Biofertilizing effects of *Anabaena cylindrica* biomass on the growth and nitrogen uptake of wheat

Rajaa Kholssi[1,2], Evan A.N. Marks[2], Jorge Miñón[2], Olimpio Montero[3], Juliana F. Lorentz[4], Abderrahmane Debdoubi[1], Carlos Rad[2]

Corresponding Author mail: Rajaa Kholssi, rajae.kholsi@gmail.com

ORCID: https://orcid.org/0000-0002-6014-1468

[1]Laboratory of Materials-Catalysis, Chemistry Department, Faculty of Science, Tetouan, Morocco.

[2] Composting Research Group, Faculty of Sciences, University of Burgos, Burgos, Spain

[3] Spanish National Research Council (CSIC), Boecillo, Spain

[4]Federal University of Viçosa, Graduate Program in CivilEngineering, Department of Civil Engineering, Campus Universitário, Viçosa, MG, Brazil.

Abstract:

There are a substantial number of studies on the biofertilization effects of cyanobacteria in rice paddy fields, mainly attributed to biological fixation of N_2, but not much attention has been given to their fertilizing capacity in aerobic soils. Few studies have used solid media (i.e. a soil) when testing the plant-growth-promoting effects of microalgae on plants, and particularly on wheat. The purpose of this study was to test the biofertilizing effect of a filamentous cyanobacterium, previously isolated from an agricultural soil, in order to evaluate the potential substitution of chemical fertilizers and to test its phyto-stimulating capacity. Seedlings of *Triticum aestivum* were grown in pots with a peat-vermiculite mixture (1:1 weight basis) in an experiment designed as a complete randomized block, consisting of four treatments and with four replicates each: a pure culture of *Anabaena cylindrica* concentrated by centrifugation to 2 g dry matter L^{-1} (treatment B); spent cyanobacteria growth medium filtered at 0.22 µm (treatment F); harvested cyanobacterial mat re-suspended in distilled water (treatment WB); and distilled water as a control (treatment W). Aboveground wheat plant mass was improved by 40% in both

36 treatments with cyanobacterial biomass (B and WB), as compared to the control (W) and
37 filtrate (F), demonstrating that the co-cultivation with living cyanobacterial biomass was
38 key to plant improvement. Chlorophyll contents were also increased by nearly 50% and
39 nitrogen by over 10% in the treatment WB, clearly indicating that nutrients in the filtrate
40 were irrelevant to the beneficial effects on plant growth.

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1. Introduction

46   Microalgae and cyanobacteria are photoautotrophic microorganisms, which are of
47 current interest as a new source of biomass to meet the increasing global demands for food,
48 feed, biofuels and chemical production that may complement agricultural crops (Giorgos
49 et al. 2014). The deployment of microalgae and cyanobacteria in agricultural soils has been
50 well documented in terms of their potential in enhancing plant growth, crop yields,
51 modulation of soil microbial activity and nutrient characteristics (Renuka et al. 2018).

52   This group of organisms can be found in widely varying habitats: in fresh and salt water
53 bodies, polluted waters of lakes, ponds, water tanks, soil, rocks and tree bark. In freshwater
54 benthic habitats, most microalgae and cyanobacteria, including *Anabaena* sp.
55 heterocystous genera, occur in consortia or communities, forming complex assemblages
56 with other organisms as periphyta (close association or attachment with submerged
57 substrates) or biofilms (Stevenson, 1996). When assemblages are applied in significant
58 quantities to an aerobic soil, the resulting community assembly may be best described as a
59 biofilm (Marks et al. 2017). Microalgae and cyanobacteria are also known to produce
60 variety of extracellular substances that have direct or indirect impact on plant growth and
61 subsequent yield such as plant growth regulators (Whitton 2000; Prasanna et al. 2010),
62 amino acids (Flynn and Gallon 1990), vitamins (Indira and Biswajit 2012), antimicrobial
63 products (Rizk 2006; Tassara et al. 2008), and polysaccharides (Maqubela 2009). Past
64 reports have suggested that cyanobacteria may be the most important nitrogen-fixing
65 agents in many agricultural soils (Nayak 2004; Asari 2008; Gavilanes et al. 2020). For this
66 reason, direct application of cyanobacterial biomass as biofertilizer may help to substitute
67 or complement the application of chemical fertilizers, just as has been practiced in rice
68 paddy systems for millennia. Keeping in mind that nitrous gas emissions resulting from
69 contemporary fertilizer use is responsible for multiple environmental problems including

70 greenhouse gas accumulation, water contamination with nitrate, and acidification of water

71 (Choudhury and Kennedy 2005; Rai 2006), reducing chemical inputs in exchange for

72 organic solutions helps ameliorate ecosystem stressors.

