

A sustainable method for enhancing plant growth and soil fertility by using microalgae as biofertilizers for the culture of peas (*Pisum sativum* L.).

Abstract

Microalgae based biofertilizers are emerging as a sustainable alternative to synthetic fertilizers, offering the potential to enhance plant growth while improving soil health. This study investigates the effectiveness of microalgae applications on the growth performance of garden peas (*Pisum sativum*), and associated changes in soil fertility. A microalgal culture was applied as a soil amendment under controlled garden conditions, and plant growth parameters—including germination rate, shoot and root length, and weekly plant growth—were monitored throughout the growing cycle. Soil samples were analyzed for nutrient content, organic matter, and microbial activity before and after treatment. Results demonstrated that microalgae application significantly improved plant vigor and yield compared to untreated controls, with notable increases in nitrogen availability and soil microbial activity. These findings highlight the potential of microalgae as an eco-friendly biofertilizer capable of supporting sustainable home garden and agricultural practices. Further research is recommended to optimize application rates and evaluate long term soil impacts.

Keywords: microalgae, biofertilizer, eco-friendly fertilizers, *pisum sativum*, sustainable agriculture, soil fertility, nitrogen enrichment

Volume 10 Issue 3 - 2026

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Received: September 21, 2026 | **Published:** October 19, 2026

Introduction

Global crop demand is projected to increase by 60–110% by 2050, placing unprecedented pressure on agricultural systems and accelerating environmental degradation.¹⁻⁵ To sustain productivity under these conditions, intensive use of chemical fertilizers has become a defining feature of modern agriculture. However, prolonged and excessive fertilizer application has resulted in escalating production costs, declining nutrient-use efficiency, soil compaction, loss of soil biodiversity, and progressive soil degradation, thereby undermining long-term agroecosystem resilience.⁶⁻⁸ In addition, nutrient surpluses frequently drive runoff and leaching losses, contributing to widespread surface- and groundwater contamination and posing serious risks to ecosystems and, consequently, human health.⁹⁻¹² Addressing the dual challenge of increasing food production while reducing environmental impacts is therefore a central priority for sustainable agriculture.¹³

Bio-based fertilizers have emerged as promising alternatives to synthetic inputs, with macroalgae and aquatic plants receiving increasing attention. Brown macroalgae such as *Turbinaria*, *Laminaria*, *Fucus*, and *Sargassum* species are rich in potassium and bioactive compounds and have demonstrated agronomic benefits across a range of cropping systems.¹⁴⁻¹⁶ Similarly, the aquatic pteridophyte *Azolla* is widely recognized as an effective biofertilizer and green manure due to its rapid growth, high biomass productivity, and broad ecological adaptability.^{17,18} Its capacity to enhance rice productivity has been extensively documented across Asia and Africa, reflecting its potential contribution to low-input and climate-resilient farming systems.

Among bio-based inputs, microalgae represent a particularly promising yet underutilized resource for agricultural applications.

Owing to their autotrophic metabolism, microalgae efficiently convert light and inorganic nutrients into lipids, proteins, pigments, and polysaccharides, enabling high photosynthetic efficiency, rapid biomass accumulation, and broad environmental tolerance.¹⁹⁻²¹ While microalgae are well established in sectors such as bioenergy, pharmaceuticals, cosmetics, animal feed, and wastewater treatment, their agronomic potential remains comparatively underexplored. Emerging studies suggest that microalgal applications can enhance crop performance by improving soil structure and nutrient availability, stimulating beneficial microbial communities, and promoting a more balanced soil micro-ecosystem.²²⁻²³ However, empirical evidence remains limited, particularly regarding the use of locally-cultivated freshwater microalgae and their effects during early crop establishment.

Against this backdrop, the present study aimed to evaluate the potential of locally- cultivated (and thus readily available) freshwater microalgae as a sustainable alternative or supplement to chemical fertilizers. Using the pea (*Pisum sativum* L.) as a model crop, we assessed the effects of microalgal inoculation on seed germination, early plant growth (plant length and biomass accumulation), and associated changes in soil properties. By focusing on early growth responses and soil dynamics, this study contributes to a growing body of evidence on the role of microalgae in advancing environmentally sustainable crop production systems.

