Canadian Journal of Soil Science
Revue canadienne de la science du sol

Bio-organic-mineral fertilizer can improve soil quality and promote the growth and quality of water spinach

Journal:	<i>Canadian Journal of Soil Science</i>
Manuscript ID	CJSS-2016-0090.R2
Manuscript Type:	Article
Date Submitted by the Author:	15-Mar-2017
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Keywords:	Bio-organic-mineral fertilizer, poor soil, quality improvement, Ipomoea aquatica, growth, chemical fertilizer

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Running head: Bio-organic-mineral Fertilizer improves quality of both soil and plants

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Keywords: Bio-organic-mineral fertilizer, poor soil, quality improvement, *Ipomoea aquatica*, growth, nutritional quality

Increasingly poor soils are used for plant cultivation involving chemical fertilizer (CF) usage which in turn lowers soil quality. To improve the quality of poor soils, promote plant growth, and reduce CF use, bio-organic-mineral fertilizer (BF) was introduced to poor soil to examine its effects on both soil and plants. Organic fertilizer (OF), CF and mineral powder (MP) were applied as controls. When cotreating water spinach with fertilizer, the soil quality was improved with the increased doses of BF, OF and MP. BF treatment produced the most pronounced improvement. Applying CF reduced soil quality. Under the CF treatment the largest plant growth-promoting effect was observed within 30days, whereas plants treated with BF showed the most favorable growth after 50days. Moreover, for BF, plants' nutritional quality was also higher, particularly in the long term. When poor soil was treated with fertilizer without plants, the soil quality changed as in previous treatment; however, the change in this treatment was more pronounced and the remaining available elements in soil were higher, particularly in the case of CF treatment. These results indicate that BF treatment without CF can be applied to improve the quality of poor soil, and meanwhile promote plant growth and quality.

Keywords: Bio-organic-mineral fertilizer, poor soil, quality improvement, *Ipomoea aquatica*, growth, chemical fertilizer

Abbreviation: AK, available potassium; AOC, active organic carbon; BF, Bio-organic-mineral fertilizer; CA, carbonic anhydrase; CF, chemical fertilizer; CK, the control; EC, electrical conductivity; MP, mineral powder; OF, organic fertilizer; Vc, vitamin C

Poor soil usually contains low concentrations of soil nutrients, has low or high pH, and low soil enzyme activity. Such soil has low fertility and yields less food. With rapid

developments in industry, agriculture, and population growth, large areas of cultivated fertile soil have been degraded into poor soil (Shu et al. 2013), and many areas of newly reclaimed soil, most of which are poor soils, have been used for plant cultivation. Measures to improve these soils are urgently required.

Many studies have shown that soil quality can be improved by applying organic matter (Barzegar et al. 2002), organic fertilizer (OF; Biau et al. 2012), dust, mineral powder (MP) (Hagos and Smit 2005; Suchodoletz et al. 2011), or by growing pasture legumes as green manure. The consensus holds that organic matter is a crucial soil component for improving the physical properties of soil, and that returning organic matter to soil can increase the content of soil active organic carbon (AOC) and improve soil vitality. However, organic matter alone, which lacks adequate available nutrients, exerts slow and variable effects on crop growth (Khaliq et al. 2006). Although applying chemical fertilizers (CFs) can promote rapid crop growth, extensive CF use has reduced soil quality worldwide (Bronick and Lal 2005; Shu et al. 2013). Hence, compound fertilizers composted with suitable microbes, organic matter, and low quantities of CF have become the most commonly used method (Liu et al. 2009; Pan et al. 2009).

Although the content of available nutrients, such as K and Ca, and Mg, in poor soil is low, their total content is high (Lian et al. 2008; Xiao et al. 2012). Most unavailable nutrients are present in minerals (Lian et al. 2008). In ecosystems with low inputs and no fertilization or soil amendment by humans, the nutrients available to plants are obtained from atmospheric inputs and weathering of soil minerals caused by water circulation and plant and microbial action (Dakora and Phillips 2002; Calvaruso et al. 2006). Microbes such as *Bacillus*

mucilaginosus (Sheng and Huang 2002; Lian et al. 2008; Basak and Biswas 2009), *Aspergillus fumigates* (Lian et al. 2008) and *A. niger* (Wang et al. 2015) can promote mineral weathering and the release of nutrients such as K and Ca from feldspar. Treatment with bio-organic-mineral fertilizer (BF) composted by organic matter, minerals and microbes, can improve K-cycling and promote crop growth (Sugumaran and Janarthanam 2007; Nishanth and Biswas 2008; Yang et al. 2012). The aforementioned studies have indicated that to improve soil quality and plant growth, the mineral weathering-promoting function of microbes might be more useful than CF.