73 In a previous study, cyanobacterium *A. cylindrica* showed high growth rates in culture

74 medium and soil, which was concomitant with an efficient production of extracellular

75 polymeric substances (Kholssi et al. 2017). Other's research have found *Anabaena* species

76 as biofertilizers in most of the paddy fields used for rice growth (Subash and Arka 2020).

77 However, no studies have been reported on the biostimulation of wheat after fertilization

78 with live *Anabaena* biomass or the culture medium using substrate. *Anabaena* has been

79 found to be a relevant member of the biofilms that are formed during certain climatic

80 conditions, but the particular contribution of *Anabaena* is not determine (Oliveira et al.

81 2015). Thus, we examine here how *Anabaena cylindrica* may contribute to the fertilization

82 effect of such biofilms. We hypothesized that adding cyanobacterial inoculation would

83 increase plant growth by improving nitrogen availability. The objective of this work was

84 to test the application of cyanobacteria biomass as bio-fertilizers and their potential to

85 replace or supplement the use of chemical fertilizers in agricultural production, reducing

86 the environmental impact associated with their use. In this way, the effects of the

87 application of this biomass on wheat growth (weight, height) in addition to the tissue

88 contents of carbon and nitrogen (C, N) and chlorophyll were evaluated, as well as on the

89 contents of total soil C and N.

90 **2. Materials and methods**

91 **2.1.Substrate characterization**

92 The substrate used in the study was prepared by mixing commercial vermiculite with

93 peat, (Pindstrup, Burgos Spain). This substrate was chosen as a neutral growth, suitable for

94 plant germination, with ability to hold microorganisms and store nutrients, adequate

95 texture, and chemically and biologically inert (Carlile et al. 2015), in order to avoid the

96 influence of factors exogenous to those set in the experimental conditions as it could

97 happen with factors regarding nutrients inherent to any solid media. And it had the

98 following basic physical and chemical properties of mixture of vermiculite and peat (1:1

99 v/v) were determined according standardized methods (TMECC, 2002). Particle size

100 distribution: 34.59 % (>2 mm), 23.83% (2 mm-0.05 mm), 37.75% (0.05 mm-2 μm) and

101 3.81% (<2 μm).Apparent density 80 mg cm^{-3}, Total carbon 15.54% and Total nitrogen

102 0.40%, using C/N TruSpec combustion analyzer (LECO); electrical conductivity (water

103   1:10 w/v, 25°C) 1.6 dS m^{-1} using a conductivimeter (Crison GLP32); pH (water 1:10 w/v)

104   6.1 using a pH-meter (Crison GLP21).

105

2.2. Culture preparation and isolation of cyanobacterial biomass

107   This cyanobacterium was chosen because it belongs to one of the groups of N$_2$-

108   fixing cyanobacteria and has been shown to promote plant growth (Saly and Gheda, 2015).

109   A strain of *Anabaena cylindrica*, heterocystous freshwater cyanobacteria, was isolated

110   from the Ap horizon of an irrigated field located at Losar de la Vera (Cáceres, Spain):

111   40º1'53'' N, 5º36'49'' W. For the isolation of the cyanobacterial strain, 1 g of soil was

112   suspended in 100 mL of BG-11 medium (pH 7.5) without NaNO$_3$ as nitrogen source

113   (BG11-N) and the content was stirred for 30 min in orbital shaking at 150 rpm until a good

114   dispersion of the soil was achieved. From this suspension, an aliquot of 100 μL was taken

115   and plated in a Petri dish with BG11-N and 2% agar. The aliquot was distributed

116   homogeneously, closing the plates to prevent drying. Purification of this strain was

117   performed by serial dilution and plating method. Strains were identified after

118   morphological examination in microscope (**Fig. 1**),

2.3. HPLC-DAD measurements

120   Photosynthetic pigments in the methanolic extract were analyzed by high

121   performance liquid chromatography with photodiode array detection (HPLC-DAD) using

122   the same chromatographic method as in Montero et al. (2016). A FINNIGAN SURVEYOR

123   PLUS chromatography system (Thermo Scientific) equipped with Quaternary LC Pump,

124   Autosampler and PDA detector was used for HPLC-DAD measurements. Pigments were

125   identified according to retention time and UV-Vis spectrum (350-700 nm). Purification and

126   sequencing of ribosomal *16S rRNA* of a sample of pure culture cyanobacteria was

127   conducted using the UltraClean kit (MO BIO Laboratories, Inc.) for identification of *A.*

128   *cylindrica*. PCR amplification of *16S rRNA* gene fragments was performed using

129   cyanobacteria-specific reverse primer CYA781R and the cyanobacteria-specific forward

130   primers CYA106F and CYA738F (Figueiredo et al., 2010). The amplification product was

131   purified by electrophoresis in agarose gel and its base sequence obtained in an ABI Prism

132   3100 Sequencer (Applied Biosystem). The similarity of the genetic sequence with respect

133   to those found in the National Center for Biotechnology Information (NCBI) database was

134   assessed using the BLAST analysis application.

