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Lab- and Pilot-Scale Effects of Spirulina (*Limnospira* sp.) Biomass Produced from Brewery Wastewater Treatment as a Biofertilizer for Barley (*Hordeum vulgare*) in Passo Fundo, Brazil

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Abstract

Microalgae have been proposed for the bioremediation of wastewater, as well as for biofertilization and biostimulation of several plant species. This study used *Limnospira* sp. biomass produced in brewery wastewater to formulate a pelletized biofertilizer. Its efficacy in promoting barley (*Hordeum vulgare*) growth was then compared with chemical fertilizers and a control group without fertilization on lab- and pilot-scale setups. On a 100-day lab-scale experiment under controlled light (260–280 µmol photons m^{-2} s^{-1}) and temperature (20 ± 2 °C), minor differences in plant growth were observed, whereas the elemental composition of the barley plants did not differ, including toxic elements. On a pilot-scale agricultural setup (5 m^2) under environmental conditions, barley productivity, protein content, and the percentage of class I grains (diameter ≥ 2.5 mm) significantly increased based

on the different dressing techniques used ($p < 0.05$). Using the microalgae-based biofertilizer for both base and top dressing increased productivity, protein content, and grain size (% class I) by 26.9%, 14.4%, and 8.78%, respectively, compared to using chemical fertilizers (NPK 5:20:20 and urea). These results indicate the great potential of using microalgae biomass from wastewater treatment as biofertilizer for more sustainable agriculture.

Keywords: phycology; microalgae; cyanobacteria; bioremediation; sustainable agriculture; agricultural fertilizer

1. Introduction

Over the last few years, conventional agriculture has been facing major challenges, such as the growing global demand for food, the environmental impact of agricultural intensification, and the uncertainty of future climate changes [1]. More sustainable agricultural practices and technologies aim to achieve the United Nations' Sustainable Development Goals (SDGs) by reducing chemical inputs and improving crop yield, among other strategies [2]. Thus, the market for biofertilizers and biostimulants has been growing rapidly, with a compound annual growth rate (CAGR) of 12.1% and is predicted to reach USD 5.6 billion by 2026 [3]. This growth rate is higher than that estimated for inorganic chemical fertilizers, which have a CAGR of about 1.5%.

Algae have received particular attention among the feedstocks commonly used as biofertilizers and biostimulants because (i) their cultivation does not require arable land, (ii) they have higher growth rates and carbon fixation efficiency than conventional crops, (iii) their biochemical composition is rich in nutritive and bioactive substances, and (iv) their biomass production can be coupled with wastewater treatment, which increases the sustainability and circularity of the entire process [4–6]. In fact, macroalgae, eukaryotic microalgae, and prokaryotic microalgae (cyanobacteria) have been extensively used in agriculture, positively affecting nutrient uptake, rooting, and tolerance to a wide array of biotic and abiotic stresses [3]. In several field and controlled trials, the performance of microalgae biofertilizers has been comparable to that of traditional fertilizers while producing distinct outcomes in crop quality and environmental parameters [7–10]. In wheat, for example, the aboveground biomass produced by algae-based fertilization is similar to that produced by urea; however, grain nitrogen is lower [7]. Yields in other crops depend on the application methods and dosage. The quality metrics of algae-fertilized crops often improve, although some outcomes are less favorable than those of conventional treatments, such as the lower crude protein in maize or wheat [8]. Environmental assessments suggest that microalgae applications can reduce greenhouse gas emissions. However, the literature presents contradictory results. For example, while Sherstra et al. [7] reported a two- to five-fold reduction in nitrogen oxide emissions from microalgae fertilization, Suleiman et al. [9] observed 4.6 times higher nitrogen oxide emissions using microalgae fertilizer. Combining microalgae with other fertilizers and using wastewater-derived microalgae biomass are novel approaches that have reportedly improved yields, reduced nitrogen losses, and saved energy costs [10].

The advantages of using wastewater for microalgae production include saving resources from the formulation of conventional mineral growth media, removing organic and inorganic contaminants from the wastewater, and producing microalgal biomass that can be used for other purposes [11]. Microalgae grown in wastewater are known for their ability to remove contaminants through mechanisms such as adsorption, direct or indirect photodegradation, bioaccumulation, biodegradation, volatilization, and hydrolysis [12,13].

However, some of these mechanisms result in the complete degradation of contaminants, while others lead to the accumulation of potentially toxic compounds in the biomass, which compromises its use in other sectors. For example, in order to be used as an agricultural biofertilizer, the resulting biomass must be chemically and biologically safe and must not lead to the accumulation of toxic elements, such as heavy metals, in the final crop. Despite the growing interest in using microalgae biomass from wastewater treatment as biofertilizer, significant gaps remain in understanding the potential transmission of toxic elements to the fertilized crop [14].