In the present study, BF, OF, CF, and MP were introduced to poor soil. A comparative study of different treatments was conducted to determine the changes in soil properties, namely pH, electrical conductivity (EC), available K (AK), AOC, soil enzyme activity, and parameters related to water spinach quality (including growth and nutritional quality), to investigate the potential applications of BF in improving soil quality and crop growth.

MATERIALS AND METHODS

This study was conducted from May to August in 2014 and 2015 in the Botanical Garden of Nanjing Normal University (118°91'E, 32°10'N).

Materials

BF was composted with chicken manure, K- containing mineral powder, corn stalk (w/w/w = 1:1:2), and mixed microbial agents. The K-containing mineral was obtained from Daoping Town, Fuquan City, Guizhou Province. Analysis of composition through X-ray fluorescence showed that K₂O, Al₂O₃, SiO₂, and CaO accounted for 9.67%, 18.06%, 57.75%, and 3.26% by mass, respectively. All constituents were not bioavailable to plants. The mineral sample was

crushed and sieved through 100–200 mesh to collect powder. The composted BF contained 1.9% available N, 1.9% available P_2O_5 , 2.5% available K_2O , and 32% organic matter. OF was composted with the same material by the same method as BF, but without K-containing mineral powder. The composted OF contained 1.7% available N, 2.5% available P_2O_5 , 1.6% available K_2O , and 45% organic matter. MP was the identical K-containing mineral powder used in BF. The effective content of CF (Aolaite Chemical Fertilizer Co., Ltd., Jiangsu, China) was 45%, with a N: P_2O_5 : K_2O ratio of 15:15:15. They were all bioavailable. The culture medium was a mixture of poor soil (obtained from 30 cm below the surface) and river sand (v/v = 1:1), the main component of which was SiO_2 . It was used for the improvement of water permeability. Seeds of *Ipomoea aquatica* Forsk, commonly known as water spinach, were purchased from Jiangsu Academy of Agricultural Science (Nanjing, China).

Experimental Design and Plant Cultivation

In experiment I, seedlings were grown in 72-hole flowerpots (peat/vermiculite = 3:2) until they reached a height of 12 cm. Seedlings with a similar height and diameter were selected, and two seedlings were transplanted into 12-cm plastic pots containing 1.5 kg of the culture medium mixed with fertilizer (Table 1). Each treatment was repeated eight times. The plants were placed inside insect nets under natural conditions with normal irrigation management and no additional fertilization. The main stem and leaves on the main stem were collected to measure growth, biomass, and nutritional quality 30 days after transplantation. The remaining aboveground parts were collected to measure biomass and nutritional quality 20 days later. Subsequently, the soil in the pots was left for another 10 days before soil sampling.

Experiment II was designed to observe the effect of fertilization on poor soil excluding the

effect of plants or leaching. Similar to experiment I, each plastic pot contained 1.5 kg of the culture medium mixed with fertilizer (Table 1), and each treatment was repeated eight times. The pots were placed indoors with no plants. The soil was watered without seepage for 60 days. At the end of the experiment, the soil was sampled and assayed.

Soil Sampling and Analysis

After removal of visible plant residue, the soil in each pot was thoroughly mixed. Half of the fresh samples were kept field-moist in a cooler at 4°C for no more than 2 weeks to determine their carbonic anhydrase (CA) activities. The remaining samples were air-dried, ground, sieved with a 2-mm sieve, and stored at room temperature to analyze their remaining soil properties. All results are expressed on a dry weight (DW) basis.

The pH and EC of the soil samples were determined in a soil/water (1:2.5 = w/v) suspension by using a PHS-3C mv/pH detector (Shanghai, China). AK was extracted with ammonium acetate, and its concentration was determined using an atomic absorption analyzer (PerkinElmerAA900F, USA). AOC was determined using the KMnO_4 oxidation method (Blair et al. 1995). The HCO_3^- content in the soil leaching solution was measured using the double indicator titration method of Verma (2004).

