertilizer regimes could increase the soil's functional microbial diversity [38,59]. In our study, the application of liquid organic fertilizers improved the AWCD of rhizospheric soil by 35.4–146.2% and −13.5–57.3% compared with the CK and NPK treatments, respectively (Figure 3). The addition of L1 exhibited the highest carbon substrate utilization, and the AWCD of L1 at the end of the incubation was 1.4–81.9% higher than those of the other liquid organic fertilizer treatments. In agreement with our results, Gomez et al. found that organic soil amendments significantly stimulated the carbon substrate utilization [60], and this mainly depended on the availability of soil carbon. The PCA showed that the fertilizer regimes significantly influenced the rhizospheric soil's functional diversity (Figure 4). Liquid organic fertilizers may induce changes in soil properties which, in turn, influence the soil's microbial functional diversity, resulting in differences in the soil carbon substrate utilization and plant growth [38,61]. Under the liquid organic fertilizer treatments, the utilization of carbohydrate, carboxylic acid, phenolic compounds, amino acid, and polymer was much greater than in the CK and NPK treatments, and an enhancement of these carbon sources was observed by 59.4–191.7% and 0.2–56.0% compared with the CK and NPK treatments, respectively (Figure 5). This was in accordance with earlier work of Romaniuk et al. in which microbial functional diversity could indicate the influence of fertilizer and management practices in organic and conventional horticulture systems [62]. The utilization of phenolic compounds showed a different tendency in our experiment and it was highest under the Non-R treatment and lowest for CK treatment. This phenomenon was possible partly for the different pH values among treatments and the pH value of the Non-R treatment was the lowest (Table 2). Additionally, phenolic compounds were the main autotoxicity substance in rhizosphere soil of many plants and it would influence the growth of plants; thus, the utilization of phenolic compounds was higher in Non-R treatment than the rhizosphere soil [63,64]. The application of L1 significantly improved the utilization of the six main carbon sources (carbohydrate, carboxylic acid, phenolic compounds, amino acid, polymer, and amine) by 76.6, 40.1, 22.4, 81.6, 29.7, and 2.9% than the NPK treatment, respectively. The Shannon index analysis also showed that the application of liquid organic fertilizer treatments (especially L1, L2, and L5) significantly improved the diversity and evenness of the soil bacterial community (Figure 6), which would be beneficial in resisting stress [61,65]. Thus, the application of liquid organic fertilizers, especially L1, significantly increased the microbial population's diversity in the rhizosphere.

5. Conclusions

Fertilizer management is of great importance in chrysanthemum production. In this study, five sources of liquid organic fertilizer were applied to promote chrysanthemum growth at the early stage. The application of liquid organic fertilizers significantly promoted the root architecture and plant growth of chrysanthemum compared with the CK and inorganic fertilizer treatment over the short term, even with a limited amount of mineral nutrient input. Among the five liquid organic fertilizers treatments applied in our experiment, the L1 liquid fertilizer proved to be effective in both root development and aboveground growth promotion, especially the root tips, SPAD value of leaves, and the aboveground biomass. The addition of L1 liquid organic fertilizer significantly stimulated the soil's microbial activity and functional diversity through the enhancement the N mineralization process at the rhizospheric level. Treating with L1 liquid organic fertilizer indicated its potential as

an effective fertilizer regime in the chrysanthemum production system. Moreover, the successful application of liquid organic fertilizers in our study suggested a rational way to reuse agricultural wastes and was effective in sustaining plant growth and the health of the soil system. In the future, more attention should be paid to quantifying the optimal fertilizer rate of the shrimp extract liquid organic fertilizer in diverse growth periods of chrysanthemum production, and further analysis is also required to clarify the key microbiologic population in the process.

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Abbreviations

NPK	chemical fertilizer
CK	no fertilizer control
EC	electric conductivity
SPAD	Soil and Plant Analyzer Development
AWCD	Average Well Color Development

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