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RESEARCH ARTICLE

Nitrogen use efficiency of microalgae application in wheat compared to mineral fertilizer

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Abstract

Background: Wastewater from sewage treatment plants contains high levels of nutrients, which can be used for plant nutrition. Classical wastewater treatment plants use complex microbial consortia of autotrophic and heterotrophic microorganisms for biological wastewater treatment. Certain autotrophic microalgae (e.g., species of the genera *Chlorella*, *Scenedesmus*, and *Pediastrum*) accumulate nutrients from wastewater very effectively.

Aims: We investigated the potential of microalgae biomass obtained from a prototype wastewater treatment plant as a source of nutrients for crops, focusing on nitrogen.

Methods: We provided wheat plants with different levels of algae biomass equivalent to 60, 120, and 180 kg N per hectare or with mineral fertilizer (N, P, and K) equivalent to the amounts contained in the algal biomass. Physiological and phenotypic traits were measured during growth, including vegetation indices, photosynthetic performance, growth, and nitrogen use efficiency (NUE). In addition, the adundances of *Bacteria*, *Archaea* and fungi and genes of ammonium oxidizing *Bacteria* and *Archaea* were determined in the rhizosphere of differently fertilized plants.

Results: Microalgal application at fertilizer levels of 120 and 180 kg N ha^{-1} showed significantly improved physiological performance, growth, yield and nutrient uptake compared to the unfertilized control. Nevertheless, their yields and NUE were lower than with the application of equal amounts of mineral fertilization, while the adundance of rhizosphere microbes and ammonia-oxidizing microorganisms were not significantly affected.

Conclusions: Microalgae from wastewater treatments form a suitable source of organic fertilizer for wheat plants with only moderate reductions in N use efficiency compared to mineral fertilizer.

KEYWORDS
algae pond, microalgae, NDVI, nutrient recycling, nutrient use efficiency, organic fertilizer, wastewater

1 | INTRODUCTION

Various residues from wastewater treatment can form a source of fertilizer for crops. In classical wastewater treatment plants, complex microbial consortia of autotrophic and heterotrophic microorganisms (especially bacteria, fungi, and protozoa) are used for biological wastewater treatment to remove organic carbon, nitrogen, and phosphate. Wastewater is mechanically aerated to regulate oxic or anoxic conditions to meet the requirements of the different microbial processes. In particular, mechanical aeration and the sometimes-required carbon introduction for denitrification processes are very energy consuming. Alternatively, certain autotrophic microalgae (e.g., species of the genera *Chlorella*, *Scenedesmus*, and *Pediastrum*) could be used for wastewater treatment because these autotrophic microalgae release oxygen and thus regulate the oxygen level of the wastewater without mechanical aeration. Microalgae are photoautotrophic organisms that occur in different ecosystems and environments, such as marine, freshwater, wastewater, and brackish water (El-Sheekh et al., 2021; Renuka et al., 2018; Salama et al., 2013). Microalgae are interesting organisms for research. Due to their fast growth rate and ability to grow in water bodies, they do not demand large cultivation areas and produce biomass highly efficiently using water, CO_2, and radiation (Ahn et al., 2020). Effluents from wastewater treatment plants are rich sources of nitrogen and phosphorus that are suitable as a cost-effective growth medium for microalgae (Renuka et al., 2015). Therefore, microalgae might provide a sustainable option for advanced wastewater treatment while producing commercially valuable products (Salama et al., 2019). They efficiently accumulate nutrients, such as nitrogen and phosphorus, removing them from wastewater (Renuka et al., 2015). The biomass of microalgae is produced by wastewater treatment plants as a byproduct and can be used as fertilizer or as feedstock for recycling various nutrients. Ideally, nutrients used in agriculture should be subject to a recycling process.

Wheat is an important source of energy and protein for human nutrition and is one of the most widely grown crops in the world (FAO, 2022). There is a high demand for nitrogen for the growth of wheat. The yield and quality of wheat depend significantly on N inputs, as these promote canopy formation required for photosynthesis, which in turn determines yield (Hawkesford, 2014; Zörb et al., 2018). The production of N fertilizers by the Haber-Bosch process and the transport of N fertilizers is very energy intensive and dependent on fossil fuels (Zörb et al., 2018). Moreover, excessive fertilizer application, favored by high availability and supply, leads to environmental problems (e.g., N pollution through nitrate in water bodies, release of N-containing greenhouse gases) in some agroecosystems (Zörb et al., 2018). To improve the currently unsustainable management of major nutrients (N, P), nutrient recovery from wastewater could be a promising solution.

Despite the high biological value of microalgae, research on their use in agriculture is not very advanced (Ahn et al., 2020). Only in recent years have an increasing number of attempts been made to recycle microalgae biomass to return nutrients to plants. Depending on the focus of the studies, different wastewater technologies as well as different microalgae strains were analyzed and investigated. Coppens et al. (2016) showed that microalgae biomass could be used as a slow-release organic fertilizer for tomato cultivation and proposed supplementing conventional fertilizers with recycled microalgae biomass. Das et al. (2019) successfully demonstrated the potential use of microalgae biomass from wastewater treatment as an organic fertilizer for wheat plants. They conducted a pot experiment with wheat plants over a period of 2 months. Wheat plant growth was higher with the NPK fertilizer compared to the unfertilized control but lower compared to the microalgae biomass. Schreiber et al. (2018) also grew wheat plants over a 46-day period. Fertilizer rates, both algae fertilizer and mineral fertilizer, were adjusted according to the amount of P used in agricultural practice (approximately 45 kg P ha^{-1}). The authors found that the plants were able to take up P and N from the algae biomass effectively but suggested that nutrients from algal biomass were taken up by plants more slowly than those from mineral fertilizers.