Urease (E.C.3.5.1.5) activity was determined using the phenol–sodium hypochlorite colorimetric method, as indicated by the mass (mg) of KNO_3 in 1 g of soil incubated for 24 h (U) (Guan et al. 1986). Alkaline phosphatase (E.C.3.1.3.1) activity was determined using disodium phenyl phosphate as a substrate and phenol as a product after incubation at 37°C for 24 h (Guan et al. 1986). CA (E.C.4.2.1.1) was assayed using the method of Brownell et al. (1991).

Plant Growth Measurement

After transplantation for 30 days, the plant height was measured on the main stem with a ruler. The main stem diameter was measured 1 cm above the ground with a Vernier caliper. The area of the fourth fully expanded leaf was measured with a multipurpose leaf area analyzer (WinFOLIA, Regent Instruments Inc., Canada). To measure the biomass of the main stem, the samples were oven-dried first at 105°C for 15 min and then at 75°C for 48 h. A total of 20 days later, the biomass of the remaining aboveground parts was reassayed. The measurement on the plant growth indices were all repeated 10 times.

Plant Nutritional Quality Measurement

On the 30th day and 50th day after transplanting, the stems and leaves were sampled and washed separately with distilled water through a fine sieve. The samples were used fresh or oven-dried, as described earlier, and powdered or frozen in liquid nitrogen and stored at -80°C until the subsequent analysis.

Soluble sugar was extracted by boiling the fresh samples in water, and the soluble sugar content was estimated using the anthrone method (Watanabe et al. 2000). A standard curve was plotted using D-glucose, and the soluble sugar content was calculated and is expressed as mg.g^{-1} fresh weight. For measurement of the vitamin C (Vc) content, samples were homogenized with ice-cold 5% (w/v) trichloroacetic acid. The Vc content was determined according to the method of Hamed et al. (2007). Carotenoids were extracted from the fresh samples by using 96% ethanol, and their content was analyzed according to the method of Porra et al. (1989). Flavonoids were extracted from the powdered samples by using methanol on a Soxhlet extractor following the method of Alothman et al. (2009). The filtrate was

separated using filter paper (Whatman number 1) and concentrated under reduced pressure in a rotator evaporator (Buchi Rotavapor R-210, Switzerland). Subsequently, the total flavonoid content was determined using the colorimetric method described by Alam et al. (2014), the results of which are expressed as $\text{mg RE.g}^{-1}\text{DW}$.

Nitrate was extracted from the fresh samples in a boiling water bath, and the content was assayed in salicylic acid–sulfuric acid (Cataldo et al. 1975), with KNO_3 as the standard. Oxalic acid was extracted from the fresh samples by using water, and the content was determined using sulfonic acid, as described by Ilarslan et al. (1997), with oxalic acid as the standard.

Statistical Analyses

The data are presented as means in all tables and were analyzed through one-way analysis of variance by using the statistical software Origin 7.0.

RESULTS

Experiment I

Soil Properties

After combined treatments with fertilizers, plants, and leaching for 60 days, the selected property indices of the soil samples were measured (Table 2). The pH of the CK samples was 7.58. The pH decreased slightly to less than 7.5 after the BF or OF treatment, decreased to less than 7.3 after the CF treatment, and remained constant after the MP treatment. The EC value of the CK was low. Application of a gradient dose of BF, OF, or MP resulted in a gradient increase of EC values in the soil. They were higher after the BF1 and BF2 treatments than those after the corresponding OF and MP treatments (Table 2). After treatment for 60

days, the EC values of the CF treatments were as low as those in the CK treatment.

With the exception of the AOC content in the CF3 treatment, the application of BF, OF, and CF increased the content of AK, AOC, and HCO_3^- in the soil. They increased with the BF or OF dose, and exhibited a more pronounced increase in the BF treatments. They were relatively low after the CF treatments, especially after the CF3 treatment. Under the MP treatment, the content of AK, AOC, and HCO_3^- increased only after the MP3 treatment.

The activity of the three soil enzymes was relatively low in the CK (Table 2). They increased with the fertilizer dose in the BF and OF treatments, and had a more pronounced increase in the BF treatments. They decreased with the CF dose, and the activity of the urease and CA only increased after the MP3 treatment.

