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Algae-based seed coatings as crop biostimulants for wheat and Persian clover: from controlled to field conditions

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Abstract

Algal biomass is a renewable source of bioactive compounds, including phytohormones, polysaccharides, amino acids, and micronutrients with potential to promote plant growth, improve stress tolerance, and nutrient use efficiency. Seed coating offers a resource-efficient delivery technology by applying active ingredients directly to the seed surface, minimising input use while maximising early root–soil contact. However, delivering whole algal biomass through seed coating remains largely unexplored beyond early seedling stages and controlled environments. This study evaluated crop-specific seed-coating formulations incorporating whole algal biomass in wheat (*Triticum aestivum* L.) and Persian clover (*Trifolium resupinatum* L.) across controlled, screenhouse, and field conditions. Wheat seeds were coated with *Tetraselmis chuii* and *Gracilaria* sp. biomass, while Persian clover seeds were coated with *Chlorella sorokiniana* biomass. Under controlled conditions, the wheat coating increased radicle length, tillering, and shoot dry biomass. In Persian clover, *C. sorokiniana* coating enhanced radicle length, shoot biomass, and nodulation. These benefits persisted beyond early development: in the screenhouse, coated wheat yielded 19% more grain, and coated Persian clover produced 43% greater cumulative shoot biomass across two cuts. In a replicated field trial, mean wheat grain yield was 13% higher in the coated treatment, although this difference was not statistically significant, while in a descriptive field-scale validation, coated Persian clover showed 39% greater cumulative dry biomass. Growth responses occurred without corresponding changes in leaf pigment indices. Overall, the positive responses observed across developmental stages and increasingly realistic growing conditions support the further development of algae-based seed coating as a scalable biostimulant delivery strategy.

1. Introduction

Modern agriculture faces growing pressure to maintain crop productivity while reducing reliance on intensive agrochemical inputs in response to climate change, soil degradation, and increasingly tightening environmental regulation. Within the European Union, policies aim to reduce nutrient losses and fertiliser use which reinforces the need for agricultural technologies that improve crop performance and resource-use efficiency. Importantly, the value of such technologies depends on their capacity to produce agronomically relevant responses under realistic cultivation environments, not only on their performance under controlled conditions, emphasizing the relevance of field trials.

Plant biostimulants have emerged as promising tools in this context because they can stimulate plant nutritional processes independently of the product's nutrient content, thereby improving traits such as nutrient-use efficiency, tolerance to abiotic stress, crop quality, or nutrient availability (Du Jardin 2015). Biostimulants can be delivered to crops through foliar application, fertigation, root drenching, and seed treatments, with the most appropriate strategy depending on the crop, formulation and intended application. Among seed-treatment approaches, seed coating enables a formulation to be deposited directly onto the seed surface without initiating germination, preserving seed dormancy and storability prior to sowing (Rocha et al. 2019). From an industrial perspective, coated seed can be supplied as a ready-to-sow product, facilitating localized delivery of relatively small quantities of biostimulant material

at the seed-soil interface, offering potential compatibility with established seed processing and sowing practices (Amirkhani et al. 2019; Rocha et al. 2019).

Among biostimulant sources, micro- and macroalgae have attracted increasing interest due to their biochemical diversity and potential to promote plant growth and stress tolerance (Kapoore et al. 2021). Algal biomass and algae-derived products contain complex mixtures of amino acids, polysaccharides, fatty acids, pigments, minerals, and hormone-like or other bioactive compounds that may influence plant development and physiological performance (Du Jardin et al. 2020; Lee and Ryu 2021; Parmar et al. 2023). Accordingly, researchers have investigated both microalgae, including *Chlorella* and *Tetraselmis* spp., and macroalgae, including *Ascophyllum nodosum* and *Gracilaria* spp., as renewable sources of plant biostimulants across diverse crop systems. However, their effects are strongly influenced by the algal source, processing and formulation, as well as by the crop, growing environment, and method of application (Fan et al. 2013; Bumandalai and Tserennadmid 2019; Chanda et al. 2019; Mannan et al. 2023). As such, the delivery strategy represents an important component in translating the biostimulant potential of algal biomass into practical crop applications.

Compared with foliar and soil applications, delivery of algae-derived treatments through seed coating remains relatively underexplored. Nevertheless, previous studies demonstrate the feasibility of this approach. Algal biomass, homogenates, and algae-derived products incorporated into seed coating formulations have been shown to influence germination and early plant development in different crops (Michalak et al. 2017; Godlewska et al. 2019; Fonseca et al. 2022). However, these studies have predominantly focused on early developmental plant responses. Field responses to cyanobacterial seed treatments have also been investigated in common bean, including effects on growth and seed production under different levels of mineral nitrogen fertilisation (Hegazi et al. 2010). Yet, this work primarily considered nitrogen-fixing cyanobacteria within a biofertilisation context, rather than algal biomass used primarily as a seed-delivered biostimulant.

Consequently, it remains unclear whether early responses to algal-based seed coatings persist through later plant development and translate into agronomically relevant responses under field conditions. Studies following the same seed-coating formulation stepwise, from controlled conditions through intermediate growing environments to field validation, remain scarce, representing an important translational gap. Whole algae or minimally processed algal biomass is particularly relevant in this context because its direct incorporation into coatings may provide a comparatively simple delivery strategy that avoids extraction and purification. However, its performance beyond early developmental stages and under increasingly realistic growing conditions remains insufficiently investigated.

Addressing this gap across contrasting crops is also important because biostimulant performance can depend on both crop and formulation. Wheat (*Triticum aestivum* L.) and Persian clover (*Trifolium resupinatum* L.) were therefore selected as representatives of two agronomically distinct crop systems. Wheat is one of the world's major cereal crops and a cornerstone of global food production, making the translation of early growth responses into grain productivity particularly relevant (Sharma and Kumar

Sharma 2025). Persian clover is a Mediterranean forage legume valued for biomass production, regrowth after cutting, and its capacity to establish symbiosis with nitrogen-fixing rhizobia, making it an important component of pasture systems and crop rotations (Capstaff and Miller 2018).

A formulation combining the microalga *T. chuii* and the macroalga *Gracilaria* sp. was evaluated in wheat, whereas a *C. sorokiniana*-based formulation was evaluated in Persian clover. The primary aim of this study was to evaluate these crop-specific seed-coating formulations incorporating whole algal biomass as a direct delivery strategy for plant biostimulation and to determine whether treatment-associated responses persist beyond early plant development under increasingly realistic growing conditions. Specifically, the study aimed to: (i) characterise the initial deposition and retention of algal biomass on coated seeds; (ii) determine the effects of coating on seed germination and establishment; and (iii) evaluate crop-specific growth and agronomic traits across controlled, screenhouse, and field conditions.

2. Materials and Methods

The study was conducted through a collaboration between InnovPlantProtect and Fertiprado, a Portuguese company specialising in the production and commercialisation of seeds for pasture and forage crops. The seed coating technology evaluated in this study was developed through this collaboration using whole algal biomass incorporated into crop-specific coating formulations and is described in a published patent application (Rato da Silva et al. 2026).

InnovPlantProtect carried out controlled-environment and screenhouse experiments with wheat and Persian clover (38°53'03.4" N, 7°08'22.0" W). The Persian clover field-validation trial was conducted by Fertiprado at Quinta de São Brás, Elvas, Portugal (38°48'33.9"N, 7°08'30.7"W), whereas the wheat field trial was conducted by InnovPlantProtect at the experimental fields of INIAV, Herdade da Comenda, Elvas, Portugal (38°53'52.0" N, 7°02'38.3" W). This coordinated experimental framework enabled evaluation of algae-based seed coating across controlled-environment, screenhouse, and field conditions.

2.1 Algal biomass materials

Three commercial producers supplied the algal biomasses used in this study following their established industrial production protocols. *Chlorella sorokiniana* (batch L2450029) and *Tetraselmis chuii* (batch L201930183) were provided by Allmicroalgae Natural Products S.A. (Leiria, Portugal) and Necton (Olhão, Portugal), respectively, whereas *Gracilaria* sp. (batch G1MFKG/G1.0130924) was supplied by ALGAplus (Ílhavo, Portugal).

The *C. sorokiniana* biomass was supplied as a fine spray-dried powder produced from fermented *Chlorella*, whereas *T. chuii* was supplied as a fine spray-dried powder. Both products consisted of 100% algal biomass without added ingredients.

The Gracilaria sp. biomass was produced in a land-based open integrated multi-trophic aquaculture (IMTA) system at the Ria de Aveiro, Portugal. After cultivation, the biomass was washed with filtered seawater, dehydrated in an industrial convective forced air-tunnel drier at 25°C to a target moisture content of 12% and then milled and sieved.

No culture-collection accession or strain number was provided in the manufacturers' product specifications. Detailed chemical characterization of the experimental biomass batches, including the concentrations of the potential bioactive compounds, was not performed as part of the present study. To ensure consistency within the study, all experiments with each algal species used biomass from the same production batch.

2.2 Seed coating process

Fertiprado developed the seed coating formulation and optimised it in collaboration with InnovPlantProtect for the experimental evaluation of algae-based seed coating technology. The coating matrix was designed to promote seed coverage and adhesion of the algal biomass. As the formulation is protected by intellectual property (Rato da Silva et al. 2026), its detailed composition is not fully disclosed.

