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Evaluation of the fertilizer potential of *Chlorella vulgaris* and *Scenedesmus obliquus* grown in agricultural drainage water from maize fields

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HIGHLIGHTS

- Maize field drainage water (ADW) was used to grew *C. vulgaris* and *S. obliquus*.
- Microalgae degraded the herbicides in the ADW to non-detectable concentrations.
- Microalgae replaced 1/2 of the N-fertilizer with a 2-fold increase in lettuce biomass.
- Soil enzymatic activities increased significantly with N-fertilization via microalgae.
- Microalgae fertilization reduces 3-times the risk of soil secondary salinity increase.

GRAPHICAL ABSTRACT

"Chlorella vulgaris and *Scenedesmus obliquus* suspensions, grown in maize drainage water, can be used on-farm, as low cost slow-release organic fertilizers, doubling lettuce fresh biomass and improving soil health"
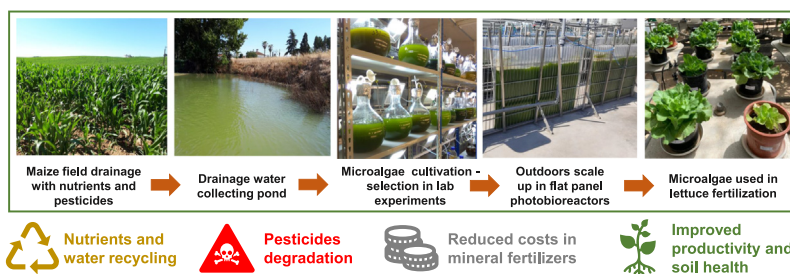
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ABSTRACT

Producing microalgae with agricultural drainage water (ADW) allows recycling water and nutrients, with the production of a biofertilizer, avoiding receiving waters' contamination. *Chlorella vulgaris* and *Scenedesmus obliquus* were cultivated using ADW and standard media supplementation and presented higher productivities, relatively to the control industrial growth medium (using freshwater). Selected strains were grown outdoors in pilot flat panel photobioreactors, reaching 2.20 g L^{-1} for *S. obliquus* and 1.15 g L^{-1} for *C. vulgaris*, and degrading herbicides in the ADW to non-quantifiable concentrations. The potential of the *C. vulgaris* and *S. obliquus* suspensions to replace 50% of nitrogen (N) mineral fertilization of lettuce (0.5 g pot^{-1}) was evaluated through a pot trial, also using a 2-times (1.0 g pot^{-1}) and 5-times (2.5 g pot^{-1}) higher dose, applied 31 days before lettuce transplanting. Even the lower dose of N, applied via *C. vulgaris* or *S. obliquus* suspensions, was able to provide significantly higher lettuce fresh matter yield, relatively to the mineral fertilized control. Soil enzymatic activities were improved, with significantly higher dehydrogenase, β-glucosidase, and acid phosphatase activities for the 2.5 g pot^{-1} dose, more marked for *S. obliquus*, which was also able to increase soil organic matter content. Both the non-fertilized control and microalgae fertilized pots led to similar soil electrical conductivities, 3-fold lower than in the N-mineral fertilized pots, evidencing the capacity of microalgae fertilizers to avoid soil secondary salinization. Results suggest benefits from using ADW from maize cultivation to produce *C. vulgaris* or *S. obliquus* suspensions, that can be further used as liquid organic slow-release fertilizer.

1. Introduction

In a scenario of increasing global population, it is necessary to deal with resource scarcity and environmental preservation, creating sustainable systems, namely in the agricultural activity. Agricultural surface drainage (agricultural runoff) consists in excess water leaving cultivated fields, relative to the infiltration rate. Subsurface drainage, on the other hand, is generated when there is a subsurface drainage system installed, to collect and drain water in excess which can be held by capillary forces, usually to avoid soil saturation and problems of root respiration. Both can be considered agricultural drainage water (ADW) and represent a major nonpoint source (NPS) of pollutants to water streams, lakes, and estuaries (Zuazo et al., 2009; Solovchenko et al., 2016; Cameira and Mota, 2017). These pollutants include nutrients, mainly nitrogen (N) as nitrates and phosphorus (P) as phosphates, and different families of pesticides (e.g., herbicides, insecticides, fungicides), which can impact the quality of receiving waters (Cai et al., 2013), causing surface water eutrophication (Solovchenko et al., 2016; Cameira and Mota, 2017) and, eventually, toxicity towards aquatic and benthic organics (Palma et al., 2018). Different actions can be taken to cope with this problem, from adoption of preventive Best Practices related to the rational use of fertilizers, pesticides, and irrigation water, to technological solutions to treat the drainage water, e.g., using membrane bioreactors (Cicek, 2003), constructed wetlands (Wang et al., 2018), or cultivated drainage ditches (Moore et al., 2010; Vymazal and Březinová, 2018). These technological solutions have the benefit of lowering the concentration of nutrients in the ADW, possibly degrading pesticides residues, thus protecting surface water systems. However, it would be important to have a solution that could reuse water and nutrients from the ADW matrix, promoting their circularity.

Microalgae cultivation needs water and nutrients to grow, stressing the importance of their production with non-potable water resources and cheap nutrients sources. Its potential to recover nutrients from different wastewater streams has been proven (Cai et al., 2013; Solovchenko et al., 2016), namely from municipal wastewater (Li et al., 2011; Cabanelas et al., 2013; Renuka et al., 2016, 2017; Geremia et al., 2021), animal production wastewater (Zhu et al., 2013; Hena et al., 2015), digestate from biogas production (Franchino et al., 2016; Xu et al., 2019), and different types of agro-industries wastewater (Cai et al., 2013; Farooq et al., 2013; Tango et al., 2018; Navarro-López et al., 2020). This is a win-win solution, since microalgae cultivation using wastewater can be considered a low-technology biological treatment (Navarro-López et al., 2020), which allows valorization of all by-products. Several studies have reported their use to treat different streams, increasing their fertilizing value and lowering ecotoxicity (e.g., digestate form biogas production; Franchino et al., 2016; Xu et al., 2019). If not for food and feed, microalgae biomass produced from wastewater streams can have other important applications, from biofuel (Cai et al., 2013), to biofertilizers and biostimulants production (Garcia-Gonzalez and Sommerfeld, 2016; Navarro-López et al., 2020; Kapoore et al., 2021). These integrated strategies are very important and can be seen as a biorefinery approach (Renuka et al., 2018; Oliveira et al., 2021; Kapoore et al., 2021).

The use of microalgae as a biofertilizer in modern agriculture is widely documented, contributing to growth and nutrition of plants, reducing the need for chemical fertilizers, and benefiting soil health (Coppens et al., 2016; Renuka et al., 2016, 2018). Renuka et al. (2018) reviewed the literature and provided several examples of the use of microalgae as biofertilizers, with additional benefits, relatively to the use of conventional mineral fertilizers, namely provision of soil organic carbon, enhanced mineralization and solubilization of nutrients (Renuka et al., 2016, 2017), microaggregate stability (Yilmaz and Sönmez, 2017), and the possibility of reducing soil erosion and nutrient-rich agricultural run-off (Renuka et al., 2018).

Nevertheless, it is important to evaluate the application doses and strategies before upscaling the use of microalgae biomass as an organic fertilizer. Garcia-Gonzalez and Sommerfeld (2016) have studied different aspects of the use of *Acutodesmus dimorphus* as a biofertilizer and

biostimulant, namely its application as dry biomass to produce tomato (*Solanum lycopersicum* var. Roma), and they concluded that earlier application, prior to transplanting (e.g., 22 days in their case), significantly enhanced plant growth. They assumed that this was necessary, for the biomass to be broken down, so that nutrients became available to the plant. Similar results were reported by Coppens et al. (2016), using *Nannochloropsis oculata* and filamentous microalgae dried biomass to produce tomatoes, obtaining similar plant growth, relatively to the use of mineral fertilizers, and better fruit quality, through an increase in sugar and carotenoid content. In their experimental setup, they considered microalgae biomass to act as a slow-release fertilizer, assuming a N mineralization rate of 33 % after 3 months (Coppens et al., 2016).

