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Growth of Creeping Bentgrass (*Agrostis palustris* Huds.) after Applying with Fish Byproduct Fermented with Effective Microorganism

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Abstract: This study was conducted to investigate changes of growth and quality of creeping bentgrass applying fish byproduct fermented with effective microorganism (FBEM), an organic agro-material. Treatments were as follows; No fertilizer (NF), control (compound fertilizer 3N a.i. g/m^2/month), FBEM-1 (control + FBEM 1 mL/m^2/month), FBEM-2 (control + FBEM 2 mL/m^2/month), FBEM-3 (control + FBEM 4 mL/m^2/month), and SFBEM (control + sterilized FBEM 2 mL/m^2/month). As compared to NF, turf color index (TCI), chlorophyll a+b content, shoot length, turfgrass clipping yield, and nitrogen (N) uptake in the FBEM treatments (FBEM-1, FBEM-2, FBEM-3, and SFBEM) were increased. Compared to control, growth factors of FBEM treatments were not significantly different. An application amount of FBEM was positively correlated to TCI, shoot length, clipping yield, N content in the tissue, and N and K uptake ($p \le 0.05$). These results indicated that FBEM application improved the quality and growth of creeping bentgrass by promoting N uptake from soil.

Keywords: Creeping bentgrass, Fish byproduct fermented with effected microorganism (FBEM), Growth, N uptake, Organic agro-materials

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Introduction

Since the enactment of the Environmentally-Friendly Agriculture Promotion Act in 1997, eco-friendly agriculture has developed in various sectors, with ongoing efforts to reduce the use of chemical fertilizers and synthetic pesticides for the production of safe agricultural products and sustainable agriculture. Domestically, to facilitate the production of eco-friendly agricultural products, a public notification system for organic agricultural materials provides farmers with a list of approved substances for crop cultivation. These organic agricultural materials supply necessary nutrients for crop growth and are utilized for pest and disease control in domestic eco-friendly farming practices. Currently, according to the National Agricultural Products Quality Management Service, as of 2025, there are 2,016 products registered as organic agricultural materials.

The development of eco-friendly agriculture has broadened awareness, encompassing both environmental preservation and the provision of high-quality turf for golf course players [1]. Key aspects identified for eco-friendly golf course management include efficient water management, reduced reliance on chemical fertilizers and pesticides, the protection of ecosystems and biodiversity, the utilization of renewable energy, and robust government policy support [1]. These elements are considered crucial for maintaining the optimal growth and quality of turfgrass on golf courses [2]. Eco-friendly management of golf course turf, which aims to reduce the use of chemical fertilizers and pesticides for promoting turf growth and controlling pests and diseases, is similar to low-input agriculture that focuses on resource recycling [2].

Various studies have been conducted on eco-friendly turfgrass management in domestic golf courses, focusing on the utilization of functional substances for turf growth or pest control to reduce the use of chemical fertilizers and pesticides [3,4]. Additionally, research on turf cultivation using the functional fertilizers such as compound fertilizers, micronutrient fertilizers, and microbial fertilizers have been published, demonstrating ongoing efforts to reduce the reliance on chemical fertilizers and pesticides in turf management. However, there has been very little research on turfgrass cultivation specifically utilizing organic agro-materials (OAM). Therefore, further research on turf management utilizing OAM is required for future golf course eco-friendly management.

The OAM selected for this study is a crop growth material fermented by microorganisms using animal byproducts and sugar byproducts. The animal byproduct used as a raw material was fish byproduct, and the sugar byproduct used was molasses. The microorganism added for fermentation was effective microorganisms (EM). A representative raw material used as fish byproduct is fish meal, which contains protein, produces amino acids during fermentation, and can be utilized as a top-dressing material during crop cultivation [5]. Molasses has been widely utilized as an industrial medium for microbial cultivation [6], and has also found application in crop cultivation [7] and forage fermentation [8]. Therefore, this study investigated changes in turfgrass growth and quality in creeping bentgrass (*Agrostis palustris*) after treatment with fish byproduct fermented with effective microorganisms (FBEM).