Water Spinach Growth and Nutritional Quality

Water spinach seedlings showed similar weak growth under the CK and MP treatments (Table 3). With BF, OF, and CF application, the seedlings showed improved growth with the increase in the dose. Thirty days after transplantation, the seedlings in the CF group exhibited the greatest height, stem diameter, and leaf area ($p < 0.05$), followed by similar seedlings growth in the BF and OF treatments (Table 3). A total of 20 days later, the aboveground biomass of the seedlings in the BF treatment had reached the biomass level of the CF treatment (Table 3). The highest total biomass was also found in the BF and CF treatments.

Plant nutritional quality was relatively low for the 30 days MP treatment (Table 4). For the other three fertilizers, most of the parameters in Table 4 increased in conjunction with the dose, with some exceptions for the CF treatment. The content of nutritional substances such as soluble sugar, soluble protein, and total flavonoids was higher for the BF and OF

treatments than for the CF treatments. By contrast, the content of antinutritional substances such as nitrate and soluble oxalic acid was lower in the BF and OF treatments than in the corresponding CF treatments. The nutritional content was not lower, and the antinutritional content was not higher in the BF treatments than in the OF treatments.

An additional 20 days later, nearly all content in Table 4 increased to varying degrees, increasing slightly in the MP treatments. The nutritional content was higher and the antinutritional content was lower in the BF and OF treatments than in the corresponding CF treatments. In addition, the content of nutritional substances such as Vc, carotenoid, and total flavonoid was higher in the BF treatments than in the OF treatments. CF treatment increased the content of Vc and carotenoid and decreased the content of soluble sugar and total flavonoid in water spinach seedlings.

Experiment II

Soil Properties

Selected property indices of the soil samples after treatment with different fertilizers for 60 days without plants and leaching were shown in Table 5. The pH of the CK samples was 7.17, which was lower than the corresponding values in experiment I (Table 2). The pH increased after the BF and MP treatments and decreased after the OF and CF treatments. The biggest increase was observed in the BF treatments, and the biggest decrease was found in the CF treatments. All EC values in Table 5 are markedly higher than those in Table 2. The EC value remained relatively low in the CK treatment, exhibiting the highest increase in the CF treatments, followed by the treatments with OF, BF and MP.

The contents of AK and AOC shown in Table 5 are significantly higher than those in

Table 2. Both BF and OF treatments increased the content of AK, AOC, and HCO_3^- in the soil dose-dependently. The most pronounced increase was found in the BF treatments. CF treatments increased the AK content and decreased the content of AOC and HCO_3^- dose-dependently. By contrast, the MP treatment only enhanced the HCO_3^- content.

The activities of the three soil enzymes in Table 5 were still significantly higher than those in Table 2. They were the lowest in the CK soil (Table 5). The BF and OF treatments increased their activities and CF treatments decreased their activities dose-dependently. Of the MP treatments, only high dose MP treatment (i.e. MP3) increased their activities.

DISCUSSION

The main function of soil is to sustain plant and animal productivity and yield food for humans (Karlen et al. 1997). Soil properties determine the soil processes that control nutrient cycling and the availability of nutrients to living organisms, thereby affecting soil quality and productivity. The pH of soil not only affects its capacity to support nutrients, but also affects its microbial balance and controls enzyme activity (Wang et al. 2014). The EC value correlates positively with the content of available nutrients in the soil. High nutrient availability in the soil can cause salt damage to living organisms (Shu et al. 2013). AOC content determines soil fertility and is a key indicator of soil quality, influencing various physical, chemical, and biological properties of soil (Blair et al. 1995). Numerous soil enzymes, which originate from plant roots and soil microorganisms and participate in organic matter decomposition, drive nutrient cycling (Dick, 1994; Waring et al. 2014). Notably, urease and phosphatase activity correlate positively with soil N and P cycling and availability (Dick 1994; Acosta-Martínez et al. 2003; Singh and Kumar 2008), and CA promotes

K-feldspar weathering and carbonate formation (Xiao et al. 2015 and 2016).

Poor soils usually exhibit low concentrations of plant-available nutrients and AOC, extreme pH values, low EC values, and low soil enzyme activity. Such soils have low fertility and produce lower yields. However, globally, human activity has resulted in large areas of fertile soil being degraded into poor soil (Ju et al. 2007; Shu et al. 2013). Measures to improve poor soils and increase food yields to accommodate population growth are urgently necessary (Zhu et al. 2015).