Of the microalgae species reported in previous studies, cyanobacteria belonging to the genus *Limnospira* (commonly known as Spirulina) are notable. Their biomass contains high amounts of proteins and bioactive substances, and they are the most widely produced and commercialized microalgae on the planet [15]. *Limnospira* spp. have been consistently used in the food, feed, and nutraceutical industries. Several studies have demonstrated their antiviral, anti-inflammatory, and antioxidant activities, as well as their potential to improve the zootechnical performance of a wide array of livestock [16–19]. Recently, our group showed the high potential of *Limnospira* sp. in treating brewery wastewater, and its wastewater-derived biomass promoted barley growth in preliminary lab-scale tests [20].

Building on these previous results, this study focused on developing a standardized biofertilizer based on wastewater-derived *Limnospira* sp. biomass and investigating its effects on lab- and pilot-scale cultivation of barley (*Hordeum vulgare*). Based on a literature review and our previous results, we hypothesized that (i) the *Limnospira* sp.-based biofertilizer could stimulate *H. vulgare* growth similarly to commonly used chemical fertilizers; (ii) results could vary between controlled lab-scale experiments and pilot-scale setups, under environmental conditions closer to real agricultural scenarios; and (iii) *Limnospira* sp. biomass produced in brewery wastewater could accumulate toxic elements, rendering it unsuitable for agricultural use. Here, we initially characterized the soil of Passo Fundo (Brazil), a region well known for barley cultivation. The soil was then used for lab-scale experiments under controlled conditions for 100 days. During this time, the growth and elemental composition of the plants were determined and compared to a control group (no fertilizer) and plants fertilized with NPK. Finally, a pilot-scale cultivation of barley (on-field 5 m^2 per treatment) was conducted according to all recommended practices [21]. This experiment aimed to compare the effects of different combinations of base and top dressing fertilization on the biochemical composition, morphology, and productivity of *H. vulgare* under environmental conditions.

2. Materials and Methods

2.1. Microalgae-Based Biofertilizer

Limnospira sp. was kindly provided by the Laboratory of Algal Biochemistry and Biotechnology (LBBAlgas), in the Chemistry Institute of the Federal University of Rio de Janeiro, Brazil. This strain was selected due to its previously reported fast and robust growth, enhanced brewery wastewater treatment capacity, and positive effects on *H. vulgare* initial growth [20]. The microalgal biomass was produced in 1.55 m^2 glass fiber open raceway ponds containing untreated brewery wastewater. The biomass was harvested by filtration and sun-dried, as described by Lima e Silva et al. [20]. The dry biomass was then used as the main ingredient and nitrogen source of a pelletized biofertilizer. The biofertilizer was produced by extruding a mix of *Limnospira* sp. biomass, malt bagasse as a structuring material, and water in a HAAKE^{TM} Rheomex PTW 16 twin-screw extruder (Thermo Fischer Scientific, Waltham, MA, USA) at room temperature. The ingredients mixture was calculated to achieve a final nitrogen concentration of 4% (m/m). The approximate final

NPK ratio of the biofertilizer was 4:0.6:0.6. The final NPK ratio was not planned, but rather a result of the ingredients mixed to achieve the desired nitrogen concentration.

2.2. Soil Characterization

The soil sample, obtained from an unidentified horizon in Passo Fundo, Rio Grande do Sul (RS), Brazil (28°14′28″ S, 52°28′32″ W), was characterized and used for lab-scale investigation of barley growth. The soil sample was subjected to granulometric classification through serial sieving, using meshes with pore sizes of 0.211, 0.053, and 0.044 mm. The minerals in the sample and their textural characteristics were determined using a scanning electron microscope (Hitachi TM3030 Plus, Tokyo, Japan) equipped with an energy dispersive X-ray spectroscopy system (Bruker Quantax, Billerica, MA, USA). Prior to the analysis, the sample was metallized with silver, which resulted in better conductivity and imaging quality than other metals in preliminary tests. X-ray diffractograms were obtained using Bruker-D8 Endeavor under the following conditions: CuKα radiation (40 kV/40 mA), a goniometer speed of 0.02° 2q per step, a counting time of 0.5 s per step, and data collection from 4 to 80° 2q using a position-sensitive detector (LynxEye, Stockholm, Sweden). Qualitative spectrum interpretations were performed by comparing them with the standards contained in the PDF02 database [22] using Bruker DifracionPlus software (DIFFRAC.SUITE11 package).

