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Enhancing Fertilizer Effect of Bioprocessed Brewers' Spent Grain by Microbial Consortium Addition

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Abstract: Brewers' spent grain (BSG) is primarily recycled as livestock feed due to its high fiber content, undegradable protein, and water-soluble vitamins. However, BSG composting represents a possible alternative to organic waste management. Adding a microbial consortium further enhances the agronomical properties of the compost intended for fertilizing applications. Microbial-based fertilizers (plant growth-promoting microorganisms, PGPM) are a means to mitigate the adverse environmental impacts of excessive or improper chemical fertilizer use, enhance the direct or indirect uptake of nutrients by plants, and add value to food waste. In a short-term pot experiment on iceberg lettuce (*Lactuca sativa* L.), this study assessed the effects of compost and pelletized compost from brewers' spent grain, both enriched with a microbial consortium. In a randomized block experiment, this study compared four organic BSG fertilizers to chemical fertilizer (NPK) and an unfertilized control treatment. The investigation indicates that BSG compost and BSG pelleted compost, with and without bio-inoculum, in general, are comparable to mineral fertilizer treatment; lettuce fresh weight was higher in pots amended with bioprocessed BSG, associated with more significant growth of soil LAB, fungi, and actinomycetes. The investigation outcomes support composting as an alternative recycling process for producing PGM for agricultural applications.

Keywords: composting; agro-industry by-product; BSG; bio-waste; pelletizing; circular economy; nutrients; inoculants; crop production

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1. Introduction

In the past decades, to achieve higher crop yields, large amounts of chemical fertilizer were applied [1]. This intensive management caused a set of negative impacts on soil, air, and water resources. Indeed, an excessive use of chemical fertilizers can lead to the degradation of soil organic carbon (SOC) and humus, the destruction of soil structure, an increase in soil erosion, biodiversity and nutrient losses, and, therefore, to a lower soil fertility, putting the ecosystem's sustainability at risk [2].

In the era of eco-sustainability, it is crucial to recognize the importance of treating the by-products of the agri-food industry in a sustainable manner, transforming what could be considered waste into a valuable resource [3]. Recently, to face climatic changes, a new business model based on the recovery and reuse of resources and waste recycling (RRR) has been defined, able to overcome the traditional linear economic model of "take, make, and dispose" [4].

The brewery industry produces large quantities of by-products, typically spent hops, yeast, and spent grain. Brewers' spent grain (BSG) accounts for 85% of the total by-products

generated during the brewing process [5], with an estimated annual worldwide production of about 37.2 million tons [6]. Nowadays, the main way of recycling BSG is through livestock feed production, due to its high content in fiber, undegradable protein, and water-soluble vitamins [7].

According to several authors [8–10], the composting process represents an efficient alternative for organic waste management, allowing for the reduction of landfill disposal and, at the same time, recycling its agronomic macronutrient content (N, P, and K) by applying the composted material to agricultural lands. The integration of compost into agricultural systems not only satisfies the waste minimization scenario proposed by major world governments, but also provides positive influences on the overall soil fertility. Indeed, it improves the SOC content directly and thus improves aggregation, hydraulic conductivity, total porosity [11], and the cation exchange capacity (CEC) [12], supplies a wide range of nutrients (N, P, K, Ca, Mg, etc.), improves the soil's physicochemical and biological characteristics, enhances the soil quality and fertility, [13] and, finally, is able to increase yields after several crop cycles [14].

The compost can be used efficiently in both organic farming and conventional farming as a constituent of a system of integrated fertilization. However, the main shortcomings of common organic amendments are the slow nutrient release, the low macronutrient availability [15], and the low bulk density, which is usually below 400 kg m^{-3} [16]. Therefore, Pampuro et al. 2018 [17] to promote the sustainable development of agriculture, suggest compacting the compost through the pelletizing process, to homogenize and further dehydrate the mass, enhancing both its uniformity and fertilizing/amending properties and, at the same time, increasing the distance that can be run in case of transport. On the other hand, Atieno et al., (2020) [18] reported the importance of using microbial-based fertilizers to overcome the deleterious effects on the environment generated by the excessive and/or improper application of chemical fertilizers and to improve the direct and/or indirect absorption of plant nutrients. Yassen et al. (2020) [19] reported that leafy vegetables, such as lettuce (*Lactuca sativa* L.) and spinach (*Spinacia oleracea*), respond well to organic fertilizers. Moreover, as reported by Radziemska et al. (2019) [20], according to test results, using lettuce presents several advantages; indeed, it is quick, simple, reliable, inexpensive, and does not require major equipment.