Crop-specific coating formulations and corresponding controls were developed for wheat and Persian clover based on each seed's characteristics (Table 1). For wheat, uncoated seeds served as the control and were compared with seeds coated with a 1:1 (w/w) mixture of *T. chuii* and *Gracilaria* sp. The wheat formulation was applied at 600 mg algal biomass per 5 g of seeds, comprising 300 mg *T. chuii* and 300 mg *Gracilaria* sp. (120 mg total algal biomass g^{-1} seed). A binder was included in the coating formulation to facilitate the adhesion of the algal biomass to the seed surface.

For Persian clover, the total coating material was kept constant between the algae treatment and its corresponding coating control, while the composition of the coating differed. The coating material was applied at 600 mg per 5 g seeds (120 mg coating material g^{-1} seed). The coating control consisted of drying agent only, whereas in the algae treatment half of the drying agent was replaced by *C. sorokiniana* biomass, resulting in equal proportions of algal biomass and drying agent, i.e., 300 mg of *C. sorokiniana* biomass and 300 mg of drying agent per 5 g of seed. This corresponded to an algal biomass application rate of 60 mg g^{-1} seed. The same binder and *Rhizobium* sp. inoculant, the latter representing Fertiprado's commercial standard for Persian clover, were applied equally to both the algae treatment and its coating control. Thus, the total amount of coating material and the application of the binder and microbial inoculant were maintained between treatments, with the replacement of half of the drying agent by *C. sorokiniana* biomass distinguishing the algae treatment from its corresponding coating control.

For the controlled-environment and screenhouse experiments, seeds were coated in batches of 5 g per treatment. For the wheat field experiment, the coating process was manually scaled up to 100 g seed batches. For the Persian clover field experiment, since larger seed quantities were required, the processing was done using an industrial-scale stainless-steel rotary seed-coating pan with

approximately 5 kg of seed coated per treatment. The same crop-specific coating formulations and corresponding controls were used in the controlled and screenhouse experiments. For the scaled-up coating procedures, the coating ingredients were applied proportionally to the seed mass to maintain the same coating application rates used for the 5 g batches.

For manual coating, the required seed mass was weighed, and a polymeric binder, prepared as an aqueous solution, was applied dropwise at 100–200 µL per 5 g of seeds. Seeds were manually mixed using a rotational motion to ensure uniform distribution of the binder over the seed surface. The crop-specific coating material was then added and mixed until coverage was visually consistent. For wheat, *T. chuii* and *Gracilaria* sp. biomasses were premixed at a 1:1 (w/w) ratio before application to the binder-treated seeds. Following coating, seeds were dried at room temperature (approximately 23°C) under ambient air circulation for 5–10 min and stored at 4°C in the dark until further use.

The biomass retention after coating and drying was estimated indirectly by quantifying the biomass that was not retained on the seeds. For each coating formulation, biomass retention was determined separately for three coating batches prepared within the same experimental run. Retention was calculated for each batch, and results are reported as the range of the three batch-level values rather than a mean. Unretained biomass remaining in the coating vessel after seed removal was recovered and weighed. Biomass retention was then calculated relative to the amount of algae biomass initially applied as

Biomass retention = [(Biomass applied – Biomass recovered after coating)/Biomass applied] x 100,

where biomass applied represents the initial mass of algal biomass used for coating, and biomass recovered represents the unretained biomass collected after the coating process. The resulting value therefore estimates the initial biomass retention rather than a direct measurement of biomass deposited on individual seeds.

Table 1
Overview of crop-specific seed coating treatments, nominal coating loads, and experimental settings.

Crop	Coating treatment	Coating composition	Application rate	Microbial inoculant	Coating control	Experimental settings
Wheat	Mixture of *T. chuii* and *Gracilaria* sp.	1:1 (w/w) algal biomass mixture with binder	600 mg algal biomass per 5 g seeds (300 mg *T. chuii* + 300 mg *Gracilaria* sp.; 120 mg total algal biomass g^{-1} seed)	-	Uncoated seeds	Controlled, screenhouse and field
Persian clover	*C. sorokiniana*	1:1 (w/w) *C. sorokiniana* + drying agent with binder	600 mg coating material per 5 g seeds (300 mg *C. sorokiniana* + 300 mg drying agent; 60 mg algal biomass g^{-1} seed)	*Rhizobium* sp., applied equally to coating treatment and control	Drying-agent coating + *Rhizobium* sp. with binder	Controlled, screenhouse and field

2.3 Experimental framework and measurements

The performance of the algae-based seed coating was evaluated in wheat (*T. aestivum* L.) and Persian clover (*T. resupinatum* L.) across three experimental systems of increasing environmental complexity: controlled environment, screenhouse, and field conditions.

Controlled experiments assessed early plant establishment and crop-specific growth responses, while the screenhouse provided an intermediate validation step before the field validation. The screenhouse consisted of a mesh-enclosed structure exposed to ambient temperature, rainfall, solar radiation, and wind, without artificial regulation of temperature, humidity, or light. The mesh limited access by larger insects, rodents, and other animals while maintaining exposure to naturally fluctuating environmental conditions.

Sections 2.4 and 2.5 describe the experimental design, growth conditions, replication, and crop-specific assessments for wheat and Persian clover. Assessment procedures common to multiple experiments are described below.

2.3.1 Seed germination and early seedling development

Seed germination and early seedling development were assessed in 9 cm glass Petri dishes lined with filter paper moistened with 4 mL of distilled water, sealed with Parafilm, and incubated under crop-specific temperature and photoperiod conditions. Germination was recorded based on the first visible

evidence of germination and expressed as the percentage of the total number of seeds assessed. Radicle length was subsequently measured in germinated seedlings as an indicator of early seedling growth. Following transplantation, seedling emergence was recorded and expressed as the percentage of transplanted seedlings that successfully established. Crop-specific incubation conditions, sample sizes, and assessment times are described in the corresponding sections below.

2.3.2 Leaf pigment and chlorophyll-related measurements

Chlorophyll content index (CCI), soil-plant analysis development (SPAD), flavonol index, and anthocyanin index were measured non-destructively using a Multi Pigment Meter (MPM-100S, Opti-Sciences, USA) on the adaxial leaf surface. Crop and experiment-specific sample sizes are described in the corresponding sections below.

2.3.3 Biomass determination

Under controlled conditions, above-ground plant biomass of wheat and Persian clover was harvested and oven-dried at 60°C until constant weight (Pérez-Harguindeguy et al. 2013), after which shoot dry biomass was determined by weighing and expressed as mg plant^{-1}. For Persian clover, 20 plants per treatment were initially established; however, five plants per treatment were excluded from shoot dry biomass determination because they were damaged at the end of the experiment, resulting in 15 plants per treatment that were included in the analysis (n = 15). Under screenhouse conditions, Persian clover shoot biomass was determined following the same drying procedure and expressed as g plant^{-1}. Shoot dry biomass was determined separately for each cut, and cumulative biomass was calculated as the sum of both cuts. The field-specific procedure used to determine Persian clover biomass is described in Section 2.5.3.

2.4 Wheat (*Triticum aestivum* L.)

Seeds of wheat cv. Roxo (INIAV, Portugal) were stored at 4°C until use. Two seed treatments were evaluated throughout the experiments: uncoated seeds and seeds coated with a formulation comprising a mixture of *T. chuii* and *Gracilaria* sp., hereafter referred to as the mix (Table 1). During formulation development, the individual algal components were initially evaluated under controlled conditions. Coating with *Gracilaria* sp. alone did not produce a detectable biostimulant response under these conditions and consequently was not advanced as an individual treatment to the screenhouse and/or field evaluations. In contrast, coating with *T. chuii* induced a positive response under controlled conditions and was therefore evaluated under controlled, screenhouse and field conditions. Results of the individual-component treatments are provided in Section 4.2.1. Based on the formulation development process, the mix was selected as the main treatment and evaluated consistently across controlled, screenhouse and field conditions.

2.4.1 Controlled conditions

For each treatment, 40 seeds (20 seeds per Petri dish) were germinated as described above at room temperature (approximately 23 ºC) under a 16 h light/8 h dark photoperiod for 4 days. Radicle length was measured at the end of the germination period, after which ten uniformly germinated seedlings per treatment were transplanted individually into alveoli (5.5 cm height x 5.5 cm width x 6.5 cm top diameter), containing Siro Relva (Portugal) substrate and maintained under controlled growth chamber conditions (22 ± 2°C, 80% relative humidity, 16 h light/8 h dark photoperiod). One plant from the uncoated control was excluded from the tiller number and shoot dry biomass assessments because it was damaged during the experiment, resulting in n = 9 for the uncoated and n = 10 for the coated treatment for these measurements. Each independently grown plant was considered one biological replicate. After six weeks of growth, leaf pigment and chlorophyll-related parameters were measured on six randomly selected plants. At the end of the experiment, tiller number was recorded, and shoots were harvested to determine dry weight.

2.4.2 Screenhouse conditions

Wheat seeds were sown directly in 3.5 L pots containing Siro Relva substrate from November 2025 to the end of June 2026. Three seeds were initially sown per pot. Following seedling emergence, plants were thinned to one plant per pot to obtain a uniform final plant density. Irrigation was supplied as needed to keep the substrate close to field capacity. Each treatment consisted of four independent pots, with each pot representing one biological replicate (n = 4 per treatment). Pots were randomly distributed within the screenhouse.

Plant development was monitored throughout the growing season by recording the number of tillers during vegetative growth. At physiological maturity, the number of spikes per plant was determined. Spikes were subsequently harvested, manually threshed, and grain production was determined for each plant.