Fertilizer application (both organic and inorganic) is a crucial management activity in agricultural production that can affect soil microbe diversity and abundance (Chen et al., 2016; Wang et al. 2015). Soil microbes are of great importance in the soil ecosystem, carrying out essential functions such as nitrogen fixation, ammonium oxidation, denitrification, and ammonification in the soil nitrogen cycle (Dincă et al., 2022). Xue et al. (2016) showed that soil fertilization with mineral and organic N (manure) increased the abundance of ammonium oxidizing *Archaea* (AOA) and *Bacteria* (AOB) in fertilized soil with a significant difference in the abundance and composition of AOA and AOB taxa in the differently fertilized soils (Xue et al., 2016).

Only a few studies have investigated the effect of microalgae as an agricultural fertilizer on soil and rhizosphere microbial communities. Alobwede et al. (2022) found that fertilization with algal biomass increased the microbial diversity and altered the relative abundance of specific microbial taxa in soil. Suleiman et al. (2020) showed that microalgae fertilization had different effects on the abundance of AOBs and AOAs in bulk soil and the rhizosphere of barley than mineral fertilization. The authors also observed that fertilization with microalgae increased N_2O and CO_2 emissions, which was linked especially to microbial nitrification processes.

This study explored the potential of microalgae as an alternative source of nutrients, especially N, for wheat plants and compared them with equivalent amounts of mineral fertilizer. The following hypotheses were tested to understand N release from algae and its uptake into wheat plants: (1) Plants supplied with N either in mineral form or as microalgae will show enhanced physiological performance compared to unfertilized plants. (2) Plants provided with microalgae and mineral fertilizer at different rates will show contrasting performance in terms of growth and nitrogen use efficiency. (3) The fertilization with different N sources, mineral versus microalgae biomass, will affect the abundance of rhizosphere microbes of the bacteria, archaea and fungi, and ammonium oxidizing bacteria and archaea in the rhizosphere of growing wheat plants in a different manner.

2 | MATERIALS AND METHODS

2.1 | Microalgal biomass

The microalgae biomass used for the experiment was generated in a prototype wastewater treatment plant at Lich (Hesse, Germany), which is maintained by the University of Applied Sciences of Central Hesse (Giessen). The open system is operated with wastewater, which means that no constant algae culture can be maintained. It is rather a mixed culture with changing genera, depending on environmental conditions. Among others, microalgal species of the genera *Chlorella*, *Scenedesmus*, and *Pediastrum* are present in the pilot plant. The microalgae were collected from the wastewater treatment plant during the winter months and dried at 40°C for several days. The microalgae dry matter had a nitrogen content of 4.45%, P content of 1.21%, and K content of 0.77%.

2.2 | Experimental design

The experiment was set up as a randomized complete block design with 6 replicates in a greenhouse as a pot experiment. Temperatures were constant at 13°C at night and 18°C during the day. After 8 weeks, the greenhouse temperature was increased to 22°C during the day and 15°C at night. Artificial lighting was used to supplement natural light for 16 h per day to ensure a minimum photosynthetically active photon flux density (PPFD) of 300 µmol m^{-2} s^{-1}, corresponding to 8 h of darkness. Plants were grown in 2-L pots, each filled with 1.5 kg of a loamy-sand LUFA standard soil F2.2 from LUFA-Speyer. This soil had a nitrogen content of 0.20%, organic carbon content of 1.72%, cation exchange capacity of 8.4 meq 100 g^{-1}, and pH value of 5.5. The experimental factors were fertilization with microalgae (A) or mineral fertilizer (M) at three factor levels each. The factor levels represented fertilizer application levels corresponding to 60, 120, and 180 kg N ha^{-1}, which were scaled down to the pot level based on the pot surface area. In addition, there was an unfertilized control (C). The mineral fertilizer treatments (M) (N, P, K) were devised to provide equivalent amounts of nutrients contained in the algae. Based on the analyses of algal composition, this corresponded to a fertilizer rate equivalent to 14.9, 32.3, and 48.4 kg P ha^{-1} and 10.2, 20.4, and 30.6 kg K ha^{-1}. Ammonium nitrate (NH_4NO_3) was used for mineral nitrogen fertilization. To provide equivalent amounts of potassium and phosphorus corresponding to the microalgae rates, potassium dihydrogen phosphate (KH_2PO_4) and sodium dihydrogen phosphate dihydrate ($H_2NaO_4P\bullet 2H_2O$) were used in the mineral fertilizer treatments. Spring wheat of the variety "Starlight" from KWS was pregerminated and then pricked into the pots to ensure homogenous plants at the beginning of the experiment.

2.3 | Growing period

All pots were watered with nutrient-free water as needed throughout the experimental period. Macro- and micronutrients ($CaCl_2$, $MgSO_4 \bullet 7H_2O$, Mo, B, Zn, Cu, Fe) were fertilized during the experimental period to ensure that plant growth would not be limited due to deficiency of these nutrients in any of the treatments. Various nondestructive measurements and physiological phenotyping were carried out during the cultivation period. Vegetation indices were recorded with the spectroradiometer PolyPen RP 410 (Photon Systems Instruments, Drásov, Czech Republic) as previously described (Begum et al., 2020) and calculated as follows:

$$\text{NDVI (Normalized Difference Vegetation Index)} = \left(R_{780} - R_{630}\right) / \left(R_{780} + R_{630}\right), \quad (1)$$

$$\text{PRI (Photochemical Reflectance Index)} = \left(R_{531} - R_{570}\right) / R_{531} + R_{570}). \quad (2)$$

The youngest and the third-youngest fully expanded leaf were measured, always with the leaf surface facing downward on the sensor. The net carbon flux rate (µmol m^{-2} s^{-1}) was measured using the LI-6800 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA). The youngest fully expanded leaf of each plant was measured for 2–3 min by clamping it in the measuring head. Wheat leaves were measured at a CO_2 reference value of 400 ppm, a leaf temperature of 23°C, a relative humidity of 60%, and a flow rate of 300 µmol s^{-1}.