135   Filaments of *A. cylindrica* were cultured in 250 mL BG11 medium without NaNO$_3$, in 1 L

136   photo-bioreactors, with a 16:8 photoperiod, photon density of 100 μmol m^{-2} s^{-1},28-18° C

137 during light-dark periods, and aeration; they were considered as stock cultures.
138 Experimental cultures were obtained by transferring aliquots of the stock cultures to 500
139 mL Erlenmeyer flasks which were stored at room temperature no more than 2 days until
140 use, with regular microscopic examination in order to monitor the culture for purity. The
141 evolution of the stock cultures was monitored by measuring absorbance of a 1 mL aliquot
142 at 750 nm on a UV-Vis spectrophotometer (GENESIS 2, Milton Roy), as well as turbidity
143 (HI93703 Turbidity Meter, Hanna).

144
145 **2.4. Experimental design**

146   *Anabaena cylindrica* biomass was harvested by centrifugation at 2,000 *g* and re-
147 suspended in appropriate amount of spent growth medium, to reach 2 g dry weight (DW)
148 L^{-1} of final biomass concentration (Treatment B). Other treatments were: spent growth
149 medium filtered through 0.22 μm (NALGENE, bottle top filter) (Treatment F); harvested
150 cyanobacterial cells re-suspended in appropriate amount of distilled water to obtain 2 g
151 DW L^{-1} of final biomass concentration (Treatment WB), and distilled water as a control
152 (Treatment W). All of these suspensions were immediately and simultaneously applied to
153 plant pots.

154   Seeds of wheat (*Triticum aestivum* Var. CAMARGO) were sterilized by immersing
155 in 5% NaClO for 2 min, rinsed several times with distilled water, and then dried between
156 two layers of filter paper. A previous germination test was carried out in order to verify the
157 percentage of germination (GP) of our seed variety (>70%) according to Eq. (1):

158
159
$$GP = \frac{\text{Seeds germinated}}{\text{Total seeds}} \times 100 \qquad (1)$$

160
161   For the pot experiment, 200 g of substrate (vermiculite and peat mixture, 1:1 v/v) was
162 introduced in 0.4 L plastic pots of dimensions 8×8×7cm **(Fig. 2).** Four pre-germinated
163 seeds of wheat (*Triticum aestivum*) were sown in each pot at equal distances and depth
164 with four replicate for each treatment and control (64 pots; 4 seeds x 4 pots x 4 treatments).
165 After the addition of 10 mL of Milli-Q water, 10 mL of the solutions of the different
166 treatments were applied on each experimental group of pots. The pots were thereafter
167 incubated in a climatic chamber for two weeks with a 16:8 photoperiod, photon density of
168 100 μmol m^{-2} s^{-1} and 28-18° C during light-dark periods, respectively, and plants were
169 irrigated every day with 15 mL of pure water to replace lost water. The arrangement of pots
170 in the climatic chamber was completely randomized.

171

2.5. Plant and substrate analysis

173   20 days after transplanting, chlorophyll contents were measured on all plant stems
174   with a handheld chlorophyll content meter (CCM-200 plus, Opti-Sciences, Hudson, USA).
175   All plant parts were harvested and carefully washed over a sieve, separating each root mat,
176   and drying them in an oven at 60 °C for 48 h, Different physiological parameters were
177   measured including root and stem lengths and total dry biomass of aboveground and
178   belowground parts. A 5 g (oven dry basis) sample of the top 0-3 cm substrate was carefully
179   taken with a spoon. The pot was carefully turned upside down and a sample of the soil in
180   the bottom portion of the pot (3-7 cm) was taken.

181   For each treatment, the plants in each pot were ground. Also, the substrate in each pot
182   for each fraction (top layer 0-3 cm and downer 3-7 cm fractions), was dried at 60° C,
183   ground, and sieved to 0.1 mm. The total contents of C and N for each pot were analyzed
184   for each replicate on a combustion analyzer (TrueSpec CN, LECO, Saint Joseph, USA)

2.6. Statistical analysis

186   The obtained results for each parameter were analyzed for statistical differences
187   between treatments using SPSS v.18.0 (SPSS, 2009). After determining of distributional
188   adequacy of the data using the Shapiro-Wilk test, the data were subjected to the
189   (parametric) one-way ANOVA with significance defined at $P<0.05$. Next, the Tukey *post*
190   *hoc* test was then used to compare treatments.