2.4.3 Field conditions

Field validation of the wheat seed coating was conducted during the 2026 growing season at Herdade da Comenda. The trial was established in March 2026 and maintained until crop maturity at the end of June 2026. Field establishment was delayed 3 months because exceptional flooding prevented earlier sowing. The same two treatments evaluated under controlled and screenhouse conditions—uncoated seeds and seeds coated with the mix formulation—were evaluated under field conditions. Seeds were mechanically sown using a Wintersteiger seeder (Austria) at a 20 cm row spacing and a sowing depth of approximately 5 cm. The sowing equipment was calibrated to ensure an equivalent seed density of 350 seeds m^{-2} for both treatments. The corresponding seed amount sown was 143 kg ha^{-1}.

Each treatment consisted of three independent plots (n = 3), with each plot representing one biological replicate. Individual plots measured 8.0 × 1.2 m (9.6 m²), corresponding to a total experimental area of 28.8 m² per treatment. The three replicate plots per treatment were randomly distributed within the experimental area to reduce potential positional effects associated with spatial variation in soil and environmental conditions. Buffer rows were maintained along the field borders to minimise edge effects.

Crop management followed standard agronomic practices for wheat cultivation. At physiological maturity, each plot was harvested independently, and grain production was determined from the harvested grain weight. Grain yield was subsequently expressed as kg ha^{-1} based on the harvested plot area.

2.5 Persian clover (*Trifolium resupinatum* L.)

Persian clover var. Resal (Fertiprado, Portugal) seeds were stored in the dark at approximately 22°C until use. Two treatments were evaluated: a coating control containing the drying agent and an algae coating containing *C. sorokiniana* biomass and drying agent in equal proportions. A *Rhizobium* sp. inoculant was applied equally to both treatments, as described in Section 2.2 (Table 1).

2.5.1 Controlled conditions

For each treatment, 50 seeds (25 seeds per Petri dish) were germinated as described and incubated in darkness for 24 h, then kept under a 16 h light/8 h dark photoperiod at room temperature (approximately 23 ºC) for three additional days. Radicle length was measured at the end of the germination period, after which twenty uniformly germinated seedlings per treatment were transplanted individually into alveoli (5.5 cm height x 5.5 cm width x 6.5 cm top diameter), containing Siro Relva (Portugal) substrate and maintained under controlled growth chamber conditions (22 ± 2°C, 80% relative humidity, 16 h light/8 h dark photoperiod). Each independently grown plant was considered one biological replicate (n = 20 per treatment). Four to five weeks after transplantation, leaf pigment and chlorophyll-related parameters were assessed on six randomly selected plants. At the end of the experiment, shoots were harvested for dry-weight determination. Root systems were examined individually, and nodulation was quantified in six plants per treatment by counting nodules per plant.

2.5.2 Screenhouse conditions

Persian clover seeds were sown directly in 3.5 L pots containing Siro Relva substrate, from November 2024 to June 2025. Three seeds were initially sown per pot. After seedling emergence, plants were thinned to one plant per pot to achieve a uniform final plant density and to allow subsequent assessment of root nodulation. Routine irrigation was not applied, although supplemental irrigation was provided during periods of extreme heat in May and June to prevent irreversible drought stress. Each treatment consisted of five independent pots, with each pot representing one biological replicate (n = 5 per treatment).

To evaluate biomass production and regrowth, plants underwent two successive cuts. The first cut was performed 11 weeks after sowing. Following regrowth, a second cut was performed five weeks later.

Leaf pigment and chlorophyll-related parameters were assessed before both biomass cuts. After the final harvest, root systems were examined for nodulation. Because the roots of the 16-week-old plants contained extensive and overlapping nodule clusters that prevented reliable counting, nodulation was assessed qualitatively by comparing nodule abundance and distribution between treatments.

2.5.3 Field conditions

Field-scale validation of the Persian clover seed coating was conducted from November 2024 until Jue 2025 at Quinta de São Brás. The same coating treatment and corresponding control evaluated under controlled and screenhouse conditions were further assessed in two adjacent field areas, with one 5,000 m² area (100 × 50 m) assigned to each treatment. Thus, the field-scale validation comprised one treatment area per condition rather than replicated field plots. Biomass production within each treatment area was characterised using three randomly selected 1 m² sampling areas at each harvest, which represented treatment subsamples rather than independent experimental replicates.

The trial was sown using an APV500 seeder with a row spacing of 12 cm and a sowing depth of approximately 0.5 cm. The sowing equipment was calibrated to ensure an equivalent seed density of 1,582 seeds m^{-2} for both treatments. The corresponding seed amount sown was 25 kg ha^{-1}. Both treatment areas were managed according to standard agronomic practices for Persian clover production.

Crop performance was evaluated through two successive harvests in March and June 2025 to assess biomass production and regrowth. At each harvest, three 1 m² sampling areas were randomly selected from the interior of each treatment area, avoiding field margins and the boundary between treatments to minimise potential edge effects. Plants were harvested approximately 10 cm above soil level, and the total fresh biomass collected from each 1 m² sampling area was weighed and used to estimate the fresh Persian clover biomass yield, expressed as kg ha^{-1}. A representative 300–500 g subsample of the harvested material was manually separated into Persian clover and weed fractions, weighed fresh and oven-dried separately at 60°C for 48 h. The dry-matter content of the Persian clover fraction was then used to calculate Persian clover dry biomass, expressed as kg ha^{-1}.

New 1 m² sampling areas were randomly and independently selected at each harvest; therefore, samples collected at the first and second harvests were not treated as paired observations. The mean of the three within-treatment subsamples was used to characterise fresh and dry biomass production for each treatment area at each harvest. Cumulative dry biomass was calculated at the treatment-area level as the sum of the mean dry biomass obtained at the first and second harvests. Because the field-scale validation included one field area per treatment, within-area samples did not provide independent treatment replication; therefore, treatment differences were evaluated descriptively.

3. Statistical analysis

Statistical analyses were performed separately for each response variable using R version 4.3.3. For continuous data, normality of model residuals and homogeneity of variances were assessed using the Shapiro–Wilk and Levene's tests, respectively. When both assumptions were met, treatment effects were analysed by one-way ANOVA followed, where applicable, by Dunnett's test for comparisons with the corresponding control. Otherwise, the Kruskal–Wallis test followed by Dunn's test with Holm adjustment

was used. Count data (tillers, spikes, and nodules) were analysed using generalised linear models (GLMs) with a log link and Poisson distribution. Overdispersion was assessed using the ratio of the Pearson chi-square statistic to the residual degrees of freedom. When the dispersion ratio exceeded 1.5, the model was refitted using a negative-binomial distribution. Overall treatment effects were assessed using likelihood-ratio chi-square tests.

Germination and seedling emergence were evaluated descriptively and are reported as percentages; no inferential statistical comparisons between treatments were performed for these parameters. For the wheat field experiment, the three independent field plots per treatment were considered experimental replicates and were included in inferential statistical analysis. In contrast, for Persian clover field-scale validation, the three 1 m² sampling areas within each treatment area were considered spatial subsamples rather than independent experimental replicates; consequently, field responses were summarised descriptively, and no inferential statistical comparison between treatments was performed. Unless otherwise stated, data are presented as individual observations together with the mean ± standard deviation (SD), with the experimental unit and number of independent replicates (n) specified for each experiment and figure. For growth, biomass, and productivity traits, treatment effects were additionally expressed as percentage change relative to the corresponding control. Statistical significance was considered at $p < 0.05$, with significance levels indicated as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).

4. Results

4.1 Characterisation of the algae-based seed coating

Visual assessment of the coated seeds showed clear, distinguishable deposition of algal biomass across crop species and coating formulations (Online Resource 1, Fig. S1). Coated seeds displayed a visible layer of algal biomass over the seed surface, with differences in coating appearance reflecting both the biomass used and the characteristics of each seed type. For each formulation, retention was determined separately from three coating batches prepared within the same experimental run, and the values are therefore reported as the range observed across the batches. The estimated biomass retention varied among the tested crop–biomass combinations. For Persian clover, *C. sorokiniana* biomass showed an estimated retention ranging from approximately 80 to 81%. For wheat, retention of the *T. chuii–Gracilaria* sp. mix ranged from 85 to approximately 89%.

4.2 Crop responses

4.2.1 Wheat seed coating performance across controlled, screenhouse, and field conditions

Under controlled conditions, germination and seedling emergence were descriptively similar between the *T. chuii–Gracilaria* sp. mix-coated and uncoated seeds. Germination was 95% in coated seeds and 97.5%

in the uncoated control, while seedling emergence was 100% and 90%, respectively. These parameters were evaluated descriptively and were not subjected to inferential statistical analysis.