Results and Discussion

Soil chemical properties

The changes in the soil of creeping bentgrass plots treated with FBEM were investigated (Table 1). At the end of the experiment, compared to the no fertilizer (NF) treatment, the soil in the FBEM treatments (FBEM-1, FBEM-2, FBEM-3, and SFBEM) showed increases in pH, electrical conductivity (EC), total nitrogen (T-N), available phosphate (Av-P_2O_5), and exchangeable potas-

Table 1. The chemical properties of soil used before and after in this study

Treatments[1)]	pH	EC	OM	T-N	Av-P_2O_5	Ex-K
	1:5	dS/m	%		mg/kg	$cmol_c$/kg
NF	7.23d[2)]	0.25a	0.59a	0.022b	28.2c	0.01d
Control	7.40c	0.30a	0.60a	0.033a	38.7bc	0.03c
FBEM-1	7.56a	0.30a	0.60a	0.033a	50.9ab	0.05b
FBEM-2	7.45bc	0.30a	0.59a	0.035a	52.7ab	0.06b
FBEM-3	7.49ab	0.31a	0.62a	0.037a	57.9a	0.06b
SFBEM	7.58a	0.28a	0.60a	0.029a	45.7ab	0.04c
Correlation coefficient[3)]	0.282^{NS}	0.707**	0.151^{NS}	0.811**	0.684*	0.756**

1) Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m^2/month), FBEM-3 (CF + FBEM 4 mL/m^2/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
2) Means with the same letters within a column are not significantly different by Duncan's multiple range test $p \leq 0.05$ level.
3) NS, *, and ** represent no significance, and a significance at the 0.05 and 0.01 probability level, respectively, for correlation coefficient between FBEM application and each chemical factor of soil.

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sium (Ex-K). As compared to control, Ex-K was also enhanced in the FBEM treatments. Kim et al. [9] reported that when fish byproduct is used as an organic fertilizer, the exchangeable potassium content in the soil increases due to the high potassium content within the fish byproduct. FBEM application rate was positively correlated with EC (R=0.707**, $p \le 0.01$), T-N (R=0.811**, $p \le 0.01$), Av-P_2O_5 (R=0.684*, $p \le 0.05$), and Ex-K (R=0.756**, $p \le 0.01$). Overall, these results suggest that FBEM application enhances soil fertility by increasing the content of nitrogen, phosphate, and potassium in turfgrass-cultivated soil.

Changes of turfgrass growth and quality

The turf color index (TCI) of creeping bentgrass was investigated after applying FBEM (Table 2). Compared to the NF, TCI of FBEM treatments increased throughout the experimental period, indicating an improvement in turf quality. While TCIs of FBEM treatments were not significantly different in measurements except for July, their average TCI was higher than that of control. High average TCI for experimental period suggests an improvement in turf quality. Correlation coefficient between FBEM application rate and TCI of June, July, and September was significantly different, positively ($p \le 0.05$). This period is summer which exceed optimal temperature conditions of creeping bentgrass, the cool-season turfgrass. Generally, creeping bentgrass tends to exhibit reduced turf quality and growth during summer; thus, these findings indicate that FBEM treatment can improve turf quality [10].

The chlorophyll content in turfgrass after FBEM treatment ranged from 1,226 to 1,712 FW μg/mL (Table 3). When compared to the NF, the content of FBEM treatments increased from May to August. However, chlorophyll content of NF could not be measured in September and October due to the inability to collect turf clippings. While an average chlorophyll content of FBEM treatments throughout all experimental period was increased by 28.2-29.7% than that of NF, it was not significantly different than that of control. The chlorophyll content of turfgrass is known to be influenced by the nitrogen content within the shoots and to affect turf growth and quality [12]. Kim et al. [13] reported that chlorophyll content of rootstocks is affected by the rooting and the water uptake, and then influencing plant response to environmental changes. As chlorophyll content in plants affects plant growth through photosynthesis, it serves as an important indicator for evaluating plant growth status and significantly influences turf quality [14]. In this study, the chlorophyll content also showed a positive correlation (R=0.962**, $p \le 0.01$) with the TCI in a Pearson correlation analysis, it improves turf quality in the FBEM treatments.