The success of the microalgae fertilizers depends not only on the economics of biomass production and harvesting technology, but also on the strategies for their commercialization and application to soil (Solovchenko et al., 2016; Geremia et al., 2021), which can be done using (i) dehydrated algal biomass (Garcia-Gonzalez and Sommerfeld, 2016), (ii) algal formulations using a suitable carrier (Renuka et al., 2016, 2017), (iii) foliar spray (Renuka et al., 2018), or (iv) simply applying the liquid cell suspension (Xu et al., 2019). Considering these possibilities, the application of microalgae biomass suspension, without solid-liquid separation and further processing, would be cheaper, avoiding costs associated with biomass harvesting and dehydration. In fact, Geremia et al. (2021), identified the large-scale feasibility and costs of biomass harvesting as the main bottleneck in using different wastewater streams for microalgae production. On the other hand, application of microalgae biomass suspension allows the use of the residual nutrients, not uptaken by microalgae, and avoids generation of further waste-streams. Of course, this strategy limits the commercialization of the microalgae biomass produced but can be an interesting solution to be applied on-farm.

In this context, the aims of the study were: (i) to evaluate the possibility of using ADW from maize cultivation as growing media to produce microalgae; (ii) to evaluate the potential of this strategy to treat ADW, enhancing degradation of undesirable substances (e.g., pesticides); and (iii) to evaluate the potential of microalgae biomass suspension to be used as slow-release organic fertilizer.

The proposed solution has socio-economic-environmental relevance and, to the best of our knowledge, recycling ADW from open field cultures for microalgae cultivation was not evaluated before. It was hypothesized that (i) it is possible to recycle water and nutrients from ADW to produce microalgae, reducing the water footprint in intensive irrigated cultures; (ii) microalgae cultivation will allow a reduction in the pollutant load of the ADW; and (iii) the use of the microalgae suspension as an organic fertilizer can reduce the application of mineral fertilizers, with benefits to soil health and to plant nutrition status.

2. Materials and methods

2.1. Water sampling and characterization

The selected maize agricultural field is located near São João da Ribeira, Tejo hydrographic region (39°17′13.6″N 8°53′37.6″W, Rio Maior, Portugal; Supplementary data: Fig. S1). During the rainy season, a subterraneous drain system collects the drainage water, which is stored, along with the runoff water, in a pond. During part of the rainy season, the pond outlet is open, to drain the surplus water into the river, and closed when the farmer wants to store water for irrigation. All the agrochemicals applied by the farmer to the maize crop during the 2021 season were registered (Supplementary data: Table S1).

A 100 L sample of ADW was collected from the pond on July 9th, 2021, to be used for microalgae cultivation. A 1 L sub-sample was transported to the laboratory in a cooler, at 4 °C, and characterized for physicochemical parameters using reference methods (Standard Methods for the Examination of Water and Wastewater, 2018): pH (by potentiometry, using a Thermo Scientific™ Orion™ 3-STAR Benchtop pH Meter); electrical conductivity (EC; by conductimetry, using a Thermo Scientific™ Orion

Table 1

Characterization of the agriculture drainage water used for microalgae cultivation (mean ± standard deviation, n = 3). EC: electrical conductivity; COD: chemical oxygen demand.

Parameter	Unit	Result
pH	Sorensen scale	7.68 ± 0.03
EC	µS cm^{-1}	1009.0 ± 0,1
Na	mg L^{-1}	64.05 ± 0.19
K	mg L^{-1}	8.41 ± 0.19
Ca	mg L^{-1}	107.24 ± 0.77
Mg	mg L^{-1}	17.22 ± 0.07
P	mg L^{-1}	0.07 ± 0.00
S	mg L^{-1}	11.99 ± 1.24
Fe	mg L^{-1}	0.02 ± 0.00
Cu	mg L^{-1}	0.010 ± 0.001
Zn	mg L^{-1}	<0.01
Mn	mg L^{-1}	0.008 ± 0.001
B	mg L^{-1}	0.032 ± 0.001
Cl$^-$	mg L^{-1}	101.6 ± 2.0
COD	mg O$_2$ L^{-1}	19.9 ± 0.1
NO$_3^-$	mg NO$_3^-$ L^{-1}	55.56 ± 0.59
NH$_4^+$	mg NH$_4^+$ L^{-1}	2.36 ± 0.03

STAR™ A212 Benchtop Conductivity Meter); mineral N (nitrates (NO$_3^-$) and ammonium (NH$_4^+$)); using a Continuous Flow Analyzer, with and UV detector, Skalar, San plus System); chemical oxygen demand (COD; by acid digestion with potassium dichromate and titration with ammonium iron (II) sulfate); chloride (Cl$^-$; by titration with silver chloride, using the Mohr's method); and total concentrations of other elements, important as macro and micronutrients (P, K, S, Na, Ca, Mg, B, Fe, Mn, Cu, and Zn; by inductively coupled plasma optical emission spectrometry, ICP-OES using an iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA, on concentrated nitric acid hot digested samples) (Table 1).

ADW was also screened of representative groups of pesticides' active substances, which can hinder microalgae growth, in an external laboratory (Eurofins), using standard quality control procedures and methodologies in accordance with DIN 38407-36:2014-09 (n.d.).

Only herbicides were detected at quantifiable concentrations in the water from the drainage pond (Table 2), some of them not reported as applied by the farmer (e.g., Glyphosate; Supplementary data: Table S1). All other pesticides that were analyzed, including the insecticide that was applied (lambda-cyhalothrin), were only, potentially, present at concentrations below the quantification limit of the method.

2.2. Microalgae cultivation using ADW

Agricultural drainage water was used for the preparation of Allmicroalgae's base medium (MNS, based on Guillard's F/2 medium, at 7.5 mM of nitrates), supplemented with 19 µM Fe. An initial screening, using different microalgae from Allmicroalgae's culture collection, was

Table 2

Pesticides quantified in the agriculture drainage water collected and used for the microalgae growth experiments (all herbicides) (mean ± standard deviation, n = 3).

Active substance	Concentration (µg L^{-1})
6-Chlor-3-phenylpyridazin-4-ol (Pyridafol)	0.36 ± 0.18
Metolachlor	0.86 ± 0.43
Nicosulfuron (*)	0.25 ± 0.13
Terbuthylazine	0.081 ± 0.041
Terbuthylazine, desethyl- (**)	0.14 ± 0.07
Glyphosate (*)	0.058 ± 0.029
AMPA (Aminomethylphosphonic acid) (***)	0.47 ± 0.24
Tembotrione	0.072 ± 0.036

(*) Herbicides not reported by the farmer as applied in the field; (**) Terbuthylazine metabolite; (***) Glyphosate metabolite.

performed, to evaluate which microalgae strains would be more suitable to grow using ADW as a culture medium, using two freshwater strains: *Chlorella vulgaris*, and *Scenedesmus obliquus* (transferred to the genus *Tetradesmus* (Wynne and Hallan, 2015), but hereafter referred by its most common name, *S. obliquus*), and two saltwater strains: *Tetraselmis chui*, and *Phaeodatylum tricornutum*. Growth screening tests were performed for 12 days, and, for the saltwater strains, salinity was adjusted to 30 g L^{-1} using NaCl and a magnesium-rich supplement.

Two selected strains were grown in three conditions: (i) using fresh water and Allmicroalgae's base medium, as a control (Control MNS); (ii) using ADW supplemented with Allmicroalgae's base medium (ADW + MNS), and (iii) using ADW (ADW). Cultures were inoculated at 0.3 g L^{-1} using 50 mL photobioreactors, under constant aeration, at 23 °C, and LED light (300 µmol photons m^{-2} s^{-1}).

A validation assay was performed, using 40 L flat panel photobioreactors at pilot industrial setting, under constant aeration and with pH control under 8.2 through pure CO$_2$ injection. *C. vulgaris* and *S. obliquus* were grown with outdoor temperature and light conditions, for 15 days, from 1st to 15th October 2021. Temperatures and radiation were registered (Supplementary data: Fig. S2 and S3, respectively). Culture growth was followed every other day, through measurement of optical density, and the dry weight was estimated using a calibration curve previously established. The nitrate level in the culture was determined twice a week according to Armstrong (1963). Biomass and supernatant were collected at the end of the assay, by centrifugation at 2500g for 15 min, to be characterized for their chemical composition, including pesticides previously quantified in the ADW. Whole suspensions were frozen (−20 °C) until use, and characterized, to ascertain application doses for lettuce growth pot experiments.