2.4 | Harvest

The final harvest took place after 15 weeks of growth. The plants were cut just above the soil surface, and the fresh and dry aboveground biomass were recorded. In addition, the total grain yield of the plants was determined. The plant samples (straw and grain) were ground to homogeneous material by means of a Retsch Mixer Mill MM 400 (Retsch GmbH, Haan). Rhizosphere soil was collected at the first harvest according to Bulgarelli et al. (2012). The total procedure was performed with gloves. Loosely attached soil was removed by carefully shaking roots. The mid part of the root mass (10–15 cm depth) was selected for subsequent analyses. Rhizosphere soil was detached from roots by shaking (180 rpm, 20 min, 21°C) in sterile 15-mL Falcon tubes containing 2.5-mL autoclaved phosphate buffer solution (pH 7.0). After roots were removed, rhizosphere soil was harvested by centrifugation (1500 × *g*, 20 min). The supernatant was discarded, and the pelleted rhizosphere soil was stored at −20°C for molecular biological analysis.

2.5 | Nutrient analyses

The nitrogen content of the microalgae and plant material was determined using an elemental analyzer (Elementar UNICUBE, Elementar Analysysteme GmbH, Langenselbold, Germany). For the determination of phosphorus and potassium, the samples were digested in 69% HNO_3 by means of a microwave (Multiwave 5000 Anton Paar,

Graz). The P measurement was based on photometric determination using the Tecan Reader Infinite M Plex multimode plate reader (Tecan Trading AG, Männedorf, Switzerland). The measurement of potassium concentration in the samples was performed on the Atomic Absorption Spectrometer (AAS) SpektrAA 220 FS from Varian (Palo Alto, California). The agronomic N use efficiency was calculated as:

$$\text{N use efficiency} = (\text{grain yield in fertilized treatment} - \text{grain yield in control}) / (\text{amount of N supplied}). \quad (3)$$

The apparent nitrogen recovery was calculated as:

$$\text{Apparent nitrogen recovery} = (\text{N uptake in fertilized treatment} - \text{N uptake in control}) / (\text{amount of N supplied}). \quad (4)$$

2.6 | Quantification of rhizosphere microbes (DNA extraction and qPCR analysis)

Total DNA was extracted from frozen rhizosphere soil (100 to 450 mg soil), dry soil (S0) used to set up the experiment and from dry algae biomass (A0) used for fertilization. The Nucleospin Soil DNA extraction kit (Macherey-Nagel GmbH & Co. KG Germany) was used according to the manufacturert's recommendations with solution SL1 and the enhance solution SX. DNA was finally eluted with 150 µL of PCR grade water preheated to 60°C. Extracted DNA was diluted 1:30 in PCR grade water for molecular analysis. Quantitative PCRs (qPCRs) were performed to determine the copy numbers of individual genes per g soil or algal biomass of bacterial and archaeal 16S rRNA genes and the ammonium oxidase genes (*amoA*) of ammonium oxidizing *Bacteria* (AOB) and ammonium oxidizing *Archaea* (AOA). qPCR was performed in a CFX 96 Cycle (Bio-Rad Laboratories, Feldkirchen, Germany) in a total reaction volume of 10 µL, including 0.2 µM of each primer, 1× SSo Fast EVA Green Supermix (Bio-Rad) and 1-µL 1:30 diluted DNA extracts. A dilution series of PCR-amplified DNA fragments with internal binding positions of the qPCR primers was used as a standard. PCR products were purified with the QiaQuick PCR purification kit (Qiagen, Hilden, Germany), and the DNA concentration was quantified with the Qubit quantification kit (Promega Quantus Fluorometer, Madison WI, USA). DNA fragment length and DNA concentrations were used to determine the concentration of target gene copy numbers according to Kolb et al. (2003). Melt curve analysis and agarose gel electrophoresis were used to control the specific size of qPCR products and to avoid the quantification of primer dimers. An overview of qPCR primer systems with specific primer concentrations, standards and thermal profiles is shown in Table S1. Box plot graphs of qPCR data were generated in SigmaPlot (Systat Software Inc., Richmond, California, USA).

2.7 | Statistical analysis

Analysis of variance (ANOVA) was performed by mixed model one-way ANOVA using the program R (R 4.2.2), packages *nlme* and *emmeans*. The fertilizers with the respective fertilizer levels were considered as a fixed factor, while the block was considered as a random effect. By using the package *multcomp*, a compact letter display (CLD) desired pairwise comparison was obtained. A significance level of $\alpha = 0.05$ according to the Tukey's distribution was used. The results are presented graphically in boxplots.

3 | RESULTS

3.1 | Physiological phenotyping

The measurements of carbon assimilation after 6 weeks of cultivation demonstrated that all fertilized treatments differed from the unfertilized control (Figure 1A). However, no significant differences occurred among in the fertilized treatments. On the other hand, NDVI, a measure of leaf greenness, differed significantly from the control only in treatments M_120 and A_180 but not at lower level of fertilizer application (Figure 1B). In addition, PRI was measured as an index reflecting the status of photosynthetic pigments involved in the xanthophyll cycle. Similar to NDVI, significant differences from the control occurred only in treatments M_120, M_180, and A_180 (Figure 1C).

3.2 | Dry matter and yield

In terms of the dry weight of straw, all treatments showed significantly higher dry weight than the control (Figure 2A). However, the mineral-fertilized variants showed higher dry matter yields than the microalgae-treated plants within the same N level. Similar results occurred in the total grain yield of spring wheat (Figure 2B), where the mineral-fertilized plants showed higher grain yields than the microalgae-treated plants at each fertilization level. M_120 showed the highest yield (7.2 g maximum yield) with significant differences from the treatments of fertilizer levels 60, A_120 and A_180.