Materials and Methods

Algal collection and culture

Freshwater microalgae were cultured in an open 100-L algal culture vessel located at Melbourne Polytechnic's Epping Campus, Victoria,

Australia. Stock cultures developed naturally, and were fertilized with organic manure (primarily cow dung). From this system, 7 L of algal suspension was collected and further propagated at Melbourne Polytechnic’s Aquaculture Training and Applied Research Centre (ATARC).

Cultivation was conducted at 22°C under a 12 h light/12 h dark photoperiod with an illumination intensity of approximately 2500 lux for two weeks. Microalgal growth was monitored to ensure cultures reached the exponential growth phase. Cell density at peak concentration was determined using a Neubauer haemocytometer (Figure 1). Species composition was identified by light microscopy using standard taxonomic keys.^{24–26} The algal community comprised *Cosmarium* sp., *Chlamydomonas* sp., *Haematococcus* sp., *Chlorella* sp., *Pseudococcomyxa* sp., *Chroococcus* sp., and *Microcystis* sp., with *Chlorella* sp. and *Pseudococcomyxa* sp. identified as the dominant taxa (Figure 2).

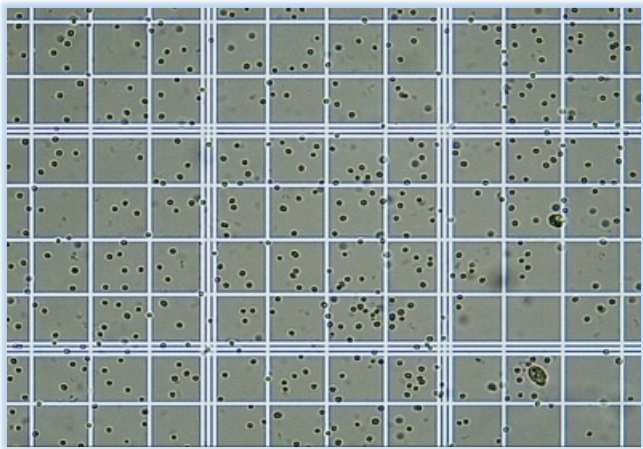


Figure 1 Algae counting using Neubauer haemocytometer (viewed at 40× magnification).

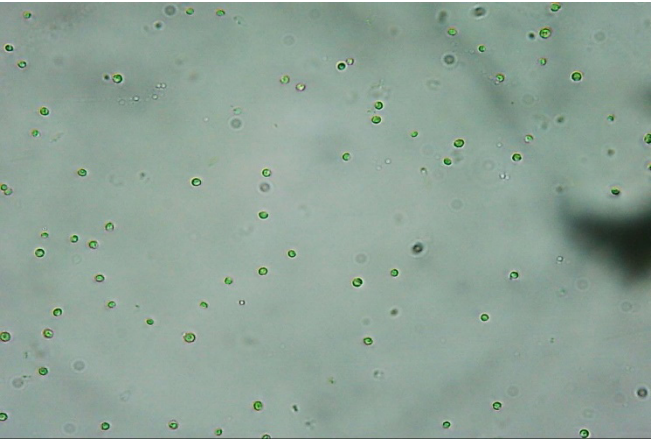


Figure 2 The dominant *Chlorella* sp. (viewed at 100× magnification).

Microalgal biomass was harvested by low-speed centrifugation (LaboGene Scanspeed 416 Clinical Centrifuge) at 4000 RPM/1500G and resuspended in sterilised distilled water to remove residual growth media.²⁷ The suspension was subsequently filtered through GF/C filters²⁸ and oven-dried using a 30L-capacity Laboratory Vacuum Oven (Selby’s Scientific) at 60°C for 48h to obtain dried algal powder.^{29,30}

Pea experimental cultivation

Glasshouse experiments (experiment code P6) were conducted at Melbourne Polytechnic’s Epping Campus over an 80-day period.

Ambient temperature was maintained at approximately 20°C using under-bench heating. Nine plastic culture vessels (34.5 cm × 25.5 cm × 14.5 cm) were used, with three vessels (replicates) allocated to each treatment: control (P6C), inorganic fertiliser (P6F), and microalgae amendment (P6A). Each vessel contained 3 kg of soil. Culture vessels were established in a randomized block design (Figure 3).