2.3. Experimental set-up to evaluate the fertilizer potential of the microalgae suspensions

Microalgae biomass potential as a slow-release organic fertilizer was assessed using lettuce in a pot experiment, with application of the liquid suspensions of *C. vulgaris* or *S. obliquus* in replacement of 50 % of the standard fertilizer recommendations for lettuce (1.0 g N plant^{-1}). Pots with 3.2 L were used (15.5 cm height and 47 cm surface diameter), with four replicates per treatment, in a complete randomized design, with a sandy loam soil (4 kg per pot), collected at Golegã (Portugal; 39°25′16.1″ N 8°27′00.9″W) (soil physicochemical characteristics in Supplementary data: Table S2). All pots, except the control (C), received a basal fertilization with 0.5 g mineral N (as ammonium nitrate). Mineral control (M) received a top-dressing fertilization with 0.5 g N pot^{-1}, as ammonium nitrate, while that part of the mineral N dose (50 %) was replaced in the others by the application of 0.5, 1.0, and 2.5 g N pot^{-1}, via microalgae suspension, leading to eight treatments: C: control without fertilization; M: mineral fertilized control; CV0.5, CV1.0, and CV2.5: *C. vulgaris* applied at 0.5, 1.0 and 2.5 g pot^{-1}, respectively; and SO0.5, SO1.0, and SO2.5: *S. obliquus* applied at 0.5, 1.0 and 2.5 g pot^{-1}, respectively.

The amount of N applied with microalgae was equal to the mineral N applied as top-dressing (0.5 g N pot^{-1}), 2-times (1.0 g), and 5-times (2.5 g) higher than that value, hypothesizing that mineralization would not be completed during lettuce growth (Coppens et al., 2016; Garcia-Gonzalez and Sommerfeld, 2016). Therefore, the application of 0.5 g N pot^{-1} via microalgae suspension (CV0.5 and SO0.5) could, eventually, correspond to a N deficit situation, when comparing with the mineral control. *C. vulgaris* and *S. obliquus* suspensions started to be applied to the soil approximately 31 days before lettuce transplanting, to allow the mineralization of a fraction of the applied biomass, rendering part of the nutrients available, but never exceeding, in each application, the volume needed to maintain the soil at 70 % of its maximum water holding capacity (WHC) (detailed description in Supplementary data: Table S3).

Lettuce (*Lactuca sativa* var. Nadine) was seeded on a non-sterile organic growing media before transplanting (one plant per pot), which occurred with 28-days grown plants. After that, pots were routinely watered with deionized water to maintain 70 % WHC. Top-dressing fertilization was

applied 28 days after transplanting, only to the mineral control (M). The experiment was maintained for 57 days after transplanting (from April 18th to June 14th, 2022), in a greenhouse without controlled conditions, evaluating the capacity of the microalgae biomass to replace mineral N provided as top-dressing, as well as other possible beneficial effects to the soil and to the plant.

2.4. Plant analysis

Chlorophyll content in the leaves was measured non-destructively using a hand-held meter (Model CL-01, Hansatech Instruments), that allows an estimation of the chlorophyll content using dual wavelength optical absorbance measurements, at 620 and 940 nm, on the leaf sample (Cassol et al., 2008). Measurements were made on a weekly basis, three weeks after transplanting. Three measurements were made on each leaf, selecting one leaf per plant (one of the youngest, totally developed), and the arithmetic mean was used.

At the end of the experiment (57 days after transplanting), each lettuce plant was cut 0.5–1.0 cm above the soil surface, eliminating the deteriorated leaves that were in contact with soils. The rest of the plant was weighted, to determine fresh biomass, rinsed with deionized water, to remove any attached particles, and oven-dried at 60 °C for 48 h. The dried aboveground biomass was grounded through a 1 mm screen, using a knife mill (Fritsch pulverisette 15 - Fritsch GmbH, Idar-Oberstein, Germany). Total content of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), boron (B), zinc (Zn), manganese (Mn), sulfur (S) and iron (Fe) was determined, after digestion with *aqua regia* (CEN, 2001), by ICP-OES (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA). Total N concentrations were determined using a DUMAS protein/nitrogen analyzer (VELP Scientific NDA 702 DUMAS Nitrogen Analyzer—TCD detector).

2.5. Soil physicochemical characterization

The whole soil was removed from the pots, separated from the plant roots, and homogenized. A subsample was preserved to be used for enzymatic activities determination (Sub-section 2.6), and the remaining sample was air-dried before sieving, to analyze the <2 mm soil fraction. Soil pH and EC were determined after 1 h of agitation of a soil:deionized water suspension (1:2.5 w/v) and measured directly on the supernatant aqueous phase after settlement (Orion 3 Star, Thermo Fisher Scientific, USA). The carbon in the soil was assessed by dry combustion at 1000 °C and infra-red detection in a total-organic-carbon analyzer (multi EA4000 TOC Analyser, AnalytikJena, Germany), converting it to organic matter content (multiplying by the factor 1.72). Total N content was determined using a DUMAS nitrogen analyzer (VELP Scientific NDA 702 DUMAS Nitrogen Analyzer—TCD detector). Mineral N ionic forms (NH_4^+ and NO_3^-) were analyzed in a segmented flow autoanalyzer (San Plus System, Skalar, Nederland), after extraction using KCl 2 M (1:5 w/v, soil:solution ratio), agitated for 1 h and centrifuged at 4000 rpm for 7 min (centrifuge 5804, Eppendorf, Germany). Extractable P and K concentrations were determined after extraction with the Egner-Riehm solution (1:20 *w/v*) (Riehm, 1958), after 2 h agitation and posterior centrifugation at 4000 rpm for seven minutes (5804, Eppendorf, Germany). The supernatant was collected, and the concentration was determined by ICP-OES (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA). The same equipment was used to analyze extractable micronutrients, Fe, Mn, Cu, and Zn, after the extraction of the soil samples for 30 min with a solution of 0.5 M acetic acid, 0.5 M ammonium acetate, and 0.02 M EDTA (1:10 w/v) (Lakanen and Ervio, 1971), and extractable B, using boiling water for 10 min (1:2 w/v) (Gupta, 1993). Cation exchange capacity (CEC) was calculated by the sum of the non-acid cations, Na, K, Ca, and Mg, obtained after the soil extraction with 1.0 M ammonium acetate (1:15 w/v). The suspension was agitated for 1 h (J.P Selecta Rotabit) and centrifuged at 4000 rpm for seven minutes (5804, Eppendorf, Germany). The extract was collected,

and Na, K, Ca, and Mg concentration was determined by ICP-OES (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA).

2.6. Soil enzymatic activities

Soil enzymatic activities were measured considering: (i) dehydrogenase, an intracellular oxidoreductase related to the phosphorylation process, often used as an overall indicator of microbial biomass, and the hydrolases (ii) β-glucosidase, an exoenzyme from the C-cycle, and (iii) acid-phosphatase, representative of the P-cycle (Alvarenga et al., 2019).

Dehydrogenase activity was measured in the moisten soil sampled at the end of the experiment, following Tabatabai (1994), with modifications, as described by Alvarenga et al. (2019). Sieved samples (<2 mm) were incubated for 16 h, at 25 °C, with 0.1 % (*w/v*) triphenyltetrazolium choride (TTC) in a Tris-buffer (0.1 M, pH 7.8 for acid soils, pH 7.6 for neutral soils). In this incubation period, the reduction of TTC to triphenylformazan (TPF) occurs, being measured spectrophotometrically at 546 nm. All measurements were carried out in duplicate, and the activity was expressed in μg TPF g^{-1} h^{-1}, on an oven-dried weight basis (105 °C, 48 h).

Hydrolases (β-glucosidase and acid phosphatase) activities were measured in sieved soil samples (<2 mm), refrigerated (4 °C) at their "field moisture content" until analysis, and the activities were also expressed on a dry weight basis (105 °C, 48 h). β-glucosidase activity was measured by incubating 1 g of soil with *p*-nitrophenyl-β-D-glucopyranoside in modified universal buffer (pH 6.5, 4 mL) at 37 °C. After 1 h, the *p*-nitrophenol (PNP) released was extracted with 0.1 M tris(hidroxymetil)aminometane-NaOH pH 12.0 and measured spectrophotometrically (Eivazi and Tabatabai, 1988; Alef and Nannipieri, 1995). Acid phosphatase activity was measured by incubating 1 g of soil with *p*-nitrophenyl phosphate in modified universal buffer (pH 6.5, 4 mL) at 37 °C. After 1 h, 0.5 M CaCl$_2$ (1 mL) was added and the PNP released was extracted with 0.5 M NaOH and measured spectrophotometrically (Eivazi and Tabatabai, 1977; Alef et al., 1995). Analytical measurements were carried out in triplicate and β-glucosidase and acid phosphatase activities were expressed in μmol PNP g^{-1} h^{-1}.