It was investigated for changes of shoot length after FBEM treatment (Table 4). While shoot length of NF gradually decreased over time, those of FBEM treatments exhibited differences according to the turfgrass's growth stage. Shoot length of creeping bentgrass applying FBEM increased in May and June, which are optimal temperature periods. In July and August, when temper-

Table 2. Changes of turfgrass color index of creeping bentgrass fertilizing FBEM

Treatments[1)]	April	May	June	July	August	September	October	Mean
NF	6.80b[2)]	6.19b	5.72b	5.42c	5.20b	4.96b	4.65b	5.56d
Control	7.37a	7.33a	6.81a	6.51b	6.81a	7.12a	7.20a	7.02c
FBEM-1	7.39a	7.35a	6.87a	6.70ab	6.97a	7.14a	7.22a	7.09ab
FBEM-2	7.40a	7.31a	6.91a	6.72ab	7.00a	7.18a	7.22a	7.11ab
FBEM-3	7.38a	7.35a	7.00a	6.80a	6.93a	7.28a	7.23a	7.14a
SFBEM	7.36a	7.33a	6.88a	6.51b	6.96a	7.22a	7.26a	7.07bc
Correlation coefficient[3)]	0.111^{NS}	0.158^{NS}	0.718**	0.698**	0.355^{NS}	0.731**	0.268^{NS}	0.870**

[1)] Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m$_2$/month), FBEM-3 (CF + FBEM 4 mL/m$_2$/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
[2)] Means with the same letters within a column are not significantly different by Duncan's multiple range test $p \le 0.05$ level.
[3)] NS, *, and ** represent no significance, and a significance at the 0.05 and 0.01 probability level, respectively, for correlation coefficient between FBEM application and each chemical factor of soil.

Table 3. Changes of shoot length of creeping bentgrass fertilizing FBEM (Unit: cm)

Treatments[1)]	April	May	June	July	August	September	October	Mean
NF	4.37b[2)]	4.50b	4.12c	3.79b	3.18b	2.91b	2.79b	3.72c
Control	4.70a	6.24a	6.78b	6.76a	6.45a	6.97a	7.03a	6.46b
FBEM-1	4.69a	6.25a	6.89ab	6.81a	6.54a	7.00a	7.01a	6.51ab
FBEM-2	4.67a	6.33a	7.09a	6.82a	6.57a	7.03a	7.03a	6.58ab
FBEM-3	4.67a	6.34a	6.99ab	6.96a	6.67a	7.13a	7.05a	6.60a
SFBEM	4.69a	6.25a	6.75b	6.77a	6.53a	7.10a	7.06a	6.49ab
Correlation coefficient[3)]	-0.315^{NS}	0.443^{NS}	0.465^{NS}	0.724**	0.728**	0.791**	0.117^{NS}	0.795**

1) Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m^2/month), FBEM-3 (CF + FBEM 4 mL/m^2/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
2) Means with the same letters within a column are not significantly different by Duncan's multiple range test p≤0.05 level.
3) NS, *, and ** represent no significance, and a significance at the 0.05 and 0.01 probability level, respectively, for correlation coefficient between FBEM application and each chemical factor of soil.

Table 4. Changes of chlorophyll content (a+b) of creeping bentgrass fertilizing FBEM (Unit: FW μg/mL)

Treatments[1)]	April	May	June	July	August	September	October	Mean
NF	1,434a[2)]	1,264b	1,226b	1,253b	1,249b	-	-	1,285b
Control	1,512a	1,624a	1,641a	1,641a	1,635a	1,702a	1,747a	1,643a
FBEM-1	1,564a	1,712a	1,682a	1,618a	1,633a	1,695a	1,764a	1,667a
FBEM-2	1,479a	1,688a	1,640a	1,624a	1,659a	1,698a	1,771a	1,651a
FBEM-3	1,497a	1,650a	1,628a	1,639a	1,674a	1,698a	1,774a	1,651a
SFBEM	1,493a	1,700a	1,597a	1,618a	1,653a	1,702a	1,769a	1,647a
Correlation coefficient[3)]	-0.162^{NS}	0.018^{NS}	-0.097^{NS}	0.027^{NS}	0.316^{NS}	-0.012^{NS}	0.297^{NS}	0.011^{NS}

1) Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m^2/month), FBEM-3 (CF + FBEM 4 mL/m^2/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
2) Means with the same letters within a column are not significantly different by Duncan's multiple range test p≤0.05 level.
3) NS represents no significance, for correlation coefficient between FBEM application and a chlorophyll content (a+b).

atures were higher than optimal, shoot length was similar to June or decreased, indicating reduced above-ground growth. Subsequently, shoot length increased again in September and October compared to August. As shoot length was influenced by environmental factors such as temperature and relative humidity, we compared its average value measured throughout the growing season in turfgrass treated with FBEM.