CONTROL	MICROALGAE	FERTILIZER
P6C1	P6A1	P6F1
MICROALGAE	CONTROL	FERTILIZER
P6A2	P6C2	P6F2
FERTILIZER	CONTROL	MICROALGAE
P6F3	P6C3	P6A3

Figure 3 Design and layout of the experiment.

For the inorganic fertiliser treatment (P6F), a multipurpose fertiliser (NPK) was applied at a rate of 2 g kg⁻¹ soil, following Khair et al.³¹ For the microalgae treatment (P6A), dried microalgal powder was incorporated at a rate of 3 g kg⁻¹ soil, consistent with previous studies.^{32,33} Control vessels received no amendments. All pots were equilibrated for two weeks prior to seed sowing.

Pea seeds (*Pisum sativum* L.) were sourced from a local retail store (Bunnings Warehouse, Australia), a major home-improvement and garden supplier commonly used by growers and researchers for horticultural materials, including seeds, substrates, and fertilizers. To ensure uniform germination, seeds were soaked in distilled water for one hour prior to sowing.³⁴

Soil sampling and analysis

Soil properties were analysed twice during the experiment. The first analysis was conducted two weeks after incorporation of NPK fertiliser and microalgal powder into the respective treatments and prior to seed sowing. The second analysis was conducted at the conclusion of the experiment for all treatments. All soil analyses were performed by Nutrient Advantage (Werribee, Victoria), a NATA (National Association of Testing Authorities)-accredited laboratory.

Seed sowing and germination

Ten healthy pea seeds were sown per vessel at a depth of 1 cm, resulting in a total of 90 seeds across all treatments. Germination percentage was calculated for each vessel following completion of germination. When seedlings reached a height of 2–3 cm, fertiliser and microalgal treatments were reapplied at the same rates as the initial application. Control vessels did not receive additional inputs.

Growth measurements

Plant growth was monitored weekly by measuring three randomly selected plants per vessel, following Dineshkumar et al.,³⁵ partially in order to compensate for the lower germination rate that was ultimately observed in the control treatments, and also to ensure consistency among treatments and to minimise measurement time without compromising representativeness. At harvest, fresh (wet) and dry biomass of shoots, roots, and leaves were measured. Dry biomass was determined after oven-drying the samples at 60 °C for 24 h. Growth performance indices, mean values, standard deviation, standard error and levels of significant difference (p = <0.05) were calculated for each treatment.

Statistical analysis

All data are presented as mean ± standard error. Statistical analyses were performed using one-way analysis of variance (ANOVA).

Differences among treatments were considered statistically significant at $p < 0.05$, with post-hoc comparisons conducted. factors related to their repair or availability. The nitrogen and phosphorus as well as other important component values are illustrated in detail in Table 1.

Results

Changes in soil properties

Soil chemical qualities include the levels and availability of nutritious mineral components for plants, as well as soil chemical

Table 1 Changes in physical and chemical characteristics of soil before and after the experiment under different treatments.

Parameter	Pre-experiment Control	Pre-experiment Fertilizer	Pre-experiment Microalgae	Post-experiment Control	Post-experiment Fertilizer	Post-experiment Microalgae
pH (1:5 H ₂ O)	7.3	6.6	7.5	7.4	7.1	6.4
pH (1:5 CaCl ₂)	6.9	6.4	7.6	6.8	6.4	6.1
Electrical conductivity (dS m ⁻¹)	0.38	1.03	1.04	0.23	0.25	1.30
Chloride (mg kg ⁻¹)	76	93	270	43	28	180
Nitrate-N (mg kg ⁻¹)	86	440	300	22	44	630
Ammonium-N (mg kg ⁻¹)	2.5	1.6	2.6	5.3	5.8	7.9
Colwell P (mg kg ⁻¹)	220	290	280	230	320	490
Phosphorus buffer index	110	74	110	72	80	84
Ca (cmol(+) kg ⁻¹)	12	12	12	14	14	14
K (cmol(+) kg ⁻¹)	1.4	1.8	3.1	0.61	1.4	1.9
Mg (cmol(+) kg ⁻¹)	3.7	4.0	3.3	3.0	3.8	4.5
Na (cmol(+) kg ⁻¹)	0.51	0.55	4.90	0.37	0.31	0.73
Ca:Mg ratio	3.5	3.0	3.6	4.7	3.7	3.1
Exchangeable Al (cmol(+) kg ⁻¹)	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
CEC incl. Al (cmol(+) kg ⁻¹)	18.5	18.6	22.8	17.6	19.0	20.8
Exchangeable Na (% of CEC)	2.8	2.9	22.0	2.1	1.6	3.5
Exchangeable Al (% of CEC)	<1	<1	<1	<1	<1	<1
Sulphur (mg kg ⁻¹)	70	110	130	54	33	160
Available K (mg kg ⁻¹)	240	550	740	530	710	1200
Al (KCl extractable, mg kg ⁻¹)	<9.0	<9.0	<9.0	<9.0	<9.0	<9.0