3.3 | N accumulation and N concentration

In terms of nitrogen accumulation in straw (g N plant^{-1}) after 15 weeks of growth, the mineral-fertilized plants were significantly different from the control (Figure 3A). Among the microalgae treatments, only A_180 differed significantly from the control. Similar results occurred for nitrogen concentration in straw (mg N g^{-1} DM), where significantly higher N concentrations were observed in the mineral fertilizer treatments (Figure 3B). The nitrogen accumulation in the grain (g N plant^{-1}) after 15 weeks of cultivation was all fertilizer treatments showed

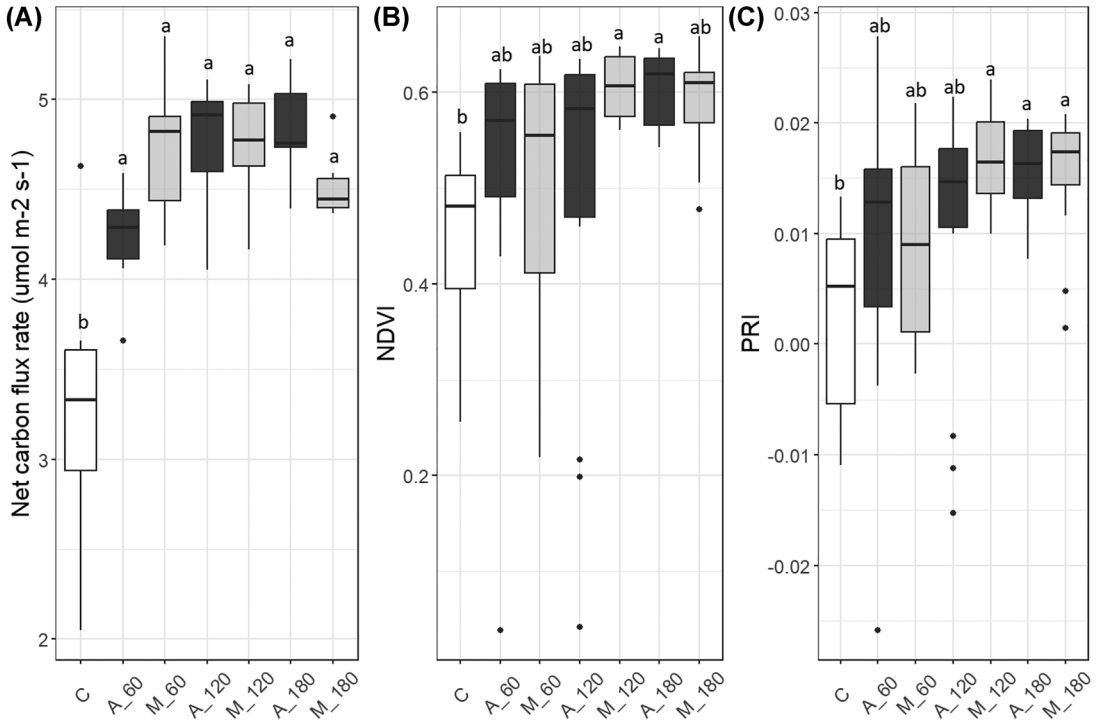

FIGURE 1 (A) Net carbon flux rate (μmol m^{-2} s^{-1}) after 6 weeks of cultivation. (B) NDVI (Normalized Difference Vegetation Index) after 3 weeks of cultivation. (C) PRI (photochemical reflectance index) after 3 weeks of cultivation. Boxplots with the same letter are not significantly different (Tukey's test, $p < 0.05$). Abbreviations: C = control, A = algae, M = mineral fertilizer; 60 = 60 kg N ha^{-1}, 120 = 120 kg N ha^{-1}, 180 = 180 kg N ha^{-1}.

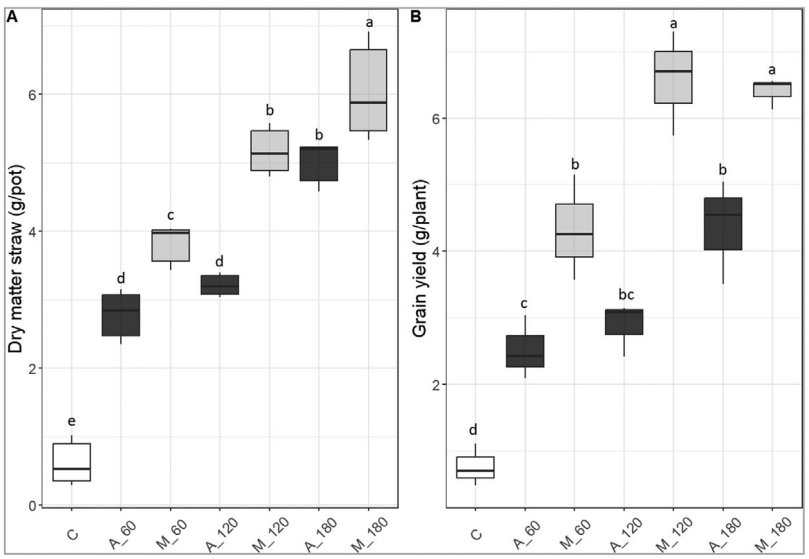

FIGURE 2 (A) Dry matter of straw in g per pot. (B) Wheat grain yield in g per plant at harvest after 15 weeks of cultivation. Boxplots with the same letter are not significantly different (Tukey's test, $p < 0.05$). Abbreviations: C = control, A = algae, M = mineral fertilizer; 60 = 60 kg N ha^{-1}, 120 = 120 kg N ha^{-1}, 180 = 180 kg N ha^{-1}.

a significantly higher N accumulation than control (Figure 3C). The mineral-treated plants showed a higher N accumulation than the plants fertilized with microalgae at all fertilization levels. Comparable results were evident for the nitrogen concentration in the grain (Figure 3D). M_180 showed the highest N concentration in the grain, while that of A_180 was significantly lower than that of M_180.