This study aimed to assess the effects of compost and pelletized compost derived from brewers' spent grain [21], both enriched with a microbial consortium, on lettuce plants in a short-term pot experiment. These organic fertilizers were compared to an NPK ternary fertilizer and to unfertilized soil used as the control treatment. We formulated several hypotheses: (i) compost derived from BSG can have a significant short-term benefit as a fertilizer (not just as an amendment); (ii) the pelletization process does not compromise the characteristics of the compost (both enriched and non-enriched); and (iii) the application of organic amendments, particularly enriched compost, leads to a more active and diverse soil microbial community compared to NPK fertilization, resulting in improved soil quality.

2. Materials and Methods

2.1. Site and Soil Description

This experiment was established under greenhouse conditions during spring 2022 at the Institute of Sciences and Technologies for Sustainable Energy and Mobility (STEMS), Italian National Research Council (CNR), in Turin, Italy (44°57′ N, 7°36′ E, 245 m above sea level).

The trial was performed using pots with a 30 cm diameter, each containing 15 kg of soil [22] and one plant of lettuce (*Lactuca sativa* L. var. iceberg). The soil was collected from the surface horizon (0–30 cm) and analyzed for physical and chemical properties. The soil was characterized by a sandy loam texture (61.7% sand, 28.4% silt, and 9.9% clay), containing 7.9 g kg^{-1} of organic matter, 0.40 g kg^{-1} of N, 11.5 mg kg^{-1} of Olsen available phosphorus (P), and 65.5 mg kg^{-1} of exchangeable potassium (K) with a pH of 8.8, measured according to the Official Methods, (1999) [23]. The cation exchange capacity

(CEC) was 8.25 meq 100 g^{-1} of dry soil, with 1.01 and 6.4 meq 100 g^{-1} of magnesium and calcium, respectively. During the whole experiment, the pots were watered regularly with distilled water to ensure that the water content was maintained around 70% of field capacity [24].

A HOBO data logger (USB micro data logger, ONSET Corp., Cape Cod, MA, USA) equipped with sensors for measuring photosynthetically active radiation (PAR), temperature, and relative humidity, was utilized throughout the whole crop cycle. The maximum temperature recorded during the day was 41.1 °C, while the minimum was 11.5 °C, recorded at night. A maximum relative humidity of 96.2% and minimum of 23.5% were recorded at night and during the day, respectively. Finally, the maximum PAR was about 681 µmol m^{-2} s^{-1}, with a minimum of about 92 µmol m^{-2} s^{-1} and an overall average of about 466 µmol m^{-2} s^{-1}.

2.2. Experimental Design

In this randomized block experiment, with four replicates, six different treatments were compared (Figure 1). The treatments included four organic fertilizers: compost (COM), compost enriched with a microbial consortium (COM+), pelleted compost (P6), and pelleted compost enriched with a microbial consortium (P6+), along with an unfertilized control treatment (TEST) and a ternary chemical fertilizer (CF, 21-8-16), which was similar to the fertilizer used in a previous study by Zandvakili et al., (2019) [25]. These organic fertilizers were produced using a co-composting process of pig slurry solid fraction and brewers' spent grain (BSG), kindly supplied by a brewery in the Province of Biella (Piedmont, Italy), as detailed in Assandri et al., (2021) [21]. Subsequently, the compost was pelletized using a laboratory-scale PLT-100 SMARTEC (Smartwood, Villafalletto City, Cuneo, Italy) flat die pellet mill with a diameter of 6 mm. The maximum temperature reached by the pellets during the pelleting process was 41.5 °C, while the flat die reached a maximum temperature of 37.6 °C.

