

## Research Article

# Enzymatic Activities of Bok Choy (*Brassica rapa* subsp. *Chinensis*) Grown Soil with the Amendment of Sandwich Compost

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Soil enzymes ensure our food security, yet they are vulnerable to abiotic stresses. Solving the global issues of food waste by amending the Sandwich compost can be a great solution to ensure food security. Food waste Sandwich compost substrate (as soil amendment) and leachate (as seed priming solution and liquid fertilizer) were used to grow Bok Choy for 4 growing cycles, where soil pH, cation exchangeable capacity, moisture content, aggregate stability, and enzyme activity were determined. The Sandwich compost substrate amendment increased soil pH close to neutral and CEC up to 1.5-fold. Anaerobic Sandwich compost substrate-amended soil reduced soil catalase activity. Still, it steadily increased during the growing cycle. The Sandwich compost substrate amendment soil sustained the aggregate stability for 4 growing cycles. On the flip side, aggregate stability without the Sandwich compost substrate amended soil declined from the growing cycle to the next growing cycle. All variables were positively correlated except catalase activity. Henceforward, Sandwich compost substrate is recommended to improve soil quality in the aspects of pH, CEC urease activity, and dehydrogenase activity.

## 1. Introduction

Soil enzymes are the vital drivers for food security. Starved of soil enzymes, the nutrient cycle will be interrupted due to the failure of the plant nutrients uptake. Soil enzyme activity is valuable to the environment, especially pollution and aeration, in which they work. Soil enzyme activity is closely correlated to the amount of soil organic matter, plant, soil, root, and microbial biomass [1]. Besides, soil enzyme activity is affected by abiotic factors such as pH, temperature, moisture content, and soil cultural management, which is largely affected by anthropogenic pollutants (such as zinc) and commercial fertilizer [2].

Catalase is a hydrogen peroxide oxidoreductase and is deemed an intracellular enzyme [3]. It is typically found in aerobic bacteria and most facultative anaerobes; however, it is absent in obligate anaerobes. The role of catalase is to defend the cells from oxidative damage [4]. Soil catalase activity is a soil pollution indicator. Hence, the studied soil is considered low in pollutants if the range lies between 0.23–0.36 mL and 0.1 mol L<sup>-1</sup> KMnO<sub>4</sub> g<sup>-1</sup> [5]; Tang et al [6]. Besides, catalase had a significant correlation to organic carbon and reduced soil depth [5, 7]. Catalase activity in subsoil (50–60 cm) has lowered by 21–43% than surface layer soil. Catalase activity is reduced by both Cr(III) and Cr(VI), however, Cr(III) has stronger inhibition of enzymatic activity than Cr(VI) [8, 18]. Lower catalase activity in urban

soil indicates lower tolerance to oxidative stress and lower soil fertility for plant growth.

Since microorganisms hydrolyze urea enzymatically, and ureases can be found in enormous quantities in biologically active soil. Being an extracellular enzyme, urease is responsible for the nitrogen (N) and carbon (C) cycles [9]. It is also vital for ammonification in the N cycle. Urease catalyzes the hydrolysis of urea to ammonia ( $\text{NH}_3$ ) and carbon dioxide ( $\text{CO}_2$ ). The substrates for ammonification are uric acid, urea, and organic N [9, 10]. High ammonium content is stated to be as high urease content. Urease activity is also affected by soil clay content and is persistent in dry soil and low temperature [11]. Urease activity is contributed by the excretion of microbes, root residues, and organic matter [12]. Urease activity is found to be positively correlated with total N [13, 14]. The low total N stored in Beijing urban soil was shown by the low urease activity [15]. Urease activity also reduced with the increased depth from ground level.

*Brassica* crops like Bok Choy provide a variety of phytonutrients, vitamins, minerals, and fiber to people [16]. Leafy vegetables have short growing cycles (3 to 4 weeks from plantation to harvest) and are demanded by people. *Brassica* sp. contains health-promoting compounds such as polyphenols, carotenoids, and glucosinolates (a group of sulfur and N-containing secondary plant metabolites [17, 18]. They play an important role in plant defense against herbivorous insects and microbial pathogens [19–21]. In Malaysia, *Brassica* sp. production was 0.15 Mt in 2019, making it 15% of the total vegetable production [22]. With the high demand and nutrients of Bok Choy, it was selected as the test crop.

Anaerobic Sandwich compost is produced with a wide range of beneficial microbes in a short period of time which is in the range of 7–21 days [23]. Sandwich compost enhanced soil urease activity in coffee production [24, 25]. Furthermore, organic matter enhanced soil aggregate stability and eventually brought about the improvement of microbial agents (W. [26]. Soil aggregate stability is affected by moisture content [27]. Therefore, the objective of this study is to determine the effect and relationship between soil pH, cation exchangeable capacity, moisture content, aggregate stability, and enzyme activity, through Sandwich compost amendment on Bok Choy.

## 2. Materials and Methods

**2.1. Study Site.** The study was carried out in a net house, Field 10, Universiti Putra Malaysia (UPM) ( $2^\circ 59' 31.4''\text{N}$   $101^\circ 42' 52.1''\text{E}$ ), Serdang, Selangor, Malaysia. The soil was collected from the study site under the following conditions (Table 1).

**2.2. Treatments.** The experimental set-up was conducted, where nine (9) treatments with three (3) replications were each carried out for four (4) growing cycles, which is the same as the

TABLE 1: The selected urban soil quality.

Physiochemical parameter	Urban soil
Texture	Clay
pH	$4.00 \pm 0.0473^*$
Soil moisture content (%)	$12.00 \pm 0.286$
Cation exchange capacity ( $\text{cmol}_c \text{ kg}^{-1}$ )	$7.6 \pm 0.216$
Catalase activity ( $\text{mL } 0.02 \text{ mol L}^{-1} \text{ KMnO}_4 \text{ g}^{-1}$ )	$0.525 \pm 0.0104$
Dehydrogenase activity ( $\text{g TPF kg}^{-1} \text{ h}^{-1}$ )	$2.31 \pm 0.338$
Urease activity ( $\text{mg NH}_3\text{-H}$ )	$1.330 \pm 0.0407$

\*Mean  $\pm$  standard error.

previous study [28]. Thus, a total of 108 experimental units were involved. The experiment was conducted as destructive sampling. The treatments evaluated are listed in Table 2.