Chemical analyses were performed using X-ray fluorescence on a PANalytical Axios Max spectrometer (Arlington, VA, USA). The samples were fused with lithium tetraborate at 1000 °C in a 1:10 ratio in a fusion machine (PANalytical EAGON 2, Arlington, VA, USA). Lithium iodide was used as a mold release agent (0.1 g). Loss-on-ignition tests were performed on a LECO TGA-701 (Duisburg, Germany) at 1000 °C using 1 g of the sample.

2.3. Lab-Scale Barley Cultivation and Characterization

Barley (*H. vulgare*) was cultivated in 7 L square plastic pots (Nutriplan). The pots were 0.33 m tall and 0.16 m wide. Thirty-three pots were filled with 4.5 ± 0.45 kg of soil from Passo Fundo (RS, Brazil) and arranged in three lines in an area that was illuminated with 260 to 280 μmol photon m^{-2} s^{-1} on a 12:12 photoperiod and maintained at 20 ± 2 °C. Five pre-germinated seeds (cultivar ABI Invicta) were planted in each pot. To avoid unviable seeds, pre-germination was conducted using sterile humidified filter papers in an oven (Eletrolab EL202, São Paulo, Brazil) at 20 ± 0.3 °C. Then, 165 seeds with an average radicle length of 5.98 ± 2.32 mm were selected for planting. The seeds were planted 3 cm deep, followed by manual homogenization of the soil surface. The 33 pots were randomly divided into three treatments (11 pots or 55 seeds per treatment): (i) a control group with no fertilization (only distilled water), (ii) a group with fertilization using NPK 10:10:10 (Dimy, Brazil), and (iii) a group with fertilization using a *Limnospira* sp.-based biofertilizer. The fertilization treatments were standardized based on the amount of nitrogen (20 kg N ha^{-1}), resulting in total masses of chemical fertilizer and biofertilizer of 338 and 735 mg per pot, respectively. All pots were watered with 240 to 300 mL of distilled water per week. The experiment lasted 100 days to encompass the flowering stage of barley plants, which takes 60 to 90 days.

At the end of the experiment, aerial portions of all plants were measured manually based on the highest leaf. The plants were removed from the soil, washed, and immediately weighed (AE 240, Mettler Toledo, Greifensee, Switzerland). After determining the fresh weight, the plants were dried in an oven (SSD-85L, Solidsteel, Piracicaba, Brazil) at 50 ± 1 °C until a constant weight was reached to determine the dry weight. Only the aerial parts of the dried plants were used to determine the elemental composition of the barley plants. Carbon and nitrogen were determined in 0.3 mg samples wrapped in tin foil using

a Flash 2000 Elemental Analyser (Thermo Fisher, Waltham, MA, USA). A calibration curve was created using acetanilide as the standard. All other elements were determined using a sequential inductively coupled plasma optical emission spectrometer (ICP-OES) with radial view (Ultima 2, Horiba Jobin Yvon, Oberursel, Germany) equipped with a MiraMist cyclonic nebulization chamber (MiraMist CE, Burgener Research Inc., Mississauga, ON, Canada), an automatic sampler (AS421, Burgener Research Inc., Mississauga, ON, Canada), and Analyst 5.4 software (Los Angeles, CA, USA) for data acquisition. Samples were first hydrolyzed in HNO_3 65% at room temperature for 12 h, followed by 40 min at 200 °C in a microwave digester (SpeedWave 4, Berghof, Eningen unter Achalm, Germany). Quantification was performed by interpolation using an analytical curve with four standard solutions for calibration. The calibration solutions were prepared by diluting a SpecSol stock standard solution (Quimlab, São Paulo, Brazil). Direct measurements (i.e., height, fresh and dry weight) were conducted on single plants, whereas elemental composition (i.e., carbon and nitrogen) was determined using pooled samples to reach an adequate sample amount.

2.4. Pilot-Scale Barley Cultivation

The pilot scale cultivation was conducted in Coxilha (RS, Brazil), which is located at 677 m above sea level. The cultivar ABI Invicta was used in a randomized block experimental design. The experimental units were plots with six rows of crops spaced 0.17 m apart and a total length of 5 m, resulting in a 5 m^2 area per experimental unit, at a sowing density of 250 plants per m^2. Four experimental units were used for each treatment. Sowing took place on 20 June 2023, and harvesting occurred on 8 November 2023. Cultivation practices followed standard procedures and were conducted according to recommended practices for barley [21].