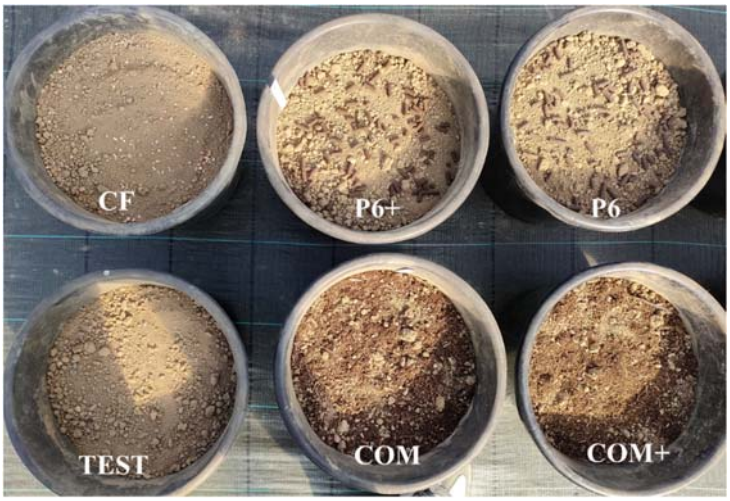

Figure 1. Details of organic fertilization before the incorporation into the soil and of the transplanting of the plants. CF—chemical fertilizer; P6—pelleted compost; P6+— enriched pelleted compost; COM—compost; COM+— enriched compost; TEST—not fertilized.

The pots were fertilized at a consistent nitrogen application rate (equivalent to 120 kg N ha^{-1}) [26]. The total nitrogen content of the organic fertilizer was about 1.91%. Therefore, 4 g pot^{-1} and 44.4 g pot^{-1} were used in the chemical fertilizer treatment (CF) and organic fertilizer treatments, respectively. Furthermore, 8 g pot^{-1} of a solution consisting of a microbial consortium composed of rhizospheric bacteria and water in a ratio of 1:20 was distributed via spray on the COM+ and P6+ treatments.

2.3. Plant Analyses

The plants were harvested 47 days after transplanting. Both yield and quality parameters were evaluated. The parameters of the phenological development stage of the plants (BBCH scale—Biologische Bundesantalt, Bundessortenamt, and CHemische Industrie, Berlin, Germany), leaf area index (LAI), and chlorophyll content were measured by using an Apogee MC-100 chlorophyll concentration meter during culture growth, and the results were expressed as the average of ten readings of different plants.

Image J 1.41 software (https://rsb.info.nih.gov/ij/, accessed on 17 September 2023) was employed for LAI analysis [27]. Additionally, measurements of plant height (cm), plant width (cm), leaf count per plant, and fresh and dry weight (g per plant) were taken at harvest time. The plant samples were cleaned, rinsed with distilled water, dried in an oven at 60 °C until a constant weight was achieved, and then stored for chemical analysis.

To determine the total N, P, and K content in the plant samples, the samples underwent digestion using a mixture comprising 350 mL of H_2O_2, 0.42 g of selenium powder, 14 g of $LiSO_4$ H_2O, and 420 mL of concentrated H_2SO_4 [28]. For total N determination, 5 mL of the digested sample was distilled with 20 mL of 40% sodium hydroxide, using a micro Kjeldahl's distilling unit, into an Erlenmeyer flask containing 10 mL of boric acid mixed indicator solution. After distillation, the total N content was determined in the distillate through titration with a standardized 0.01 of N sulfuric acid [29]. The total phosphorus content was determined spectrophotometrically using the stannous chloride phosphor molybdic acid method in a sulfuric acid system. The total K content was determined using the flame photometer method [22].

The content of chlorophyll a, b, ab (mg g^{-1}), carotenoids (mg g^{-1}), phenolic compounds (ppm of fresh weight), nitrates (ppm of fresh weight), and brix degrees was also measured after harvest. The chlorophyll and carotenoid contents were measured in the same way as reported by Slamet et al., (2017) [30]. Specifically, fresh leaves were ground in a mortar to obtain 500 mg, and then 2 mL of 80% acetone was added to the mixture. Subsequently, 10 mL of 80% acetone solution was added, and the resulting solution was filtered using Whatman paper 42. Three milliliters of the filtrate were transferred into a cuvette, and its absorbance was measured using a spectrophotometer. The spectrophotometric chlorophyll assay was conducted at wavelengths of 663 nm (A663) and 645 nm (A645). The chlorophyll concentration was determined using the following formula:

$$\text{Total chlorophyll} = 8.02\ (\text{A. } 663) + 20.2\ (\text{A. } 645)\ \text{mg l}^{-1} \quad (1)$$

The contents of the phenolic compounds and nitrates was determined spectrophotometrically using the Folin–Ciocalteu reagent [31] and the Griess–Ilosvay reagent [32], respectively. Finally, the °Brix was measured directly using a digital refractometer (Hanna Instruments, Padova, Italy) at room temperature.