2.7. Statistical treatment of the data

The differences among the treatments were evaluated using one-way ANOVA analysis of variance. Whenever significant differences were found, a post hoc Tukey Honest Significant Difference (HSD) test was used to further elucidate differences among means, at a P level 0.05.

3. Results and discussion

3.1. Microalgae cultivation using ADW

C. vulgaris, *S. obliquus*, *T. chui*, and *P. tricornutum*, grew in ADW supplemented with Allmicroalgae's base medium, with no significant differences for their global and maximum productivities (Table 3), meaning that they all could be grown using maize ADW. Considering these results, the two freshwater strains were selected: *C. vulgaris* and *S. obliquus*, as these strains would not require salt supplementation to be grown (i.e., lower production

Table 3
Global and maximum productivity of the four strains (mean ± standard deviation, n = 3) grown in ADW supplemented with Allmicroalgae's base medium. Values in the same column marked with the same letter are not significantly different (Tukey HSD test, p > 0,05).

Strain	Global productivity (g L^{-1} day^{-1})	Maximum productivity (g L^{-1} day^{-1})
T. chui	0.340 ± 0.046 a	0.690 ± 0.110 a
C. vulgaris	0.431 ± 0.022 a	0.767 ± 0.110 a
P. tricornutum	0.352 ± 0.064 a	0.546 ± 0.072 a
S. obliquus	0.442 ± 0.075 a	0.807 ± 0.169 a

costs), enabling the reuse of the treated water, recovered after biomass harvest, without salinity problems.

The next experiment was designed to evaluate (i) if ADW alone had sufficient nutrients for microalgae growth, and (ii) if there were any drained chemicals (e.g., nutrients, pesticides) which could either inhibit or potentiate microalgae growth, compared to the control using freshwater in standard media preparation. ADW characterization (Tables 1 and 2) together with the global productivities (Table 4) suggests there were no relevant inhibitory compounds that affected microalgae growth; on the contrary, supplemented ADW allowed increased algae productivities compared to the use of freshwater (Table 4; Growth curves for *C. vulgaris* and *S. obliquus* in Supplementary data, Fig. S4). These results suggest microalgae can uptake nutrients from ADW, allowing their recycling, which could be a cheap nutritive alternative for microalgae growth, instead of freshwater.

The next step was to grow *C. vulgaris* and *S. obliquus* at pilot scale industrial settings, to obtain enough biomass to be used in the pot assays. During 15 days of cultivation, *S. obliquus* grew from 0.35 to 2.2 g L^{-1}, with a global productivity of 0.123 g L^{-1} day^{-1}, while *C. vulgaris* grew from 0.32 g L^{-1} to 1.15 g L^{-1}, with a global productivity of 0.055 g L^{-1} day^{-1} (Supplementary Material, Fig. S5). At the end of the trials, biomass and supernatant were collected and analyzed, as described in Materials and Methods, including the herbicides which were previously quantified in the ADW (Table 2). Analysis of the supernatant after microalgae cultivation suggests that this strategy could be applied for ADW treatment (Supplementary data, Table S5), since none of the herbicides which were quantified in the ADW were detected in the supernatant, which indicates that they were degraded during the microalgae growth to concentrations below the quantification limit of the method, <0.05 μg L^{-1}. This corresponded to a reduction in the concentrations to >17-times for Metolachlor, 9-times for AMPA, 7-times for Pyridafol, and 5-times for Nicosulfuron, the substances found at higher concentrations in the ADW sample. An advantage of applying a microalgae-based treatment to ADW, is that treatment can be installed at the points of ADW discharge to the collecting pond, e.g., using shallow raceway ponds (Oliveira et al., 2021; Geremia et al., 2021), or before the discharge to the river, diminishing the impact on surface water and its potential pollution.

Biomass suspensions of both microalgae strains were analyzed for their characteristics that could be important to their use in replacement of conventional mineral fertilizers, namely considering their macro and micronutrients content (Table 5). Despite the different microalgae productivities, total N concentration in both cultures was similar ($\cong$ 2 g N L^{-1}), possibly because mineral N was supplemented to the growth media as nitrates, and some was immobilized by the biomass, but some remained in the growth media.

3.2. Effects of microalgae biomass application on plant parameters

Microalgae application promoted a two-fold higher lettuce biomass fresh weight, compared to that obtained when using mineral-N fertilizer, similar for all application rates (0.5, 1.0 and 2.5 g pot^{-1}), and without significant differences between *C. vulgaris* and *S. obliquus* (Fig. 1A). Therefore, 31 days allowed sufficient microalgae biomass mineralization, without the need to increase the microalgae application beyond what is necessary to

Table 4
Global productivity (g L^{-1} day^{-1}) of *C. vulgaris* and *S. obliquus grown* in: (i) freshwater medium (Control MNS); (ii) medium prepared using agricultural runoff water (ADW + MNS); and (iii) agricultural runoff water alone (ADW) (mean ± standard deviation, n = 3). Values in the same column marked with the same letter are not significantly different (Tukey HSD test, p > 0,05).

	Global productivity (g L^{-1} day^{-1})	
	C. vulgaris	*S. obliquus*
Control MNS	0.145 ± 0.026 ab	0.263 ± 0.039 b
ADW + MNS	0.196 ± 0.021 a	0.358 ± 0.021 a
ADW	0.071 ± 0.007 b	0.062 ± 0.007 c

Table 5
Characterization of the microalgae cultures obtained outdoors (mean ± standard deviation, n = 3).

	C. vulgaris	*S. obliquus*
pH	7.30 ± 0.01	7.84 ± 0.02
EC (μS cm^{-1})	2603.0 ± 2.5	2994 ± 0.4
Dry matter (g L^{-1})	3.92 ± 0.01	3.17 ± 0.02
N$_{total}$ (mg L^{-1})	2079 ± 42	2005 ± 24
P$_{total}$ (mg L^{-1})	31.47 ± 0.55	16.49 ± 0.18
K (mg L^{-1})	346.7 ± 17.2	410.6 ± 16.5
Na (mg L^{-1})	248.9 ± 10.0	301.7 ± 9.8
Ca (mg L^{-1})	96.7 ± 5.3	24.3 ± 0.6
Mg (mg L^{-1})	18.0 ± 1.3	11.0 ± 0.5
S (mg L^{-1})	69.0 ± 5.0	72.3 ± 1.7
Fe (mg L^{-1})	4.58 ± 0.37	1.96 ± 0.85
Cu (mg L^{-1})	0.062 ± 0.004	0.032 ± 0.005
Zn (mg L^{-1})	0.130 ± 0.032	0.067 ± 0.031
Mn (mg L^{-1})	0.357 ± 0.009	0.102 ± 0.017
B (mg L^{-1})	0.089 ± 0.017	0.144 ± 0.005

supply the recommended N dose. Higher plant yield in the microalgae fertilized pots was not accompanied by an increase in N content in lettuce, which was significantly higher in the mineral control (M), where N was not limitative of its growth (Fig. 1A).

Results suggest N was the limiting nutrient in control soil (C), where a very reduced growth was observed, and where N concentration in lettuce was significantly lower than for the other treatments (Fig. 1B). Contrarywise, P and K concentrations in lettuce grown in control soil (C) were higher, without significant differences to the higher concentrations observed (Fig. 1C and D, respectively), indicating these were not limiting nutrients in the soil.

Due to this high level of extractable P in the soil (194 mg P$_2$O$_5$ kg^{-1}; Supplementary Material, Table S2), even without P-fertilization, biomass obtained in the mineral-N fertilized pot (M) was 4-times higher than in the control soil (C), which exerted a dilution effect on the P concentration in lettuce (Fig. 1C). In the case of the microalgae-fertilized pots, plants presented higher P concentration than the mineral control (M) (except for the lettuce fertilized with CV0.5), because they were able to absorb extractable P from the soil, together with P provided by the microalgal biomass.

Regarding K, results suggest that microalgal application made a difference in a soil where the level of extractable K was medium. In this case, lettuce presented a significantly higher K content when higher doses of *C. vulgaris* and *S. obliquus* were applied (Fig. 1D).

For the other nutrients (Ca, Mg, S, Fe, Cu, Zn, Mn, and B; Supplementary data, Table S5), their concentrations in lettuce biomass were not statistically different between the mineral fertilized pots and the ones that received microalgae fertilization, except for Ca, which was statistically higher in the mineral fertilized pots, and for S, which presented statistically higher concentrations in plants fertilized with the higher doses of *C. vulgaris* or *S. obliquus*.

