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Growth Performance of Varieties of Bean (*Phaseolus vulgaris*) Seedlings in Earthworm-Bioturbated Soil and Crab-Bioturbated Soil

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A B S T R A C T

Earthworms and crabs are known to influence the physicochemical state of their respective soil habitats through their bioturbation actions. While earthworm-bioturbated soils have been well documented to positively affect plant growth, not much is known about the effects of crab-bioturbated soil on plant growth. In this study, we compared the growth performance of four varieties of *Phaseolus vulgaris* (bean) seedlings in earthworm-bioturbated soil, crab-bioturbated soil, and unbioturbated soil collected within the same proximity of a wetland habitat. Seeds of *Phaseolus vulgaris* were planted in replicates in each soil type, and allowed to grow for 15 days. Physical growth was measured using a metre rule. Biochemical growth parameters were measured using standard procedures. The differences in stipule length of bean seedlings grown in all the soil types were generally not significant ($p > 0.05$). However, seedlings grown in earthworm-bioturbated soil and crab-boturbated soil recorded significantly higher ($p < 0.05$) stipule weight, relative to those grown in unbioturbated soil. Bean seedlings grown in earthworm-bioturbated soil recorded the highest and significant ($p < 0.01$) concentrations of chlorophyll, total sugar, starch, nitrogen, and crude protein, relative to those grown in crab-bioturbated and unbioturbated soils. This was followed by seedlings grown in crab-bioturbated soil which recorded significantly higher ($p < 0.01$) concentrations of these biochemical parameters, relative to those grown in unbioturbated soil. The significantly higher biochemical and marginally better physiological growth recorded for seedlings in bioturbated soils indicate that earthworms and crabs both contribute significantly to wetland productivity, through their bioturbatiion activities.

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INTRODUCTION

Bioturbation is a biological reworking and remixing of soil sediments by the activities of plants and animals. Bioturbation activities by living organisms, especially burrowing animals, are a powerful driver of nutrients and carbon cycling across soil sediments and layers. Bioturbation significantly impacts soil quality, nutrients availability, and other ecosystem services [1]. Plants, especially trees, may also bioturbate the soil through their rooting and falling actions [2, 3].

The contributions of earthworms to soil nutrient enrichment, plant growth, and crop productivity have, for long, been significantly appreciated, researched, and documented, such that earthworms are figuratively referred to as ‘friends of farmers’ [4, 5]. Earthworms are so much considered important to soil health and quality that their presence has become a major yardstick for determining the fertility of soil. Earthworms impact soil quality and contribute to plant growth and crop yield through their bioturbation activities of burrowing, feeding, foraging, and hormone and enzyme secretions. Burrowing, feeding and casting are a three-in-one soil-impacting bioturbation process of many earthworm species. During feeding, earthworms eat through the soil or soil organic matter, creating burrows in the process. As the soil and organic matter passes through the alimentary canal of the earthworm, they are ground and mixed with

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digestive enzymes and microorganisms, and are eventually egested as vermicasts. Earthworm casts or vermicasts have been found to be richer in plant growth-promoting hormones and microorganisms, than their surrounding soil [4, 6, 7].

Similarly, burrowing crabs have been identified as one of the most essential macroinvertebrates in many marshy wetlands, where they are often present in large numbers [5, 8, 9]. The burrowing and feeding activities of these crabs have been found to influence many wetland ecosystem functions, including carbon transformation, nutrients cycling, and sediments remixing [3, 10, 11]. Although, studies on the potential of crab-bioturbated soil as a plant growing medium are scarcely found, researches conducted to examine the quality of crab-bioturbated soil have suggested that they would serve as good plant-growing medium [12, 13]. Crab burrows can selectively trap sediments that have high organic matter concentrations, finer grain size and low density, which can increase nutrient availability and thus, promote the growth of plant and beneficial microorganisms [12, 13].