Sandwich compost was prepared with cooked and uncooked food waste [25, 28, 29]. The Sandwich compost consist of substrate and leachate (Table 3). The 280 dwarf types of Bok Choy seed were from the green eagle [30]. One g of seed was primed in 500 mL of overnight tap water (Table 3) with the addition of 1 mL of Sandwich compost leachate (0.2%) for 3 hours [31, 32] before being sown in peat moss. The mixed urban soil with Sandwich compost substrate (1 : 1 ratio) was filled with a weight of 1.3 kg per polybag in a  $10 \times 10$  cm polybag, covered with 0.7 kg urban soil, and covered with a layer of plastic gunny bag. Sandwich compost substrate amended soil was incubated for 45 days. The seedlings were transplanted into the soil after seven (7) days of emerging. A 0.2% of Sandwich compost leachate [33] was applied at every five-day interval beginning from 8 days after transplanting (DAT).

The oven-dried Sandwich compost sample was ground with an electrical stainless-steel coffee grinder and deposited in a well-packed plastic bag for further analysis. The sample (0.25 g) was digested using a 1 : 1 ratio of  $\text{H}_2\text{SO}_4$  and  $\text{H}_2\text{O}_2$  at  $350^\circ\text{C}$  until the content turns colorless. After cooling the contents, the volume is made up of the distilled water and filtered through No. 1 filter paper for further analysis including N, P, K, Mg, Ca, Fe, and Al. The concentration was determined by using ICP except N. Nitrogen was determined by distillation and titration. A 10 mL sample and 10 mL 30% NaOH were added to the distillation apparatus. An indicator solution, 10 mL of boric acid mixed indicator, was added to an Erlenmeyer flask. The distillation process had changed the color of the 2% boric acid mixed indicator from purple color to green color. About 50 mL of green solution was ready for the next step. The green color solution will be titrated with 0.01 N HCl to give purple color and the used HCl will be recorded.

**2.3. Soil Physiochemical Analysis.** The soil moisture content was determined gravimetrically [34]. A 20 g of fresh soil was oven-dried in a crucible at  $105^\circ\text{C}$  for 24–36 hours, cooled in a desiccator, and weighed. The data were expressed as %. The remaining soil sample was air-dried and crushed using a mortar and pestle in an anticlockwise orientation. The sample was sieved gently with a 2 mm sieve for further analysis.

TABLE 2: The treatments.

Treatment	Substrate as a soil amendment	Leachate as a seed priming solution	Leachate as a liquid fertilizer
T000	No	Dry seed	No
T001	No	Dry seed	Yes
T009	No	Dry seed	No
T010	No	Yes	No
T011	No	Yes	Yes
T100	Yes	Dry seed	No
T101	Yes	Dry seed	Yes
T110	Yes	Yes	No
T111	Yes	Yes	Yes

TABLE 3: Physiochemical parameters of tap water, Sandwich compost substrate, and leachate.

Physiochemical parameter	Tap water	Sandwich compost substrate	Sandwich compost leachate
pH	6.98 ± 0.02*	—	4.78 ± 0.011
Total N (%)	0.00056 ± 1.63 × 10 <sup>-18</sup>	1.722 ± 0.2560	0.2135 ± 0.0052
P (mg kg <sup>-1</sup> )	0.0447 ± 0.0197	16397 ± 543	5833 ± 223
K (mg kg <sup>-1</sup> )	3.64 ± 0.0415	20799 ± 1230	3941 ± 131
Ca (mg kg <sup>-1</sup> )	13.8 ± 0.150	5935 ± 183	528 ± 18.6
Mg (mg kg <sup>-1</sup> )	1.01 ± 0.0111	4870 ± 218	1249 ± 53.9
Fe (mg kg <sup>-1</sup> )	0.306 ± 0.015	360 ± 37.3	160 ± 42.5
Al (mg kg <sup>-1</sup> )	Not detected	10050 ± 94.4	770 ± 510

\*Mean ± standard error with 4 replications.

$$\text{Moisture \%} = \frac{\text{fresh soil weight} - \text{oven-dried soil weight}}{\text{oven-dried soil weight}} \times 100. \quad (1)$$

Soil pH was determined using a 1:2.5 (w/v) soil-water extract [34] with a glass electrode HI2211 pH/ORP meter. A 100 mL plastic vial with a cap was filled with 10 g of soil and 25 mL of distilled water and shaken using an orbital shaker for 30 minutes at 180 rpm. To set down the soil, it stood for 1–24 hour(s) and the PH was tested with a pH meter.

Aggregate stability (%) was analyzed with the wet sieving method [35]. A 5 g sample of 1 to 2 mm air-dried soil was weighed and placed in the wet sieving sieve. The sample was premoisturized with a water sprayer. The can was then filled with three-quarters of distilled water. The sample was wet sieved for 10 minutes. The sample remaining on the sieve was transferred to a dish with slow and continuous flow of tap water. The sample was oven-dried at 105°C for 24 hours and the dry aggregate was weighed (W). The sample was washed under tap water on the 0.25 mm sieve until the color turned clear. The sample remaining on the sieve was oven-dried at 105°C for 24 hours and weighed (S). The calculation is as follows:

$$\text{aggregate stability\%} = \frac{W - S}{5 - S} \times 100. \quad (2)$$

Cation exchange capacity was determined by using the leaching method [36]. The leaching tube was layered with glass wool, filter paper, 10 g of air-dried soil, and filter paper. A 100 mL of pH 7.1 N ammonium acetate was leached for 10 ± 3 seconds per drop to a 100 mL volumetric flask. The extractant was made up of pH 7.1 N ammonium acetate. After that, 100 mL of 95% ethanol leached for 10 ± 3 seconds per drop. To deform the cation exchange capacity, 100 mL of 0.05 M K<sub>2</sub>SO<sub>4</sub> was leached with speed. The extractant was then determined using a distillation method. A 10 mL of 30% NaOH and 10 mL of extractant were distilled with 10 mL of boric acid with an indicator mixture. The purple color of boric acid with the indicator mixture was then turned green and collected after distilling up to 50 mL. The green color solution was then titrated with 0.01 N HCl. The used HCl was recorded. The calculation is as follows:

$$\text{CEC} = \frac{\text{volume of titrant (mL)}}{\text{volume of distill (mL)}} \times \text{volume of 0.01N HCl (mL)} \times \text{concentration of HCl} \left( \frac{\text{mmol}}{\text{mL}} \right) \times \frac{1000\text{g}}{1\text{kg}} \times \frac{1}{10\text{g}} \times \frac{1\text{cmol}}{10\text{mmol}} \quad (3)$$