Numerous studies have indicated that the application of organic matter (Barzegar et al. 2002), OF (Biau et al. 2012), microbes (Nisha et al. 2007), and even dust or MP (Hagos and Smit 2005; Suchodoletz et al. 2011) can improve the quality of poor soil. However, to rapidly improve the quality of both soils and plants, compound fertilizers composted with suitable microbes, organic matter, and a certain amount of CF have become the most common approach (Liu et al. 2009; Pan et al. 2009). Nevertheless, to implement the “Action Plan of Zero Growth on Chemical Fertilizers by 2020” (Ministry of Agriculture of the People’s Republic of China 2015), developing fertilizers without any added CF to improve both soil and plant quality is essential.

In ecosystems without any fertilization or soil amendment by humans, plants show favorable growth, and nutrients are mainly obtained from atmospheric inputs and the weathering of soil minerals (Van Breemen et al. 2000; Dakora and Phillips, 2002; Calvaruso et al. 2006). Dust or MP application can improve soil quality (Hagos and Smit 2005; Suchodoletz et al. 2011). The actions of plants and microbes are the main biological causes for the weathering of soil minerals (Dakora and Phillips 2002; Calvaruso et al. 2006).

Microbes such as *B. mucilaginosus* (Sheng and Huang 2002; Lian et al. 2008; Basak and Biswas 2009) and *A. fumigates* (Lian et al. 2008) and *A. niger* (Wang et al. 2015) exhibit high mineral-weathering activities. Hence, through suitable composting and application, nutrient release can be promoted from minerals by microbes, thereby enhancing crop growth (Sugumaran and Janarthanam 2007; Nishanth and Biswas 2008; Yang et al. 2012). On the basis of the aforementioned findings, we hypothesize that BF composted with organic matter, MP, and microbes with high weathering activity, are more effective than OF in improving soil and providing available nutrients for plants, and that CF can effectively be replaced by minerals and microbes. The contents of N, P, and K were higher in the BF than in the OF, which was composted identically to the BF, but without K-containing minerals (Table 1). This indicates that the microbes promoted the release of K and P, and the preservation of N during composting (Yang et al. 2012).

The CK soil investigated in the present study was slightly alkaline poor soil (Table 2). A total of 60 days after treatment with different fertilizers, nutrient absorption by water spinach, and leaching, the changes in the soil properties varied among the investigated samples. Although CF can rapidly supply available nutrients, nutrient absorption by plants and leaching for 60 days caused soil to be depleted of most of the added nutrients. Moreover, the CF treatment decreased the pH value and soil enzyme activity significantly. All of the preceding observations further demonstrated the marked soil degradation effect of CF (Shu et al. 2013). Although the initial nutrient content was relatively low, the soil quality improved after 60 days of treatment with BF, OF, and a high MP dose. The most marked improvement was observed in the BF treatments and the lowest in the MP treatments (Table 2). This

finding indicated that although natural microbes in soil could promote mineral weathering (Hagos and Smit 2005; Suchodoletz et al. 2011), their efficiency was relatively low. When the BF was added the poor soil, the cooperation of vigorously growing microbes and K-containing minerals enhanced mineral weathering and promoted AOC accumulation in the soil (Table 2).

Water spinach, botanically classified as *I. aquatica*, is a semiaquatic, tropical plant grown as a vegetable for its tender shoots and leaves. On the basis of the vitamin content of certain varieties, it is considered a tonic, and is used to treat stomach and intestinal disorders in much of southeastern Asia (Nagendra Prasad et al. 2008). In the present study, the growth and yield of water spinach was determined by a combination of soil quality and available mineral content (Table 3). Seedlings showed weak growth after the CK and MP treatments and favorable growth after the CF, BF, and OF treatments; the strongest growth was observed after the CF treatments, followed was the BF and OF treatments. These results indicate that, although CF can promote plant growth over a short period, it reduced soil quality. Moreover, although the growth-promoting effect of BF was lower than that of CF at the initial stage (30 days), it was higher than that of CF over a long term (50 days; Table 3). BF improved soil quality stably and more rapidly than did OF (Table 2), which in turn promoted plant growth more effectively.