From the beginning of its measurement until six weeks of growth, chlorophyll values measured on the leaves of lettuce which received fertilization, mineral or organic, were similar and above those of the non-fertilized plants (C) (Fig. 2). This was expected, since all pots, except the control (C), received N-mineral basal fertilization with half the recommended dose, which leveled the N needs during the first stages of growth. However, on day 44 after transplanting, chlorophyll estimated content of lettuces that received the other half of N via microalgae application, started to decrease, which was evident by the chlorophyll values registered (Fig. 2). This trend was not observed in lettuces which received N-mineral top-dressing, which continued to increase, ending with statistically higher values. Plants fertilized with *C. vulgaris* and *S. obliquus* biomass most likely became dependent on the N resulting from their mineralization, which can only act as a slow-release fertilizer. These chlorophyll values are in accordance with the results for the N content in lettuce at the end of the experiment, with significantly higher N content in the mineral control (Fig. 1B). Nevertheless, the lower N content in the plants from microalgae fertilized pots were not limitative of their growth, as was obvious from plant productivity results (fresh weight; Fig. 1A).
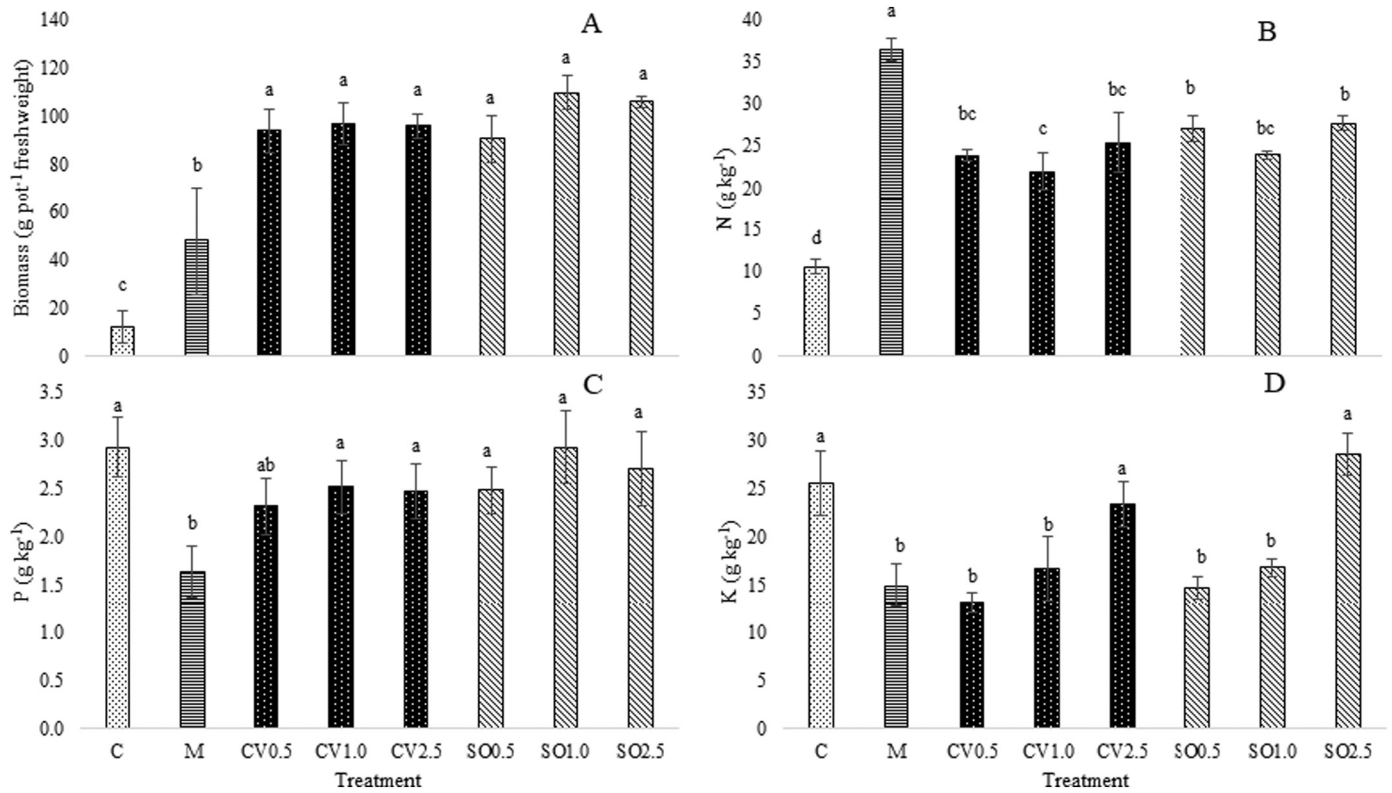
Fig. 1. Effects of the treatments on the: (A) lettuce fresh biomass; (B) N total content; (C) P total content; and (D) K total content (mean ± standard deviation, n = 4). Concentrations for N, P, and K are reported for a dry matter basis. Columns marked with the same letter are not significantly different (Tukey test, p > 0.05). C: control soil; M: mineral fertilized soil; CV0.5, CV1.0, and CV2.5: pots fertilized with *C. vulgaris* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively; SO0.5, SO1.0, and SO2.5: pots fertilized with *S. obliquus* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively.

3.3. Effects of microalgae biomass application on soil properties

As for the physicochemical soil properties, application of mineral fertilizer led to a significant decrease in soil pH (Fig. 3A), a consequence of its chemical formulation (ammonium nitrate), but soil remained slightly alkaline, with pH values ranging between 7.89 and 8.40. However, more important, mineral fertilizer application led to a very marked increase in soil EC values, relatively to the control (approximately, a 3-fold increase) and to the pots with microalgae fertilizer application. Higher dose of *C. vulgaris* and *S. obliquus*, 2.5 g N pot^{-1}, also led to a significant increase
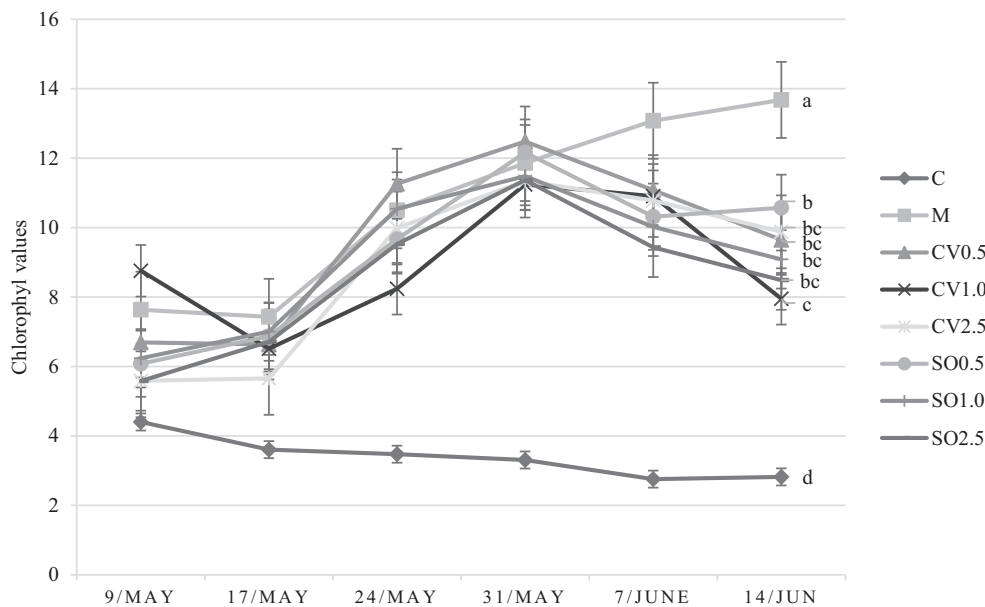