2.4. Efficiency Coefficients

The nitrogen efficiency coefficients were calculated as follows:

- R/F, or removal to fertilizer ratio, was calculated according to the following equation:

$$\text{R/F} = \frac{\text{Nremoval}}{\text{Nfertilizer}} \quad (2)$$

  where Nremoval is the nitrogen taken up as yield, and Nfertilizer is the total quantity of nitrogen applied.
- AR, or apparent recovery, was calculated as suggested by Zavattaro et al., (2016) [33]:

$$\text{AR} = \frac{\text{Nremoval} - \text{Nremoval TEST}}{\text{Nfertilizer}} \quad (3)$$

where Nremoval and NremovalTEST are the amounts of N removed by a fertilized treatment and the TEST (not fertilized) treatment, respectively.

2.5. Microbiological Analysis

Ten grams of each fertilizer, bare soil, and soil amended with the different fertilizers were dissolved in 90 mL of sterile distilled water, homogenized for 2 h, and serially diluted in peptone water. From these samples, the total population of cultivable fungi and bacteria was estimated after plating on Wallerstein Laboratory Nutrient agar (WL; VWR International Srl, Milan, Italy) + 0.01% chloramphenicol, and brain heart infusion (BHI; VWR International Srl, Milan, Italy) + 0.01% cycloheximide, after incubation for 48 h at 26 ± 2 °C and 30 ± 2 °C, respectively. The population of *Lactobacillaceae* was estimated on Man Rogosa and Sharpe (MRS; VWR International S.r.l., Milan, Italy) agar plates added with 0.01% cycloheximide after 48 h of incubation at 30 ± 2 °C. Cultivable *Actinomycetes* spp. and *Pseudomonas* spp. were determined on Actinomycete Isolation agar (AIA, Millipore and Sigma-Aldrich, Oakville, ON, Canada; St. Louis, MO, USA) and Pseudomonas Selective agar (PSA, VWR International Srl, Milan, Italy), respectively, after incubation for 48 h at 30 ± 2 °C.

The enumeration of *Escherichia coli* was performed on Tryptone Bile X-GLUC agar plates (TBX, Microbiol, Cagliari, Italy) after incubation at 37 °C for 4 h, followed by incubation at 44 °C for 21 ± 3 h [34]. The contamination by Salmonella spp. was evaluated as previously described [35]. Briefly, 25 g of vermicompost were suspended in 225 mL of peptone water at 37 °C for 18 h, and *Salmonella* spp. enrichment was carried out in Rappaport Vassiliadis broth (RVS, Microbiol, Cagliari, Italy) and in Muller Kauffmann tetrathionate/novobiocin broth (MKTTn, Microbiol, Cagliari, Italy) for 24 h at 42 °C and 37 °C, respectively. Finally, the enriched samples were streaked on xylose lysine deoxycholate (XLD) agar and in Salmonella Shigella agar and incubated at 37 °C for 24 h.

Microbiological analyses were carried out in triplicate for each sample and are reported as colony-forming units (CFU) × dilution factor × sample weight (g^{-1}).

2.6. Statistical Analysis

All data were analyzed by a one-way analysis of variance (ANOVA), performed using R statistic software (R-4.0.3). Differences between means were compared using a Bonferroni post hoc test at $p = 0.05$. The data were previously tested for normal distribution using the Shapiro–Wilk test, and the homoscedasticity using Levene's test. The results are presented as mean ± standard deviation (s.d.). The differences among microbial communities were evaluated by computing the standardized mean difference (Cohen's d) and the confidence intervals between the control (TEST) and treatments, as implemented in the R package "effsize" (ver.0.8.1).