High yields do not represent high plant quality. Although the growth-promoting effect of CF was the highest in the short term, the main nutrient content of water spinach in Table 4 was not improved with increased doses. Moreover, the content of two antinutritives, namely nitrate and soluble oxalic acid, was enhanced using the CF treatment (Table 4). The main

nutrient content produced by the BF and OF treatments was similar within 30 days of treatment. However, the Vc and total flavonoid content obtained with the BF treatments was higher than that from the corresponding OF treatments. Thus, the BF treatment not only improved growth, but also enhanced nutritional quality (Tables 3 and 4).

Soil fertility may decline after plant cultivation, owing to direct uptake into harvest materials or through indirect leaching (Debiase et al. 2016). To explain these effects and investigate methods for rapidly improving soil, the poor soils in experiment II were treated as in experiment I, with the exception that nutrient absorption by plants and leaching through watering were not included. The results indicate that the changing trend of soil quality produced by fertilizers without plants and leaching was the same as that with plants and leaching (Tables 2 and 5). In addition, the soil-degrading effect of CF, and the soil-improving effect of BF, OF, and MP were more substantial without plants and leaching. This demonstrates that applying BF without plants and in combination with mulch treatment to avoid leaching is the most effective method for rapidly improving poor soil.

CONCLUSIONS

1. CF treatment can promote plant growth rapidly and substantially, but reduces soil quality considerably.
2. Treatment with BF, OF, and a high MP dose can improve soil quality and increase plant growth and nutrient content. Of these fertilizers, BF had the largest comprehensive effects.
3. The plant growth-promoting effect of BF is lower than that of CF within a 30-day treatment period, but higher over the longer term.

4. BF can be applied to simultaneously improve poor soil, promote plant growth, and plant quality without the addition of CF. Furthermore, poor soil can be improved more quickly through BF treatments without plants.

ACKNOWLEDGEMENTS

This work was jointly supported by grants from the National Science Foundation of China (Grant No. 41373078), the Key Project of Natural Science Research in Colleges and Universities in Jiangsu Province (Grant No. 16KJA180003), the Jiangsu Key Laboratory for Eco-Agricultural Biotechnology around Hongze Lake (HZHL1504) and the National Fund for Fostering Talents in Basic Science (Grant No. J1210025).

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Tables

Table 1. Fertilizer combinations and available nutrients added in different treatments

Treatment ^z	BF (g kg ⁻¹)	OF (g kg ⁻¹)	CF (g kg ⁻¹)	MP (g kg ⁻¹)	OM (g kg ⁻¹)	N (g kg ⁻¹)	P ₂ O ₅ (g kg ⁻¹)	K ₂ O (g kg ⁻¹)	C (g kg ⁻¹)
CK	0	0	0	0	0	0	0	0	0
BF1	67	0	0	0	21.44	1.27	1.27	1.67	12.44
BF2	134	0	0	0	42.88	2.53	2.53	3.33	24.87
BF3	200	0	0	0	64	3.80	3.80	5.00	37.12
OF1	0	47.64	0	0	21.44	0.81	1.19	0.76	12.44
OF2	0	95.29	0	0	42.88	1.62	2.38	1.52	24.87
OF3	0	142.22	0	0	64	2.42	3.56	2.28	37.12
CF1	0	0	1	0	0	0.15	0.15	0.15	0
CF2	0	0	2	0	0	0.30	0.30	0.30	0
CF3	0	0	3	0	0	0.45	0.45	0.45	0
MP1	0	0	0	16.75	0	0	0	0	0
MP2	0	0	0	33.50	0	0	0	0	0
MP3	0	0	0	50.00	0	0	0	0	0

Note: ^zCK, control without fertilizer; BF, bio-organic-mineral fertilizer; OF, organic fertilizer; CF, chemical fertilizer; MP, mineral powder.

The amounts of organic matter (OM) in the respective treatments of BF and OF were same, and the amounts of MP in the respective treatments of BF and MP were same.