Compared to the NF, the shoot length of the FBEM treatments increased by 74.9-77.2%, while they were not significantly different than that of control. No significant difference in turfgrass shoot length was detected between the FBEM-2 and SFBEM treatments, indicating that EM microorganisms had a negligible effect on elongation. Turfgrass shoot growth is affected by soil environment [15] and nutrient supply [16]. In this study, soil conditions and nutrient supply were maintained identically. Correlation analysis showed a positive relationship between FBEM application rate and turfgrass shoot length (R=0.795**, p≤0.01).

Turfgrass growth was measured by measuring turfgrass clippings after FBEM treatment (Table 5). Throughout the experimental period, total clipping yield ranged from 34.63 to 374.46 g/m^2. Clippings in the FBEM treatments were 10.5-10.8 times higher than in the NF treatment, but did not differ significantly from the control. In turfgrass growth, the clipping yield is known to be influenced by nitrogen fertilization rate [17]. In this study, turfgrass clippings were greater in the fertilized treatments (control, FBEM, and SFBEM) than in the NF treatment. A positive correlation was observed between FBEM application rate and clipping yield (R=0.821**, p≤0.01), although no significant increase was detected in the FBEM treatment. Furthermore, no

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Table 5. Clipping yield of creeping bentgrass fertilizing FBEM (Unit: g/m^2)

Treatments[1)]	April	May	June	July	August	September	October	Total
NF	6.98b[2)]	12.90b	5.79b	4.08b	3.82b	1.05b	-	34.63b
Control	15.80a	56.09a	48.72a	52.27a	54.25a	56.88a	81.11a	365.11a
FBEM-1	15.27a	56.09a	47.66a	53.98a	53.98a	56.22a	83.87a	367.09a
FBEM-2	15.80a	59.25a	46.35a	54.38a	54.64a	57.93a	82.16a	370.51a
FBEM-3	13.96a	55.43a	51.09a	55.43a	55.96a	58.20a	84.40a	374.46a
SFBEM	15.67a	58.99a	45.82a	54.38a	54.38a	56.88a	81.50a	367.61a
Correlation coefficient[3)]	-0.313^{NS}	-0.039^{NS}	0.269^{NS}	0.378^{NS}	0.356^{NS}	0.544^{NS}	0.394^{NS}	0.821**

[1)] Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m^2/month), FBEM-3 (CF + FBEM 4 mL/m^2/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
[2)] Means with the same letters within a column are not significantly different by Duncan's multiple range test $p \le 0.05$ level.
[3)] NS and ** represent no significance, and a significance at the 0.01 probability level, respectively, for correlation coefficient between FBEM application and a clipping yield.

statistically significant difference was observed between SFBEM and FBEM treatments, suggesting that EM may have a limited or negligible effect on turfgrass growth under the conditions tested.

The content of nitrogen (N), phosphorus (P), and potassium (K) in the turfgrass tissue was 1.52-2.87%, 0.13-0.18%, and 0.59-1.44%, respectively (Table 6). Compared to the NF, the N and K content increased in the FBEM treatments, while P in the FBEM-3 treatment only increased. As compared to the control, the content of N, P, and K in FBEM treatments was not significantly different. Nevertheless, a positive correlation was observed between the FBEM application rate and the N content in the turfgrass tissue (R=0.618*, $p \le 0.05$), suggesting that FBEM application may increase the N content in the turfgrass tissue.

The uptake amounts of N, P, and K in the turfgrass ranged from 0.52-9.15 g/m^2, 0.04-0.58 g/m^2, and 0.20-4.58 g/m^2, respectively. Compared to the NF, the uptake of N, P, and K increased by 14.5-17.4 times, 10.1-13.2 times, and 19.3-22.6 times, respectively. As compared to the control, the N, P, and K uptake in the FBEM treatment were not significantly different. In the correlation analysis between the FBEM application rate and nutrient uptake, it was a positively correlated N uptake (R=0.632*, $p \le 0.05$) and K uptake (R=0.579*, $p \le 0.05$), and suggested that FBEM treatment is considered to enhance the N and K uptake.