191

3. Results

193   Biofertilizing experimental treatments revealed visible differences in the growth of
194   *Triticum aestivum L.* plants; this was confirmed by the results of the statistical tests. For
195   plant biometric parameters, shoot weight and length did not show statistical differences.
196   Conversely, Tukey multiple contrasts revealed differences for every of the others
197   parameters. Root weight (**Fig. 3A**) was lower in the control treatment (W) which was 26.84
198   mg but without significant differences with the filtrate treatment (F). The highest
199   enhancement in root length (**Fig. 3B**) was recorded in the treatments (WB) and (B) with
200   values of 31.69 and 34.42 cm, respectively, but without significant differences between the
201   treatments (WB) and (F). Regarding shoot weight (**Fig. 3A**), only the treatment W showed
202   a value significantly lower than the other treatments (B, WB and F), whereas no significant
203   differences between treatments were found for shoot length (**Fig. 3B**).

The effect of cyanobacterial treatments on the chlorophyll content is shown in **figure 3C**. Among the treatment variables analyzed, only a significant increase of 48% in chlorophyll content was observed when biomass was removed from its filtrate (WB) compared to control treatment.

In the cyanobacterial treatments (B and WB), biofilm growth was visible around the pot edges, demonstrating that the liquid cyanobacterial fertilizer continued to live throughout the experimental duration and release its nutrients for plant uptake. The amounts of C and N in plants (**Table 1**) are quite appreciable, considering total amounts of carbon in control that was significantly lower than all other treatments. However, plant N contents showed a similar pattern to chlorophyll, with contents in (WB) plants being significantly higher than in (W) plants, but without significant differences of these plants with the plants of the other two treatments. Results of the correlation analysis revealed a significant positive correlation between the plant N content and dry shoot weight (Pearson's $r=0.65$, $t=3.2$, $p<0.01$).

Finally, according to the data in **Figure 4**, total N and C contents showed significant differences ($p < 0.001$) only for filtrate treatment (F) in both the top and bottom soil fractions with values of 0.73% and 28%, respectively.

4. Discussion

Overall, both cyanobacteria biomass treatments showed consistent improvements for wheat growth over the water control. There are very few studies that have assayed the effects of *Anabaena* on cereal growth in a solid medium such this substrate (vermiculate and peat). Regarding this effect of biofertillizers, similar studies have revealed that wheat plants obtained from grains of wheat grown in inoculated soil showed significant increase in most morphological plant characters including grain yield, straw yield and weight of 1000 grains by *Anabaena oryzae* (Boghdady and Ali 2013). Similar to this result; the effect of the application of cyanobacterial culture on rice, chickpea and wheat seeds germination under low temperature conditions were investigated and the germination percentages, root-stem length, number of leaves, chlorophyll amount found more than the control (Khushwaha and Banerjee 2015)

Therefore, since the filtrate (F) treatment barely had no significant effect on plant growth over the control, it seems that nutrients or bioactive compounds in that medium were not at all effective in improving the growth of wheat as compared to actual algal

238 biomass. On the contrary, the average root: shoot ratio of filtrate treatments was 0.75, value

239 significantly higher than that of all other treatments (0.54; 0.51; and 0.55 for W; WB; and

240 W respectively; multiple Tukey contrasts with p<0.01). This large deviation in the

241 root/shoot ratio of filtrate treatment (F) from the control (W) may indicate that the filtrate

242 treatments induced some sort of stress in the wheat plants (Fageria and Moreira, 2011). For

243 instance due to allelopathic chemicals in the filtrates which has been described for the

244 *Anabaena* genus as well as others (Leão et al. 2009; Dias et al. 2017), whereas water

245 contaminated with cyanotoxins is known to affect the physiology of higher plants

246 (Bittencourt-Oliveira et al. 2016; Jia et al. 2018). For plant growth, the positive effect of

247 the living cyanobacterial biomass might have outweighed any stressors in the filtrates since

248 the treatment (B) improved plant growth over the control (W) and filtrate (F).

249 Total N content was only improved when biomass was removed from its filtrate

250 (WB treatment; **Table 1**). Obreht et al. (1993) demonstrated significant enhancement in

251 plant nitrogen and root/shoot length in co-cultivation experiment for 15 days (using

252 *Nostoc/Anabaena*) in three different wheat varieties in glass vessels with aqueous media -

253 that is, exposed to "low levels" of incident light in glass bottles with liquid media. These

254 authors reported that their *Anabaena* strain had no nitrogenase activity in darkness, in

255 accordance with other studies (Gantar et al. 1995), and that this genus does not tend to be

256 capable of using C-containing exudates, though root elongation nevertheless occurred in

257 darkness. Apart from close association with roots, benefits, therefore, might be related to

258 plant growth regulation. Babu et al. (2014) also explored the effect of four *Anabaena* strains

259 on plant root metabolic products in assays conducted in a hydroponic experiment (liquid

260 medium). In this set-up, three of the four *Anabaena* strain treatments contributed to greater

261 plant N fixation (expressed as acetylene-reducing activity or ARA), as well as greater plant

262 dry weight. Gantar et al. (1995) reported a positive impact of *Anabaena* strain in nitrogen

263 economy on wheat plants assayed.