**2.4. Soil Enzyme Activity Analysis.** Back-titrating residual  $\text{H}_2\text{O}_2$  measured catalase activity with  $\text{KmnO}_4$  [12], p. 323; [5, 37]. A 2 g of soil sample was added to 40 mL of distilled water with 5 mL of 0.3%  $\text{H}_2\text{O}_2$  solution, shaken for 20 min (180 rpm), and then 5 mL of 1.5 mol/L of  $\text{H}_2\text{SO}_4$  was added. The solution was filtered and titrated using 0.02 mol  $\text{L}^{-1}$  of  $\text{KmnO}_4$ . The reacted amount of 0.02 mol  $\text{L}^{-1}$  of  $\text{KMnO}_4$ , calculated per gram of dry soil, was used to express catalase activity.

Dehydrogenase activity was measured using the classical triphenyl tetrazolium chloride method [38]. A 5 g of sieved soil, 0.4 g of  $\text{CaCO}_3$ , 1 mL of 1.5% 2,3,5-triphenyl tetrazolium chloride (TTC), and 2.5 mL of pure water were added and mixed well in a test tube. The tubes were sealed tightly and incubated for 24 h at 37°C in the dark. The product 1,3,5-triphenyl formazan (TPF), from the reduction of TTC, was extracted by using methanol, and additional methanol was added to make the sample volume of 50 mL. The TPF concentration was measured by the spectrophotometric method at 485 nm, and methanol was used in the reference cell. The DHA activity was expressed as  $\mu\text{g TPF g}^{-1} \text{h}^{-1}$ .

Urease activity was determined using urea as the substrate [12], p. 296; [39]. The soil mixture, including 5 g of soil, 1 mL of toluene, 10 mL of 10% urea solution, and 20 mL of citrate buffer pH 6 was incubated at 37°C for 24 h. A 4 mL of sodium phenolate (20 mL of phenolate solution, 62.5 mL of liquefied phenol, 2 mL of methanol, 187.5 mL of acetone and made up with ethanol to 100 mL), 20 mL of 27% NaOH made up with  $\text{H}_2\text{O}$  in a 100 mL volumetric flask, 3 mL of 0.9% sodium hypochlorite, and 20 mL  $\text{H}_2\text{O}$  were added to the 3 mL filtrate, the mixture is left to stand for 20 min until a blue color appeared. Ammonium sulfate was used as a calibration curve. A 0.4717 g of ammonium sulfate in 1000 mL contained 0.1 mg of  $\text{N mL}^{-1}$ . The released  $\text{NH}_3\text{-N}$  was measured spectrophotometrically at 578 nm within an hour. The urease release rate was expressed as mg  $\text{NH}_3\text{-N}$ .

**2.5. Statistical Analysis.** The recorded data were analyzed with a two-way analysis of variance (ANOVA) using package “agricolae” under R studio, version 4.2.1 [40]. When F was significant at the  $p < 0.05$  level, treatment means were compared and separated using Duncan Multiple Range Test (DMRT). Pearson’s correlation was analyzed by the package “corrplot” [41]. The results were expressed as a mean  $\pm$  standard error of measurement.

### 3. Results

**3.1. Soil Physicochemical Properties.** Soil pH had significant interaction between the growing cycle and Sandwich compost amendment (Figure 1). Soil pH of Sandwich compost substrate amended soil significantly increased and was maintained along with the four growing cycles of growing.

Cation exchange capacity (CEC) has no significant interaction between the growing cycle and the Sandwich compost amendment (Figure 2). CEC has significantly

decreased in the fourth growing cycle (Figure 2(a)). CEC of Sandwich compost amended soil was significantly higher than unamended soil (Figure 2(b)).

Soil moisture content has no significant difference between the growing cycle and Sandwich compost amendment. The second and fourth growing cycles showed significantly higher soil moisture content (Figure 3(a)). Sandwich compost amended soil showed significantly higher soil moisture content (Figure 3(b)) as well.

Soil aggregate stability had significant interaction between the growing cycle and the Sandwich compost amendment. Aggregate stability of unamended soil significantly decreased along with the growing cycle period (Figure 4). Soil aggregate stability of T000 has significantly decreased during the growing period, especially during cycle 4. Soil aggregate stability in commercial fertilized soil (T009) also decreased along the growing cycle. Sandwich compost amended soil (T100, T101, T110, and T111) has significantly stronger aggregate stability along the 4 growing cycles.

**3.2. Soil Enzyme Activity.** Soil enzyme activity such as catalase, dehydrogenase, and urease activities were affected by both Sandwich compost amendment and growing cycles. Catalase activity was significantly stable in the soil without Sandwich compost substrate amendment along with the growing cycles (Figure 5). Sandwich compost substrate amended soil has significantly lower catalase activity compared to unamended soil along with the four growing cycles. Soil catalase activity of Sandwich compost substrate amended soil has significantly increased along with the growing cycles.

Urease activity and dehydrogenase activity have significantly increased with the Sandwich compost substrate amendment. Nevertheless, urease activity decreased during the fourth growing cycle (Figure 6). The application of Sandwich compost leachate has not significantly affected soil urease activity. Dehydrogenase activity of Sandwich compost substrate unamended soil significantly decreased compared to preamended soil (Figure 7).

**3.3. Correlation of Soil pH, Moisture Content, Aggregate Stability, CEC, and Enzyme Activity.** All variables were positively correlated to one another except for catalase activity (Figure 8). Catalase activity was significantly negatively correlated to all variables except aggregate stability and soil moisture content. Aggregate stability was significantly positively correlated to pH, CEC, dehydrogenase activity, and urease activity.