In our previous work [3], we carried out physicochemical and microbial analysis of earthworm-bioturbated soil and crab-bioturbated soil collected from a wetland habitat and reported improved qualities in the two soil types, relative to unbioturbated soil. In this study however, we aimed to compare the growth performance of four varieties of *Phaseolus vulgaris* (bean) seedlings in earthworm-bioturbated soil and crab-bioturbated soil, collected from the same proximity of a wetland habitat.

MATERIALS AND METHODS

Sample collection and experimental design

The present study was conducted in the main campus of the University of Lagos, Nigeria, on longitude 3° 24' E and latitude 6° 30' N. The location is a marshy wetland, in which earthworms and crabs are always active, especially in the rainy season. Three soil samples [earthworm-bioturbated soil (earthworm casts), crab-bioturbated soil (dug-out soil), and unbioturbated soil (undisturbed soil)] were collected within a radius of one meter (1m). Twelve plastic containers were obtained and divided into three groups (four per group). The first, second, and third groups of plastic containers were filled with earthworm-bioturbated soil, crab-bioturbated, and unbioturbated soil, respectively. Each container was filled with 500 mg soil, to three-quarter capacity.

Four varieties of *Phaseolus vulgaris* (Ife Brown, Oloyin, White Bean, Olo), purchased from an open market in Lagos state, Nigeria were used for the experiment. Five seeds of each variety of bean seeds were sown in each soil type. Each experimental container was irrigated weekly with distilled water. After 15 days, three seedlings were randomly harvested from each experimental container and evaluated for the growth performance.

Determination of physical and biochemical growth of bean seedlings

Physical growth parameters of seedlings, namely stipule length and stipule weight were measured using a metre rule and weighing balance, respectively. To measure biochemical growth, bean seedling tissues were homogenized using a mortar and pestle. Homogenized samples were thereafter analyzed for chlorophyll a, chlorophyll b and total chlorophyll, starch, total sugar, protein, total nitrogen, using the procedures adapted from the Association of Official Analytical Chemists [14], as briefly described hereunder.

Chlorophyll a, b and total chlorophyll

To determine chlorophyll a, chlorophyll b, and total chlorophyll, fresh leaf samples were weighed, put separately in 80 % N/V acetone (15 ml for each gram) and homogenized with the Phillip type harmonizer at 1000 rpm for one minute. Thereafter, 0.5 g of pure sand, plus 1.0 g of anhydrous sulphate was added to the sample. The homogenate was filtered through a two-layered cheesecloth and centrifuged using the Signs centrifuge, at 2500 rpm for ten minutes. The supernatant was separated, and the absorbance were read at spectrophotometer. Chlorophyll a was read at wavelength 663 nm and 645 nm, while chlorophyll b was read at 645 nm and 663 nm [15, 16], Chlorophyll a, chlorophyll b, and total chlorophyll were calculated as indicated below.

$$\text{Chlorophyll a (Chl a)} = 12.7\text{A663}) - 2.69\ (\text{A645}) \quad (1)$$

$$\text{Chlorophyll b (Chl b)} = 22.9\ (\text{A645}) - 4.68\ (\text{A663}) \quad (2)$$

$$\text{Total chlorophyll} = (\text{Chl a} + \text{Chl b}) \quad (3)$$

Total nitrogen and crude protein

The crude protein of each sample was determined using the Kjeldahl nitrogen method. Two grams of each sample with two tablets of digestion catalyst were subjected to reaction with 15ml sulphuric in a Kjeldahl tube. The tubes were set up in a digestion block heater (370ºC) until a clear green colour was obtained. The organic nitrogen in the sample was converted to ammonium sulphate. The digest was cooled to room temperature and diluted to 100ml with distilled water, which was further distilled in an alkaline condition of 40% sodium hydroxide. The liberated ammonium hydroxide was trapped in a 2% boric acid solution and titrated against a 0.1 N hydrochloric acid solution [1, 17, 18]. Total nitrogen was then calculated using the following formula:

$$Total\ Nitrogen = \frac{1.4V*N}{W} \quad (4)$$

where, V is acid used in titration (ml), N stand for normality of standard acid and W is the weight of sample (g).