3. Results

3.1. Plant Growth

The chlorophyll concentration, measured by MC-100, did not show significant differences between treatments, while there was a significant effect on all other parameters (Table 1). In detail, lettuce plants showed higher growth characteristics in the fertilized whit compost (COM) than those grown in the TEST treatment ($p < 0.05$). The plants fertilized with enriched compost (COM+) showed no differences with those fertilized with the COM or CF treatments ($p > 0.05$); however, the statistical analysis showed significant differences between the COM and CF treatments in fresh weight parameter. The treatments with pelleted compost (P6) and enriched pelleted compost (P6+) showed no significative differences between them ($p > 0.05$), but intermediate growth characteristics between the COM and TEST treatments, with significant differences in both fresh weight and BBCH parameters, with only differences in the LAI parameter and leaf number for P6 and P6+, respectively.

Table 1. Growth characteristics of lettuce plants. Values represent the mean (n = 4) ± standard deviation. Letters a, b and c indicate significant differences between treatments in the post-hoc Bonferroni.

Parameter	Unit	Treatments						F-Value	*p*-Value
		COM	COM+	CF	P6	P6+	TEST		
Diameter	(cm)	28 ^{a} ± 0.85	27 ^{a} ± 0.85	27 ^{a} ± 0.63	26 ^{a} ± 0.50	27 ^{a} ± 0.75	22 ^{b} ± 0.00	34.53	0.000
MC-100		12.2 ^{a} ± 2.45	10.6 ^{a} ± 2.69	10.1 ^{a} ± 1.84	11.0 ^{a} ± 2.59	9.8 ^{a} ± 1.28	10.9 ^{a} ± 0.85	0.66	0.66
BBCH		49 ^{a} ± 0.00	48 ^{ab} ± 0.50	48 ^{ab} ± 0.82	47 ^{b} ± 0.58	48 ^{b} ± 0.58	44 ^{c} ± 0.00	39.75	0.000
Fresh weight	(g plant^{-1})	461.8 ^{a} ± 67.4	331.1 ^{ab} ± 61.9	311.9 ^{b} ± 48.2	273.5 ^{bc} ± 26.4	264.0 ^{bc} ± 50.0	155.3 ^{c} ± 42.0	11.78	0.000
Leaf	(number)	29 ^{a} ± 2.65	26 ^{ab} ± 0.00	28 ^{ab} ± 0.58	26 ^{ab} ± 1.15	25 ^{b} ± 0.96	22 ^{c} ± 1.15	11.59	0.000
Dry matter	(% g plant^{-1})	0.06 ^{b} ± 0.01	0.08 ^{ab} ± 0.01	0.07 ^{ab} ± 0.01	0.07 ^{ab} ± 0.01	0.08 ^{ab} ± 0.00	0.08 ^{a} ± 0.01	3.85	0.02
LAI	(cm^{2})	630 ^{a} ± 32.2	551 ^{ab} ± 33.6	607 ^{ab} ± 50.7	523 ^{b} ± 13.1	567 ^{ab} ± 33.0	418 ^{c} ± 7.1	16.65	0.000

Apogee MC-100 chlorophyll concentration meter; BBCH—phenological development stages of plants; LAI—leaf area index.

The parameters of diameter, BBCH, number of leaves, and LAI were lower ($p < 0.05$) in plants grown in the TEST treatment than those grown in all other treatments. Considering the fresh weight parameter, significant differences were found between the COM, COM+, and CF treatments, while in the dry matter parameter, the TEST treatment only resulted higher than and significantly different from the COM treatment.

3.2. Quality Characteristic

Significant differences were found in Chl. A, carotenoid content, °Brix, C, and K, while there was no significant effect on Chl. B, phenolic content, NO_3-N, N, and P (Table 2).

Table 2. Quality characteristics of lettuce plants. Values represent the mean (n = 4) ± standard deviation. Letters a, b, c and d indicate significant differences between treatments in the post-hoc Bonferroni.