264 It is expected that soil inoculation with N_2-fixing cyanobacteria can also lead to

265 increases in soil organic carbon (SOC), total N, and available nutrients in the top substrate,

266 as demonstrated in other studies (Jeffries et al. 1992; Lange et al. 1994; Malam et al. 2001,

267 Mulat et al 2019). However, given the particular substrate utilized in this experiment with

268 high organic matter contents and therefore relatively rich in total nutrients compared to a

269 mineral soil, it is not surprising that no clear effects on soil C and N were seen for the short

270 length of this laboratory study. We are unable to offer a clear explanation for why the

271 filtrate (F) treatments had greater C and N in both the top and bottom fractions (**Fig. 4**).

272 One possible reason may be that those plants, stressed (see above), produced greater root

273   mass and turnover of fine root hairs, since the F treatment also exhibited the greatest

274   average root mass.

275   **Conclusion**

276   The results of our study show that cyanobacterial biomass improved the growth and

277   fitness of young wheat plants grown in a peat substrate - specifically, that *A. cylindrica*

278   biomass – not extracts, which, to the contrary, may have induced plant stress - was key for

279   increasing growth and N nutrition in an aerobic substrate. While as a first step the study

280   indicates that N nutrition did in fact increase, the specific mechanism was not studied, and

281   this should be addressed in the future. While the objective of this work was to study the

282   effects on N nutrition, future work should confirm the interesting dichotomy between

283   cyanobacterial filtrates and slurries with living algal cells.

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Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest

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References

Asari, N., Ishihara, R., Nakajima, Y., Kimura, M., Asakawa, S. 2008. Cyanobacterial communities of rice straw left on the soil surface of a paddy field. Biol. Fertil. Soils. 44, 605–612.

Babu, S., Prasanna, R., Bidyarani, N., Singh, R. 2014. Analyzing the colonization of inoculated cyanobacteria in wheat plants using biochemical and molecular tools. J. Appl. Phycol. 27, 327–338.

Bittencourt-Oliveira, M. C., Cordeiro-Araújo, M. K., Chia, M. A., Arruda-Neto, J. D. T., de Oliveira, Ê. T., dos Santos, F. 2016. Lettuce irrigated with contaminated water: Photosynthetic effects, antioxidative response and bioaccumulation of microcystin congeners. Ecotoxicol. Environ. Saf. 128, 83–90.

Boghdady, M.S., Ali S. A. 2013. Comparison between effect of *Azospirillum brasilense* and *Anabaena oryzae* on growth, yield and anatomical characters of wheat plants; J. Appl. Sci. Res. 9, 627–637,

Carlile, W., Cattivello, C., Zaccheo, P. 2015. Organic Growing Media: Constituents and Properties. Vadose Zone J. 14, 1–13.

Choudhury, A. T. M., Kennedy, I. R. 2005. Nitrogen fertilizer losses from rice soils and control of environmental pollution problems. Comm. Soil Sci. Plant Anal. 36, 1625–1639.

Dias, F., Antunes, J. T., Ribeiro, T., Azevedo, J., Vasconcelos, V., Leão, P. N. 2017. Cyanobacterial allelochemicals but not cyanobacterial cells markedly reduce microbial community diversity. Front. Microbial. 8, 1495.

Fageria, N. K., Moreira, A., 2011. The Role of Mineral Nutrition on Root Growth of Crop Plants. Adv. Agron. 110, 251–331.

Figueiredo, D. R, Artur A, Mário J. P., António, C. 2010. Molecular characterization of bloom-forming Aphanizomenon strains isolated from Vela Lake (Western Central Portugal), Journal of Plankton Research. 32: 239–252.

322 Flynn, K. J., Gallon, J. R. 1990. Changes in intracellular and extracellular x-amino acids in
323   Gloeothece during N_2-fixation and following addition of ammonium. Arch.
324   Microbiol. 153, 574–579.

325 Gantar, M., Karby, N., Rowell, P., Obreht, Z., Scrimgeour, C. 1995. Colonization of wheat
326   (*Triticum vulgare* L.) by N_2-fixing cyanobacteria: IV. Dark nitrogenase activity and
327   effects of cyanobacteria on natural ^{15}N abundance in the plants. New Phytol. 129,
328   337–343.

329 Gavilanes, F.Z., Andrade, D.S., Zucareli, C., Horácio, E.H., Yunes, J.S., Barbosa, A.P.,
330   Ribeiro Alves, L.A., Cruzatti, L.G., Maddela, N.R., de Fátima Guimarães, M., 2020.
331   Co-inoculation of *Anabaena cylindrica* with *Azospirillum brasilense* increases maize
332   grain yield. Rhizosphere. 100224

333 Giorgos, M., Dries, V., Koenraad, M. 2014. Microalgal and cyanobacterial cultivation: The
334   supply of nutrients. Water Res. 65, 186–202.