### 4. Discussion

The significant shifting of soil pH between 6.0 and 7.0 makes most of the nutrient available to plants [42, 43]. The pre-amended soil pH (4.0) was not suitable for most plants to grow (Table 1). However, organic matter has the potential to replace liming in the effort of lowering Al toxicity. Therefore, its amendment of the Sandwich compost substrate can be an alternative to liming activity. Soil pH with Sandwich

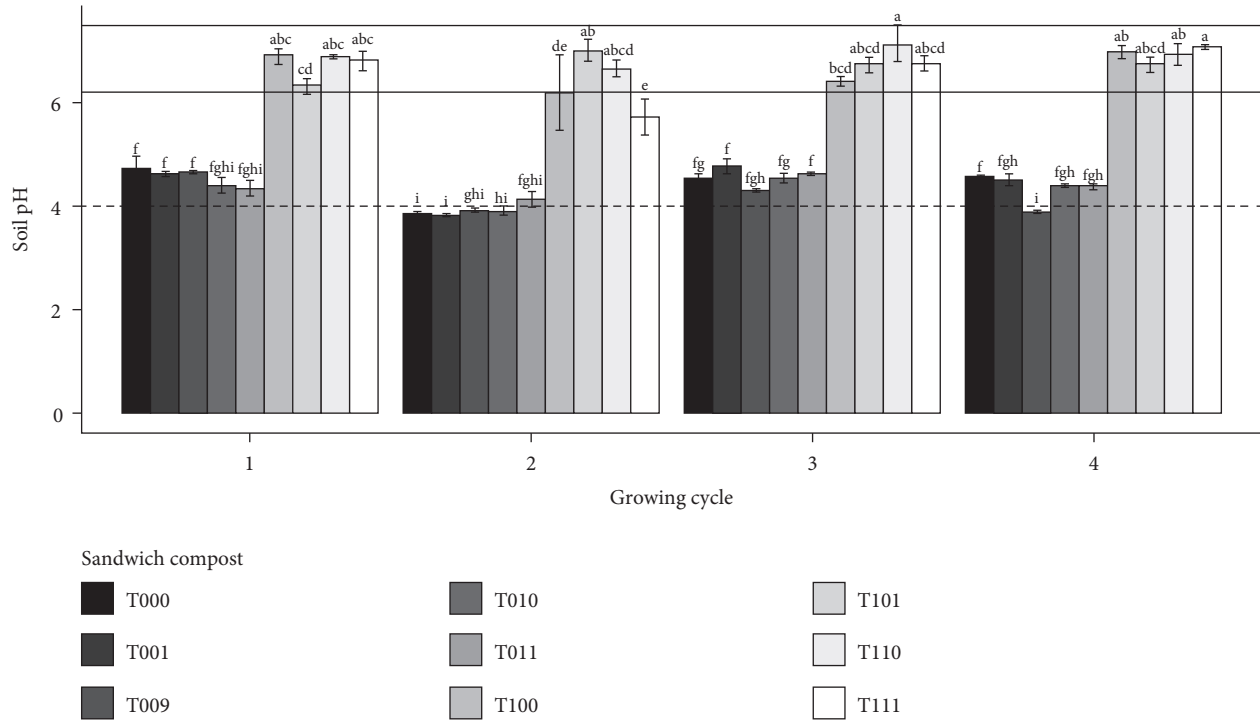


FIGURE 1: Effect of the growing cycle and Sandwich compost amendments on soil pH. Mean ± standard error with different letters is significantly different at  $P < 0.05$  when using DMRT. The dotted line is referred to as the original soil pH 4. The solid line is referred to as the optimum soil pH of 6.2–7.

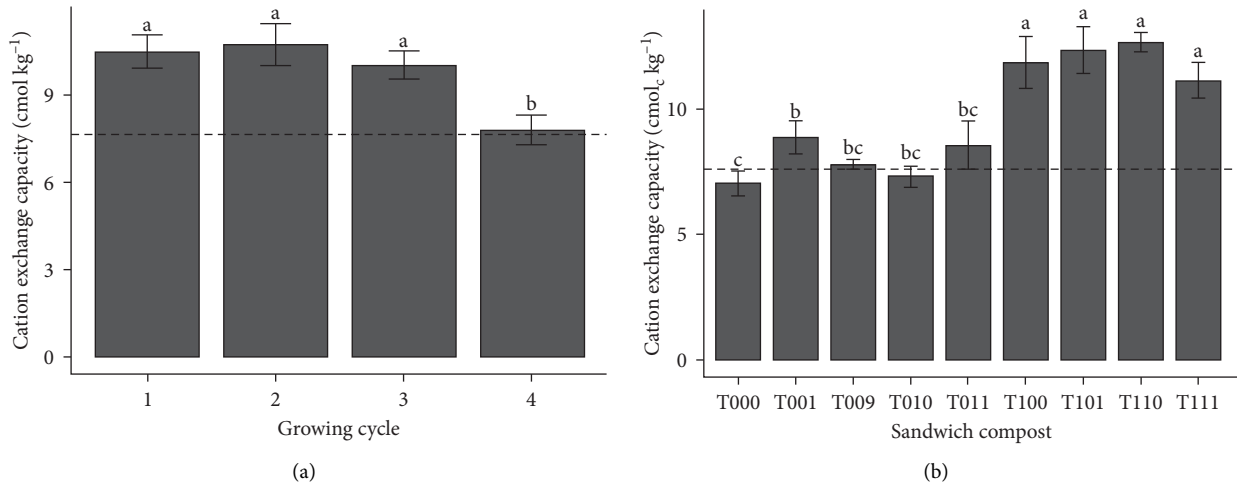


FIGURE 2: (a) Effect of the growing cycle on cation exchangeable capacity ( $\text{cmol}_c \text{kg}^{-1}$ ). (b) Sandwich compost amendments on cation exchangeable capacity ( $\text{cmol}_c \text{kg}^{-1}$ ). Mean ± standard error with different letters is significantly different at  $P < 0.05$  when using DMRT. The dotted line is referred to as the original cation exchange capacity ( $7.6 \text{ cmol}_c \text{kg}^{-1}$ ).

compost substrate amended soil exhibited an optimum plant growth pH range and they sustained four growing cycles [44–46]. Moreover, another soil amendment such as biochar was also able to stabilize the soil pH under drought conditions [47].