Parameter	Unit	Treatments						F-Value	*p*-Value
		COM	COM+	CF	P6	P6+	TEST		
Chl. A	(mg g^{-1})	0.61 ab ± 0.09	0.56 ab ± 0.14	0.56 ab ± 0.07	0.89 a ± 0.07	0.55 ab ± 0.16	0.46 b ± 0.22	3.64	0.03
Chl. B	(mg g^{-1})	0.24 a ± 0.07	0.19 a ± 0.05	0.19 a ± 0.03	0.28 a ± 0.09	0.15 a ± 0.09	0.21 a ± 0.11	1.27	0.32
Caroten.	(mg g^{-1})	43.2 ab ± 12.4	27.0 b ± 8.9	30.5 ab ± 6.7	49.9 a ± 3.9	33.5 ab ± 8.1	21.9 b ± 8.3	4.87	0.008
Phenolic	(µg g^{-1} FW)	242.1 a ± 18.2	247.5 a ± 38.4	249.3 a ± 32.9	209.6 a ± 23.6	262.0 a ± 33.7	199.8 a ± 47.9	2.02	0.13
NO3	(mg kg^{-1} FW)	22.9 a ± 6.2	25.2 a ± 4.3	24.3 a ± 4.3	18.2 a ± 7.7	21.5 a ± 5.6	27.1 a ± 2.8	1.31	0.31
°Brix		4.00 bc ± 0.44	6.50 a ± 0.68	5.06 b ± 0.25	3.33 cd ± 0.33	3.42 c ± 0.62	2.17 d ± 0.30	37.54	0.000
N	(% DM)	1.66 a ± 0.13	1.62 a ± 0.26	1.96 a ± 0.14	1.73 a ± 0.25	1.88 a ± 0.16	1.58 a ± 0.24	2.32	0.09
C	(% DM)	39.4 ab ± 0.16	39.6 a ± 0.50	38.9 ab ± 0.11	39.2 ab ± 0.32	39.2 ab ± 0.24	38.6 b ± 0.28	4.50	0.009
P	(% DM)	0.32 a ± 0.02	0.30 a ± 0.02	0.32 a ± 0.02	0.31 a ± 0.02	0.32 a ± 0.01	0.31 a ± 0.01	1.06	0.41
K	(% DM)	2.49 b ± 0.13	1.97 c ± 0.11	3.27 a ± 0.25	3.44 a ± 0.14	3.51 a ± 0.20	3.36 a ± 0.38	35.25	0.000
R/F		0.49 a ± 0.07	0.45 a ± 0.12	0.53 a ± 0.10	0.44 a ± 0.19	0.46 a ± 0.08		0.35	0.84
AR		0.23 a ± 0.06	0.20 a ± 0.05	0.27 a ± 0.06	0.18 a ± 0.12	0.20 a ± 0.15		0.54	0.71

R/F—removal to fertilizer ratio; AR—apparent recovery.

The nitrogen efficiency coefficients (R/F and AR) did not show significant differences, probably due to the same N content between treatments.

Considering the qualitative parameters, the P6 and P6+ treatments did not show significant differences between them ($p > 0.05$), highlighting low °Brix values, with the exception of the plants grown in the TEST treatment, which showed the lowest °Brix values.

The differences between COM and COM+ emerged in the °Brix and K content, the first higher in COM+ than COM, while inverted for the second parameter.

Statistical analysis showed significant differences between the organic fertilizer and CF treatments in the °Brix and K content parameters. In detail, the higher values of °Brix were found in COM+, followed by CF and COM, while higher values of K content were found in all treatments, except in COM and COM+, with their whit values being intermediate and the lowest, respectively.

3.3. Microbial Growth

The different substrates used as fertilizers showed different microbial loads (Figure 2).