3.4 | Nitrogen use efficiency

Agricultural nitrogen use efficiency represents the ratio between the net increase in plant grain weight with and without N fertilization and total fertilizer-N (Figure 4A). The highest nitrogen use efficiency occurred in the M_60 variant, which was significantly different from

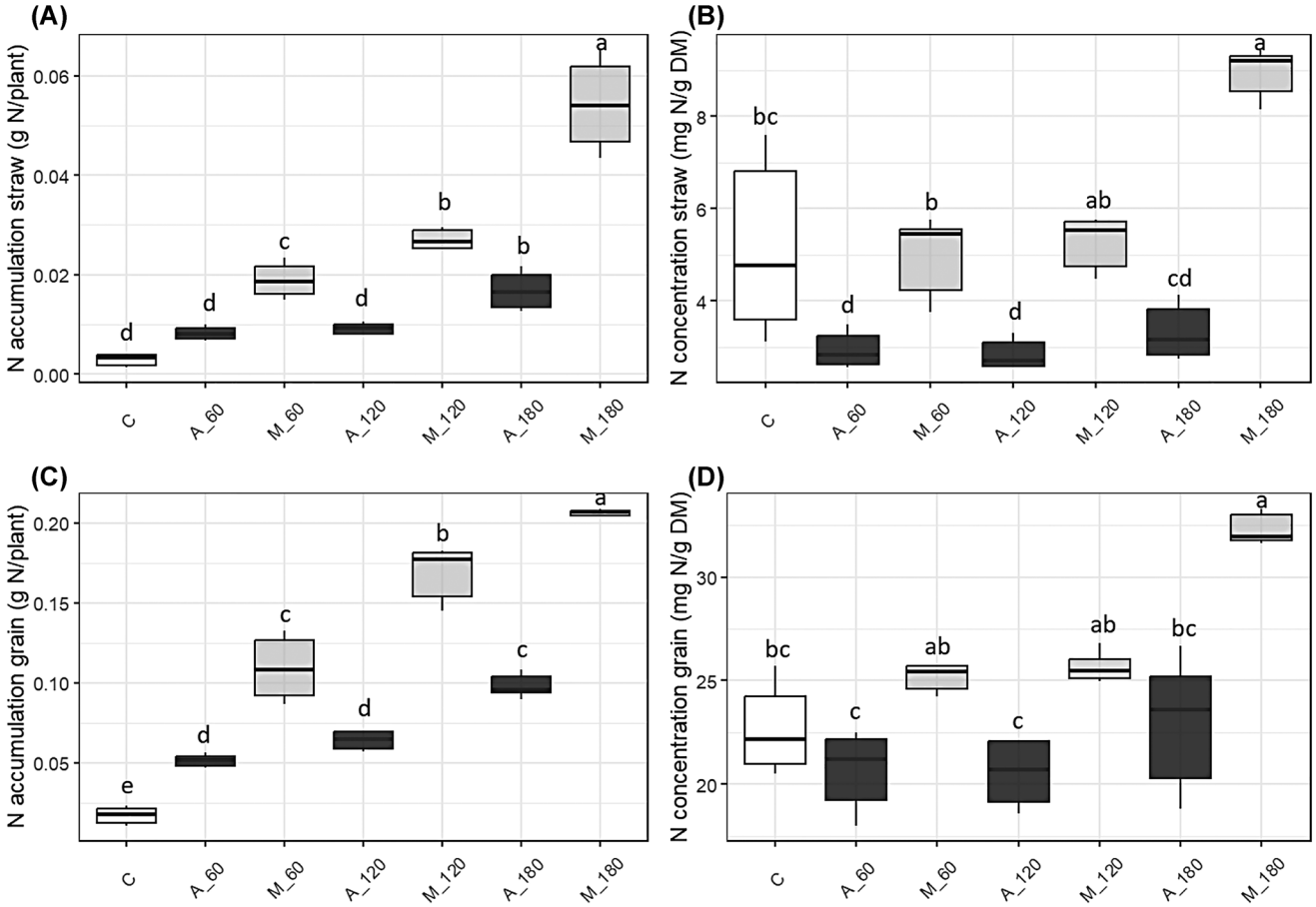

FIGURE 3 (A) N accumulation in straw in g N per plant. (B) N-concentration straw in mg N per g dry matter. (C) N accumulation in grain in g N per plant. (D) N-concentration grain in mg N per g dry matter. Boxplots with the same letter are not significantly different (Tukey's test, $p < 0.05$). Abbreviations: C = control, A = algae, M = mineral fertilizer; 60 = 60 kg N ha^{-1}, 120 = 120 kg N ha^{-1}, 180 = 180 kg N ha^{-1}.

the microalgae fertilized variants. There were no significant differences between the variants fertilized with microalgae. Variant A_180 had the lowest nitrogen use efficiency. The apparent recovery rate (%) showed similar results (Figure 4B) and reflected the increased net total uptake of N by the plant with and without N fertilization in relation to the total amount of fertilized N. The highest apparent utilization rate was observed in M_60 after 15 weeks of cultivation. N utilization in microalgae-treated plants did not differ significantly among fertilizer levels ($p < 0.05$)

3.5 | Concentrations of *Bacteria*, *Archaea*, fungi, and ammonium oxidizers in the rhizosphere of differently fertilized wheat plants

Quantification of bacterial and archaeal 16S rRNA gene and fungal 18S rRNA gene targets in the rhizosphere of differently fertilized plants showed no significant differences (Figure 5A–C). The same result occurred for AOB (Figure 5C), while a slight but not significantly different increase in the abundance of AOA *amoA* targets was obtained in the rhizosphere of fertilized plants compared to control plants (Figure 5D). No differences were obtained between mineral and algal biomass fertilized plants. Both, the soil used for the setup of the experiment (S0) and the microalgae biomass used as fertilizer (A0) contained gene targets of all quantified taxa and *amoA* genes. Interestingly, the abundance of AOA *amoA* genes in the microalgae biomass was in the range of the *amoA* gene concentrations determined in the rhizosphere, while the concentration in the used soil was much lower.