The treatments differed on the fertilizers used on the base dressing (application prior to sowing) and the top dressing (application after plant emergence): the control group used NPK 5:20:20 (Agroadubo, São Paulo, Brazil) in base dressing and urea in top dressing; treatment 1 (T1) used NPK 5:20:20 (Agroadubo, São Paulo, Brazil) in base dressing, and *Limnospira* sp.-based biofertilizer in top dressing; and treatment 2 (T2) used *Limnospira* sp.-based biofertilizer in base and top dressing. Samples were collected for post-harvesting analyses of productivity (ton ha^{-1}), grain size (class I), and biochemical composition (starch, proteins, and moisture). The grain size was determined by passing samples of barley grains through hardened brass sieves with 2.8, 2.5, and 2.2 mm slots for five minutes using vibration. Class I grains were defined as those with a diameter of 2.5 mm or greater. The biochemical composition was determined in whole grains using near infrared spectroscopy as described by EBC Analytica [23].

2.5. Statistical Analysis

Granulometric and mineralogical analyses of the soil were performed on a single soil sample collected from the experimental site in Coxilha (RS, Brazil). All biological measurements were carried out in at least three biologically independent replicates ($n \geq 3$). The reported data show the mean and standard deviation (±SD) of the values. A one-way analysis of variance (ANOVA) with a 5% significance level ($\alpha = 0.05$) was performed to evaluate the effect of a categorical factor on the dependent variables. Tukey's honest significant differences (HSD) post hoc tests were carried out to determine significant differences among the datasets. All statistical analyses were carried out on GraphPad Prism 10.2.3.

3. Results

3.1. Soil Granulometry and Composition

The soil from Coxilhas (RS, Brazil) was characterized by low granulometric values, with 57.9% of the soil fraction below 0.211 mm and 41.3% below 0.044 mm (Figure 1). The high concentration of silicon, aluminum, and iron (Table 1), combined with the low granulometry, indicated the argillaceous nature of the soil. Its mineral composition primarily consisted of quartz, kaolinite, muscovite, feldspar, goethite, and other smectite minerals. The texture and other physical characteristics of the clay minerals were demonstrated by scanning electron micrography (see Supplementary Material).

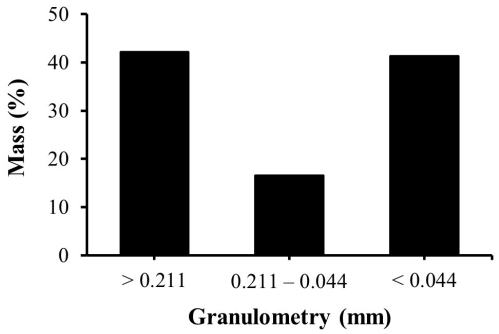

Figure 1. Granulometric classification of the soil from Passo Fundo, Brazil. Size classes were based on serial sieving using meshes +65, +270, +325, and −325.

Table 1. Chemical composition of the soil from Passo Fundo, Brazil.

Oxides	Composition (%)
SiO_2	40.5
Al_2O_3	17.9
Fe_2O_3	17.0
K_2O	7.00
TiO_2	3.70
MgO	0.32
CaO	0.32
P_2O_5	0.21
MnO	0.17
SO_3	<0.1
ZrO_2	<0.1
LOI *	12.7

* Losses on ignition.

3.2. Lab-Scale Barley Growth and Composition

The lab-scale barley cultivation under controlled conditions lasted 100 days. By the end of the experiments, the barley plants had grown to between 25.2 ± 7.34 cm and 33.6 ± 12.5 cm. On average, plants fertilized with *Limnospira* sp.-based biofertilizer were taller, although the difference between treatments was not significant ($p > 0.05$). Similarly, the carbon and nitrogen contents were essentially the same in all treatments, ranging from 37.5 ± 2.42% to 40.0 ± 4.12% and 5.01 ± 0.96% to 5.94 ± 1.08%, respectively (Figure 2). In contrast, the fresh and dry weights of the barley plants were significantly affected by the different fertilization treatments ($p < 0.05$). The fresh weights of plants fertilized with NPK and biofertilizer were not significantly different (on average, 174 mg) but were higher than those of the control group (114 ± 10.5 mg). However, the dry weights of plants fertilized with the biofertilizer were the lowest (24.1 ± 1.01 mg) and significantly lower than those of the other treatments ($p < 0.05$; Figure 2). After 100 days of cultivation, the elemental composition of the barley plants subjected to different fertilization treatments was essentially the same with regard to macrominerals (Table 2), trace minerals (Table 3),

and toxic elements (Table 4). Arsenic was not detected in the NPK treatment, but was detected in low amounts in the other treatments.