Neutralization of pH in Sandwich compost substrate amended soil has improved the efficiency uptake of Fe and Mn in plants [45]. The pH neutralization also reduced the

toxicity of the soil. Besides, earthworm survival percentage can be affected by the low pH ( $\text{pH}_{\text{KCl}} 3.4$ ) and the high concentration of  $\text{Al}_2(\text{SO}_4)_3$  [48]. Thus, the neutralization of pH in the soil will positively impact the survival of earthworms. Besides, soil pH has a positive correlation with microbial biomass [49]. Hence, the shifting of soil pH in Sandwich compost substrate amended soil would increase microbial biomass.

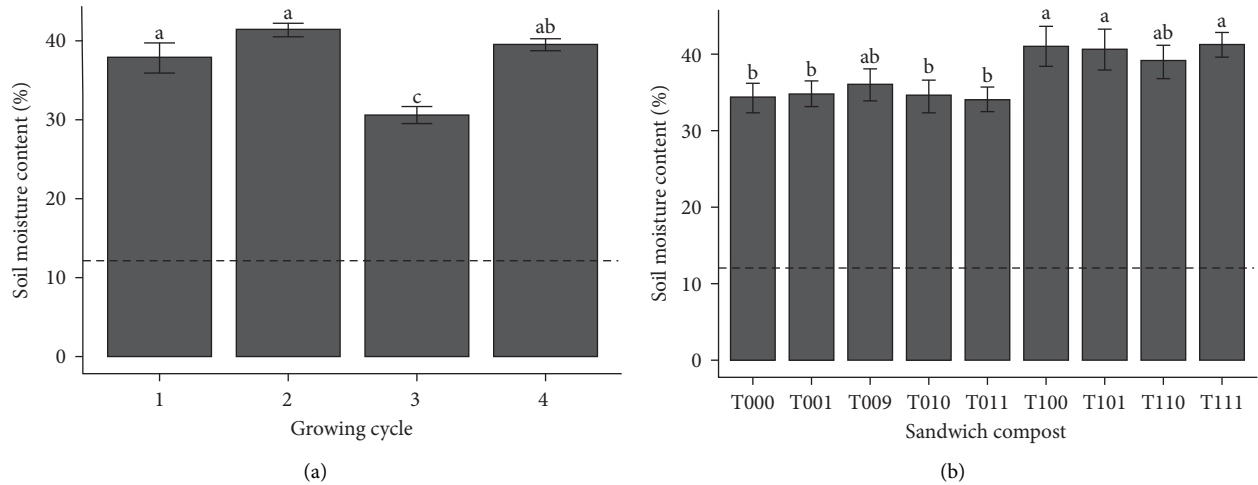


FIGURE 3: (a) Effect of the growing cycle on soil moisture content (%). (b) Sandwich compost amendments on soil moisture content (%). Mean  $\pm$  standard error with different letters is significantly different at  $P < 0.05$  when using DMRT. The dotted line is referred to as the original soil moisture content (12%).

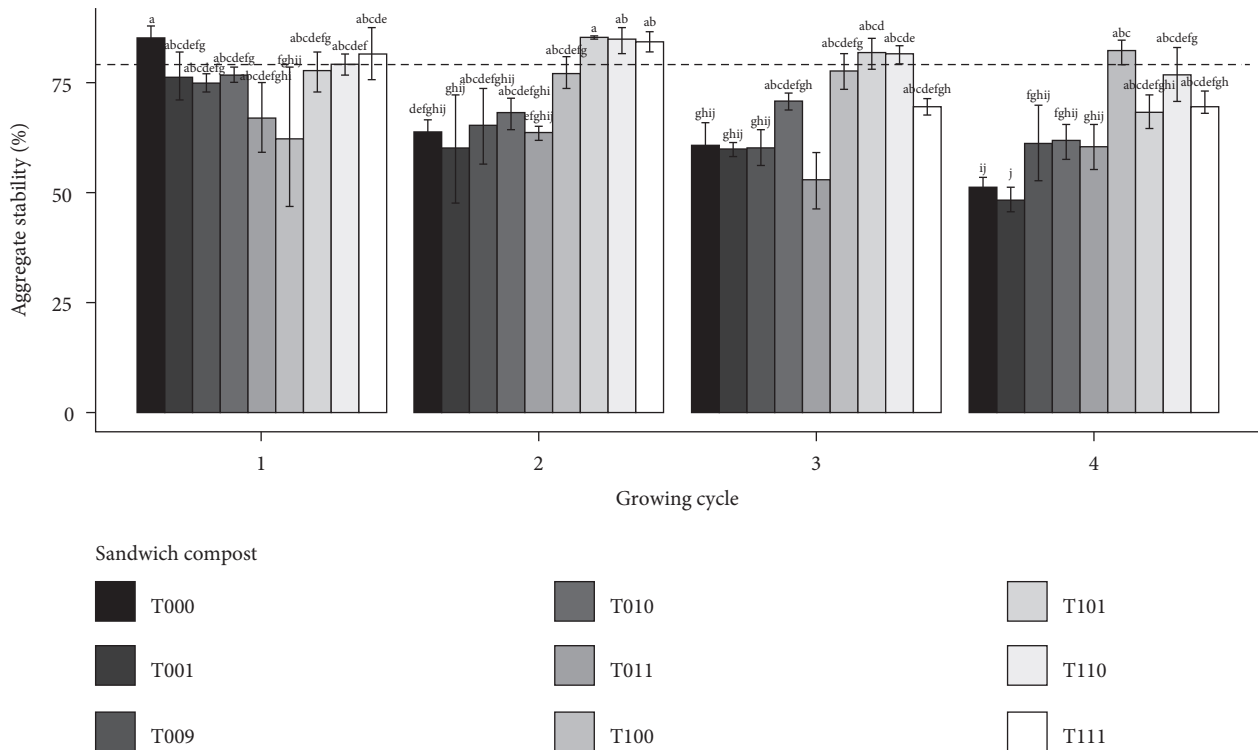


FIGURE 4: Effect of the growing cycle and Sandwich compost amendments on soil aggregate stability (%). Mean  $\pm$  standard error with different letters is significantly different at  $P < 0.05$  when using DMRT.