4 | DISCUSSIONS

To investigate the physiological performance of wheat plants treated with different nutrient sources, several measurements or phenotyping were carried out over the cropping period. The NDVI value has previously been shown to be a reliable indicator for determining the N status of cereal plants (Rambo et al., 2010). After 3 weeks of culture, the NDVI value of the highest fertilizer level (180) was significantly different from that of the control (Figure 1B), regardless of the nutrient source (A, M). This result is consistent with the findings of Kizilgeci et al. (2021), who reported elevated NDVI values at higher N treatments, while 50 kg N ha^{-1} had the lowest NDVI values. PRI is an

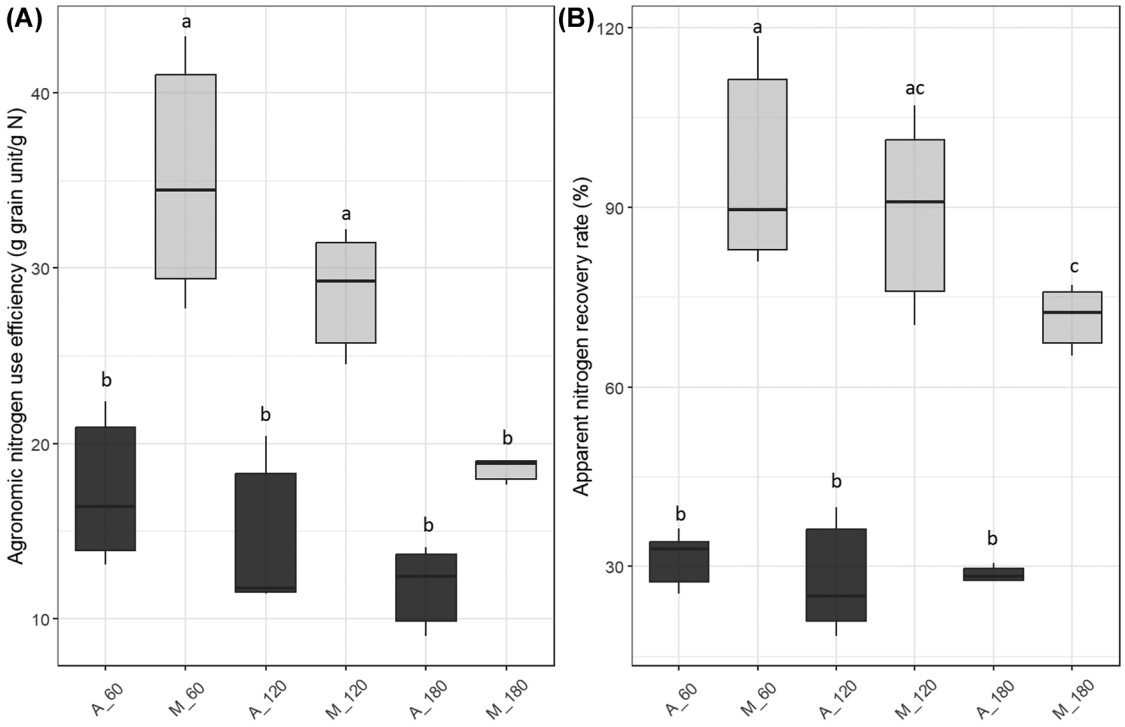

FIGURE 4 (A) Agronomic nitrogen use efficiency in g grain unit per g N. (B) Apparent nitrogen recovery rate in%. Boxplots with the same letter are not significantly different (Tukey's test, $p < 0.05$). Abbreviations: C = control, A = algae, M = mineral fertilizer; 60 = 60 kg N ha^{-1}, 120 = 120 kg N ha^{-1}, 180 = 180 kg N ha^{-1}.

indicator of short-term changes in photosynthetic activity because it is coupled with the epoxidation state of pigments from the xanthophyll cycle (Gamon et al., 1992). The PRI captures the use efficiency of the absorbed light energy and showed similar results to the NDVI after 3 weeks of cultivation (Figure 1C). Significant differences from the control were evident at the highest fertilizer level (180), indicating higher photosynthetic efficiency. Similar to NDVI, the significantly higher PRI values can be explained by the increased N supply at fertilizer level 180 (Gamon et al., 1992; Kizilgeci et al., 2021). Furthermore, the net carbon flux rate or CO_2 flux rate was measured after 6 weeks of culture as a direct measurement of photosynthetic efficiency. Here, all fertilized variants differed significantly from the control. There were no significant differences between fertilization levels and nutrient sources. With insufficient or excessive nutrient and water supply, there is a reduction in the CO_2 assimilation rate, and photosynthetic output is limited as observed in our control treatment. In addition to the function of nitrogen as a component for chlorophyll, it is also required to synthesize important plant enzymes, nucleotides, and proteins. This explains the lower carbon assimilation in plants without nitrogen fertilization (Figure 1A). In summary, a higher nitrogen supply indeed improved the physiological performance of the plants, although for some parameters, significant differences became evident only at the highest fertilizer level.

After 15 weeks of culture, all variants differed significantly in DM from the control. Within fertilizer levels, there was a significant difference in DM between the microalgae-treated plants and the mineral-fertilized plants (Figure 2A). With increasing N fertilization (fertilizer level), higher DM was observed, which can be attributed to photosynthetic performance, where N acts as an important component of chlorophyll (Sarma & Gogoi, 2017).