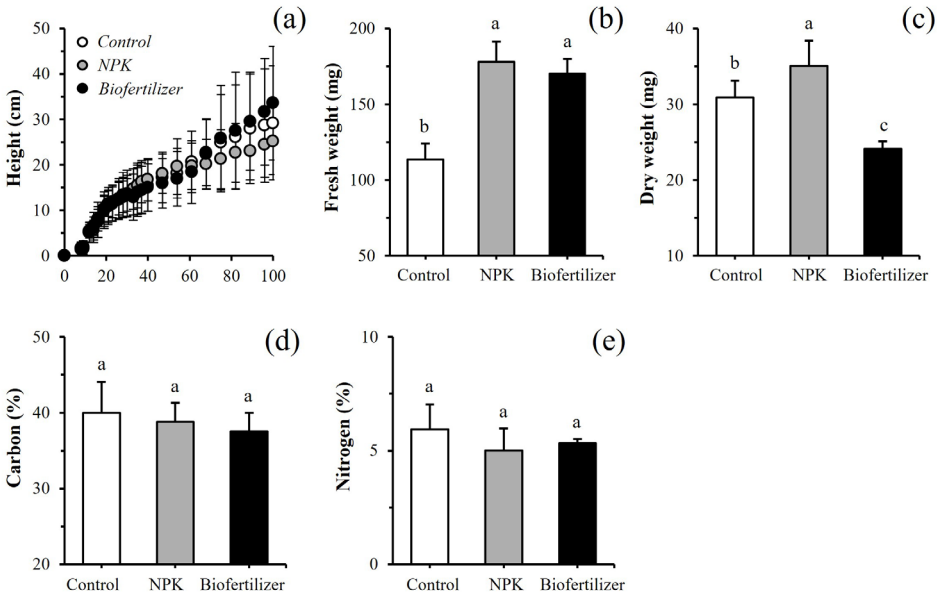

Figure 2. Height (**a**), fresh weight (**b**), dry weight (**c**), carbon (**d**), and nitrogen (**e**) contents of barley (*Hordeum vulgare*) plants grown at 20 ± 2 °C and 270 µmol photon m^{-2} s^{-1} without chemical fertilization (control; white circles and bars) or fertilized with NPK 10:10:10 (gray circles and bars) or *Limnospira* sp.-based biofertilizer (black circles and bars). The reported data are the means of at least three biologically independent experiments (fresh and dry weights: n = 25 whole plants; carbon and nitrogen: n = 3–6 pooled samples). Error bars show the standard deviation (± SD). Different letters above the bars represent statistically significant differences (one-way ANOVA; $p < 0.05$).

Table 2. Concentration of macrominerals in the biomass of barley (*Hordeum vulgare*) plants grown at 20 ± 2 °C and 270 µmol photon m^{-2} s^{-1} without chemical fertilization (control), fertilized with NPK 10:10:10, or *Limnospira* sp.-based biofertilizer.

Treatment	Macrominerals (g kg^{-1})				
	Ca	Mg	Na	K	P
Control	6.16 ± 2.39	1.90 ± 0.36	2.61 ± 1.42	44.09 ± 9.57	2.52 ± 0.82
NPK	4.28 ± 1.16	2.03 ± 0.06	3.31 ± 0.83	49.60 ± 5.81	2.17 ± 0.87
Biofertilizer	8.50 ± 3.44	2.35 ± 0.35	3.52 ± 0.35	32.86 ± 15.6	2.48 ± 1.02

The reported data are the means and standard deviation (±SD) of three biologically independent experiments ($n \geq 3$). No statistically significant differences were observed between the treatments.

Table 3. Concentration of trace minerals in the biomass of barley (*Hordeum vulgare*) plants grown at 20 ± 2 °C and 270 µmol photon m^{-2} s^{-1} without chemical fertilization (control), fertilized with NPK 10:10:10, or *Limnospira* sp.-based biofertilizer.

Treatment	Trace Minerals				
	Fe (g kg^{-1})	Zn (ppm)	Cr (ppm)	Cu (ppm)	Mn (ppm)
Control	1.39 ± 0.70	43.22 ± 4.19	34.31 ± 7.61	<LOD *	63.20 ± 35.18
NPK	1.56 ± 0.74	761.33 ± 913.98	32.07 ± 4.75	<LOD *	122.19 ± 68.04
Biofertilizer	1.36 ± 0.52	56.65 ± 28.57	32.89 ± 7.88	<LOD *	103.21 ± 17.20

The reported data are the means and standard deviation (± SD) of three biologically independent experiments ($n \geq 3$). No statistically significant differences were observed between the treatments. * LOD = limit of detection, according to INMETRO [24].

Table 4. Concentration of toxic elements in the biomass of barley (*Hordeum vulgare*) plants grown at 20 ± 2 °C and 270 µmol photon m^{-2} s^{-1} without chemical fertilization (control), fertilized with NPK 10:10:10, or *Limnospira* sp.-based biofertilizer.