Notes: Soil chemical properties were measured before and after the experimental period under control, fertilizer, and microalgae treatments. pH was measured in a 1:5 soil–solution ratio using distilled water or CaCl₂. Exchangeable cations and cation exchange capacity (CEC) were determined using ammonium acetate extraction. Values preceded by ‘<’ indicate concentrations below the analytical detection limit.

Seeds germination in different treatments

Seed germination rates differed among treatments (Figure 4). The control treatment (P6C) showed the lowest germination, with a mean of 5.33 ± 0.58 seeds germinated out of ten. Germination was moderately higher under the P6F treatment, with a mean of 6.33 ± 0.58 seeds. The highest germination was observed in the P6A treatment, which resulted in a mean of 7.67 ± 0.58 seeds germinated.

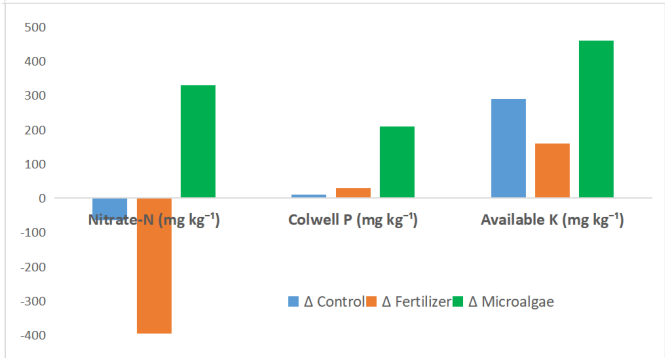


Figure 4 Observed differences according to treatment type (post-experiment minus pre-experiment values) in soils for nitrate, phosphorus and potassium.

Overall, both treatments improved seed germination compared with the control, with P6A showing the strongest positive effect. The relatively small standard deviations indicate consistent germination responses across replicates within each treatment.

Plant growth data were analysed using a repeated-measures framework. A linear mixed-effects model was fitted with Treatment, Week, and their interaction as fixed effects, and individual plant as a random effect to account for repeated measurements over time (Figure 5). Model assumptions were checked prior to analysis. Statistical significance was assessed at $\alpha = 0.05$.

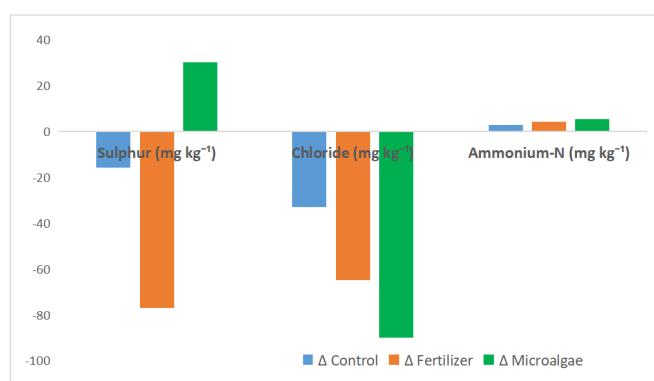


Figure 5 Observed differences according to treatment type (post-experiment minus pre-experiment values) in soils for sulphur, chloride and ammonium.