The crude protein was then calculated from the total nitrogen in the sample using the conversion factor 6.25, based on the premise that the average protein contains about 16% nitrogen [14].

Total soluble soluble sugar and starch content

Total sugars and starch were estimated according to the anthrone-sulphuric acid reported by Sung et al. [19], with slight modification. Homogenized sample extract (0.1ml) was pipetted into duplicate test tubes and 0.9 ml of distilled water was added. The test tube contents were further diluted by taking 0.1 ml from each of the tubes, and adding 0.9 ml distilled water (making 1% extract solution), which was also made in triplicates. A blank was prepared using 1ml of distilled water. Four millilitres (4ml) of Anthrone reagent (0.2% anthrone in concentrated H_2SO_4) was added to each of the test tubes. The tubes were shaken and left in boiling water for ten minutes. The tubes were cooled under tap water and the optical density was read using 620nm wavelengths. The concentration of total soluble sugar was calculated, using the concentration factor 1.0 mg/ml glucose as standard, with the formula:

$$\text{Total sugar} = \frac{Std.conc}{Std.\ abs.}\ x\ \frac{sample\ abs.\ x\ 100}{weight\ of\ sample} \qquad (5)$$

where,
Std. abs. = Standard absorbance, and
Sample abs. = Sample absorbance.

The concentration of starch was then determined by multiplying the obtained value by 0.9; converting the glucose value to starch content [20, 21].

Statistical analysis of data

The data generated from the measurement of physical growth and laboratory analysis of biochemical parameters were subjected to Analysis of Variance (ANOVA). Mean differences were separated using Duncan Multiple Range Test, at 5% level of significance ($p < 0.05$). All analyses were done using IBM SPSS v 26 (IBM Corporation, New York).

Results

Stipule length and stipule weight of *Phaseolus vulgaris* seedlings grown in earthworm-bioturbated, crab-bioturbated, and unbioturbated soils

The stipule length and stipule weight of *Phaseolus vulgaris* seedlings grown in crab-bioturbated, earthworm-bioturbated and unbioturbated soils are presented in Table 1. The differences in stipule length of bean seedlings grown in all the soil types were generally not significant ($p > 0.05$). The stipule weight of Olo and oloyin varieties of *Phaseolus vulgaris* grown in earthworm-bioturbated soil (Olo: 5.57±1.01 g; Oloyin: 5.83±0.29 g) and crab-boturbated soil (Olo: 4.83±0.29 g; Oloyin: 5.00±0.50 g) were significantly higher ($p < 0.05$), relative to those grown in unbioturbated soil. Both stipule lengths and weights of the White bean variety showed no significant

Table 1. Stipule length and stipule weight of *Phaseolus vulgaris* seedlings grown in earthworm-bioturbated, crab-bioturbated and unbioturbated soils

Stipule length and stipule weight of varieties of bean seedlings				
Stipule length (cm)				
Soil type	**Ife brown**	**Olo**	**Oloyin**	**White bean**
Unbioturbated soil	10.00 ± 1.00 ^{a}	10.17 ± 0.29 ^{a}	10.57 ± 0.51 ^{a}	10.17 ± 1.04 ^{a}
Crab-bioturbated soil	10.93 ± 0.12 ^{a}	10.90 ± 0.17 ^{a}	11.50 ± 0.50 ^{a}	11.23 ± 0.64 ^{a}
Earthworm-bioturbated soil	10.00 ± 0.50 ^{a}	11.06 ± 1.51 ^{a}	11.67 ± 0.58 ^{a}	11.67 ± 1.41 ^{a}
F value	2.27^{ns}	1.93 ^{ns}	3.73 ^{ns}	2.08 ^{ns}
Stipule weight (g)				
Soil type	**Ife brown**	**Olo**	**Oloyin**	**White bean**
Control (Unbioturbated soil)	4.07 ± 0.81^b	3.83 ± 0.29 ^{b}	4.37 ± 0.71 ^{b}	4.33 ± 1.26 ^{a}
Crab-bioturbated soil	5.20 ± 0.61 ^{a}	4.83 ± 0.29 ^{ab}	5.00 ± 0.50 ^{ab}	5.17 ± 0.58 ^{a}
Earthworm-bioturbated soil	4.83 ± 0.76 ^{b}	5.57 ± 1.01^a	5.83 ± 0.29 ^{a}	5.50 ± 0.87 ^{a}
F value	1.862^{ns}	5.77*	5.82*	1.29^{ns}