CEC significantly reduced at the fourth growing cycle may be due to soil organic matter (Sandwich compost substrate) content which has reduced after three growing cycles. Sandwich compost substrate is being fully degraded by microbes. In contrast, the longer the composting, the higher the CEC value (51.7–59.8 meq 100 g<sup>-1</sup>) [50]. Moreover, CEC decreases with soil depth [51].

CEC of Sandwich compost substrate amended soil showed significantly greater than Sandwichcompost substrate unamended ones [52]. CEC was largely affected by the

amount of organic matter [53–55] including Sandwich compost substrate. Therefore, large percentages of organic matter contribute to more negative sites (e.g., lignin derivatives including humic-like substances) and thus, a high CEC value [56]. Furthermore, the humification of the Sandwich compost substrate contributed to the phenolic and carboxylic groups and thus increased the CEC [50, 57].

Sandwich compost substrate possibly consisted of high urea for urease to work on it. Nevertheless, the Sandwich compost substrate amendment may be needed to sustain the

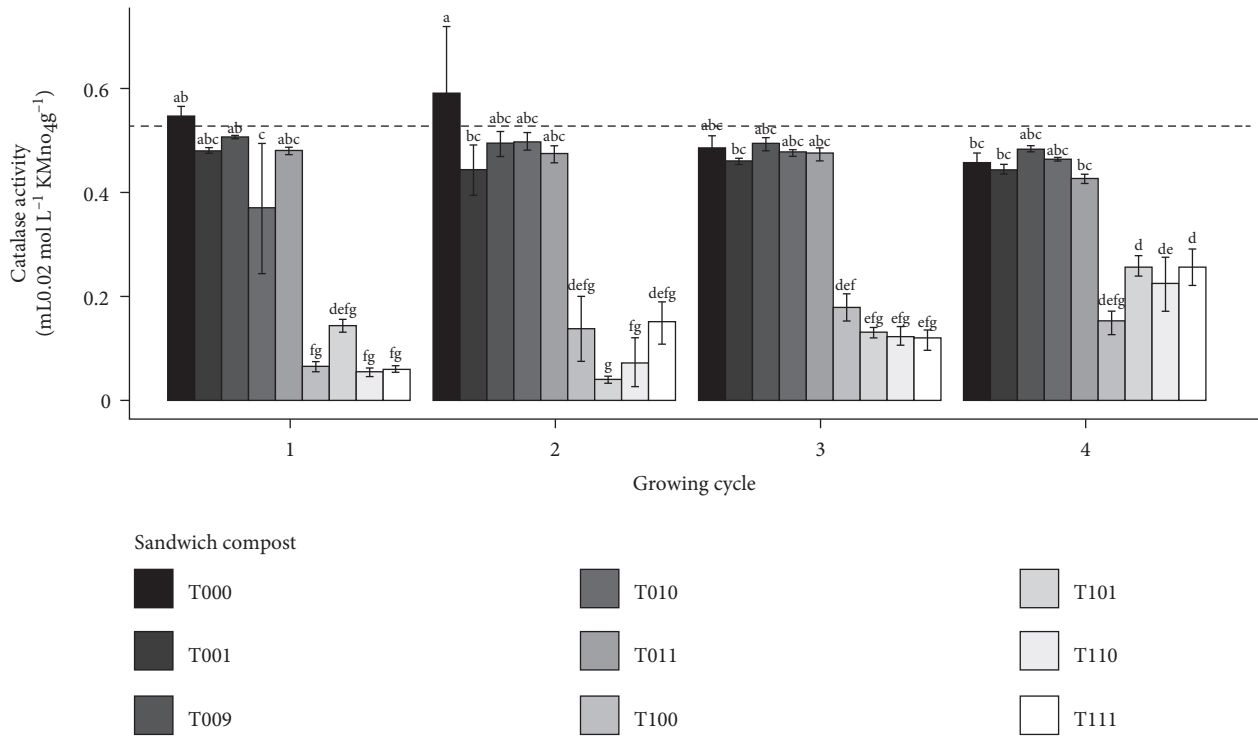


FIGURE 5: Effect of the growing cycle and Sandwich compost amendments on catalase activity (mL 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub> g<sup>-1</sup> soil). Mean ± standard error with different letters is significantly different at *P* < 0.05 when using DMRT. The dotted line is referred to as the original soil catalase activity (0.525 mL 0.02 mol L<sup>-1</sup> KMnO<sub>4</sub> g<sup>-1</sup> soil).