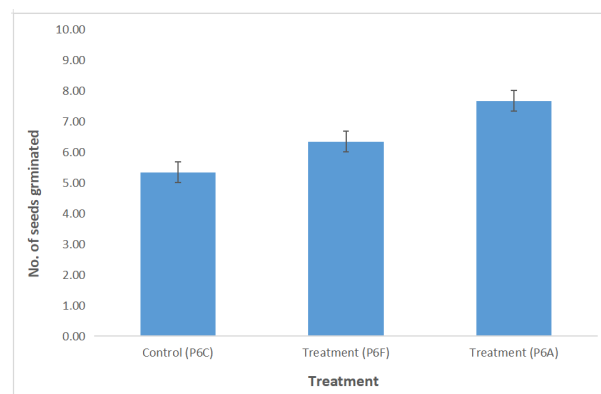


Figure 6 Mean ($\pm$ SE) number of seeds germinated under P6C (Control), P6F (Fertiliser), and P6A (Microalgae) treatments (n = 3 treatments, 3 replicates, 10 seeds sown in each tub).

Plant growth and biomass parameters of peas were significantly influenced by treatment (Table 2).

Table 2 Effect of different treatments on growth and biomass of peas

Treatment	Shoot height (cm)	Root length (cm)	Whole plant fresh weight (g)	Whole plant dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf fresh weight (g)	Leaf dry weight (g)
Control	24.74 $\pm$ 2.04 c	4.33 $\pm$ 0.76 c	1.70 $\pm$ 0.78 c	0.85 $\pm$ 0.09 c	0.39 $\pm$ 0.12 c	0.03 $\pm$ 0.02 c	1.00 $\pm$ 0.05 c	0.43 $\pm$ 0.12 c
Inorganic	27.31 $\pm$ 0.83 b	5.34 $\pm$ 0.40 b	4.53 $\pm$ 0.50 b	1.01 $\pm$ 0.11 b	0.77 $\pm$ 0.17 b	0.12 $\pm$ 0.02 b	2.44 $\pm$ 0.35 b	0.97 $\pm$ 0.13 b
Microalgae	39.08 $\pm$ 0.77 a	6.98 $\pm$ 0.52 a	7.67 $\pm$ 0.93 a	2.29 $\pm$ 0.37 a	1.28 $\pm$ 0.24 a	0.32 $\pm$ 0.06 a	4.33 $\pm$ 0.44 a	1.63 $\pm$ 0.25 a

Values are mean $\pm$ SD (n = 9). Different letters within a column indicate significant differences among treatments according to Tukey's HSD test at $p \leq 0.05$.

One-way ANOVA revealed highly significant differences among treatments for shoot height, root length, whole plant fresh and dry weight, root fresh and dry weight, and leaf fresh and dry weight ($p \leq 0.001$ for all variables). Plants treated with freshwater microalgae consistently exhibited the greatest values for all measured parameters, followed by those receiving inorganic fertiliser, while control plants showed the lowest performance. Mean shoot height and root length were significantly higher in the microalgae treatment compared with both inorganic fertiliser and control treatments. Similarly, whole plant

biomass, including fresh and dry weights of shoots, roots, and leaves, was markedly enhanced under microalgae application. Tukey's HSD post-hoc analysis confirmed significant differences among all three treatments for each parameter, indicating a clear ranking of freshwater microalgae > inorganic fertiliser > control. These results demonstrate that freshwater microalgae application substantially improves pea growth and biomass accumulation compared with conventional inorganic fertiliser and untreated controls.