After establishment, plants from coated seeds showed enhanced growth from early seedling development onwards. The first clear response was observed in root development, with mean radicle length increasing from approximately 38.35 ± 13.78 mm in uncoated seedlings (n = 39) to 98.10 ± 48.85 mm in coated seedlings (n = 38) (Kruskal–Wallis test: $\chi^2(1) = 28.50$, $p < 0.001$, Dunn's post-hoc test with Holm adjustment: $z = 5.34$, adjusted $p < 0.001$; Fig. 1a). This early stimulation was followed by enhanced vegetative growth, as coated plants produced 2.90 ± 0.32 tillers plant^{-1} (n = 10) compared with 1.33 ± 0.50 tillers plant^{-1} in the uncoated control (n = 9). Tiller number was significantly influenced by the coating treatment (Poisson GLM, likelihood-ratio χ^2 test: $\chi^2(1) = 5.59$, $p = 0.0181$; Fig. 1b). Greater vegetative development was also reflected in shoot dry biomass, which increased from 237.28 ± 54.18 mg plant^{-1} in uncoated plants (n = 9) to 467.44 ± 85.68 mg plant^{-1} in coated plants (n = 10), representing a 97% increase (one-way ANOVA: $F(1,17) = 47.63$, $p < 0.001$ followed by Dunnett's multiple-comparison test: $t(17) = 6.90$, adjusted $p < 0.001$; Fig. 1c). These differences in plant development are visually apparent, with coated plants displaying greater shoot development and tillering than the uncoated control (Fig. 1d).

Despite the pronounced effects of the mix seed coating formulation on wheat growth, no significant differences were detected in the measured leaf pigment and chlorophyll-related parameters under controlled conditions. Anthocyanin and flavonol indices were comparable between coated and uncoated plants, while CCI and SPAD values, although numerically lower in coated plants, did not differ significantly from the uncoated plants (Online Resource 1, Fig. S2a-d). Overall, the enhanced root development, tillering, and biomass accumulation observed following seed coating occurred without significant changes in the measured leaf pigment and chlorophyll-related traits.

Individual-algae biomass treatments evaluated during the formulation development showed environment-dependent responses (Online Resource 1, Fig. S3). Under controlled conditions, *T. chuii* applied individually as a seed coating significantly increased the number of tillers from 1.33 ± 0.50 tillers plant^{-1} in the uncoated control (n = 9) to 3.00 ± 0.00 tillers plant^{-1} in coated plants (n = 10) (Poisson GLM, likelihood-ratio $\chi^2(1) = 6.19$, $p = 0.0128$; Online Resource 1, Fig. S3a(i)). Shoot dry biomass also increased from 237.28 ± 54.18 mg plant^{-1} in the uncoated control (n = 9) to 462.89 ± 42.34 mg plant^{-1} in coated plants (n = 10), corresponding to a 95% increase (one-way ANOVA: $F(1,17) = 103.46$, $p < 0.001$ followed by Dunnett's multiple-comparison test: $t(17) = 10.17$, adjusted $p < 0.001$; Online Resource 1, Fig S3a(ii)). In contrast, *Gracilaria* sp. coating alone did not significantly affect tiller number (Poisson GLM, likelihood-ratio $\chi^2(1) = 0.057$, $p = 0.8111$; Online Resource 1, Fig. S3a(iii)) or shoot dry biomass under controlled conditions (one-way ANOVA: $F(1,18) = 1.18$, $p = 0.2910$; Dunnett's test: $t(18) = -1.09$, adjusted $p = 0.2910$; Online Resource 1, Fig. S3a(iv)) and was therefore not selected as an individual treatment for the subsequent screenhouse and field evaluations.

Tetraselmis chuii was further evaluated under screenhouse conditions. No significant differences were detected in the number of tillers (Poisson GLM with a log link, likelihood-ratio $\chi^2(1) = 0.1085$, $p = 0.7419$; Online Resource 1, Fig. S3b(i)) or spike number (Poisson GLM with a log link, likelihood-ratio $\chi^2(1) = 0.0169$, $p = 0.8964$; Online Resource 1, Fig. S3b(ii)) relative to the uncoated control. However, grain weight increased significantly from 9.52 ± 0.50 g grain plant^{-1} in the uncoated control (n = 4) to 12.03 ± 1.31 g of grain plant^{-1} in the *T. chuii* coated plants (n = 4), corresponding to a 26% increase (one-way ANOVA: $F(1,6) = 12.87$, $p = 0.0115$; Dunnett's test: $t(6) = 3.59$, adjusted $p = 0.0115$; Online Resource 1, Fig. S3b(iii)).

This increase in grain weight was not maintained under field conditions, where mean grain yield was comparable between the *T. chuii* coated plants and uncoated plants with 2,865 ± 303 and 2,875 ± 587 kg ha^{-1}, respectively (n = 3 independent field plots per treatment; one-way ANOVA: $F(1,4) = 0.0007$, $p = 0.9795$ followed by Dunnett's test: $t(4) = -0.0273$, $p = 0.9795$; Online Resource 1, Fig. S3c).

Considering the positive performance of the mix formulation under controlled conditions, it was subsequently evaluated under screenhouse conditions. Coated plants showed greater grain weight, with 11.32 ± 0.87 g of grain plant^{-1} (n = 4) compared with 9.52 ± 0.50 g grain plant^{-1} in the uncoated control (n = 4), which corresponds to a 19% increase (one-way ANOVA: $F(1,6) = 13.06$, $p = 0.0112$; Dunnett's multiple-comparison test: $t(6) = 3.61$, adjusted $p = 0.0112$; Fig. 1e).

In contrast to the increased tillering observed under controlled conditions, the number of tillers and spikes did not significantly differ between coated and uncoated plants under screenhouse conditions. The number of tillers averaged 10.75 ± 0.50 tillers plant^{-1} in the uncoated control and 9.25 ± 2.06 tillers plant^{-1} in the mix coating treatment (n = 4 independent pots per treatment), with no significant difference between treatments (Poisson GLM with a log link, likelihood-ratio $\chi^2(1) = 0.45$, $p = 0.5021$; Online Resource 1, Fig. S2e). Similarly, spike number was comparable between uncoated and coated plants, averaging 7.25 ± 0.96 and 6.75 ± 0.96 spikes plant^{-1}, respectively (Poisson GLM with a log link, likelihood-ratio $\chi^2(1) = 0.07$, $p = 0.7892$; Online Resource 1, Fig. S2f). Thus, the greater grain weight observed in plants coated with the coating mix was not associated with an increase in tiller or spike number.

To assess whether this productivity response extended to field conditions, the mix coating formulation was further evaluated in a field trial. Plants established from coated seeds reached a mean grain yield of 3,236 ± 259 kg ha^{-1} (n = 3 independent field plots) compared to 2,875 ± 587 kg ha^{-1} for the uncoated control (n = 3 independent field plots), corresponding to a 13% higher mean yield. However, this difference was not statistically significant (one-way ANOVA: $F(1,4) = 0.95$, $p = 0.3845$ followed by Dunnett's multiple-comparison test: $t(4) = 0.98$, adjusted $p = 0.3845$; Fig. 1f).

Overall, the sequential evaluation showed a significant stimulation of early root and vegetative growth under controlled conditions, followed by a significant increase in grain production under screenhouse conditions, while mean field grain yield was numerically higher but did not differ significantly between treatments.

4.2.2 Persian clover performance across controlled, screenhouse, and field conditions

Under controlled conditions, germination and seedling emergence were descriptively similar between *C. sorokiniana*-coated seeds and the coating control. Germination was 94% in coated seeds and 96% in the coating control, while seedling emergence following transplantation reached 100% in both treatments. These parameters were evaluated descriptively and were not subjected to inferential statistical analysis.

After establishment, the first growth-promoting response to the coating was observed during early seedling development, with seedlings from *C. sorokiniana*–coated seeds developing longer radicles than those from the coating control. Mean radicle length increased from 14.13 ± 8.28 mm in control seedlings (n = 47) to 18.51 ± 10.34 mm in coated seedlings (n = 47). Radicle length differed significantly between treatments (Kruskal–Wallis test: $\chi^2(1) = 4.22$, $p = 0.0401$; Dunn's post-hoc test with Holm adjustment: z = 2.05, adjusted $p = 0.0401$; Fig. 2a).

As plants developed, this early response was followed by greater shoot biomass accumulation. Seed coating with *C. sorokiniana* significantly increased shoot dry weight from 154.47 ± 42.65 mg plant^{-1} in the coating control (n = 15) to 229.33 ± 70.62 mg plant^{-1} in coated plants (n = 15), corresponding to an increase of approximately 48% (Kruskal–Wallis test: $\chi^2(1) = 10.20$, $p = 0.0014$; Dunn's post-hoc test with Holm adjustment: $z = 3.19$, adjusted $p = 0.0014$; Fig. 2b). Enhanced shoot development was also visually apparent at the end of the experiment, with coated plants displaying greater above-ground growth than plants from the coating control (Fig. 2c), consistent with the quantitative biomass measurements.

As observed for wheat, despite the effects of *C. sorokiniana* seed coating on radicle development and shoot biomass, no significant differences were detected in the measured leaf pigment and chlorophyll-related indices under controlled conditions. Anthocyanin index did not differ significantly between coated and coating-control plants (one-way ANOVA: $F(1,10) = 4.00$, $p = 0.0734$; Dunnett's multiple-comparison test: $t(10) = 2.00$, adjusted $p = 0.0734$; Online Resource 1, Fig. S4a(i)). Similarly, no significant difference was detected for flavonol index (one-way ANOVA: $F(1,10) = 2.37$, $p = 0.1547$; Dunnett's multiple-comparison test: $t(10) = -1.54$, adjusted $p = 0.1547$; Online Resource 1, Fig. S4a(ii)). CCI and SPAD values were also unaffected by the coating treatment (Kruskal–Wallis test: $\chi^2(1) = 0.00$, $p = 1.000$; Dunn's post-hoc test with Holm adjustment: $z = 0.00$, adjusted $p = 1.000$ for both CCI and SPAD; Online Resource 1, Fig. S4a (iii-iv)).