Treatment	Toxic Elements				
	Al (g kg^{-1})	As (ppm)	Cd (ppm)	Sn (ppm)	Hg (ppm)
Control	1.29 ± 0.78	9.17 ± 0.92	<LOD *	19.70 ± 0.00	<LOD *
NPK	1.46 ± 0.65	<LOD *	<LOD *	9.29 ± 0.38	<LOD *
Biofertilizer	1.26 ± 0.72	13.9 ± 6.08	<LOD *	18.45 ± 12.51	<LOD *

The reported data are the means and standard deviation (± SD) of three biologically independent experiments ($n \geq 3$). No statistically significant differences were observed between the treatments. * LOD = limit of detection, according to INMETRO [24].

3.3. Pilot-Scale Growth and Composition of Barley Using Limnospira sp.-Based Biofertilizer for Base and/or Top Dressing

Pilot-scale barley cultivation experiments under environmental conditions lasted 141 days. Post-harvest samples were analyzed for starch, protein, moisture content, grain size, and productivity, all of which are relevant parameters for general agriculture and brewing. While there were no significant differences in starch content or moisture between treatments, all other parameters were significantly affected ($p < 0.05$; Figure 3). The average starch and moisture values across all treatments were 60.6% and 13.7%, respectively. Similar results were found for protein content and productivity of barley in pilot-scale cultivation, where T2 showed the highest values, which were significantly higher than the lowest values found in the control group ($p < 0.05$). T1 showed intermediate values that were not statistically different from the control group or T2. The highest protein content and productivity were 13.6 ± 0.71% and 3.77 ± 0.47 ton ha^{-1}, respectively, whereas the lowest were 11.8 ± 0.76% and 2.97 ± 0.54 ton ha^{-1}, respectively. Grain size, measured as the proportion of grains with a diameter greater than or equal to class I, did not differ statistically between T1 and T2 (averaging 79.6%), but was significantly higher than the control group (74.0 ± 2.00%; $p < 0.05$).

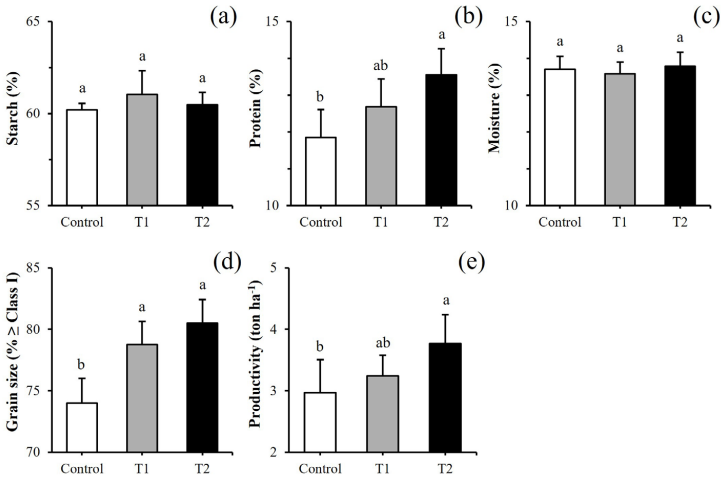

Figure 3. Macromolecular composition (starch (**a**), proteins (**b**), and moisture (**c**)), grain size (**d**), and productivity (**e**) of barley (*Hordeum vulgare*) cultivated in a pilot-scale setup (see Section 2.4). The fields were fertilized with NPK 5:20:20 in base dressing, and urea in top dressing (control; white bars), NPK 5:20:20 in base dressing, *Limnospira* sp.-based biofertilizer in top dressing (T1; gray bars), or *Limnospira* sp.-based biofertilizer in base and top dressing (T2; black bars). The reported data are the means of four experimental units ($n = 4$). Error bars show the standard deviation (± SD). Different letters above the bars represent statistically significant differences (one-way ANOVA; $p < 0.05$).

4. Discussion

4.1. Passo Fundo Soil Under Controlled Conditions (Lab-Scale Trials)

Barley production in Passo Fundo (RS, Brazil) has increased due to the establishment of malting facilities that are interested in the region's ability to produce high-quality grain. The Passo Fundo soil has been characterized as red latosol soil due to its low granulometry (Figure 1) and mineral composition (Figure 2; Supplementary Material). Different classes of latosol soils are distinguished by their Fe_2O_3 content and SiO_2: Al_2O_3 ratio. Latosols are ideal for growing annual and perennial crops, pastures, and reforestation. They are typically found on flat to gently undulating terrain, which makes agricultural mechanization easier. These soils are deep, porous, well-drained, permeable, and friable, even when clayey. They are also easy to prepare. Despite their generally low fertility, good yields can be obtained with adequate application of soil amendments and fertilizers. The present study examined the cultivation of barley on a lab scale under controlled conditions. No differences in elemental composition were observed between fertilization treatments, whereas differences in metrics such as plant height and weight were noted (Figure 2; Tables 2–4). These results suggest that, depending on the environmental conditions, soil amendments may not be necessary for optimal production in Passo Fundo.