Table 2. Soil properties under treatments with fertilization, water spinach and leaching

Treatment	pH	EC ^z ($\mu\text{S cm}^{-1}$)	AK ^z (mg g^{-1})	AOC ^z (mg g^{-1})	HCO ₃ ⁻ (%)	Urease (mgN g^{-1})	Alkaline phosphatase ($\text{mgP}_2\text{O}_5 \text{ g}^{-1}$)	Carbonic anhydrase (U g^{-1})
CK	7.583e	109.37a	0.148a	0.114a	0.139a	0.196c	1.194de	1.579cd
BF1	7.437d	170.37c	0.522d	0.456c	0.208d	0.458f	1.785f	3.573f
BF2	7.467d	196.90e	0.664f	0.629e	0.222e	0.696g	1.984h	4.531g
BF3	7.420d	229.33f	0.788g	0.942g	0.286g	0.752h	2.062i	7.140i
OF1	7.440d	164.90c	0.484c	0.438c	0.206d	0.224d	1.705f	3.163e
OF2	7.473d	177.87d	0.528d	0.543d	0.219e	0.316e	1.906g	3.872f
OF3	7.487d	195.30e	0.577e	0.716f	0.253f	0.345e	1.912g	5.606h
CF1	7.257c	110.63a	0.165a	0.143b	0.167c	0.161b	0.853c	1.493c
CF2	7.143b	106.23a	0.180b	0.131b	0.164c	0.116a	0.788b	1.252b
CF3	7.053a	107.83a	0.170ab	0.119a	0.153b	0.108a	0.723a	1.108a
MP1	7.557e	131.27b	0.151a	0.116a	0.139a	0.186c	1.189d	1.478c
MP2	7.553e	179.20d	0.157a	0.128a	0.136a	0.199c	1.199de	1.578cd
MP3	7.550e	226.33f	0.166a	0.137b	0.147b	0.228d	1.207e	1.695d

Note: Results in the table were from the soils treated with fertilizers, plants and leaching for 60days.

Means followed by the same letter within a column are not significantly different at $P\leq 0.05$ (LSD).

^zEC, electrical conductivity; AK, available potassium; AOC, active organic carbon.

Table 3. Water spinach growth condition under different fertilization treatments

Treatment	¹ Height (cm)	¹ Stem diameter (cm)	¹ Area (cm ² leaf ⁻¹)	¹ Main stem biomass (g plant ⁻¹)	² Aboveground biomass (g plant ⁻¹)	¹⁺² Aboveground biomass (g plant ⁻¹)
CK	12.93a	5.506a	13.832a	0.990b	1.100a	2.090a
BF1	17.50bc	7.244c	24.234de	1.620e	3.335c	4.955d
BF2	18.13bc	7.329c	22.928cd	1.667f	3.489c	5.156de
BF3	17.90bc	8.460e	20.866c	1.675f	4.335d	6.010f
OF1	16.50b	7.469c	21.599cd	1.526d	2.564b	3.773b
OF2	16.63b	7.548c	20.309c	1.538d	2.235b	4.090c
OF3	17.28bc	7.626cd	22.600cd	1.570d	2.577b	4.147c
CF1	19.38cd	8.056d	25.597ef	1.755g	3.243c	4.998d
CF2	18.94bc	8.490e	27.079f	1.751g	3.652c	5.403e
CF3	21.10d	8.976f	31.093g	2.464h	3.751c	6.215f
MP1	14.17a	5.959ab	14.144a	0.958a	1.042a	1.900a
MP2	14.00a	6.148b	13.298a	1.137a	0.961a	2.098a
MP3	14.38a	6.237b	13.382a	1.061a	1.205a	1.791a

Note: ¹, results were from the main stem after the seedling transplanted for 30days; ², results were from the seedling after the main stem was taken away for 20days.

¹⁺²: sum of 1 and 2. Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ (LSD).

Table 4. Nutrient and anti-nutrient contents in water spinach under different fertilization treatments