Chlorella sorokiniana seed coating also significantly enhanced root nodulation. Coated plants developed 17.67 ± 6.31 nodules plant^{-1} (n = 6) compared with 6.33 ± 5.72 nodules plant^{-1} in the coating control (n = 6). Nodule number was significantly affected by the coating treatment (negative-binomial GLM with a log link, likelihood-ratio χ^2 test: $\chi^2(1) = 7.03$, $p = 0.0080$), with the estimated nodule count being 2.79-fold higher in coated plants than in the coating control (treatment-to-control ratio = 2.79, $z = 2.67$, $p = 0.0077$; Fig. 2d). Figure 2e shows representative root systems illustrating nodulation in Persian clover at the end of the experiment.

The coating performance was subsequently evaluated under screenhouse conditions, where the positive effect on biomass production was maintained across successive cuts. At the first cut, shoot-dry biomass increased from 3.76 ± 1.61 g plant^{-1} in the coating control (n = 5) to 6.10 ± 1.07 g plant^{-1} in coated plants (n = 5), corresponding to a 62% increase (Kruskal–Wallis test: $\chi^2(1) = 3.94$, $p = 0.0472$; Dunn's post-hoc test with Holm adjustment: $z = 1.98$, adjusted $p = 0.0472$; Fig. 3a(i)). The increase in biomass remained evident after cutting and regrowth, with coated plants producing 26.07 ± 5.17 g plant^{-1} at the second cut compared with 18.71 ± 2.72 g plant^{-1} in the coating control, corresponding to a 39% increase (one-way ANOVA: $F(1,8) = 7.95$, $p = 0.0225$; Dunnett's multiple-comparison test: $t(8) = 2.82$, adjusted $p = 0.0225$; Fig. 3a(ii)). When both cuts were considered together, cumulative shoot dry biomass was 43% higher in coated plants, increasing from 22.47 ± 3.52 to 32.18 ± 4.95 g plant^{-1} (one-way ANOVA: $F(1,8) = 12.80$, $p = 0.0072$; Dunnett's multiple-comparison test: $t(8) = 3.58$, adjusted $p = 0.0072$; Fig. 3a(iii)).

Leaf physiological responses under screenhouse conditions were limited and varied with growth stage. Before the first cut, the flavonol index was significantly higher in coated plants than in the coating control (one-way ANOVA: $F(1,8) = 5.83$, $p = 0.0422$; Dunnett's multiple-comparison test: $t(8) = 2.41$, adjusted $p = 0.0422$; Online Resource 1, Fig. S4b(ii)). In contrast, anthocyanin index ($F(1,8) = 0.00$, $p = 1.000$), CCI ($F(1,8) = 0.15$, $p = 0.7118$), and SPAD ($F(1,8) = 1.40$, $p = 0.2712$) did not differ significantly between treatments (Online Resource 1, Fig. S4b(i) and (iii-iv)). During regrowth before the second cut, the anthocyanin index was significantly lower in coated plants than in the coating control (one-way ANOVA: $F(1,8) = 8.52$, $p = 0.0193$; Dunnett's multiple-comparison test: $t(8) = -2.92$, adjusted $p = 0.0193$; Online Resource 1, Fig. S4c(i)). Flavonol index remained comparable between treatments ($F(1,8) = 3.62$, $p = 0.0937$), while no significant differences were detected for CCI or SPAD (Kruskal–Wallis test: $\chi^2(1) = 0.011$, $p = 0.9168$ for both parameters; Online Resource 1, Fig. S4c(ii–iv)). Overall, these measurements indicate that the substantial increase in biomass was not accompanied by consistent changes in the measured chlorophyll- and pigment-related indices.

At the end of the screenhouse experiment, nodules were abundant throughout the root systems of both treatments. The extensive development and overlapping of nodule clusters prevented reliable quantification; therefore, the nodulation was assessed qualitatively. Visual examination indicated greater nodule abundance in plants from *C. sorokiniana*-coated seeds than in the coating control (Fig. 3b).

Overall, the sequential evaluation of Persian clover showed a consistent growth-promoting response to *C. sorokiniana* seed coating across controlled and screenhouse conditions. Under controlled conditions, treatment initially promoted root development, nodulation, and shoot biomass accumulation, while in screenhouse the biomass response remained evident through cutting and subsequent regrowth, resulting in a 43% increase in cumulative shoot dry biomass.

To assess whether the positive responses observed under controlled and screenhouse conditions extended to more complex agronomically representative conditions, *C. sorokiniana*-coated Persian clover seeds were further evaluated in a field-scale validation.

At the first cut, mean fresh biomass was 10,011 ± 3,735 kg ha⁻¹ in the coated treatment and 9,553 ± 2,354 kg ha⁻¹ in the coating control, corresponding to a 5% numerical difference (Fig. 4a(i)). After regrowth, the difference was more pronounced at the second cut, with mean fresh biomass of 37,513 ± 4,322 kg ha⁻¹ in the coated treatment compared with 27,953 ± 528 kg ha⁻¹ in the coating control, corresponding to a 34% numerical difference (Fig. 4a(ii)). Cumulative fresh biomass across both cuts was 47,524 kg ha⁻¹ in the coated treatment and 37,507 kg ha⁻¹ in the coating control, corresponding to a 27% numerical difference and approximately 10,018 kg ha⁻¹ greater cumulative fresh biomass in the coated treatment area (Fig. 4a(iii)).

A similar pattern was observed for dry biomass. At first cut, mean dry biomass was 1,394 ± 466 kg ha⁻¹ in the coated treatment compared with 1,021 ± 593 kg ha⁻¹ in the coating control, corresponding to a 37% numerical difference (Fig. 4b(i)). At the second cut, mean dry biomass was 4,637 ± 412 kg ha⁻¹ in the coated treatment compared with 3,305 ± 354 kg ha⁻¹ in the coating control, corresponding to a 40% numerical difference (Fig. 4b(ii)). Cumulative dry biomass across both cuts was 6,031 kg ha⁻¹ in the coated treatment and 4,326 kg ha⁻¹ in the coating control, representing a 39% numerical difference and approximately 1,706 kg ha⁻¹ greater cumulative dry biomass in the coated treatment area (Fig. 4b(iii)). Because the field-scale validation consisted of a single field area per treatment, with three 1 m² sampling areas per treatment and harvest representing spatial subsamples rather than independent field replicates, treatment differences were evaluated descriptively and no inferential statistical comparison between treatments was performed.

5. Discussion

5.1 Algal coating performance and biomass retention

Visual characterisation of the coated seeds confirmed algal biomass deposition on both wheat and Persian clover despite differences in seed morphology. The algal biomass formed a visually distinguishable layer over the seed surface, with coating appearance varying according to the biomass used and the characteristics of each seed type. This visual evidence was complemented by the high proportion of biomass retained after coating and drying, estimated at approximately 85–89% for the *T. chuii–Gracilaria* sp. mixture on wheat and 80–81% for *C. sorokiniana* on Persian clover. Differences between crop-formulation combinations may reflect interactions among seed characteristics, biomass properties, and the coating process (Pedrini et al. 2017; Rocha et al. 2019; Paravar et al. 2023).

These results demonstrate an effective initial deposition of the whole algal biomass and provide the basis for evaluating its biological performance. Importantly, retention was determined immediately after coating and drying and therefore represents initial coating performance rather than coating durability. Evaluation after storage, transport, and mechanical sowing will be needed to determine the formulation´s stability under practical handling conditions.

5.2 Biostimulant performance of the *Tetraselmis chuii* and *Gracilaria* sp. coating mixture in wheat

In wheat, the *T. chuii–Gracilaria* sp. biomass coating did not compromise crop establishment, as germination and seedling emergence remained comparable to those of uncoated seeds. Instead, the first clear response occurred after germination, with increased radicle elongation, followed by enhanced tillering and shoot biomass under controlled conditions. Similar stimulation of early root and vegetative development in wheat has been reported following microalgae- and seaweed-derived treatments, although responses vary with algal source, formulation, and concentration (Supraja et al. 2020; Minaoui et al. 2024; Mutum et al. 2024; Chabili et al. 2026). Early root development may be agronomically relevant by increasing the capacity of young plants to explore the soil for water and nutrients, although roots were not followed beyond the seedling stage in the present study.

Previous studies have demonstrated the feasibility of delivering algae-derived biostimulants through seed coating. *Dunaliella salina* biomass incorporated into a bioplastic coating influenced germination, early plant growth, and secondary metabolite accumulation in coriander (Fonseca et al. 2022), while *Spirulina platensis* homogenate applied as a seed coating produced dose-dependent growth responses in radish (Godlewska et al. 2019). Algae-derived seed coatings have also promoted early shoot and root development in wheat (Michalak et al. 2017). The present study extends these approaches by incorporating whole microalgal and macroalgal biomass into crop-specific seed-coating formulations and evaluating their biological performance beyond germination and early seedling development through controlled, screenhouse and field conditions.