In general, microalgae promote plant growth by improving plant physiological responses. They produce a wide array of bioactive molecules, such as phytohormones and antioxidants, that boost photosynthetic performance and inhibit oxidative stress [25]. The efficiency of *Limnospira* spp. extracts, rather than a standardized dry biofertilizer, has been extensively investigated in a wide array of plant types in small-scale setups under controlled conditions. Interestingly, *H. vulgare* has not been extensively studied, despite its immense commercial relevance [3]. *Limnospira* spp. extracts have been studied at different concentrations and application methods. They have been shown to positively affect the growth of *Lepidium sativum*, *Solanum lycopersicum*, *Cynara cardunculus*, *Zea mays*, and *Beta vulgaris*, as well as other more specific traits, such as the photosynthesis rate and flavonoid levels of *Cucumis sativus* and *Lactuca sativa* [26–30]. In *H. vulgare*, *Limnospira platensis* extract positively affected germination and seedling growth [31]. Using a different approach, Lima e Silva et al. [20] observed faster growth in *H. vulgare* with similar final plant height when using whole *Limnospira* sp. dry biomass from the remediation of brewery wastewater. This contrasts with the results presented here, which show a similar growth rate but slightly taller plants in the biofertilizer treatment (Figure 2). Interestingly, the fresh and dry weights of barley plants fertilized with *Limnospira* sp. had opposing results, being significantly higher and lower than the control group, respectively ($p < 0.05$). This indicates an overaccumulation of water rather than carbon fixation in plants grown with the biofertilizer. The mechanistic reasons for this outcome should be further investigated. Notably, even when the controlled parameters are similar, the results in the literature are highly heterogeneous due to intraspecific differences among microalgae and plant strains. Therefore, results should be compared with caution.

4.2. Limnospira sp.-Based Biofertilization in the Field (Pilot-Scale Trials)

The heterogeneity of the results is especially apparent in field and/or pilot-scale setups, where application methods, dosing, timing, and unpredictable environmental parameters strongly affect the results, increasing variability [3]. For example, soil application of an *L. platensis*-based commercial biofertilizer reduced eggplant (*Solanum melongena*) yield at higher concentrations in a greenhouse [32], whereas the foliar application of *L. platensis* hydrolysates increased the yield of *Petunia x hybrida* [33]. Additionally, foliar spraying of *Limnospira* suspensions has been shown to have no effect on *Carica papaya* growth, whereas soil application can improve it [34]. Rather than directly comparing application methods,

this study examined different combinations of base and top dressings using conventional chemical fertilizers and a *Limnospira* sp.-based biofertilizer in soil. To the best of the authors' knowledge, this is the first report of this approach in the scientific literature.

Basal and top dressing are commonly used in agriculture for different purposes. While the first provides essential nutrients for the initial growth and development of a plant, mainly establishing a robust root system, the latter supplements nutrients required by the emerged plant, replenishing those depleted from the soil. In this study, using a *Limnospira* sp.-based biofertilizer in both the base and top dressings (T2) resulted in significantly higher protein content, grain size, and productivity ($p < 0.05$). Using the biofertilizer only in the top dressing (T1), however, only differed from the control group in terms of grain size (Figure 3). Interestingly, slightly higher values of protein, grain size, and productivity were found in T2 when compared to T1 (Figure 3). Although they were not statistically significant ($p > 0.05$), this finding invites further research on the nutrient release mechanisms of base and top dressing using microalgae. For commercially relevant crops such as barley, increased productivity is clearly beneficial. Similarly, an increased percentage of grains with a diameter above 2.5 mm (class I) is highly desirable for brewers. Evenly sized grains are necessary for good malting. Market standards are set based on the percentage of grains retained over a screen. In most countries, the standard is 90% retention over a 2.5 mm screen [35]. Therefore, this parameter strongly influences the market value of grains. Broken grains and grains smaller than 2.2 mm in width are removed from the malting process and can be used as animal feed, for example [36]. However, the economic impact of the observed 15% increase in protein in T2 should be interpreted with caution. While barley grains with a higher protein content may be desirable for animal feed applications, they are generally undesirable for brewing [37]. Barley grains have been reported to contain 8–30% crude protein. These values are used to predict malting and brewing quality [38]. The ideal protein content for malting barley is between 10% and 12%. Higher protein content is strongly correlated with lower carbohydrate content. Conversely, a lower protein content may be insufficient for yeast nutrition and decrease enzymatic activity. Both cases are detrimental to the malt extract yield [37]. The protein content of barley varies greatly depending on the cultivar, the environmental conditions, and the fertilization method. For example, hot, dry, nitrogen-rich environments increase barley's protein content, often exceeding the level suitable for brewing [39]. Interestingly, the control group in this study had a protein content at the upper limit for brewing purposes ($11.9 \pm 0.76\%$), suggesting that the environmental conditions during the pilot-scale trials were not optimal for brewing-directed cultivation of barley (see Supplementary Material). Nevertheless, the use of the *Limnospira* sp.-based biofertilizer clearly increased the protein content of barley in this study.