Pearson correlation analysis was conducted to evaluate the effects of FBEM application rate on turfgrass growth (Table 7). Growth factors showing significant correlations with FBEM application rate included shoot length, clipping yield, N content, N

Table 6. Nutrient content and uptake in the creeping bentgrass fertilizing FBEM

Treatments[1)]	Nutrient content (%)			Nutrient uptake (g/m^2)		
	N	P	K	N	P	K
NF	1.52b[2)]	0.13b	0.59b	0.52c	0.04b	0.20b
Control	2.59a	0.16ab	1.25a	8.10b	0.51a	3.92a
FBEM-1	2.54a	0.16ab	1.32a	7.92b	0.50a	4.11a
FBEM-2	2.64a	0.16ab	1.32a	8.34ab	0.52a	4.16a
FBEM-3	2.87a	0.18a	1.44a	9.15a	0.58a	4.58a
SFBEM	2.45a	0.14ab	1.26a	7.60b	0.44a	3.91a
Correlation coefficient[3)]	0.618*	0.247^{NS}	0.508^{NS}	0.632*	0.298^{NS}	0.579*

[1)] Treatments were follows. NF: no fertilizer, Control (compound fertilizer, CF; N-P_2O_5-K_2O=21-17-17, 3N a.i g/m^2/month), FBEM-1 (CF + FBEM 1 mL/m^2/month), FBEM-2 (CF + FBEM 2 mL/m^2/month), FBEM-3 (CF + FBEM 4 mL/m^2/month), and SFBEM-1 (CF + sterilized FBEM 2 mL/m^2/month). Compound fertilizer was applied on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were fertigated every week from April 4. CF, FBEM, and SFBEM was diluted with tap water. FBEM presents a fish byproduct fermented with effected microorganism.
[2)] Means with the same letters within a column are not significantly different by Duncan's multiple range test $p \le 0.05$ level.
[3)] NS and * represent no significance, and a significance at the 0.05 probability level, respectively, for correlation coefficient between FBEM application and a clipping yield.

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Table 7. Correlation coefficient between each growth factor in the creeping bentgrass fertilizing FBEM

Factors[1)]	TCI	SL	CY	NC	NU	KU
TCI	1.000	0.715**	0.762**	0.620*	0.624*	0.698**
SL		1.000	0.546	0.331	0.342	0.320
CY			1.000	0.726**	0.784**	0.421
NC				1.000	0.993**	0.604*
NU					1.000	0.573*
KU						1.000

* and ** represent a significance at the 0.05 and 0.01 probability level, respectively, for correlation coefficient between each growth factor in the creeping bentgrass applying FBEM.
[1)] TCI: turf color index, SL: Shoot length, CY: clipping yield, NC: nitrogen content in the plant tissue, NU: nitrogen uptake, KU: potassium uptake.

uptake, and P uptake, as indicated by their correlation coefficients with TCI ($p \leq 0.05$). Notably, N uptake was positively correlated with clipping yield, N content, and K uptake ($p \leq 0.05$), suggesting that N uptake may play a central role in mediating turfgrass growth responses to FBEM treatment. In contrast, shoot length did not show significant correlations with other growth factors, implying that elongation may be less sensitive to changes in nutrient content or FBEM application.

FBEM is an OAM produced by the microbial fermentation of fish by-products and molasses. As fermented the fish by-products containing protein, it produced essential and inorganic nutrients for plant growth. Fish meal is a representative raw material for fish by-products, and it is widely utilized as a protein source for feed or fertilizer [14]. Organic material containing fish meal produces various fermented substances by microbial fermentation, which have diverse nutrients and functional materials [18].

Amino acids are representative functional substances derived from proteins sources [19]. When fermented liquid fertilizer is applied to turfgrass, it is known to promote spring green-up, thereby improving leaf color and growth [20]. Amino acids are known to enhance the utilization of carbon dioxide in plants, leading to increased photosynthesis [21], promote nitrogen absorption in plants to improve turfgrass growth [20], and increase ground cover rate [22]. When amino acids are utilized as functional fertilizers, supplying them as complex amino acids, rather than single amino acids, enhances the turf density of creeping bentgrass [23], consequently improving turf growth and ground cover rate [24]. However, the enhancement in turf density due to amino acid fertilizer application does not always lead to increase its growth and ground covering. Furthermore, turf growth is influenced by the elongation of turfgrass depending on the type of fertilizer [23].