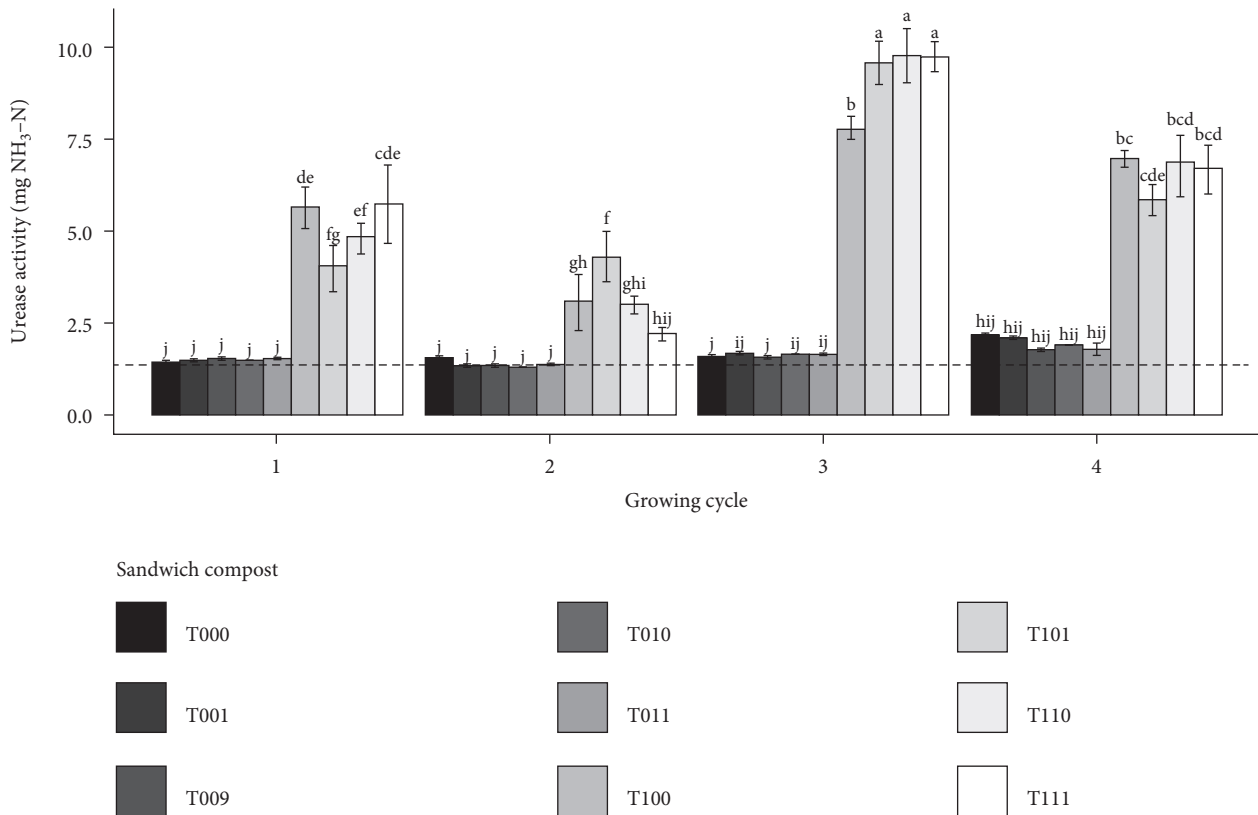


FIGURE 6: Effect of the growing cycle and Sandwich compost amendments on urease activity (mg NH<sub>3</sub>-N). Mean ± standard error with different letters is significantly different at *P* < 0.05 when using DMRT. The dotted line is referred to as the original soil urease activity (1.33 mg NH<sub>3</sub>-N).

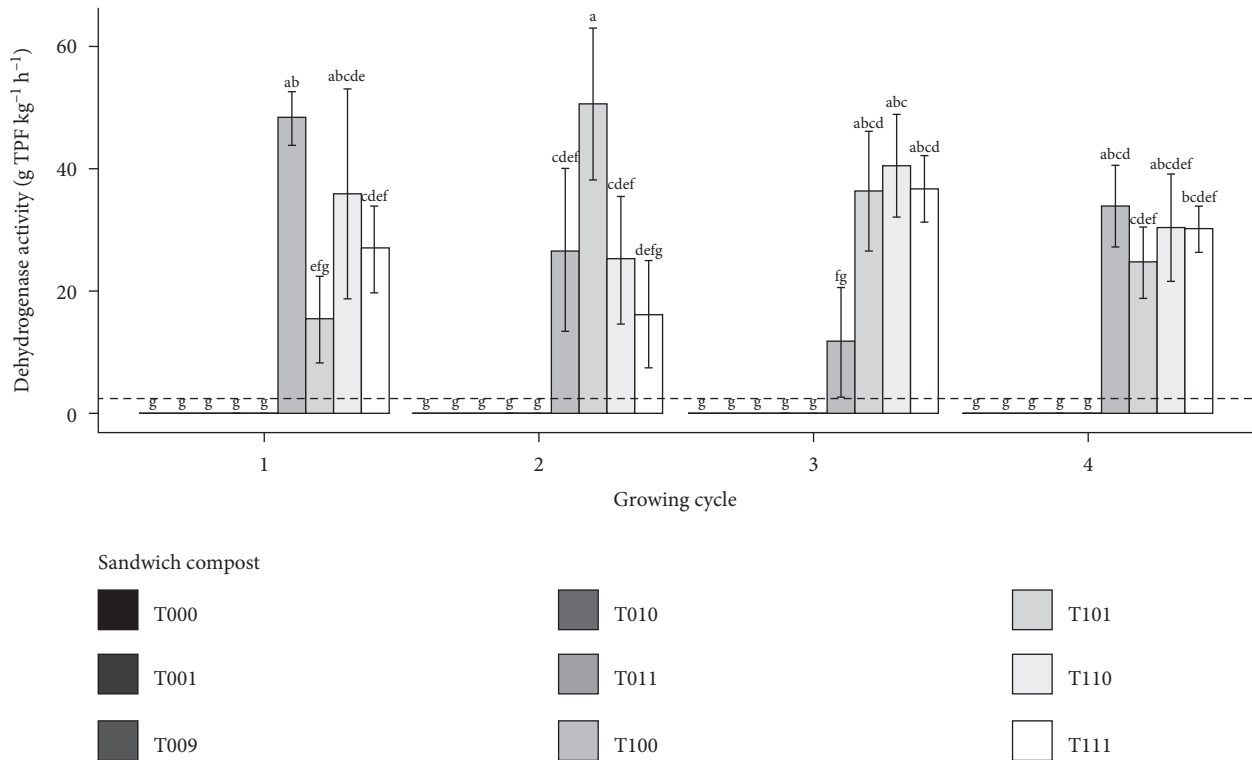


FIGURE 7: Effect of the growing cycle and Sandwich compost amendments on dehydrogenase activity ( $\text{g TPF kg}^{-1} \text{h}^{-1}$ ). Mean  $\pm$  standard error with different letters is significantly different at  $P < 0.05$  when using DMRT. The dotted line is referred to as the original dehydrogenase activity ( $2.31 \pm 0.338 \text{ g TPF kg}^{-1} \text{h}^{-1}$ ).