Fig. 2. Measurement of the chlorophyll value on the lettuce leaves during the five last weeks of growth (mean ± standard deviation, n = 4). Results measured on 14 of June marked with the same letter are not significantly different (Tukey test, p > 0.05). C: control soil; M: mineral fertilized soil; CV0.5, CV1.0, and CV2.5: pots fertilized with *C. vulgaris* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively; SO0.5, SO1.0, and SO2.5: pots fertilized with *S. obliquus* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively.
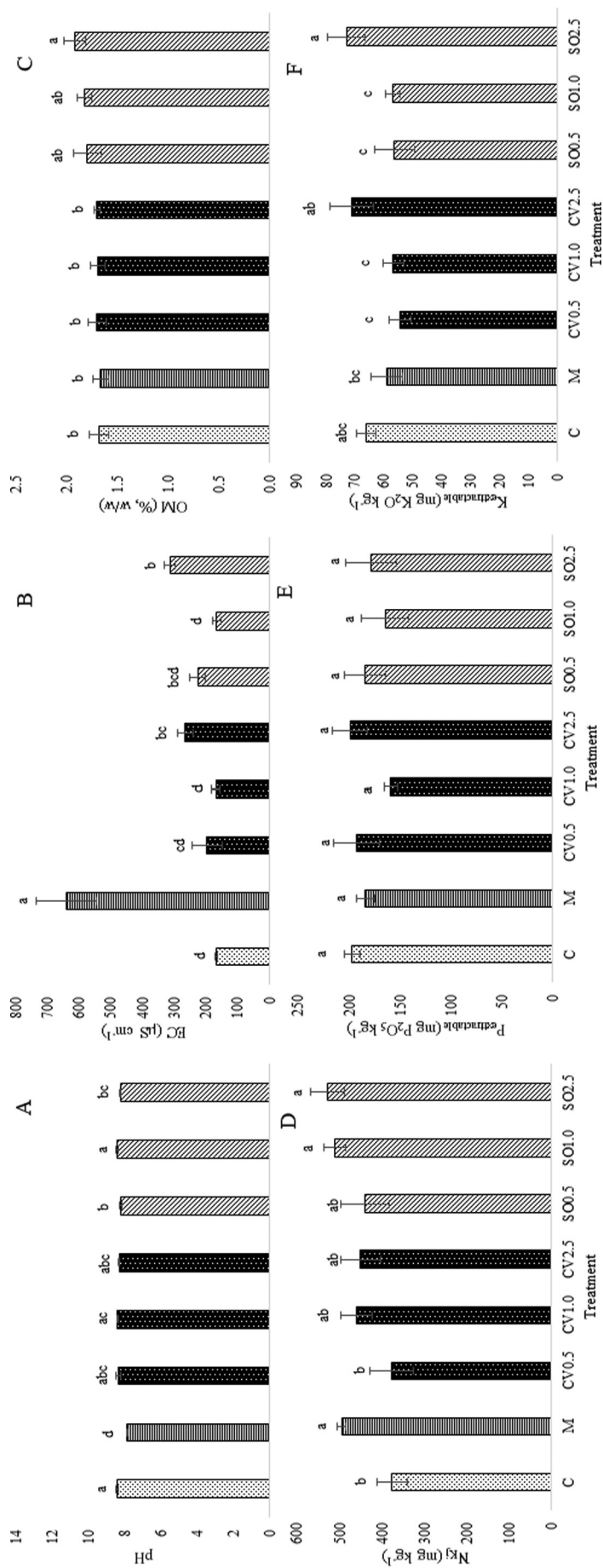
Fig. 3. Effects of the treatments on the: (A) soil pH; (B) electrical conductivity (EC); (C) organic matter (OM); (D) N Kjeldahl; (C) extractable P; and (F) extractable K concentrations (mean ± standard deviation, n = 4). Columns marked with the same letter are not significantly different (Tukey test, p > 0.05). C: control soil; M: mineral fertilized soil; CV0.5, CV1.0, and CV2.5: pots fertilized with C. vulgaris biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively; SO0.5, SO1.0, and SO2.5: pots fertilized with S. obliquus biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively.

in soil EC values, relatively to the control (Fig. 3B), but the values were only 1.5 to 2.0-times higher than that of the control. Secondary salinization of the soil, which can be induced by repeated application of mineral fertilizers, can, in fact, be avoided by the application of organic fertilizers, as microalgae-based fertilizers, if the doses remain only those necessary to fulfill plant nutritional needs.

As for the soil OM, application of *S. obliquus* biomass led to higher contents than the application of *C. vulgaris*, relatively to control (C) and to mineral fertilized soil (M), but results were only statistically different for the higher dose of application (Fig. 3C), and the soil still remained with a low OM content (between 1.1 and 2.0 % w/w; LQARS, 2006). The fact that mineralization occurs after microalgae application to soil, hinders a higher impact on that soil property, at least with a single application, as was the case. However, results obtained for *S. obliquus* were promising.

C. vulgaris for the higher application doses, 1.0 and 2.5 g pot^{-1}, was also able to significantly increase soil N content, relatively to control, and without significant differences from mineral N-fertilizer application (Fig. 3D).

As for extractable P concentrations (Fig. 3E), since the soil that was used had high levels of P (LQARS (2006); 194 mg P_2O_5 kg^{-1}, as reported in Table S2 Supplementary Material), microalgae application did not impact its concentration, but the contrary was true for extractable K concentrations (Fig. 3F). The soil that was used had a medium value of extractable K (LQARS (2006); 89 mg K_2O kg^{-1}, Table S2 Supplementary Material). Consequently, in microalgae fertilized pots, where plant biomass yield was higher, K uptake was also higher, which led to a slight decrease in extractable K concentrations in the soil in those pots, but without significant differences to (C) or (M). Significant differences were only observed for the higher application dose of both microalgae biomasses (2.5 g pot^{-1}) which led to a significant increase of that parameter in the soil, relatively to results obtained for the lower microalgae doses (0.5 and 1.0 g pot^{-1}).

The CEC and extractable Fe, Cu, and Zn concentrations in the soil were not affected, without significant differences among the results, and only slight differences were obtained for extractable Mn and B, but without a marked trend (Supplementary Material; Table S6).

3.4. Effects of microalgae biomass application on soil enzymatic activities

Soil quality improvement in microalgae fertilized pots, relative to control and to mineral N-fertilizer application, was evident by the increase in some soil enzymatic activities (Fig. 4).

The most relevant positive impact was for soil dehydrogenase activity (Fig. 4A). In this case, enzymatic activity increased with the increase in the application doses of either microalga, with statistically higher values, relatively to the mineral control, for the higher application dose of *C. vulgare* and *S. obliquus*, 2.5 g N pot^{-1}. This is an important result, indicating that, globally, soil microbial activity was significantly enhanced by the application of microalgae biomass, which was able to improve soil quality and act beyond just a fertilizer in their role of providing nutrients to fulfill plant needs. In fact, dehydrogenase is an intracellular oxidoreductase, which is only present in viable cells, making it possible to use its activity as representative of the overall microbial population of a soil (Tabatabai, 1994), and has been used by different authors as an indicator of soil health (Alvarenga et al., 2019).

As for β-glucosidase activity (Fig. 4B), an enzyme from the C-cycle, its activity was also positively affected by the microalgae application, with significantly higher activities, relative to soil and to N-mineral control (except for the application of the lower application rate of *C. vulgaris*, 0.5 g N pot^{-1}, which was higher, but not significantly different from the controls), indicating a higher activity to degrade the organic compounds added trough the microalgae biomass.

For acid phosphatase (Fig. 4C), only the higher application dose of *S. obliquus*, 2.5 g N pot^{-1}, was able to significantly increase its activity, relatively to N-mineral fertilized control, the same treatment which significantly increased soil OM content (Fig. 3C). This better performance of *S. obliquus*, relatively to *C. vulgaris*, may be a consequence of the fact that *S. obliquus* reached a higher productivity when growing in similar

conditions in ADW (2.2 g L^{-1} *for S. obliquus* versus 1.15 g L^{-1} for *C. vulgaris*), turning *S. obliquus* more interesting to be used on-farm to treat ADW.

4. Conclusions

ADW, used to cultivate *S. obliquus* and *C. vulgaris*, allowed higher productivities than just using conventional growth medium, despite the concentrations of herbicides found in the ADW, which did not hinder the microalgae growth. Microalgae cultivation led to degradation of the herbicides in ADW, to below their limits of quantification, which supports this bioremediation strategy to capture pollutants that can cause eutrophication and, eventually, ecotoxicity, when runoff is directly sent to surface receiving waters.

Microalgae suspensions were able to replace 50 % of the N mineral fertilizer applied to lettuce, with significantly higher biomass production (2-fold increase), meaning that an equal dose of N applied via the microalgae biomass was sufficient to satisfy the plant's needs, if applied at least 31 days before transplanting.