Different letters: statistically significant
* Signoficant at P< 0.05
** Significant at P < 0.01
^{ns} Not significant

difference, across the three soil types. Olo seedlings in earhtworm-bioturbated soil recorded significantly higher ($p < 0.05$) weight (5.57 ± 1.01mg/g) than those in unbioturbated soil (3.83 ± 0.29mg/g). Oloyin seedlings in earthworm-bioturbated soil had significantly higer ($p < 0.05$) weight (5.83 ± 0.29mg/g) than the seedlings in unbioturbated soil (4.37 ± 0.71mg/g).

Chlorophyll a, b and total chlorophyll of *Phaseolus vulgaris* seedlings grown in earthworm bioturbated, crab-bioturbated, and unbioturbated soils

Phaseolus vulgaris grown in bioturbated soil recorded significantly higher Chlorophyll a, b and total chlorophyll, relative to those grown in unbioturbated soil. Seedlings grown in earthworm–bioturbated soil recorded the highest chlorophyll a, b and total chlorophyll, followed by those grown in crab-bioturbated soil. Seedlings grown in unbioturbated soil recorded the least of these parameters (Table 2). The chlorophyll b in White bean and Oloyoin seedlings showed no significant difference ($p > 0.05$), across the three soils. The difference observed between the chlorophyll a of Oloyin seedlings grwon in crab-bioturbated soil (29.11±1.66mg/g) and those grown in earthworm-bioturbated soil (29.00±1.84mg/g) was not significant ($p > 0.05$). However, they were both significantly higher than chlorophyll a in Oloyin seedling grown in unbioturbated soil (19.93±2.39mg/g). The same pattern was observed for chlorophyll a of White bean; there was no significant difference between the pigments in crab-bioturbated soil (29.11±1.66mg/g) and earthworm-bioturbated soil (30.64±0.42mg/g). However, they were both significantly higher ($p < 0.05$) than those in unbioturbated soil (21.61±1.91mg/g). Total chlorophyll in White bean seedlings grown in crab-bioturbated soil (42.64±1.50mg/g) and earthworm-bioturbated soil (42.16±0.57mg/g) showed no significant deifference, but were both significantly higher ($p < 0.05$) than the total chlorophyll in White bean grown in unbioturbated soil (34.50±3.11mg/g).

Total soluble sugar, starch, nitrogen, and protein in *Phaseolus vulgaris* seedlings grown in earthworm bioturbated, crab-bioturbated, and unbioturbated soils

Bean seedlings grown in earthworm-bioturbated soil recorded the highest and significant ($p < 0.01$) concentrations of total soluble sugar, starch, nitrogen, and crude protein, relative to those grown in crab-bioturbated and unbioturbated soils. Similarly, seedlings grown in crab-bioturbated soil recorded significantly higher ($p < 0.01$) concentrations of these biochemical parameters, relative to those grown in unbioturbated soil ($p < 0.01$) (Table 3).