335 Indira, P., Biswajit, R.N., 2012. Commercial and industrial applications of microalgae. J.
336   Algal Biomass Utln. 3, 89–100.

337 Jeffries, D. L., Klopatek, J. M., Link, S. O., Bolton, H. J., 1992. Acetylene reduction by
338   cryptogamic crusts from a blackbrush community as related to resaturation and
339   dehydration. Soil Biol. Biochem. 24, 1101–1105.

340 Jia, Y., Li, H., Qu, Y., Chen, W., Song, L., 2018. Phytotoxicity, bioaccumulation and
341   potential risks of plant irrigations using cyanobloom-loading freshwater. Sci. Total
342   Environ. 624, 704–712.

343 Kholssi R., Evan A. N. M., Montero, O., Pascual, A., Debdoubi, A., Rad, C. 2017. The
344   growth of filamentous microalgae is increased on biochar solid supports. Biocat.
345   Agric. Biotech. 13, 182–185.

346 Khushwaha M., Banerjee M. 2015. A Novel Method of Seed Germination and Growth of
347   Three Staple Crop Plants: Effect of Low Temperature and Cyanobacterial Culture
348   Addition. J. Algal Biomass Utln. 6, 26–32.

349 Lange, O. L., Meyer, A., Zellner, H., Heber, U. 1994. Photosynthesis and water relations
350   of lichen soil crusts: field measurements in the coastal fog zone of the Namib Desert.
351   Funct. Ecol. 8, 253–264.

352 Leão, P. N., Vasconcelos, M. T. S. D., Vasconcelos, V. M. 2009. Allelopathy in freshwater
353   cyanobacteria. Crit. Rev. Microbiol. 35, 271–282.

354 Malam, I. O., Bissonnais, L. Y., Defarge, C., Trichet, J. 2001. Role of a cyanobacterial
355   cover on structural stability of sandy soils in the Sahalian part of western Niger.
356   Geoderma. 101, 15–30.

357 Maqubela, M. P., Mnkeni, P. N. S., Issa, M. O., Pardo, M. T., D'Acqui L. P. 2009. *Nostoc*
358   cyanobacterial inoculation in South African agricultural soils enhances soil structure,
359   fertility and maize growth. Plant Soil. 315, 79–92.

360   Marks, E. A. N., Miñón, J., Pascual, A., Montero, O., Navas, L. M., Rad, C. 2017.
361   Application of a microalgal slurry to soil stimulates heterotrophic activity and
362   promotes bacterial growth. Sci. Total Environ. 605–606, 610-617.

363   Montero, O., Porta, J. M., Porta, J., Martínez, G., Lubián, L. M. 2011. Characterization of
364   two *Synechococcus* sp. PCC7002-related cyanobacterial strains in relation to 16S
365   rDNA, crtR gene, lipids and pigments. Phycological Res. 59, 47–155.

366   Montero, O., Velasco, M., Sanz-Arranz, A., Rull, F. 2016. Effect of Different Broad
367   Waveband Lights on Membrane Lipids of a Cyanobacterium, *Synechococcus* sp., as
368   Determined by UPLC-QToF-MS and Vibrational Spectroscopy. *Biology* 5, 22 (17
369   pp.)

370   Mulat A, Girma W., Mekiso Y., Solomon Y., Endalkachew W., Alemayehu C., Jessica
371   G.D. 2019. Comparison of cyanobacterial bio-fertilizer with urea on three crops and
372   two soils of Ethiopia. Afr. J. Agric. Res. 14, 588-596.

373   Nayak, S., Prasanna, R., Pabby, A., Dominic, T. K., Singh, P. K. 2004. Effect of urea, blue
374   green algae and Azolla on nitrogen fixation and chlorophyll accumulation in soil
375   under rice. Biol. Fertil. Soils 40, 67–72.

376   Obreht, Z., Kerby, N. W., Gantar, M., Rowell, P. 1993. Effects of root-associated N2-fixing
377   cyanobacteria on the growth and nitrogen content of wheat (Triticum vulgare L.)
378   seedlings. Biol. Fertil. Soils. 15, 68–72.

379   Oliveira, P., Martins, N. M., Santos, M., Couto, N. A., Wright, P. C., Tamagnini, P. 2015.
380   The Anabaena sp. PCC 7120 Exoproteome: Taking a Peek outside the Box. Life
381   (Basel). *5*, 130–163.

382   Prasanna, R., Joshi, M., Rana, A., Nain, L. 2010. Modulation of IAA production in
383   cyanobacteria by tryptophan and light. Pol. J. Microbiol. 59, 99–105.

384   Rai, M. K. 2006. Handbook of Microbial Biofertilizers. Food Products Press, an imprint of
385   The Haworth Press. Inc. Binghamton, New York.

386   Renuka, N., Guldhe, A., Prasanna, R., Singh, P., Bux, F. 2018. Microalgae as multi-
387   functional options in modern agriculture: current trends, prospects and challenges.
388   Biotechnol. Adv. 36, 1255–1273.