AS	0.52	0.52	0.53	0.33	0.15	-0.42
0.52	pH	0.60	0.86	0.82	0.23	-0.78
0.52	0.52	CHC	0.63	0.47	0.28	-0.58
0.53	0.86	0.63	DHA	0.83	0.34	-0.83
0.33	0.82	0.47	0.83	ure	0.25	-0.78
0.15	-0.23	0.28	0.34	0.25	smc	-0.46
-0.42	-0.78	-0.58	-0.83	-0.78	-0.46	CAT

FIGURE 8: Pearson’s correlation for aggregate stability (AS), pH, CEC, dehydrogenase activity (DHA), urease activity (ure), soil moisture content (smc), and catalase activity (CAT) from Bok Choy amended Sandwich compost, with 4 growth cycles. The “\*” was indicated as the significant level.

high urease activity [58, 59]. Organic matter content and microbial biomass improved urease activity [60]. Urease is generally found in intercellular living cells and on extracellular clay organic matter (Sandwich compost substrate) surfaces [61].

Landfill leachate toxicity was negatively correlated to enzyme activity, including urease activity [62]. Yet, Sandwich compost leachate was not significantly affected by urease activity. Therefore, Sandwich compost leachate is

believed to be nontoxic to soil enzyme activity. However, high urease activity in the topsoil was immobilized by the microbial biomass due to a surplus of hydrolyzed urea-N [11]. Therefore, the plant root morphology and plant nutrient content were significantly lower than the preamended Sandwich compost substrate.

Dehydrogenase activity was strongly suppressed under soil 1.5–4.5 [63]. Low soil pH of Sandwich compost substrate unamended soil suppressed the potential enzyme activity [64] by damaging ion and hydrogen bonds in the enzyme center [65].

Soil pH was significantly positively correlated to CEC (Figure 8) [66, 67]. Sandwich compost substrate amended soil has high CEC and, thus, it has a high  $\text{H}^+$  buffering capacity. Additionally, urease activity and CEC were positively correlated, which is supported by the previous findings [68, 69]. This would be reduced by the ammonia volatilization from urea [70]. However, these findings were contradictory to wetland urease activity [71].

Soil urease activity and pH were significantly positively correlated (Figure 8). Soil pH that is close to neutral demonstrated significantly high urease activity [2, 72]. Increasing soil pH by liming significantly increased soil urease activity and the growth of urease-producing microbes [73]. In older studies, urease activity and pH were not significantly correlated [71]. Soil urease activity was significantly negatively correlated to catalase activity (Figure 7), which is contrary to the findings of the previous study [2]. This may be due to the anaerobes that were predominant in the Sandwich compost substrate amended soil.



Soil moisture plays a vital role in the hydrological cycle during land surface processes. High soil moisture content promotes plant growth. Hence, soil moisture stress deteriorated the plant physiology parameters [74]. On the other hand, the application of plant growth regulators reversed the plant physiology measurement [74]. Therefore, Sandwich compost substrate was the key to improving soil moisture content.

Soil moisture content can directly reflect the soil water holding capacity and indirectly reflect the field capacity [75]. The selected soil type was clay. Clay theoretically holds large amounts of water. Nonetheless, the presence of Sandwich compost substrate in high amounts will shift the key player of water retention. The amount of soil clay content and organic matter (Sandwich compost) is the major control of water retention. For instance, large amounts of clay content with low amounts of organic matter significantly affected the soil water retention and vice versa [76–78]. Sandwich compost substrate as a soil amendment may be able to increase water retention under high soil degradation [76].

Soil aggregate stability provides water storage and filtering, nutrient storage and recycling, as well as physical support and stability [79]. Continuous harvesting affects the soil aggregate. In general, field production soil has significantly lower aggregate stability compared to forest soil [80]. Sandwich compost substrate amended soil showed stronger aggregate stability because of the increased soil organic matter storage by the development of soil aggregate [79, 81]. Amendment of organic matter and plant root length density enhanced soil aggregate stability [82, 83]. However, the low root length of Bok Choy in Sandwich compost substrate amended soil has significant aggregate stability.

Low catalase activity may be due to the production of Sandwich compost substrate in anaerobic conditions. Thus, the anaerobes were predominant in the soil. Moreover, the amendment of organic matter in low doses will not improve soil catalase activity [84]. One-third of the Sandwich compost substrate may be deemed a low dose for improving catalase activity. However, catalase activity was increased with organic matter [60]. The aeration of compost production will affect the catalase activity.

High soil catalase activity of Sandwich compost substrate amended soil during the growing cycles may be due to the soil air increasing along with the growing cycles. High air-filled porosity, oxygen diffusion rate and redox potential, and low water amount and  $Fe^{2+}$  content resulted in high soil catalase activity [85, 86]. Moreover, soil water-filled pore space and soil pore size distribution indirectly affect the soil enzyme activity because they affect the fungal and bacterial biomass [87].

Catalase activity was significantly negatively correlated to pH (Figure 8) [88]. Nevertheless, catalase activity was significantly negatively correlated to CEC, which contrasts with the aforementioned findings [88] because catalase may be predominantly present in aerobic organisms [89]. Soil aggregate stability was positively correlated to soil enzyme activity [90].

There were some limitations in this study. Firstly, the soil incubation environment was aerobic without any disturbance, such as tillage, before growing Bok Choy. Thus, the catalase activity was low under the Sandwich compost substrate amendment. Second, the soil texture was limited to clay soil. Third, the tested crop (Bok Choy) was a short-term crop that is demanded by people.

## 5. Conclusion

The crucial player in soil quality was the Sandwich compost substrate amendment. Aggregate stability was positively correlated to pH, CEC, dehydrogenase activity, urease activity, and soil moisture content. Nevertheless, catalase activity was negatively correlated to aggregate stability, pH, CEC, dehydrogenase activity, urease activity, and soil moisture content. Aggregate stability was reduced with the growing cycle since continuous harvesting disturbs the soil structure significantly. Soil urease activity, dehydrogenase activity, pH, and CEC were significantly improved with the Sandwich compost substrate amendment. Hence, Sandwich compost substrate is proposed to improve soil quality.

## Data Availability

The figure data used to support the findings of this study have been deposited in the figshare repository (10.6084/m9.figshare.20493879).

## Conflicts of Interest

The authors declare no conflicts of interest.

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