In terms of grain yield, the variants differed significantly from each other. As expected, the control showed the lowest grain yield and was significantly different from the other treatments (Figure 2B). At all fertilizer levels, the mineral fertilization showed significantly higher grain yield than the microalgae-treated plants. It is possible that the nitrogen supply or plant available nitrogen was insufficient compared to the mineral fertilizer. In previous experiments, in which plants were fertilized with microalgae biomass, the duration of the cultivation period was usually only a few weeks, which is why (grain) yield was often not one of the parameters recorded (Ahn et al., 2020; Das et al., 2019; Mau et al., 2022). Similar results in total yield were reported by Coppens et al. (2016). They compared microalgae fertilizer with an alternative organic fertilizer and an inorganic fertilizer on tomato plants. They reported lower tomato yield in plants treated with microalgae compared to other fertilizers (Coppens et al., 2016). The mineral fertilizer ammonium nitrate has the advantage that nitrogen is present in both nitrate and ammonium forms (Schubert, 2006). Organically bound nitrogen in microalgae must be mineralized in the soil before it can be absorbed by plants. The total grain yield (Figure 2B) and the difference in dry matter of the wheat plants (Figure 2A) showed similar results as those of Coppens et al. (2016), suggesting that the mineral fertilizer ammonium nitrate was more readily available and mobile than the nitrogen contained in the microalgae.

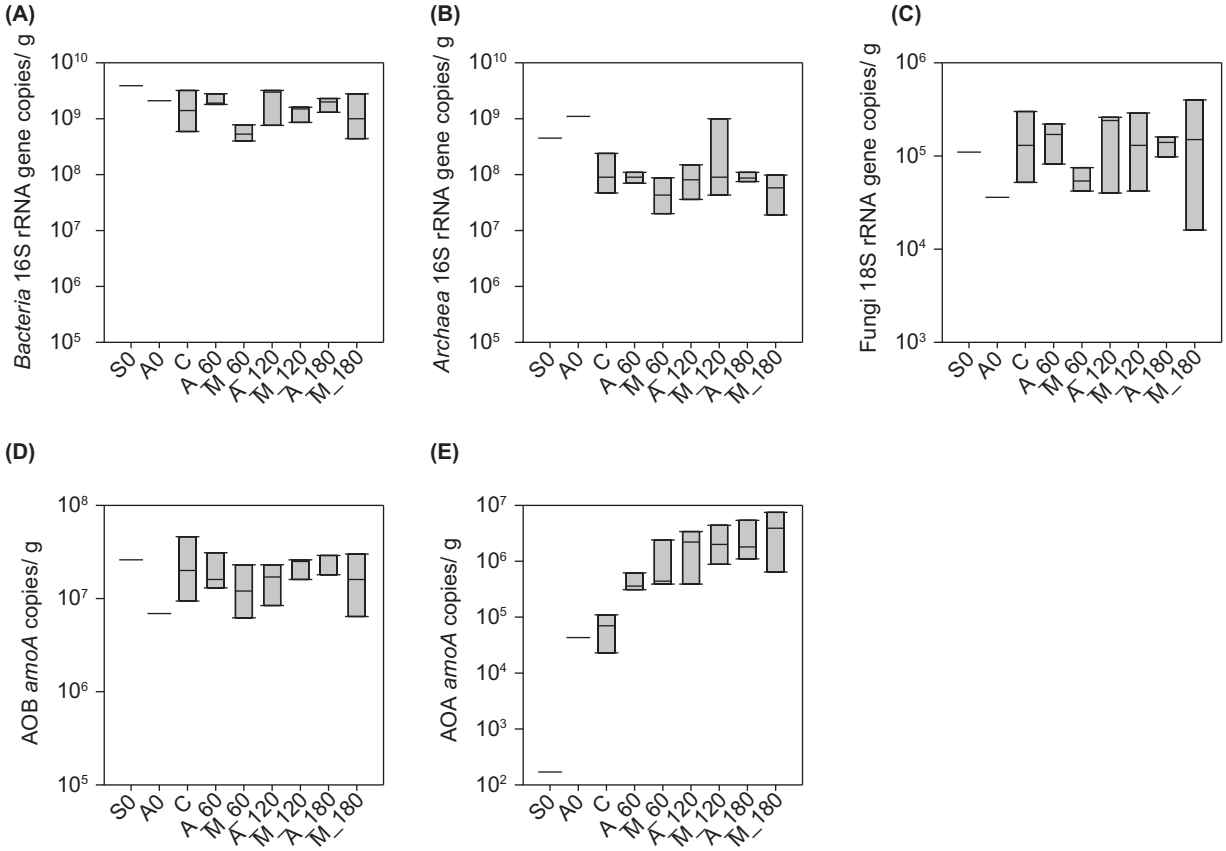

FIGURE 5 Concentrations of bacterial (A) and archaeal 16S rRNA gene copies (B), fungal 18S rRNA gene copies (C) and *amoA* gene copies of ammonia-oxidizing *Bacteria* (AOB) (D), and ammonia-oxidizing *Archaea* (AOA) (E) per g rhizosphere soil of differently fertilized wheat plants after 7 weeks of growth. S0, A0: Soil and algal biomass used to set up the experiment. Boxplots based on analysis of rhizosphere soil harvested from individual plants of three different pots. ANOVAs, performed in SigmaPlot, did not show significant differences among the rhizosphere samples (p values > 0.05). S0: dry LUFA soil used to set up the experiment; A0: dried algal biomass used as fertilizer. Variants: A_ fertilized with A0 to obtain a N fertilization of 60, 120, or 180 kg N ha^{-1}. Abbreviations: C = control, A = algae, M = mineral fertilizer.