The individual-component experiments provide additional context for interpreting performance of the combined wheat coating formulation. *Tetraselmis chuii* showed clear biostimulant activity when applied alone, whereas *Gracilaria* sp. did not trigger a detectable response during the initial controlled-conditions screening. Although a positive response to *T. chuii* alone persisted under screenhouse conditions as increased grain production, this response was not maintained in the field, suggesting that responses observed during early vegetative stage screening do not necessarily translate across increasingly variable growth environments. Interestingly, the combined formulation showed a different response pattern, retaining a positive, although statistically non-significant, yield response under field conditions. This observation suggests that an algal component's contribution in a mixture may differ from its performance when applied individually. One possible explanation is that combining algal biomasses modifies the overall balance of potentially bioactive compounds delivered by the formulation, which could influence the resulting plant response (Vaghela et al. 2023). However, the present experiments were not designed to investigate interactions between the two biomasses, and the specific contribution of *Gracilaria* sp. within the mixture cannot be separated from that of *T. chuii.* Accordingly, the results should not be interpreted as evidence of synergy between the two biomasses, but rather as an indication that formulation composition deserves consideration alongside the performance of individual components when developing algae-based seed coatings. This interpretation is consistent with the

broader algae-biostimulant literature, in which plant responses depend on algal source, processing, formulation, application strategy, and environmental conditions (Pérez-Oñate et al. 2026).

The response to the combined formulation extended beyond vegetative development. Under screenhouse conditions, coated plants produced significantly more grain (19%) than the uncoated control despite comparable tiller and spike numbers. Thus, the increased grain production cannot simply be attributed to the enhanced tillering observed under controlled conditions. Because grain number per spike, grain weight per spike, or thousand-grain weight were not determined, the specific yield component responsible for this response cannot be identified. Nevertheless, increased wheat growth and productivity following algae-derived treatments have previously been reported, with responses influenced by cultivar and application timing (Bakhoum et al. 2023; Mutum et al. 2024).

Under field conditions, mean grain yield was approximately 13% higher in the coated treatment, although the difference was not statistically significant, and should therefore be interpreted as a numerically higher mean yield rather than evidence of a treatment effect. This contrasts with the significant increase in grain production observed under screenhouse conditions and illustrates the variability that can occur when biostimulant formulations are transferred to agronomic environments (Takács et al. 2019; Bakhoum et al. 2023; Pérez-Oñate et al. 2026). Confirmation of this field response will therefore require replicated trials across different years and locations.

A limitation of the wheat experiment is that the reference treatment consisted of uncoated seeds rather than seeds coated with the same formulation matrix without algal biomass. Consequently, the observed responses represent the effect of the complete algae-containing coating relative to the uncoated seed and cannot be attributed exclusively to algal biomass independent of the coating matrix or coating process.

Overall, the wheat results demonstrate beneficial responses at different developmental stages, from early root and shoot growth to grain production, but do not establish a causal progression between these responses. Rather, they demonstrate that the response associated with the combined formulation extended beyond early crop establishment and highlight both formulation composition and growing environment as important determinants of algae-based biostimulant performance.

5.3 Performance of *Chlorella sorokiniana*-based seed coating in Persian clover across growing systems

Persian clover responded to *C. sorokiniana*–based coating by enhancing nodulation and sustaining biomass production after cutting. As for wheat, crop establishment was not compromised, and the first detectable response occurred post-germination, as indicated by the greater radicle length followed by increased shoot biomass. Importantly, the response initiated following seed coating was not restricted to early plant development. More importantly for a forage crop, greater biomass production persisted through successive harvests under screenhouse conditions, and the same response direction was observed during field-scale validation. This persistence is agronomically relevant because Persian clover

productivity depends not only on successful establishment but also on biomass accumulation following cutting and regrowth.

Enhanced nodulation added another dimension to the growth response. Under controlled conditions, *C. sorokiniana*-coated plants developed significantly more nodules than the coating control, while greater nodule abundance was also visually apparent at the end of the screenhouse experiment. Because the same *Rhizobium* sp. inoculant was applied to both treatments, the algal biomass may have influenced root development or conditions associated with nodule formation. *Chlorella sorokiniana* has been shown to synthesise and release indole-3-acetic acid (IAA) (Khasin et al. 2018), while auxin and cytokinin signalling are important regulators of root development and rhizobia nodule organogenesis (Boivin et al. 2016). Extracellular polysaccharides also participate in the establishment of successful *Rhizobium*-legume interactions (Castellani et al. 2021). Our observations provide plausible biological contexts for the concomitant enhancement of radicle development and nodulation, but they do not establish the mechanism operating in the present study. Neither the bioactive composition of the algal biomass nor nodule activity or biological nitrogen fixation were determined; consequently, increased nodule number should not be interpreted as evidence of enhanced symbiotic efficacy or nitrogen fixation.

The most agronomically relevant feature of the Persian clover response was its persistence during regrowth. Under screenhouse conditions, the coated treatment maintained greater biomass production after the first cut, resulting in 43% greater cumulative dry biomass. The field-scale evaluation supported this pattern: although the difference in fresh biomass was small at the first harvest, the coated area showed a larger numerical advantage after regrowth, while dry biomass remained consistently higher across both harvests and was 39% higher cumulatively. Rather than demonstrating an increasing treatment effect over time, these results indicate that the response initiated following a single seed-coating application remained detectable beyond establishment, cutting, and regrowth. This is particularly relevant for forage crops, for which cumulative production across successive harvests is more informative than performance at a single developmental stage.

The field results nevertheless require cautious interpretation. Treatments were established in single field areas, and the three 1 m² sampling areas represented spatial subsamples rather than independent field replicates. Consequently, the observed biomass differences provide descriptive field-scale validation rather than statistical confirmation of treatment efficacy. They indicate that the response remained detectable under agronomically representative conditions but cannot establish a replicated treatment effect. Replicated trials across years and locations will be required to establish the robustness of the response. Taken together, however, the combination of enhanced early root development, increased nodulation, and sustained biomass production after cutting indicates that the effects associated with seed delivery of whole *C. sorokiniana* biomass extended beyond early plant development. These findings support whole *C. sorokiniana* biomass seed coating as a promising biostimulant delivery strategy for Persian clover.

5.4 Cross-crop performance and agronomic implications of algae-based seed coatings

Across both crops, growth stimulation occurred without consistent changes in CCI, SPAD, flavonol, or anthocyanin indices. A similar dissociation between growth responses and leaf pigment-related measurements has been reported following microalgal biomass application by Mutum et al. (2023). The absence of consistent differences in these indices does not demonstrate that the photosynthetic performance was unaffected, as gas exchange, chlorophyll fluorescence, and carbon assimilation were not directly measured. Rather, the results indicate that the growth responses observed here cannot be readily explained by persistent changes in the measured leaf pigment-related traits.

More broadly, algae-based seed coating was associated with distinct agronomic responses in the two crop systems evaluated. In wheat, the *T. chuii–Gracilaria* sp. formulation enhanced early vegetative development and was subsequently associated with greater grain production under screenhouse conditions, whereas in Persian clover, the *C. sorokiniana* formulation was associated with enhanced nodulation and sustained forage biomass production through successive harvests. Because the crops were evaluated using different formulations and crop-specific agronomic endpoints, the responses cannot be directly compared between the species. Nevertheless, the results indicate that algae-based seed coating can generate crop-specific agronomic outcomes rather than a uniform response across different crops. Importantly, the responses observed during early development were, for both crop-formulation combinations, followed by agronomically relevant responses under progressively more realistic growing conditions.

The algal biomasses contain proteins, carbohydrates, lipids, minerals, and other constituents according to the manufacturer's specifications (Section 2.1); therefore, biomass-derived nutrients cannot be excluded as putative contributors. However, because nutrient release and plant nutrient uptake were not quantified, the present study cannot determine the relative contribution of nutritional or non-nutritional mechanisms.

From an application perspective, seed coating enables localised delivery of whole algal biomass through a single application at the beginning of crop development. Taken together, our results show that seed coating can provide an effective route for delivering whole algal biomass and that biological responses observed during early development can, for selected crop-formulation combinations, remain detectable under increasingly realistic growing conditions. At the same time, the differences observed between crops, formulations, and experimental environments emphasise that algae-based biostimulant performance cannot be extrapolated solely from controlled-conditions screening. Nevertheless, practical implementation will require confirmation of coating stability during storage and mechanical handling and, importantly, replicated multi-year and multi-location field trials to establish the reproducibility of crop responses under variable agronomic conditions. These aspects are particularly important given the known dependence of biostimulant performance on crop, formulation, soil, climate, and management conditions (Li et al. 2022; Ördög et al. 2025). Further development should therefore combine formulation

optimisation and coating-durability assessment with replicated field validation to identify crop-algal biomass combinations that provide reproducible agronomic benefits under practical production conditions.

Conclusions

Beyond what we report, using algal biomass as a seed-coating material has major agronomic and sustainability implications, since it delivers bioactive compounds directly to the seed and developing rhizosphere rather than dispersing them across the field. Compared with foliar or soil applications, seed coating requires significantly less material per unit area, which could lower production and transport costs. Because the application is localised, it may also reduce losses from spray drift, runoff, and compound degradation, allowing more efficient use of the biological material. Together, this combination of the algal biomass intrinsic properties with a low-dose, targeted delivery method positions seed coating as a promising route for incorporating algae-derived products into more sustainable cropping systems. The actual extent of these benefits, however, will vary with crop species, coating formulation, biomass composition, and application rate, which, of course, require field-scale trials.

Declarations

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Competing interests: RMC, SA, CR, and SC are named inventors on the patent application covering the seed-coating technology evaluated in the study. The remaining authors declare no other conflicts of interest.

Availability of data and material: Most data supporting the findings of this study are included in this paper and its Supplementary Information files (Online Resource 1) or can be made available upon request to the corresponding author.