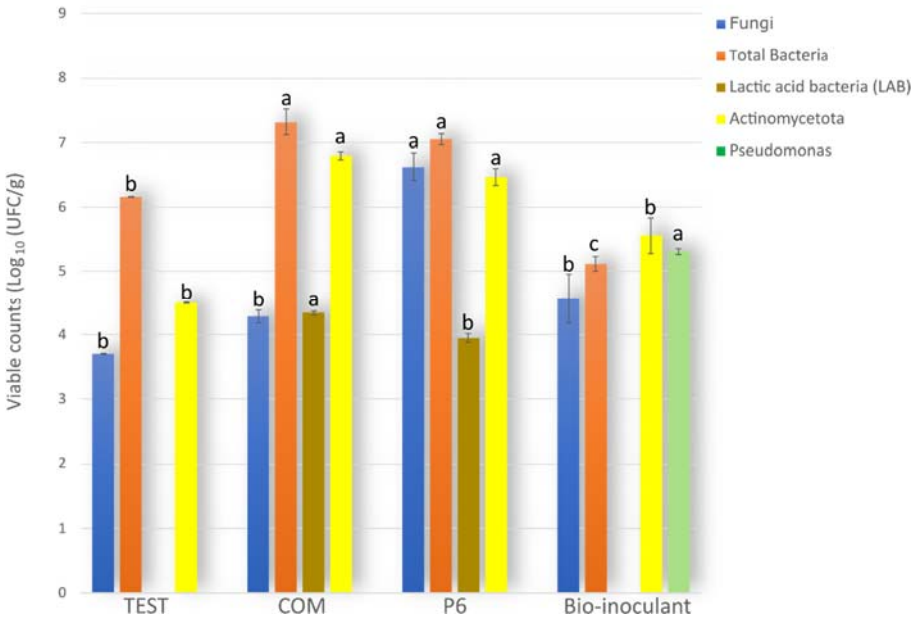

Figure 2. Viable counts of microbial groups in the different substrates. For each microbial group, different letters indicate significant differences among substrates, as determined by ANOVA followed by Bonferroni post hoc test ($p < 0.05$).

Particularly, the total bacteria, lactic acid bacteria (LAB) and *Actinomycetes*, were detected at significantly higher populations in the COM and P6 fertilizers. Interestingly, compost pelletizing had a significant effect on the abundance of fungi, which were detected at significantly higher concentrations in P6. The bio-inoculant was characterized by the presence of *Pseudomonas* spp. and the absence of LAB.

To evaluate the effect of the addition of the different fertilizers, the microbial communities of the bare soil and treated soils (CF, COM, COM+, P6, and P6+) were evaluated before lettuce transplanting and after lettuce harvesting (Figure 3).

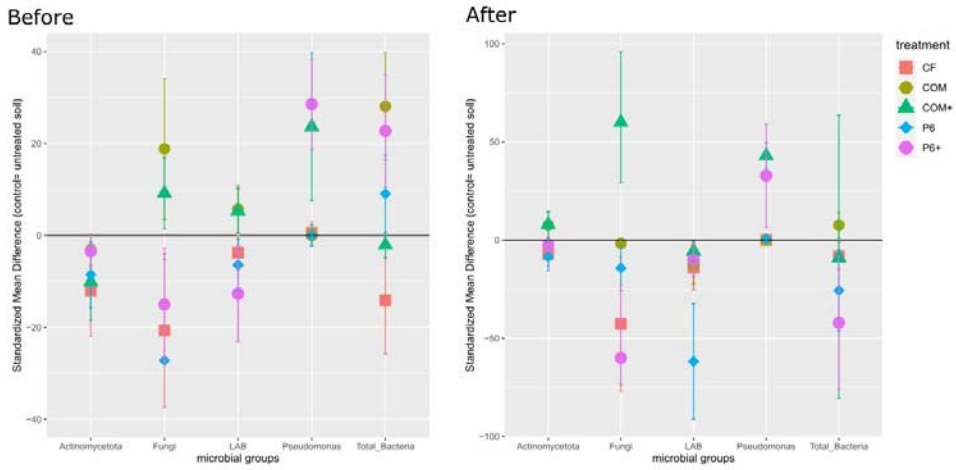

Figure 3. Standardized mean difference effect sizes (95% CI) before lettuce transplanting (Before) and after lettuce harvesting (After). For each microbial group, the effect of each fertilizer was determined in comparison to the untreated soil: values > 0 indicate viable counts higher in fertilized than in untreated soil.

Overall, the chemical fertilization before lettuce transplanting reduced the microbial abundance of the soil, the standardized mean difference in the CF samples always being lower than zero. A negative effect on the viable microbial counts was also observed following P6 addition to the bare soil. On the contrary, the fungal and bacterial counts were higher in the soil treated with the compost (COM) than in the TEST soil. A stimulating effect was particularly evident following the addition of the bio-inoculant to the compost and the pelleted compost. Indeed, the total bacterial population and particularly *Pseudomonas* counts were higher in soil fertilized with COM+ and P6+ than in all the other treatments. The ability of the analyzed microbial communities to thrive and establish in soil was evaluated by analyzing fertilized and untreated soil samples after lettuce harvest. At this time point (47 days), the abundance of all the microbial groups was generally higher in the TEST pot than in the fertilized pots. An exception was represented by the COM+ treatment that resulted in higher viable fungal and *Pseudomonas* counts. Similarly, high *Pseudomonas* counts were observed in P6+, suggesting the significant effect of the addition of the bio-inoculant to both the composted and pelleted BSG, in providing microbial taxa more adapted to the soil environment.