As hypothesized by the authors, differences were observed between the results obtained from lab- and pilot-scale experiments. The main observed difference was the moisture content of the barley plants. At the lab scale, the moisture content was higher when comparing the biofertilizer to the chemical fertilizer, but it was essentially the same for all treatments at the pilot scale. This led to a lower dry weight in plants fertilized with *Limnospira* sp. in the laboratory, contrasting with the higher productivity observed in the pilot-scale setup (Figures 2 and 3). This discrepancy may be due to the different experimental conditions, the adaptability of natural soil microorganisms, or the kinetics of nutrient release in the d iverse dressing methods. Direct comparison of the results presented here is limited due to significant methodological differences between the setups. The mechanistic reasons for the observed outcomes should be the subject of future research.

4.3. Microalgae Wastewater Treatment and Safe Use of the Biomass

The use of microalgae for wastewater treatment has several advantages over conventional treatment methods, including lower operational costs and energy consumption [14]. Additionally, microalgae efficiently recover nutrients (e.g., carbon, nitrogen, phosphorus) from wastewater to produce biomass that can be commercially exploited, thereby increasing the economic viability of the entire process [40]. The feasibility of commercial-scale microalgae-based wastewater treatment is demonstrated in a number of industrial facilities currently operating in Europe. However, the use of microalgae biomass derived from wastewater treatment remains challenging, mainly due to contaminants and/or toxic elements present in the wastewater and possibly in the resulting biomass [11]. Several studies have reported promising results regarding the removal of nutrients, heavy metals, and pharmaceuticals from wastewater using microalgae. Still, the assimilation and metabolization of these compounds in the microalgal biomass, as well as their implications for further biomass applications, have not been fully investigated [11]. For example, Wuang et al. [41] demonstrated the potential of *L. platensis* to remove ammonia and nitrate from aquaculture wastewater and the ability of the resulting biomass to promote the growth of leafy plants such as *Eruca sativa*, *Amaranthus gangeticus*, and *Brassica rapa*. However, the authors did not address the toxic contaminants potentially present in the microalga or plant biomass.

By contrast, Morillas-España et al. [14] showed that the green microalga *Scenedesmus almeriensis* was able to remove and metabolize pharmaceutical contaminants of emerging concern without accumulating them in the resulting biomass. They further showed the biostimulant activity of the microalga in *Lepidium sativum*, *Glycine max*, and *Cucumis sativus*. The results presented here show that there were no significant differences in the elemental composition of barley plants fertilized with a biofertilizer formulated with *Limnospira* sp. produced in brewery wastewater, including toxic elements (Tables 2–4). Although the arsenic value was below the limit of detection only in the group fertilized with NPK, the other values were close to the lower limit of detection, indicating similarity. The higher aluminum values, which did not differ between treatments, can be explained by the soil's high clay mineral content, primarily composed of hydrous aluminum silicates. These findings indicate that *Limnospira* sp. biomass produced in brewery wastewater, as described by Lima e Silva et al. [20], can be safely used as a plant biofertilizer.

5. Conclusions

Using the rich soil from a well-established barley-producing region in Brazil, this study showed that the biomass of *Limnospira* sp. produced in brewery wastewater can be efficiently used as a biofertilizer for barley (*H. vulgare*). The outcomes of *Limnospira* sp. fertilization on barley growth and composition were comparable or superior to those from conventionally used chemical fertilizers. The results of lab-scale experiments under controlled conditions and those of pilot-scale trials using an agricultural setup differed mainly in the moisture content of barley plants, and the mechanistic reasons for that should be further investigated. The use of *Limnospira* sp. biomass derived from brewery wastewater treatment did not lead to an increase in toxic elements in the resulting barley plants, implying no restrictions for its final use. However, a substantial increase in protein content indicates that brewing quality is negatively affected.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture15222397/s1, Figure S1: X-ray diffractogram of Passo Fundo (RS, Brazil) soil sample.; Figure S2: Scanning electron micrographs of Passo Fundo (RS, Brazil) soil sample; Figure S3: Meteorological conditions of Passo Fundo during the pilot-scale fertilization experiment.

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