FBEM contains not only fish byproducts but also molasses. This mixture is an OAM that is fermented using microorganisms. Fermentation is facilitated when various organic sources such as fish meal, bone meal, rice bran, and oil cake, are mixed with molasses and EM are added [18,25]. It is known that application of these OAMs enhances the growth of Chinese leek (*Allium tuberosum*), Chinese cabbage (*Brassica rapa*), and red pepper (*Capsicum annuum*) [18,25,26].

FBEM used in this study contained EM in addition to fish by-products and molasses. Although there were only negligible changes in turfgrass growth and quality after applying EMs, these microorganisms can still improve the soil's physical properties by decomposing thatch, which may lead to increased turfgrass root growth [27]. However, in this study, the application of FBEM had no significant effect on the growth and quality of creeping bentgrass. This is because the impact on plant growth relies on the specific microbial species and their properties. Also, no significant difference was observed in the growth and quality of creeping bentgrass between the FBEM and SFBEM treatment, suggesting that EM had a minimal effect on turfgrass growth [28].

Although applying FBEM had no effect on increasing turfgrass growth, positive correlation coefficient between its application rate and each growth factor, suggesting to improve turfgrass growth (Table 7). Path analysis of each growth factor of grass was performed after FBEM treatment (Figure 1). In creeping bentgrass growth, the standardized coefficient between FBEM application rate and nitrogen uptake was 0.63* ($p \leq 0.05$). The standardized coefficient between nitrogen uptake and TCI was 0.61* ($p \leq 0.05$), and between nitrogen uptake and clippings yield was 0.78** ($p \leq 0.01$), indicating that nitrogen uptake significantly influenced turfgrass quality and growth. The standardized coefficient between N uptake and shoot length was -0.26NS, and between TCI

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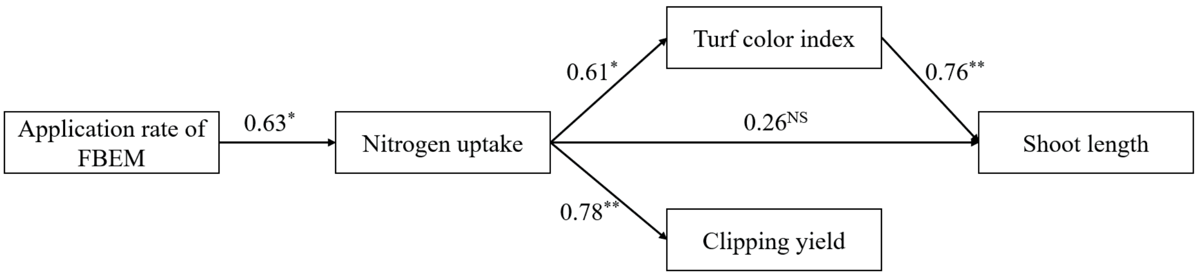

Figure 1. Path analysis of growth factors in the creeping bentgrass treated FBEM.

and shoot length 0.76** (p≤0.01). The FBEM application rate showed a direct effect on nitrogen uptake. Nitrogen uptake, in turn, had direct effects on the TCI and clippings yield. Although N uptake did not directly affect shoot length, it indirectly promoted shoot length through the TCI, indicating a mediating effect on shoot length changes. In summary, these results suggest that FBEM application increases N uptake in creeping bentgrass, consequently enhancing turfgrass quality and growth.

Materials and Methods

Materials

This study was conducted for approximately seven months, from April to October 2024, in a glasshouse located at the Daegu University Attached Farm. Creeping bentgrass, commonly used for golf greens, was utilized as the experimental turfgrass, and the cultivar 'Penn A1' was donated from company A. The experimental soil used sand matched on the United States Golf Association (USGA) sand particle size distribution specifications (Table 8). The pH and electrical conductivity (EC) were 6.37 and 0.31 dS/m, respectively, which were suitable for turfgrass experiments [10]. For the experimental fertilizers, a compound fertilizer (CF; N-P_2O_5-K_2O=21-17-17, Namhae Chemical Co., Ltd., Yeosu, Korea) was purchased from an agricultural supply store. Additionally, the FBEM (N-P_2O_5-K_2O=1.35-0.36-0.89, Public Announcement-2-2-126, Nature and Future Chemical Co., Ltd., Gokseong, Korea) was donated from company B. After filling the 5-inch test plastic pot (diameter 12.7 cm, depth 13 cm) with test sand, tap water was used to compact the soil. After compaction was completed, the gravity water was removed, and 10 g/m^2 of creeping bentgrass seeds were sown on May 7, 2023, and cultivated during approximately 9 months for this experiment.