4. Discussion

Chemical fertilizers offer quick results after application, but their continuous use and overexploitation can result in numerous environmental issues, such as soil degradation, leading to long-term damage to agricultural lands. In contrast, organic fertilizers release nutrients gradually, allowing plants to absorb them in accordance with their nutritional needs, while simultaneously reducing the risk of nutrient leaching. The nitrogen use efficiency coefficients (R/F and AR) are influenced by the nitrogen supply. The R/F indicator quantifies the amount of nitrogen removed by the plant per unit of fertilizer. A value exceeding 1.00 is attainable when the plant relies on natural reserves from soil mineralization. Conversely, values below 1.00 arise when the plant's nitrogen needs are satisfied by the nitrogen supplied through fertilization. The AR coefficient offers a more comprehensive description of nitrogen use efficiency, as it estimates soil supply based on the control and then subtracts it. Both coefficients reveal low efficiency, underscoring reduced nitrogen uptake by plants.

Soils are viewed as complex communities of organisms that are continually changing in response to soil characteristics, climatic and management factors, and especially in response to the addition of organic matter [36].

Despite the short duration of the crop cycle (47 days), the synergy between the organic fertilizers and PGPR (plant growth-promoting rhizobacteria) showed positive effects on the growth, yield, and quality characteristics of lettuce plants. This could also be ascribed to the total nitrogen content (about 2%) in the BSG compost, which made the nitrogen readily available for microbial growth without depleting it from the plants' initial needs.

The chlorophyll physiological adjustments induced by the FCA treatment appeared to be linked to an increase in photosynthesis, as the proteins related to this metabolism accumulated in the shoots.

A recent study showed that the PGPR inoculation of lettuce increased productivity and mineral uptake, resistance to salt stresses, and root development [37]. Angelina et al. (2020) [38] showed that management history of the soil (the substrates of lettuce growth) influences the effect of microbial inoculation. Here, composted and pelleted BSG samples were enriched in a microbial consortium consisting of bacteria (including LAB and actinomycetes), fungi, and yeasts, in accordance with the already observed increased occurrence of microbial taxa during the composting process [39]. These microbial groups have been considered among plant growth-promoting microorganisms (PGPM). Particularly, LAB can regulate phosphate in soil, fix atmospheric nitrogen, act as biocontrol agents, and increase the shelf life of the amendment [40]. Yeasts have been described as antagonists of various plant pathogens and are currently considered for biocontrol applications [41]. Thus, it is conceivable that composted and pelleted BSGs have a microbial

consortium that is stable and effective on lettuce growth. A recent study [42] showed that the core bacterial community of compost is represented by the ubiquitous *Thermobifida*, while *Lactobacillus* prefers vegetable waste.

Pseudomonas, which was not detected in the control soil or in the composted and pelleted BSG samples, but was included in the bioinoculant, plays an important role in improving crop yield directly or indirectly. Particularly, *Pseudomonas* spp. and *Bacillus* spp. are the most widely known plant growth-promoting rhizobacteria (PGPR), frequently commercialized as they can survive different conditions and promote plant accessibility to macronutrients during root colonization. Among the various PGPR genera, *Pseudomonas* is considered an ideal bioinoculant due to its ability to promote plant growth and control phytopathogen occurrence. These properties are related to siderophore production, phosphate solubilization, nitrogen fixation, antibiotic synthesis, and the induction of plant systemic resistance. In addition, *Pseudomonas* regulates plant growth and phytopathogen resistance by secreting phytohormones (auxins and gibberellins), secondary metabolites (flavonoids), and enzymes (aminocyclopropane-1-carboxylate and phenylalanine ammonia-lyase) [43]. These activities have been described in detail in *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas syringae* [43].

5. Conclusions

Organic fertilizers play a vital role in organic and sustainable agriculture as alternatives to inorganic fertilizers. This study found that both BSG composts and enriched composts performed equally well in qualitative parameters, and even better when bio-inoculum was present, likely because of the increased growth of beneficial microorganisms. As a result, these findings endorse the ongoing use of BSG composts in agriculture, contributing to food waste recycling efforts.

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