Table 2. Chlorophyll a, b and total chlorophyll of *Phaseolus vulgaris* seedlings grown in earthworm bioturbated, crab-bioturbated, and unbioturbated soils

Chlorophyll a, b and total chlorophyll in varieties of bean seedlings				
Chlorophyll a (mg/g)				
Soil type	**Ife brown**	**Olo**	**Oloyin**	**White bean**
Unbioturbated soil	$19.84±1.73^{c}$	$22.71±1.26^{c}$	$19.93±2.39^{b}$	$21.61±1.91^{b}$
Crab-bioturbated soil	$29.56±1.79^{b}$	$28.08±1.26^{b}$	$29.11±1.66^{a}$	$30.44±1.16^{a}$
Earthworm-bioturbated soil	$31.33±1.07^{a}$	$30.45±0.74^{a}$	$29.00±1.84^{a}$	$30.64±0.42^{a}$
F value	141.10^{**}	113.70^{**}	625.04^{**}	138.94^{**}
Chlorophyll b (mg/g)				
Unbioturbated soil	$16.32±0.67^{b}$	$16.05±0.52^{b}$	$14.52±0.22^{a}$	$12.89±2.13^{a}$
Crab-bioturbated soil	$19.59±0.71^{a}$	$15.96±0.84^{b}$	$15.27±0.93^{a}$	$12.61±1.11^{a}$
Earthworm-bioturbated soil	$20.10±1.04^{a}$	$17.33±0.96^{a}$	$14.53±2.33^{a}$	$11.51±0.27^{a}$
F value	55.52^{**}	8.33^{**}	0.781^{ns}	2.43^{*}
Total chlorophyll (mg/g)				
Unbioturbated soil	$36.14±3.36^{b}$	$37.64±3.05^{c}$	$34.45±2.54^{c}$	$34.50±3.11^{b}$
Crab-bioturbated soil	$50.05±2.91^{a}$	$44.04±2.08^{b}$	$44.39±2.26^{b}$	$42.64±1.50^{a}$
Earthworm-bioturbated soil	$51.42±2.01^{a}$	$47.78±1.48^{a}$	$43.46±3.97^{a}$	$42.16±0.57^{a}$
F value	109.63^{**}	44.68^{**}	29.62^{**}	46.04^{**}

Different letters: statistically significant
* Signoficant at $P< 0.05$
** Significant at $P < 0.01$
^{ns} Not significant

Table 3. Total soluble sugar, starch, nitrogen, and protein in *Phaseolus vulgaris* seedlings grown in earthworm bioturbated, crab-bioturbated, and unbioturbated soils

Total soluble sugar, starch, nitrogen, and protein in varieties of bean seedlings				
Total soluble sugar (mg/g)				
Soil type	**Ife brown**	**Olo**	**Oloyin**	**White bean**
Unbioturbated soil	54.76±1.60^{c}	53.96±1.20^{c}	56.46±0.77^{c}	57.97±0.69^{c}
Crab-bioturbated soil	64.05±0.95^{b}	60.81± 0.86^{b}	61.30± 0.81^{b}	62.12± 2.15^{b}
Earthworm-bioturbated soil	65.71± 0.51^{a}	63.87± 0.63^{a}	66.12± 0.62^{a}	66.52± 0.87^{a}
F value	253.41**	246.70**	382.35**	83.96**
Starch (mg/g)				
Unbioturbated soil	34.75±0.97^{c}	35.75± 0.33^{c}	35.35± 0.10^{c}	35.79± 0.19^{c}
Crab-bioturbated soil	36.49±0.20^{b}	36.82±0.46^{b}	37.19±0.27^{b}	37.83± 0.23^{b}
Earthworm-bioturbated soil	38.71± 0.22^{a}	38.61± 0.21^{a}	39.19± 0.26^{a}	39.30± 0.08^{a}
F value	103.83**	55.14**	658.32**	866.99**
Nitrogen (mg/g)				
Unbioturbated soil	3.47± 0.07^{c}	3.22± 0.06^{c}	3.34± 0.04^{c}	3.23± 0.02^{c}
Crab-bioturbated soil	3.99± 0.08^{b}	3.84± 0.07^{b}	3.88± 0.11^{b}	4.02± 0.05^{b}
Earthworm-bioturbated soil	4.48± 0.10^{a}	4.27± 0.08^{a}	4.32± 0.04^{a}	4.46± 0.04^{a}
F value	316.54**	551.43**	430.43**	2355.66**
Protein (mg/g)				
Unbioturbated soil	21.69± 0.47^{c}	20.14± 0.35^{c}	20.83± 0.35^{c}	20.23± 0.12^{c}
Crab-bioturbated soil	24.96± 0.49^{b}	23.99± 0.42^{b}	24.21± 0.63^{b}	25.10± 0.29^{b}
Earthworm-bioturbated soil	27.93± 0.58^{a}	26.70± 0.49^{a}	27.02± 0.24^{a}	27.81± 0.26^{a}
F value	332.75**	555.31**	451.08**	2360.64**