Important benefits arise from the fertilization with microalgae biomass, namely avoiding the increase in soil secondary salinity, which was evident by a 3-times higher EC in the soil which received mineral fertilizer (ammonium nitrate), or leading to increased soil enzymatic activities.

Results validate the use of ADW from maize cultivation to produce *C. vulgaris* or *S. obliquus* suspensions, that can be used on-farm as low cost slow-release organic fertilizers, improving soil quality, and avoiding surface water pollution with nutrients and herbicides. Further studies would be important, to validate the alternative use of dehydrated biomass, because, despite the increase in operation costs, it would allow the storage, transport, and commercialization of the microalgae biomass produced.

CRediT authorship contribution statement

Paula Alvarenga: Conceptualization, Investigation, Project administration, Resources, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Marta Martins:** Investigation, Writing – review & editing. **Henrique Ribeiro:** Methodology, Supervision, Writing – review & editing. **Mariana Mota:** Methodology, Supervision, Writing – review & editing. **Inês Guerra:** Investigation, Writing – review & editing. **Helena Cardoso:** Investigation, Methodology, Validation, Writing – review & editing. **Joana Laranjeira Silva:** Investigation, Methodology, Validation, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.160670.
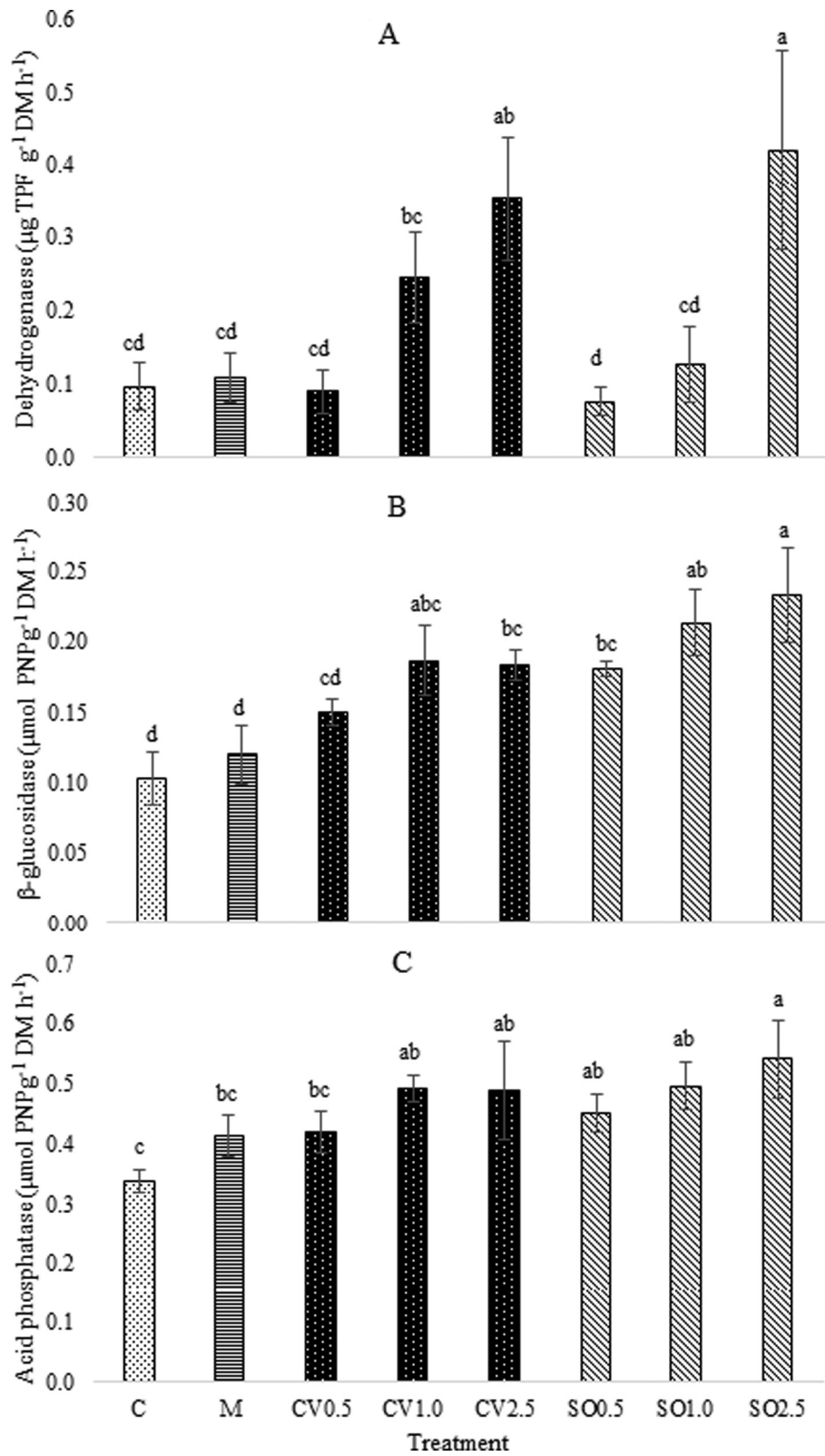
Fig. 4. Effects of the treatments on the soil enzymatic activities of: (A) Dehydrogenase, (B) β-glucosidase, and (C) Acid phosphatase (mean ± standard deviation, n = 4). Columns marked with the same letter are not significantly different (Tukey test, p > 0.05). C: control soil; M: mineral fertilized soil; CV0.5, CV1.0, and CV2.5: pots fertilized with *C. vulgaris* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively; SO0.5, SO1.0, and SO2.5: pots fertilized with *S. obliquus* biomass, 0.5, 1.0, and 2.5 g pot^{-1}, respectively.

References

Alef, K., Nannipieri, P., 1995. β-Glucosidase activity. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 350–352.

Alef, K., Nannipieri, P., Trasar-Cepeda, C., 1995. Phosphatase activity. In: Alef, K., Nannipieri, P. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 335–344.

Alvarenga, P., Rodrigues, D., Mourinha, C., Palma, P., de Varennes, A., Cruz, N., Tarelho, L.A.C., Rodrigues, S., 2019. Use of wastes from the pulp and paper industry for the remediation of soils degraded by mining activities: chemial, biochemical and ecotoxicological effects. Sci. Total Environ. 686, 1152–1163. https://doi.org/10.1016/j.scitotenv.2019.06.038.

Standard Methods for the Examination of Water and Wastewater. 23rd edition. American Public Health Association (APHA), American Water Works Association (AWWA), Water Environment Federation (WEF), New York.

Armstrong, F.A.J., 1963. Determination of nitrate in water by ultraviolet spectrophotometry. Anal. Chem. 35, 1292–1294. https://pubs.acs.org/doi/10.1021/ac60202a036.

Cabanelas, I.T.D., Ruiz, J., Arbib, Z., Chinalia, F.A., Garrido-Pérez, C., Rogalla, F., Nascimento, I.A., Perales, J.A., 2013. Comparing the use of different domestic wastewaters for coupling microalgal production and nutrient removal. Bioresour. Technol. 131, 429–436. https://doi.org/10.1016/j.biortech.2012.12.152.

Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: Status and prospects. Renew. Sustain. Energy Rev. 19, 360–369. https://doi.org/10.1016/j.rser.2012.11.030.

Cameira, M., Mota, M., 2017. Nitrogen related diffuse pollution from horticulture production —mitigation practices and assessment strategies. Horticulturae 3, 25. https://doi.org/10.3390/horticulturae3010025.

Cassol, D., De Silva, F.S.P., Falqueto, A.R., Bacarin, M.A., 2008. An evaluation of non-destructive methods to estimate total chlorophyll content. Photosynthetica 46, 634–636. https://doi.org/10.1007/s11099-008-0109-6.

CEN, 2001. EN13650 - Soil Improvers and Growing Media - Extraction of Aqua Regia Soluble Elements. European Committee for Standardization, Brussels.

Cicek, N., 2003. A review of membrane bioreactors and their potential application in the treatment of agricultural wastewater. Can. Biosyst. Eng. 45, 13.

Coppens, J., Grunert, O., Van Den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G., De Gelder, L., 2016. The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl. Phycol. 28, 2367–2377. https://doi.org/10.1007/s10811-015-0775-2.