The results described above (physiological phenotyping, DM, grain yield) were also confirmed by elemental analysis of wheat plants. Nitrogen was released from the microalgae cells, and plant growth was supported but at a lower level compared to the mineral fertilizer. After 15 weeks of cultivation, the control plants and the microalgae-treated plants at fertilizer levels of 60 and 120 had the significantly lowest N accumulation (Figure 3A). The mineral-fertilized plants showed significantly higher N uptake than the microalgae variants at every fertilization level. This suggests that the amount of nitrogen released from the microalgae did not ideally match the requirements of the plants. While inorganic nutrients such as nitrate are readily available to the plant, organically bound N in microalgae needs to be mineralized, leading to slower release (Coppens et al., 2016). However, the growth of crops following in the rotation could benefit from the slower release of organically bound nitrogen from the microalgae into the soil. Therefore, due to the slower release of nutrients from organic fertilizers, a long-term fertilizing effect of microalgae can be expected (Hernandez et al., 2021). The N concentration (Figure 3B) in the plant dry matter also showed significant differences between the mineral fertilizer and the organic microalgae fertilizer. Here, the control showed a higher N concentration than the fertilized variants (with the exception of M_180), which can be attributed to the low growth and especially the lower grain yield due to N limitation. Most likely, a larger amount of organically bound N from microalgae remained in the soil after harvest.

N accumulation in the grain was significantly higher in the mineral-fertilized plants than in microalgae-treated plants (Figure 3C). This indicates that the mineral nitrogen was directly available to the plants and sufficient for grain filling. The plants fertilized with microalgae showed significantly higher N accumulation in the grain than the control. There was a shift of nitrogen from the shoot to the grain. N plays an important role in determining the concentration of storage protein in grains (Zörb et al., 2018). As expected, N accumulation increased with the applied fertilizer rate. Studies have shown that grain N accumulation is controlled by the applied N fertilizer rate in wheat, with grain protein concentration increasing with increasing N fertilizer application (Langenkämper et al., 2006; Barraclough et al., 2010; Zörb et al., 2018).

There are different ways to represent N use efficiency in cereals, depending on whether grain yield of the plant or N recovery efficiency in terms of the amount of nitrogen fertilizer applied is considered

(Doyle & Holford, 1993). Agronomic nitrogen use efficiency focuses on the economic portion of the crop (crop yield) and indicates how much productivity is improved by the application of N compared to an unfertilized control (Congreves et al., 2021). Significantly higher agronomic N use efficiency was observed with mineral fertilization as compared with microalgae fertilization (Figure 4A). Another approach to calculate and represent nitrogen use efficiency is the apparent nitrogen recovery rate of the N fertilizer (Doyle & Holford, 1993). The apparent N recovery rate reflects the efficiency with which nitrogen is accumulated by the plant (Craswell & Godwin, 1984). Again, the mineral-fertilized wheat plants showed improved apparent N recovery efficiency, especially with a low application rate of M_60 (Figure 4B). On the other hand, the N recovery rates of microalgae by spring wheat were approximately 27%–30% of mineral N fertilizer. Looking at the results of biomass, grain yield, and N accumulation in straw and grain, these are in agreement with the apparent N use efficiency. While the N use efficiency in microalgae treatments was generally relatively low due to slow N release, it showed no decreasing trend with higher application rates as opposed to mineral N treatments, in which a diminishing marginal benefit of fertilizer application was evident (Figure 4).

The abundance of rhizosphere microbes (*Bacteria*, *Archaea*, and fungi) and AOA and AOBs was not differentially affected by microalgae and mineral fertilization. The results were similar to quantitative data of bacteria, archaea, AOA, and AOB performed for a barley fertilization experiment (Suleiman et al., 2020). In contrast to our study, the authors obtained a higher abundance of AOA instead of AOB in their system. This discrepancy can be due to the different soil types and plant types, which can have strong effects on shaping microbial communities in the rhizosphere of agricultural plants (Breitkreuz et al., 2021). We have not performed community profiling, which may have shown differences in the composition of the rhizosphere microbiota depending on the respective treatments (Suleiman et al., 2020). The high abundance of gene targets of *Bacteria*, *Archaea*, fungi, AOA, and AOB in the microalgae biomass used for fertilization is a clear indication for the introduction of new microbes, including wastewater treatment-associated nitrifiers, into the system.

If those microbes are still active, they may have efficiently colonized the rhizosphere after fertilization. Future studies must be focused on microbial activity and the composition of the quantified microbial taxa to understand the overall effect of microalgae fertilization on the soil and plant microbiota.

5 | CONCLUSIONS

In this work, the use of microalgae biomass derived from a prototype wastewater treatment plant as a potential source of nutrients for wheat plants was investigated in a pot experiment. On the one hand, microalgae application at different rates significantly improved growth, yield, and nutrient uptake compared to the control and thus formed a suitable source of fertilizer for wheat. Thus, our first hypothesis can be accepted. However, the performance was mostly inferior compared to equivalent rates of mineral fertilization. Nevertheless, the second hypothesis that plants with different amounts of fertilizer show different performance in terms of growth and nitrogen use efficiency can be confirmed. The third hypothesis must be rejected, as the different N sources did not affect the abundance of rhizosphere microbes of bacteria, archaea, and fungi. This result offers various perspectives for the use of microalgae as a source or nutrient for crops. First, combinations of microalgae with reduced mineral fertilizer application might be helpful to better coordinate plant demand and N availability. Such regimes would reconcile the agronomic efficiency of mineral fertilizer application with the ecological advantage of recycling nutrients from innovative sewage treatment. Second, unmineralized N from microalgae remaining in the soil after wheat harvest should be used by subsequent cover crops, requiring adequate planning of crop rotations. These aspects should be explored in further experiments, including field studies and investigations of possible contaminants arising from sewage residues such as microalgae.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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