Different letters: statistically significant
* Signoficant at P< 0.05
** Significant at P < 0.01
^{ns} Not significant

DISCUSSION

In this study, seedlings performance in earthworm-bioturbated and crab-bioturbated soils, in comparison with unbioturbated soil, show significant improvements in all the measured biochemical and physiological growth parameters, expect for stipule weight whose difference was only marginal. The improved seedling growth in earthworm-bioturbated soil readily corroborates many previous studies including Tomati et al. [22] and Xaio et al. [23] who reported improved growth of *Raphanus sativus* and *Brassica rapa* respectively, in earthworm casts. Both earthworm-bioturbated and crab-bioturbated soils are known to possess improved physicochemical qualities that can potentially promote plant growth and development. Earthworm-bioturbated soils are characterized by decreased bulk density and increased soil water content, organic matter, soil organic and inorganic carbon, nitrogen, phosphorus and beneficial microorganisms [4, 6]. These qualities are also associated with crab-bioturbated soil [9, 11]. These qualities can be related to the better growth performance of seedling grown in bioturbated soil.

The chlorophyll content of a plant is directly related to the amounts of soil nutrients availabe for the plant's absorbtion, as these nutrients serve as building blocks for the plant tissues [24, 25]. Therefore, the observed increase in chlorophyll content of *P. vulgaris* seedings grown in bioturbated soils can be linked to the relatively abundanrt nutrients in the soils [3]. Total soluble sugar, starch, nitrogen and protein are essentially photosynthetic products; hence, the higher concentrations recorded for these products in seedlings grown in bioturbated soil can be associated with their elevated levels of chlorophyll, which must have conferred on them, improved photosynthesis efficiency. Similarly, the significantly and marginally higher stipule weight and stipule length respectively, recorded for seedlingsown in bioturbated soils are complimentary to the observed increased biochemical growth and chlorophyll efficiency. The stipule growth-promoting potential of bioturbated is of remarkable importance because it contributes substantially to primary productivity in plants. It is on record that stipules can contribute as much as 30% to plant photosynthesis, which is occasionally more than the contribution of leaves [26]. Stipule growth also determines the flowering time of a plant, an indicator of plant pod production [27]. In other words, stipule growth substantially determines seed production.

The higher growth performance recorded for seedlings grown in earthworm-bioturbated soil, relative to those grown in crab-bioturbated soil is a reflection of the long-known positive impact of earthworms to soil improvement and crop production. It is also in tandem with our earlier work [3]. where we compared the physicochemical and biochemical qualities of earthworm-bioturbated soil and crab-bioturbated soil and reported that the former, earthworm-bioturbated soil, contained more quantities of nitrogen, phosphorus, organic carbon and total organic matter. The relative extra abundance of these elements must have positively influenced the better growth recorded by the seedlings grown in earthworm-bioturbated soil in this study. Nevertheless, these results indicate that the bioturbating activities of earthworms and crabs both contribute significantly to wetland productivity.