Code availability: Not applicable

Authors' Contributions: RMC conducted the wheat controlled-environment experiments; designed and conducted the screenhouse trials for wheat and Persian clover and the wheat field trial; performed data analysis and interpretation; and wrote the original draft. SA designed and conducted the Persian clover field trial, performed data analysis and interpretation and contributed to manuscript preparation. CR optimised the germination and coating protocols, performed the controlled-environment experiments for wheat and Persian clover and contributed to data analysis and interpretation, as well as to manuscript preparation. RF assisted with the controlled environment, screenhouse, and field experiments for wheat and contributed to the experiments of Persian clover in controlled and screenhouse conditions experiments. RC assisted with and supervised the wheat field trial. AC and IO provided associated technical information for *Gracilaria* sp. biomass and contributed to manuscript preparation. JN provided associated technical information for *T. chuii* biomass. SC and CA coordinated data interpretation and research activities. CA contributed to manuscript writing, reviewing, and editing. All authors reviewed and approved the final manuscript.

Patent: The seed-coating technology evaluated in this study is covered by patent application WO2026/159618A1 dated 30 July 2026, entitled "Coated Seeds, Methods and Uses Thereof". The intellectual property covers coated seeds and seed coating composition comprising algal biomass for plant biostimulation and bioprotection against biotic stresses. The disclosed seed coating composition also comprises at least one binder, optionally in combination with at least one drying agent, at least one microbial inoculum, and/or at least one additive. The experimental work reported here evaluates selected formulations derived from that technology under controlled, screenhouse, and field conditions.

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Figures

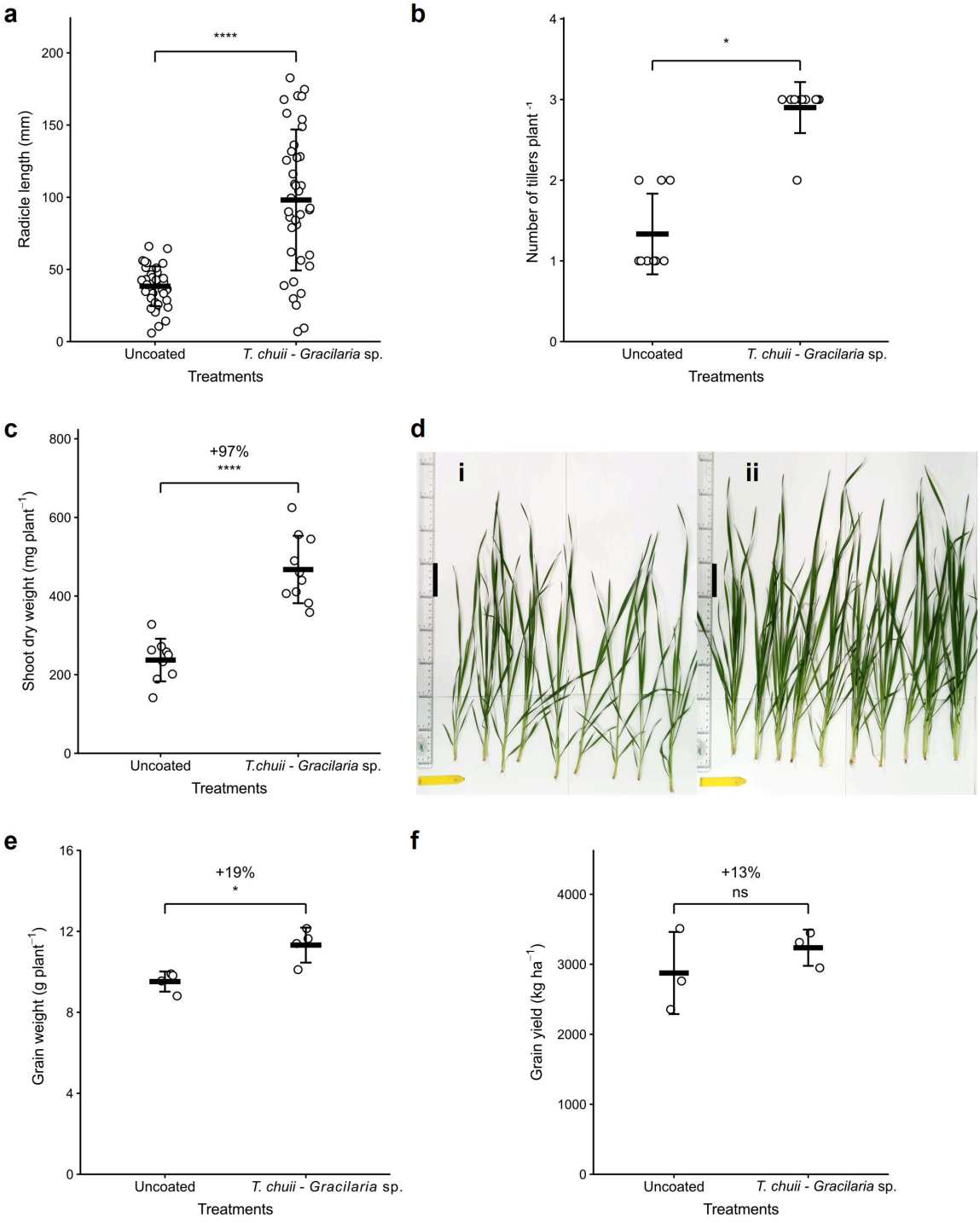


Fig. 1

Figure 1

Effect of the *Tetraselmis chuii–Gracilaria* sp. mix seed coating formulation on wheat growth and productivity across controlled, screenhouse, and field conditions. (a) Radicle length under controlled conditions (uncoated, n = 39 seedlings; coated, n = 38 seedlings). Treatment effects were assessed using the Kruskal–Wallis test ($\chi^2(1) = 28.50$, $p < 0.001$), followed by Dunn's post-hoc test with Holm adjustment ($z = 5.34$, adjusted $p < 0.001$). (b) Tiller number per plant under controlled conditions

(uncoated, n = 9 plants; coated, n = 10 plants). Count data were analysed using a Poisson generalised linear model (GLM) with a log-link function; the overall treatment effect was assessed by a likelihood-ratio chi-square test ($\chi^2(1) = 5.59$, $p = 0.0181$). (c) Shoot dry weight under controlled conditions (uncoated, n = 9 plants; coated, n = 10 plants). Treatment effects were assessed by one-way ANOVA ($F(1,17) = 47.63$, $p < 0.001$), followed by Dunnett's multiple-comparison test against the uncoated control ($t(17) = 6.90$, adjusted $p < 0.001$). (d) Representative wheat plants grown under controlled conditions from: (i) uncoated and (ii) *T. chuii–Gracilaria* sp. mix-coated seeds, illustrating differences in shoot development and tillering. Scale bar = 5 cm. (e) Grain weight per plant under screenhouse conditions (n = 4 independent pots per treatment, one plant per pot). Treatment effects were assessed by one-way ANOVA ($F(1,6) = 13.06$, $p = 0.0112$), followed by Dunnett's multiple-comparison test against the uncoated control ($t(6) = 3.61$, adjusted $p = 0.0112$). (f) Grain yield under field conditions (n = 3 independent field plots per treatment). Treatment effects were assessed by one-way ANOVA ($F(1,4) = 0.95$, $p = 0.3845$), followed by Dunnett's multiple-comparison test against the uncoated control ($t(4) = 0.98$, adjusted $p = 0.3845$). Individual points represent independent seedlings, plants, pots, or field plots, as specified for each panel. Horizontal bars and error bars indicate mean ± SD. Statistical significance is indicated as * $p < 0.05$, **** $p < 0.0001$; ns, $p \geq 0.05$.