Treatment establishment

Treatments of creeping bentgrass after FBEM were follows. No fertilizer treatment (NF), control (CF 3N a.i. g/m^2/month), FBEM-1 treatment (control + FBEM 1 mL/m^2/month), FBEM-2 treatment (control + FBEM 2 mL/m^2/month), FBEM-3 treatment (control + FBEM 4 mL/m^2/month), and SFBEM treatment (control + sterilized FBEM 2 mL/m^2/month). The respective treatments were arranged with 5 replicates using the completely randomized design. The treatments of CF, FBEM, and SFBEM were diluted with tap water, and 1,000 mL/m^2 of the diluted solution was applied as a foliar spray with a portable sprayer (Trigger sprayer

Table 8. Particle size distribution of sand used in this study

Item	Particle size (mm)						
	> 4.00	2.00-4.00	1.00-2.00	0.50-1.00	0.25-0.50	0.15-0.25	0.15-0.053
Sand	0%	19.3%	38.4%	28.7%	9.2%	3.7%	0.7%
USGA standard	0%	< 10%	> 60%		> 20%		< 10%

USGA: United States Golf Association

700, Apollo Industrial Co., Ltd., Siheung, Korea). CF was applied a total of seven times on April 4, May 2, May 30, June 27, July 25, August 22, and September 19. FBEM and SFBEM were applied 31 times, at 7-day intervals, starting from April 4. During the experimental period, no pesticides such as insecticides or fungicides were applied because no disease or insect infestations occurred. Considering the extent of turfgrass growth and weather condition, irrigation was performed once or twice daily.

Investigating turfgrass growth and analyzing soil and plant

Turfgrass growth was assessed by measuring the TCI, chlorophyll (a+b) content, shoot length, and clippings yield. The turf color index was measured using a turf color meter (TCM 500, Spectrum Technologies, Inc., Plainfield, IL, USA) at weekly intervals from April 4, for a total of 31 measurements, and monthly averages were calculated. Clippings yield and shoot length were measured every 14 days from April 4, totaling 15 times. Turfgrass clippings were harvested using scissors sterilized with 70% ethanol at a height of 30 mm above the soil surface, and the total monthly clipping yield after fertilization was used to evaluate turf growth. Clippings were dried for 48 hours in an oven set at 70°C (Thermostable OF-W155, Daihan Scientific Co., Ltd., Gangwon-do, Korea) and their dry weight was measured. Chlorophyll content was determined by extracting a portion (0.1 g fresh weight) of clippings collected 14 days after fertilizer application in 95% ethanol, following the method described by Kim et al. [28].

Soil samples for analysis were collected before the experiment (May 1, 2023) and after the experiment (October 31, 2024). The soil samples were dried in the shade at room temperature, passed through a 2 mm sieve, and then analyzed. The soil analysis parameters included soil acidity (pH), electrical conductivity (EC), organic matter (OM), total nitrogen (T-N), available phosphate (Av-P_2O_5), and exchangeable potassium (Ex-K). Soil analysis was conducted with the soil chemical analysis methods prescribed by the Rural Development Administration (RDA) of Korea.

The nutrient content of turfgrass tissue was analyzed with an aliquot digesting 0.2 g of dry biomass with 20 mL of sulfuric acid and 1 mL of perchloric acid, following the Kjeldahl digestion method in the plant analysis method established by RDA. N was analyzed by the Kjeldahl distillation method, P by the vanadomolybdate method, and K by atomic absorption spectrophotometry. The nutrient uptake amount in the turfgrass tissue was calculated with the dry biomass and nutrient content [28].

Statistical analysis

Statistical analysis was performed using SPSS (ver. 29, IBM, NY, USA). Significant differences in mean values among treatments were evaluated using Duncan's multiple range test. Pearson’s correlation analysis was conducted to investigate the correlation between each growth factor and FBEM application rate. Additionally, path analysis was performed for each growth factor.

Data Availability: All data are available in the main text or in the Supplementary Information.

Author Contributions: Y.-S. Kim and M.-H conceived and designed the research; Y.-S Kim performed the statistical analysis, wrote the first manuscript, and revised the manuscript, provide critical feedback; S.-J. Lim led the growth experiment, and collected the data.

Notes: The authors declare no conflict of interest

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