Treatment	Soluble sugar		Soluble		Vc		Carotenoids		Total		Nitrate		Oxalic acid	
	(mg g ⁻¹ DW)		protein		(mg g ⁻¹ FW)		(mg g ⁻¹ FW)		flavonoids		(µg g ⁻¹ FW)		(mg g ⁻¹ FW)	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
CK	2.973a	2.802c	2.480a	2.146a	9.703b	7.157a	0.165a	0.240c	1.699c	1.971de	42.731a	60.786a	0.682a	0.8530a
BF1	7.121d	4.291d	2.771c	2.522d	11.444c	11.945e	0.178b	0.238c	2.023e	2.341f	60.786bc	75.231b	0.927c	1.133d
BF2	9.365e	4.980e	3.335g	2.675e	13.134e	13.917g	0.189b	0.277d	2.400g	2.602g	93.287d	111.343d	1.168e	1.309e
BF3	11.410g	7.039f	3.535h	2.689e	16.828g	17.970h	0.185b	0.332f	2.498h	3.020h	107.7325e	136.622e	1.394f	1.657g
OF1	6.417c	4.995e	2.741c	2.641e	11.007c	8.909c	0.192b	0.238ab	1.994e	1.991e	60.786bc	76.131b	1.014d	1.307e
OF2	9.410e	8.876g	3.077e	2.813f	12.650d	11.413e	0.208c	0.287d	2.382f	2.407f	104.122e	114.954d	1.211e	1.635g
OF3	10.587f	9.187h	3.216f	3.274g	14.105f	13.714g	0.242ef	0.273d	2.374f	2.371f	129.400f	140.233e	1.400f	1.672g
CF1	3.713b	2.602bc	2.449a	2.328c	12.665d	9.786d	0.227de	0.249b	1.563b	1.777b	71.620c	96.898c	1.350f	1.552f
CF2	3.321ab	2.476b	2.759c	2.239b	14.167f	11.664e	0.243ef	0.280d	1.543b	1.791b	169.122g	176.345f	1.406f	1.676g
CF3	3.336ab	1.676a	2.943d	2.108a	18.706h	12.978f	0.253f	0.318e	1.215a	1.304a	190.789h	203.429g	1.774g	1.802h
MP1	3.454ab	2.750c	2.683b	2.374c	9.426a	7.423b	0.218cd	0.210b	1.749d	1.948cd	49.953ab	68.731ab	0.734b	0.912b
MP2	3.817b	2.756c	2.678b	2.449c	9.837b	7.767b	0.220cd	0.222b	1.793d	1.928c	53.564ab	68.009ab	0.758b	0.936bc
MP3	3.750b	2.854c	2.695b	2.433c	9.942b	7.579b	0.214cd	0.211b	1.847d	1.996e	60.780bc	73.064b	0.755b	0.962c

Note: ¹, results were from the main stem after the seedling transplanted for 30days; ², results were from the seedling after the main stem were taken away for 20days. Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ (LSD).

Table 5. Soil properties under fertilization treatments without water spinach and leaching

Treatment	pH	² EC ($\mu\text{S cm}^{-1}$)	² AK (mg g^{-1})	² AOC (mg g^{-1})	HCO_3^- (%)	Urease (mgN g^{-1})	Alkaline phosphatase ($\text{mgP}_2\text{O}_5 \text{ g}^{-1}$)	Carbonic anhydrase (U g^{-1})
CK	7.167e	552.33a	0.235a	0.282b	0.167b	0.728d	4.678c	1.857b
BF1	7.270fg	876.33d	0.936f	0.664d	0.230d	0.817ef	6.438f	3.654e
BF2	7.307g	980.00e	1.883g	0.873e	0.268e	0.882g	9.986i	5.441g
BF3	7.420h	1399.00h	2.708h	1.137f	0.341f	1.125h	13.598g	8.093i
OF1	7.147de	726.33b	0.380c	0.461c	0.225d	0.774e	5.299d	3.377d
OF2	7.167e	941.67e	0.589d	0.676d	0.244d	0.791e	7.487g	3.956f
OF3	7.063b	1115.33g	0.843e	0.908e	0.275e	0.823f	9.377h	6.262h
CF1	7.187e	839.00c	0.265a	0.293b	0.160b	0.694c	3.655b	1.835c
CF2	7.113d	1050.33f	0.330b	0.241a	0.152b	0.645b	3.046a	1.565b
CF3	6.873a	1576.67i	0.402c	0.212a	0.133a	0.594a	2.813a	1.292a
MP1	7.247f	715.67b	0.249a	0.282b	0.184c	0.713cd	4.742c	1.847b
MP2	7.310g	750.33b	0.243a	0.305b	0.189c	0.722d	5.299d	1.924bc
MP3	7.268d	940.67e	0.255a	0.294b	0.192c	0.821f	5.636e	2.014c

Note: Results in the table were from the soils treated with fertilizers indoors without plants and leaching for 60days. Means followed by the same letter within a column are not significantly different at $p \leq 0.05$ (LSD).

²EC, electrical conductivity; AK, available potassium; AOC, active organic carbon.