DIN 38407-36:2014-09, . German standard methods for the examination of water, waste water and sludge - Jointly determinable substances (group F) - Part 36: Determination of selected active substances of plant protection products and other organic substances in water - Method using high performance liquid chromatography and mass spectrometric detection (HPLC-MS/MS or -HRMS) after direct injection (F 36). Deutsches Institut für Normung e. V. Available at https://www.beuth.de/en/standard/din-38407-36/208008665.

Eivazi, F., Tabatabai, M.A., 1977. Phosphatases in soils. Soil Biol. Biochem. 9, 167–172. https://doi.org/10.1016/0038-0717(77)90070-0.

Eivazi, F., Tabatabai, M.A., 1988. Glucosidases and galactosidases in soils. Soil Biol. Biochem. 20, 601–606. https://doi.org/10.1016/0038-0717(88)90141-1.

Farooq, W., Lee, Y.-C., Ryu, B.-K., Kim, B.-H., Kim, H.-S., Choi, Y.-E., Yang, J.-W., 2013. Two-stage cultivation of two Chlorella sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. Bioresour. Technol. 132, 230–238. https://doi.org/10.1016/j.biortech.2013.01.034.

Franchino, M., Tigini, V., Varese, G.C., Mussat Sartor, R., Bona, F., 2016. Microalgae treatment removes nutrients and reduces ecotoxicity of diluted piggery digestate. Sci. Total Environ. 569–570, 40–45. https://doi.org/10.1016/j.scitotenv.2016.06.100.

Garcia-Gonzalez, J., Sommerfeld, M., 2016. Biofertilizer and biostimulant properties of the microalga Acutodesmus dimorphus. J. Appl. Phycol. 28, 1051–1061. https://doi.org/10.1007/s10811-015-0625-2.

Geremia, E., Ripa, M., Catone, C.M., Ulgiati, S., 2021. A review about microalgae wastewater treatment for bioremediation and biomass production - a new challenge for Europe. Environments 8 (12), 136. https://doi.org/10.3390/environments8120136.

Gupta, U.C., 1993. Chapter 12: boron, molybdenum, and selenium. In: Carted, R.M. (Ed.), Soil Sampling and Methods of Analysis. Canadian Society of Soil Science.

Hena, S., Fatimah, S., Tabassum, S., 2015. Cultivation of algae consortium in a dairy farm wastewater for biodiesel production. Water Resour. Ind. 10, 1–14. https://doi.org/10.1016/j.wri.2015.02.002.

Kapoore, R.V., Wood, E.E., Llewellyn, C.A., 2021. Algae biostimulants: a critical look at microalgal biostimulants for sustainable agricultural practices. Biotechnol. Adv. 49, 107754. https://doi.org/10.1016/j.biotechadv.2021.107754.

Lakanen, E., Ervio, R., 1971. Comparison of eight extractants for the determination of plant available micronutrients in soils. Acta Agralia Fenn. 123, 223–232.

Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., Zhu, J., Ruan, R., 2011. Characterization of a microalga Chlorella sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. Bioresour. Technol. 102 (8), 5138–5144. https://doi.org/10.1016/j.biortech.2011.01.091.

LQARS, 2006. Manual de Fertilização de Culturas. Laboratório Químico Agrícola Rebelo da Silva, Instituto Nacional de Investigação Agrária e Veterinária – INIAV, Oeiras, Portugal (In Portuguese).

Moore, M.T., Kröger, R., Locke, M.A., Cullum, R.F., Steinriede, R.W., Testa III, S., Lizotte Jr., R.E., Bryant, C.T., Cooper, C.M., 2010. Nutrient mitigation capacity in Mississippi Delta, USA drainage ditches. Environ. Pollut. 158, 175–184. https://doi.org/10.1016/j.envpol.2009.07.024.

Navarro-López, E., Ruíz-Nieto, A., Ferreira, A., Acién, F.G., Gouveia, L., 2020. Biostimulant potential of Scenedesmus obliquus grown in brewery wastewater. Molecules 25 (3), 664. https://doi.org/10.3390/molecules25030664.

Oliveira, C.Y.B., Oliveira, C.D.L., Prasad, R., Ong, H.C., Araujo, E.S., Shabnam, N., Gálvez, A.O., 2021. A multidisciplinary review of Tetradesmus obliquus: a microalga suitable for large-scale biomass production and emerging environmental applications. Rev. Aquacult. 13, 1594–1618. https://doi.org/10.1111/raq.12536.

Palma, P., Matos, C., Alvarenga, P., Köck-Schulmeyer, M., Simões, I., López de Alda, M.J., Barceló, D., 2018. Ecological and ecotoxicological responses in the assessment of the ecological status of freshwater systems: a case-study of the temporary stream Brejo of Cagarrão (South of Portugal). Sci. Total Environ. 634, 394–406. https://doi.org/10.1016/j.scitotenv.2018.03.281.

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

Renuka, N., Prasanna, R., Sood, A., Bansal, R., Bidyarani, N., Singh, R., Shivay, Y.S., Nain, L., Ahluwalia, A.S., 2017. Wastewater grown microalgal biomass as inoculants for improving micronutrient availability in wheat. Rhizosphere 3, 150–159. https://doi.org/10.1016/j.rhisph.2017.04.005.

Renuka, N., Prasanna, R., Sood, A., Ahluwalia, A.S., Bansal, R., Babu, S., Singh, R., Shivay, Y.S., Nain, L., 2016. Exploring the efficacy of wastewater-grown microalgal biomass as a biofertilizer for wheat. Environ. Sci. Pollut. Res. 23 (7), 6608–6620. https://doi.org/10.1007/s11356-015-5884-6.

Riehm, H., 1958. Die ammoniumlaktatessigsaure-methode zur bestimmung der leichtloeslichen phosphosaure in karbonathaltigen boden. Agrochimica 3, 49–65.

Solovchenko, A., Verschoor, A.M., Jablonowski, N.D., Nedbal, L., 2016. Phosphorus from wastewater to crops: an alternative path involving microalgae. Biotechnol. Adv. 34 (5), 550–564. https://doi.org/10.1016/j.biotechadv.2016.01.002.

Tabatabai, M.A., 1994. Soil enzymes. In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis. Part 2 - Microbiological and Biochemical Properties. Soil Science Society of America, Madison, Wisconsin, pp. 775–833.

Tango, M.D., Calijuri, M.L., Assemany, P.P., do Couto, E.A., 2018. Microalgae cultivation in agro-industrial effluents for biodiesel application: effects of the availability of nutrients. Water Sci. Technol. 78 (1-2), 57–68. https://doi.org/10.2166/wst.2018.180.

Vymazal, J., Březinová, T.D., 2018. Removal of nutrients, organics and suspended solids in vegetated agricultural drainage ditch. Ecol. Eng. 118, 97–103. https://doi.org/10.1016/j.ecoleng.2018.04.013.

Wang, M., Zhang, D., Dong, J., Tan, S.K., 2018. Application of constructed wetlands for treating agricultural runoff and agro-industrial wastewater: a review. Hydrobiologia 805, 1–31. https://link.springer.com/article/10.1007/s10750-017-3315-z.

Wynne, M.J., Hallan, J.K., 2015. Reinstatement of Tetradesmus G.M. Smith (Sphaeropleales, Chlorophyta). Feddes Repert. 126, 83–86. https://doi.org/10.1002/fedr.201500021.

Xu, Z.-M., Wang, Z., Gao, Q., Wang, L.-L., Chen, L.-L., Li, Q.-G., Jiang, J.-J., Ye, H.-J., Wang, D.-S., Yang, P., 2019. Influence of irrigation with microalgae-treated biogas slurry on agronomic trait, nutritional quality, oxidation resistance, and nitrate and heavy metal residues in Chinese cabbage. J. Environ. Manag. 244, 453–461. https://doi.org/10.1016/j.jenvman.2019.04.058.

Yilmaz, E., Sönmez, M., 2017. The role of organic/bio–fertilizer amendment on aggregate stability and organic carbon content in different aggregate scales. Soil Tillage Res. 168, 118–124. https://doi.org/10.1016/j.still.2017.01.003.

Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. Water Res. 47 (13), 4294–4302. https://doi.org/10.1016/j.watres.2013.05.004.

Zuazo, V.H.D., Pleguezuelo, C.R.R., Flanagan, D.C., Martínez, J.R.F., Raya, A.M., 2009. Agricultural runoff: new research and trends – chapter 2. In: Hudspeth, C.A., Reeve, T.E. (Eds.), Agricultural Runoff, Coastal Engineering and Flooding, Environmental Science, Engineering and Technology Series. Nova Science Publishers, New York, pp. 27–48.