389   Rizk, M. A. 2006. Growth activities of the sugarbeet pathogen *Sclerotium rolfsii* Sacc.,
390   *Rhizoctonia solani* Khun. and *Fusarium verticilloides* Sacc. under cyanobacterial
391   filtrate stress. Plant Pathol. J. 5, 212–215.

392   Saly, F. G., Gheda, A. 2015. Improved soil characteristics and wheat germination as
393   influenced by inoculation of *Nostoc kihlmani* and *Anabaena cylindrica*. Rend. Fis.
394   Acc. Lincei. 26, 121–131.

395   SPSS Inc. 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.

396   Stevenson, R. 1996. An introduction to algal ecology in freshwater benthic habitats, in:
397   Stevenson R. J., Bothwell M. L., Lowe R. L.. (Eds.), Algal Ecology: Freshwater
398   Benthic Ecosystems. Academic, San Diego, pp. 3–30.

399   Subash K.G., Arka P. C. 2020. Cyanobacterial Biofertilizer for sustainable agriculture and
400   environment. Journal of Creative Research Thoughts.8, 2320-2882

401

402   TMECC. 2002. Test Methods for the Examination of Composting and Composts. US
403   Composting Council: Holbrook, NY, USA.

404   Tassara, C., Zaccaro, M., C., Storni, M., M., Palma, M., Zulpa, G. 2008. Biological control
405   of lettuce white mold with cyanobacteria. Int. J. Agric. Biol. 10, 487–492.

406   Whitton, B. A. 2000. Soils and rice-fields, in: Whitton B.A., Potts M. (Eds.), Ecology of
407   Cyanobacteria: Their Diversity in Time and Space, Kluwer, Dordrecht, pp. 233–255.

408

409 **Table 1**. Total carbon and nitrogen contents for the above-ground matter of *Triticum*
410 *aestivum* L. plants grown in the study.

	Treatments			
	W	**B**	**F**	**WB**
C (%)	39.50 ± 0.30 b	37.83 ± 0.20 a	38.30 ± 0.23 a	38.50 ± 0.20 a
N (%)	4.54 ± 0.10 a	4.84 ± 0.11 ab	4.80 ± 0.07 ab	5.07 ± 0.05 b

411 Treatments: culture of *Anabaena cylindrica* (B); spent cyanobacteria growth medium
412 filtered through 0.22 µm (F); harvested cyanobacterial mat re-suspended in distilled water
413 (WB); distilled water as a control (W).Statistically significant differences between
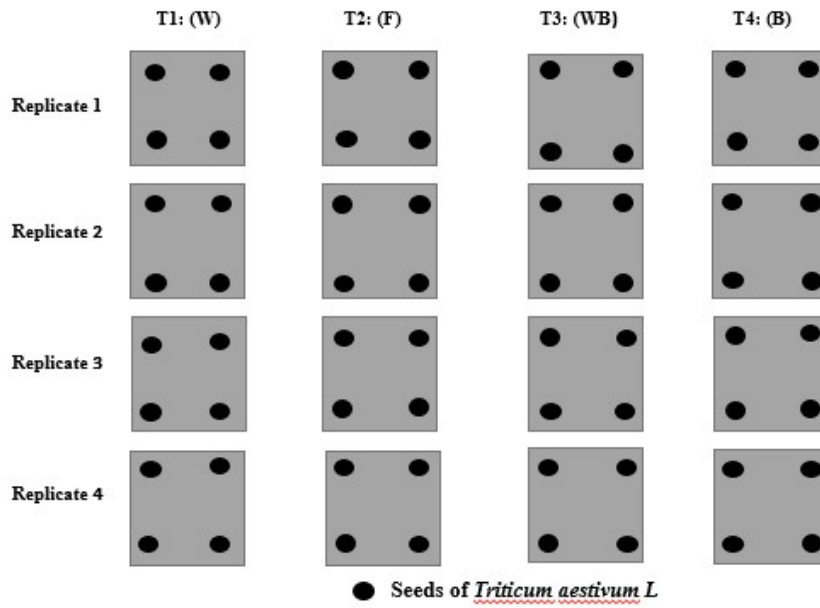414 treatments are indicated by different letters.