It was also observed that the influence of the soil type on the bean seedlings was not uniform for all the four varieties. Ife brown and Olo bean seedlings were somewhat evenly influenced, and were the most responsve to soil type. However, White bean variety showed the least response to soil type, followed by Oloyin. This uneven response to soil type can be attributed their strucural difference (size, texture and colour). This assumption is in line with the reports of Fenner [28] and El-Abady [29] that posited that the structure of seeds influences their seedling germination and growth.

CONCLUSION

We compared the growth performance of four varieties of *Phaseolus vulgaris* (bean) seedlings in earthworm-bioturbated soil, crab-bioturbated soil, and unbioturbated soil collected within the same proximity of a wetland habitat. Seedlings grown in earthworm-bioturbated soil and crab-boturbated soil recorded significantly higher physical and biochemical growth, relative to those grown in unbioturbated soil. In the same vein, bean seedlings grown in earthworm-bioturbated soil performed better than those in crab-bioturbated. Earthworm bioturbated soil have long been documented to positively improve plant growth, but the same cannot be said of crab-bioturbated soil. Therefore, the relatively improved performance recorded for seedlings grown in crab-biotubated soil is not only remarkable, but calls for research attention.

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CONFLICT OF INTREST

The authors declare that there was no conflict of interest related to this article.

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Persian Abstract

چکیده

کرم‌های خاکی و خرچنگ‌ها به‌عنوان تأثیرگذار بر وضعیت فیزیکوشیمیایی زیستگاه‌های خاکی مربوطه خود از طریق اقدامات بیوتورباسیون خود شناخته شده‌اند. در حالی که خاک‌های بیوتورباته شده با کرم خاکی به‌خوبی مستند شده‌اند که بر رشد گیاه تأثیر مثبت می‌گذارند، اطلاعات زیادی در مورد اثرات خاک‌های بیوتورباته شده با خرچنگ بر رشد گیاه شناخته نشده است. در این مطالعه، ما عملکرد رشد چهار گونه از نهال‌های (*Phaseolus vulgaris*) لوبیا را در خاک با کرم‌های خاکی، خاک بیوتورباته شده با خرچنگ، و خاک بدون توربوتی که در همان مجاورت یک زیستگاه تالاب جمع‌آوری شده‌اند، مقایسه کردیم. بذر ***Phaseolus vulgaris*** به صورت تکرار در هر نوع خاک کاشته شد و به مدت ۱۵ روز رشد کرد. رشد فیزیکی با استفاده از قانون متر اندازه گیری شد. پارامترهای رشد بیوشیمیایی با استفاده از روش های استاندارد اندازه گیری شد. تفاوت در طول نهال لوبیا رشد یافته در تمام انواع خاک به طور کلی معنی دار نبود ($p > ۰/۰۵$). با این حال، نهال‌های رشد یافته در خاک با کرم‌های خاکی و خاک خرچنگ بوته‌شده به طور قابل‌توجهی بالاتر ($p < ۰/۰۵$) وزن stipule، نسبت به کسانی که در خاک unbioturbated رشد کرده‌اند، ثبت کردند. نهال‌های لوبیا رشد کرده در خاک بیوتورباته شده با کرم خاکی، بالاترین و معنی‌دارترین ($p < ۰/۰۱$) غلظت کلروفیل، قند کل، نشاسته، نیتروژن و پروتئین خام را نسبت به آنهایی که در خاک‌های خرچنگ و ارده‌ای نشده رشد می‌کنند، ثبت کردند. این توسط نهال رشد کرده در خاک خرچنگ bioturbated که به طور قابل توجهی بالاتر ($p < ۰/۰۱$) غلظت این پارامترهای بیوشیمیایی، نسبت به کسانی که در خاک unbioturbated رشد ثبت شده است. رشد بیوشیمیایی و فیزیولوژیکی به طور قابل توجهی بالاتر ثبت شده برای نهال‌ها در خاک‌های بیوتوربیت شده نشان می‌دهد که کرم‌های خاکی و خرچنگ‌ها هر دو از طریق فعالیت های بیوتوربایی خود به طور قابل توجهی به بهره‌وری تالاب کمک می‌کنند.