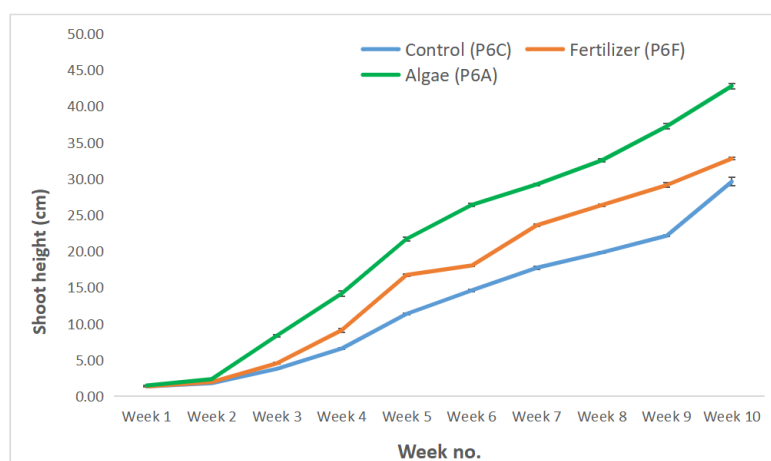


Figure 7 Weekly growth (cm per plant) of peas (*Pisum sativum*) under P6C (Control), P6F (Fertiliser), and P6A (Microalgae) treatments shown as mean $\pm$ SE (n = 9). Repeated-measures analysis revealed a significant effect of Week and a significant Treatment $\times$ Week interaction ($p < 0.001$).

Root length exhibited a positive relationship with root weight across all treatments (Figure 8).

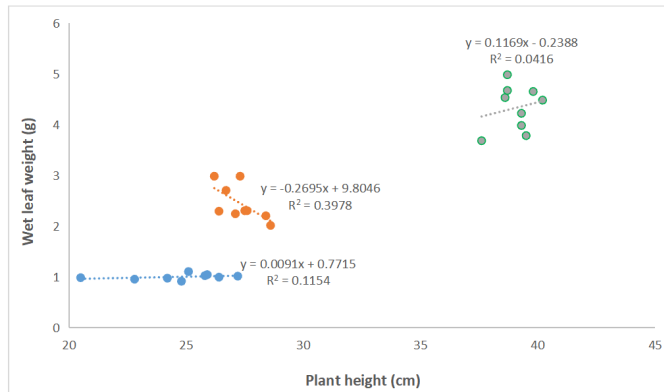


Figure 8 Relationship between root length and root weight of pea plants under control, inorganic fertilizer, and freshwater microalgae treatments. Points represent individual plants, and solid lines indicate fitted linear regression models for each treatment.

In control plants, root length ranged from 3.2 to 5.6 cm plant⁻¹, while root weight remained low (0.2–0.5 g plant⁻¹), indicating a weak length–weight relationship and limited biomass accumulation with increasing root elongation. Inorganic fertilizer application resulted in both longer and heavier roots compared with the control, with root length ranging from 4.7 to 5.9 cm plant⁻¹ and root weight from 0.6 to 1.1 g plant⁻¹. The fitted regression line showed a steeper slope relative to the control, reflecting enhanced root biomass accumulation per unit increase in root length. The freshwater microalgae treatment produced the greatest root development, with root length extending from 5.3 to 7.9 cm plant⁻¹ and corresponding root weight increasing from 0.8 to 1.5 g plant⁻¹. This treatment exhibited the strongest length–weight relationship, demonstrating a pronounced increase in root biomass with increasing root length. Overall, regression analysis indicates that freshwater microalgae application most effectively enhanced root growth and biomass accumulation, followed by inorganic fertilizer, while control plants showed the weakest response.

Discussion

This study demonstrates that microalgal biomass functions as an effective biofertilizer for peas, significantly enhancing plant height, root biomass, shoot biomass, and soil NPK retention. These findings are consistent with previous studies reporting the growth-promoting effects of microalgae on a wide range of crop species. For example, Kholssi et al.³⁶ observed significant increases in both fresh and dry root and shoot biomass following the application of algal extracts. Similarly, Taha and Youssef³⁷ reported improved growth performance in *Zea mays* plantlets treated with liquid formulations of *Coelastrella oocystoides* and *Chlorella minutissima*, indicating that the beneficial effects of microalgae are not limited to peas.

The stimulatory influence of microalgae on early plant development has also been well documented. Faheed and Abd-el Fattah³⁰ demonstrated that *Chlorella vulgaris* acted as an effective germination promoter for *Lactuca sativa*, resulting in faster growth and higher pigment content. In wheat, Abd et al.³⁸ reported increased chlorophyll-a production following the application of seawater supplemented with extracts from *Chlorella ellipsoidea* and *Spirulina maxima*, compared with seawater irrigation alone. Enhanced photosynthetic pigment accumulation may partially explain the improved biomass production observed in the present study. Furthermore, Grzesik et al.³⁹ showed

that foliar application of *Chlorella* spp. improved both growth and physiological performance in willow, highlighting the versatility of microalgae-based inputs across plant types and application methods.