415

417 **Fig. 1** Filaments of the cultured strain of *Anabaena cylindrica* seen under a microscope at
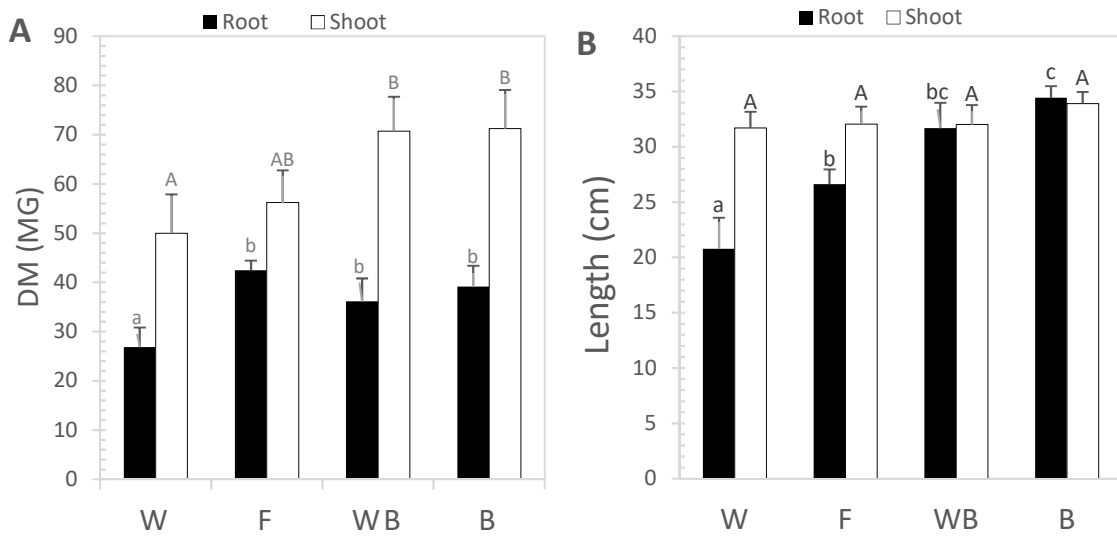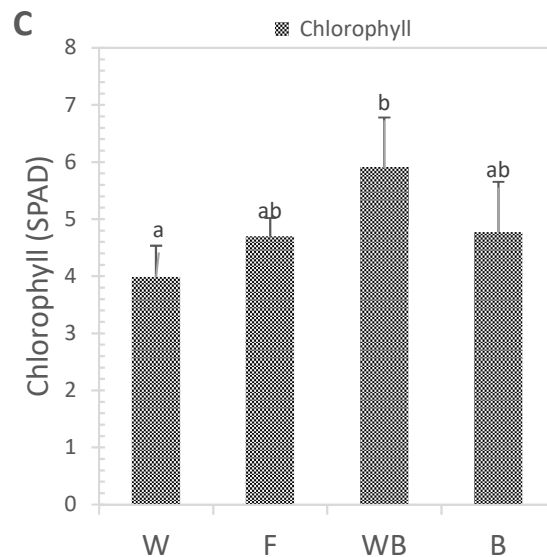418 40x. Cyanobacterial culture. Turbidity was 380 FTU.

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421   **Fig. 2** Pot experimental design. All four treatments were repeated four times (four seeds

422   per replicate).

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Fig.3 Biometric properties of harvested wheat (*Triticum aestivum L.*) plants under the different experimental algal treatments. Error bars display 95% of confidence interval of four replicates. Panel (1) is above plant dry matter (DM); panel (2) is length; and panel (3) is chlorophyll concentration of both roots and shoots. Treatment codes, in the X axis as in Table 1. Statistically significant differences between treatments are indicated by different letters, lower-case or capitals for roots and shoots, respectively.

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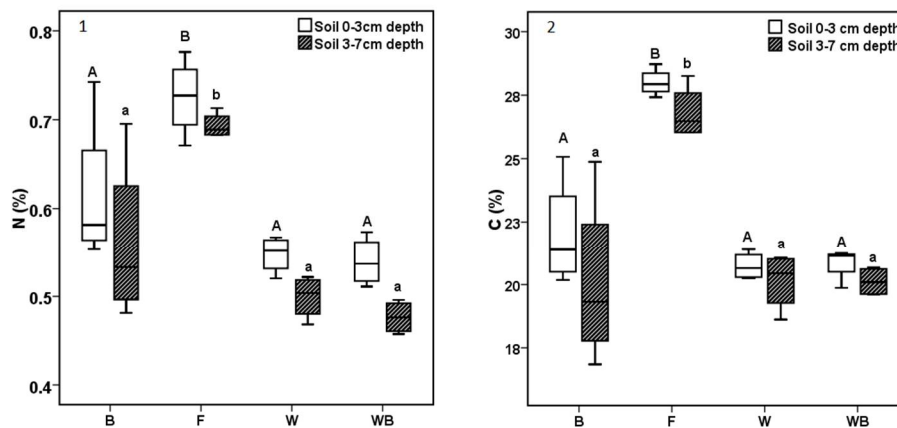
Fig.4 Total C (panel 1) and N (panel 2) in the two soil depth fractions at the end of the experiment. Treatment codes in the X axis as in Table 2. Error bars display 95% confidence interval of four replicates. Statistically significant differences between treatments are indicated by different letters, capitals or lower-case for soil depths 0-3 cm and 3-7 cm, respectively.

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