Comparable growth responses have also been reported for other organic and alternative fertilizers. Eifediyi and Remison⁴⁰ found that inorganic fertilizers and farmyard manure produced similar effects on plant height and shoot weight, while Wuang et al.⁴¹ demonstrated that *Spirulina platensis* increased leaf number, plant height, and root length. Collectively, these findings support the conclusion that microalgal biomass represents a highly effective biofertilizer capable of matching or surpassing conventional nutrient sources.

A wide range of green microalgae, including *Acutodesmus dimorphus*, *Chlorella vulgaris*, *Scenedesmus quadricauda*, *Chlamydomonas reinhardtii*, *Chlorella sorokiniana*, *Asterarcys quadricellulare*, *Dunaliella salina*, and *Chlorella ellipsoidea*, have been investigated for their fertilizer potential. The positive effects of algal biomass and extracts on plant growth are largely attributed to their content of phytohormones, exopolysaccharides, and readily available nutrients. These compounds enhance nutrient uptake, stimulate root development, and improve soil biological activity, thereby promoting overall plant performance.

The growing interest in microalgal biofertilizers is also driven by concerns regarding the excessive use of chemical fertilizers. Rahman and Zhang⁸ reported that over-application of synthetic fertilizers can reduce crop productivity while substantially increasing soil and environmental pollution. Consequently, there is a strong need for innovative agricultural technologies that improve yields while reducing input intensity and environmental impacts. Biofertilizers, including algae-based formulations, offer a sustainable alternative by improving soil chemical and biological properties, stimulating plant growth, and restoring soil fertility.

Microalgae are particularly attractive as biofertilizers because of their rich biochemical composition, which includes proteins, carbohydrates, lipids, phytohormones, carotenoids, vitamins, and both macro- and micronutrients. These components contribute to enhanced nutrient absorption, biomass accumulation, and yield formation. In addition to being environmentally benign and economically viable, microalgal biofertilizers have the potential to partially or fully replace chemical fertilizers, thereby reducing nutrient losses and environmental pollution.

Beyond yield improvement, microalgal biofertilizers contribute to improved soil health by enhancing soil structure, increasing water retention, reducing nutrient leaching, and promoting carbon sequestration. Although the use of microalgae in ecological crop production, soil remediation, and climate-resilient agriculture has gained increasing global attention, many microalgal species with biofertilizer potential remain underexplored.

In the present study, freshwater microalgae—predominantly *Chlorella* spp. and *Pseudococcomyxa* spp.—significantly improved pea growth and soil nutrient status. These results provide further evidence supporting the use of microalgae as sustainable biofertilizers and highlight their potential to reduce reliance on chemical fertilizers in modern agricultural systems.

Conclusion

Microalgae contain a wide range of bioactive compounds that enable them to function as effective biofertilizers, while also contributing to improvements in soil quality. Long-term reliance on inorganic fertilizers in Australia has contributed to soil degradation

under conventional farming systems. This study investigated the potential of freshwater microalgae as biofertilizers to enhance nutrient availability during the germination of peas and early seedling growth under pot culture conditions. Pea seedlings grown in microalgae-amended soils exhibited significantly improved establishment and vigour compared with those grown under conventional fertilizer application, or without any amendment. The research question is therefore conclusively addressed, demonstrating that freshwater microalgae possess a dual capacity to supply essential nutrients that promote plant growth while simultaneously enhancing soil nutrient retention. Future research should focus on scaling up these findings through field-based validation trials, optimizing algal species selection and application rates, and evaluating long-term impacts on soil health and economic feasibility. Overall, microalgal biofertilizers represent a sustainable and environmentally sound strategy for improving soil nutrient balance and enhancing crop productivity in horticultural and agricultural systems.

Author Contributions

S Awal: experiment conduction, data analysis, and manuscript drafting.

A Christie: transportation of samples, assistance with data analysis, manuscript review and editing.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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