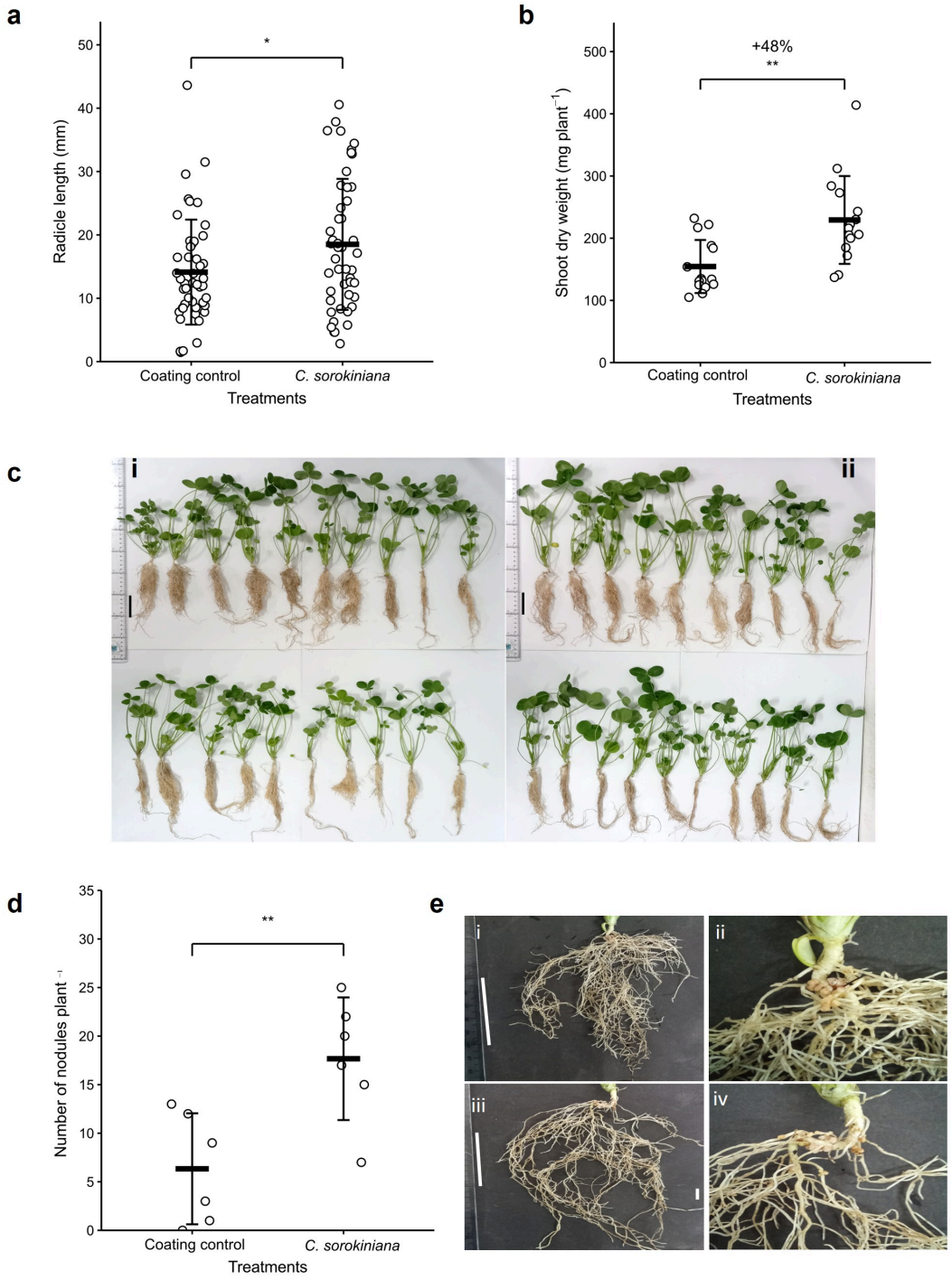


Fig. 2

Figure 2

Effect of *Chlorella sorokiniana* seed coating formulation on Persian clover growth and nodulation under controlled conditions. (a) Radicle length (n = 47 seedlings per treatment). Treatment effects were assessed using the Kruskal–Wallis test ($\chi^2(1) = 4.22$, $p = 0.0401$), followed by Dunn's post-hoc test with Holm adjustment ($z = 2.05$, adjusted $p = 0.0401$). (b) Shoot dry weight (n = 15 plants per treatment). Treatment effects were assessed using the Kruskal–Wallis test ($\chi^2(1) = 10.20$, $p = 0.0014$), followed by

Dunn's post-hoc test with Holm adjustment (z = 3.19, adjusted p = 0.0014). (c) Representative Persian clover plants grown from: (i) coating-control and (ii) *C. sorokiniana–coated* seeds, illustrating differences in shoot development between treatments. Scale bar = 5 cm. (d) Root nodulation expressed as the number of nodules per plant (n = 6 plants per treatment). Nodule counts were analysed using a negative-binomial generalized linear model (GLM) with a log-link function; the overall treatment effect was assessed using a likelihood-ratio chi-square test ($\chi^2(1) = 7.03$, p = 0.0080). The estimated nodule count was 2.79-fold higher in coated plants than in the coating control (treatment-to-control ratio = 2.79, z = 2.67, p = 0.0077). (e) Representative root systems of Persian clover plants showing: (i) nodulation in coating-control plants, (ii) close-up of nodules from coating-control plants, (iii) nodulation in *C. sorokiniana*–coated plants and (iv) close-up of the nodules from *C. sorokiniana*–coated plants. Scale bar = 5 cm. Individual points in panels A, B, and D represent individual seedlings or plants, as specified for each panel, and horizontal bars and error bars indicate the arithmetic mean ± SD. Statistical significance is indicated as * $p < 0.05$, ** $p < 0.01$.

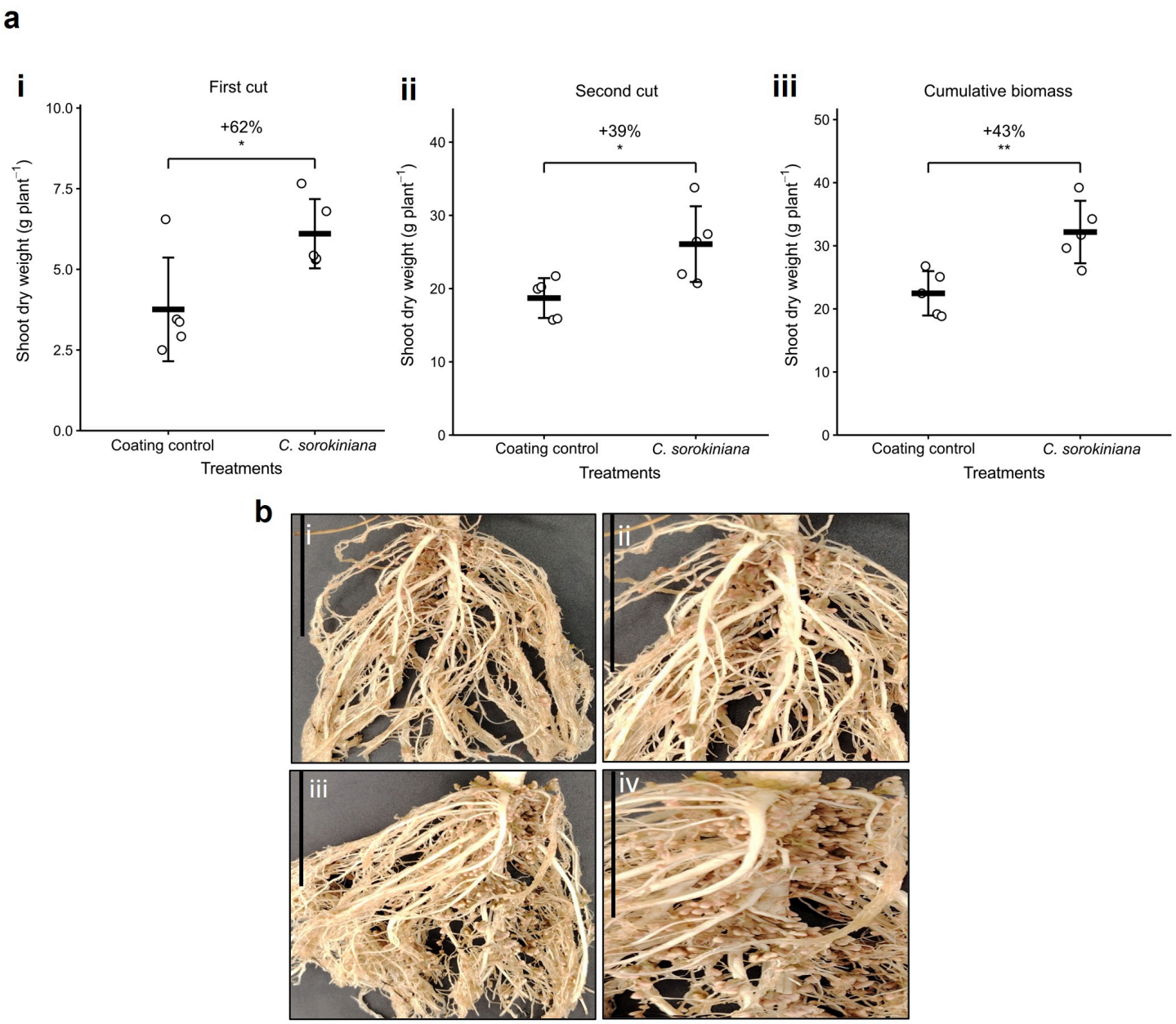


Fig. 3

Figure 3

Effect of *Chlorella sorokiniana* seed coating formulation on Persian clover biomass production and nodulation under screenhouse conditions. (a) Shoot dry biomass: (i) first cut, (ii) second cut following regrowth, and (iii) cumulative dry biomass across both cuts (n = 5 independent pots per treatment). First-cut biomass was analysed using the Kruskal–Wallis test ($\chi^2(1) = 3.94$, $p = 0.0472$), followed by Dunn's post-hoc test with Holm adjustment ($z = 1.98$, adjusted $p = 0.0472$). Second-cut biomass was analysed by one-way ANOVA ($F(1,8) = 7.95$, $p = 0.0225$), followed by Dunnett's multiple-comparison test against the coating control ($t(8) = 2.82$, adjusted $p = 0.0225$). Cumulative biomass was analysed by one-way ANOVA ($F(1,8) = 12.80$, $p = 0.0072$), followed by Dunnett's multiple-comparison test against the coating

control ($t(8) = 3.58$, adjusted $p = 0.0072$). Individual points represent individual plants grown in independent pots (one plant per pot), and horizontal bars and error bars indicate mean ± SD. (b) Representative images of Persian clover root systems: (i) nodulation in coating-control plants, (ii) close-up of nodules from coating-control plants, (iii) nodulation in *C. sorokiniana*–coated plants, and (iv) close-up of nodules from *C. sorokiniana*–coated plants. Scale bar = 5 cm. Nodulation under screenhouse conditions was assessed qualitatively because extensive and overlapping nodule clusters prevented reliable quantification. Statistical significance is indicated as * $p < 0.05$ and ** $p < 0.01$.

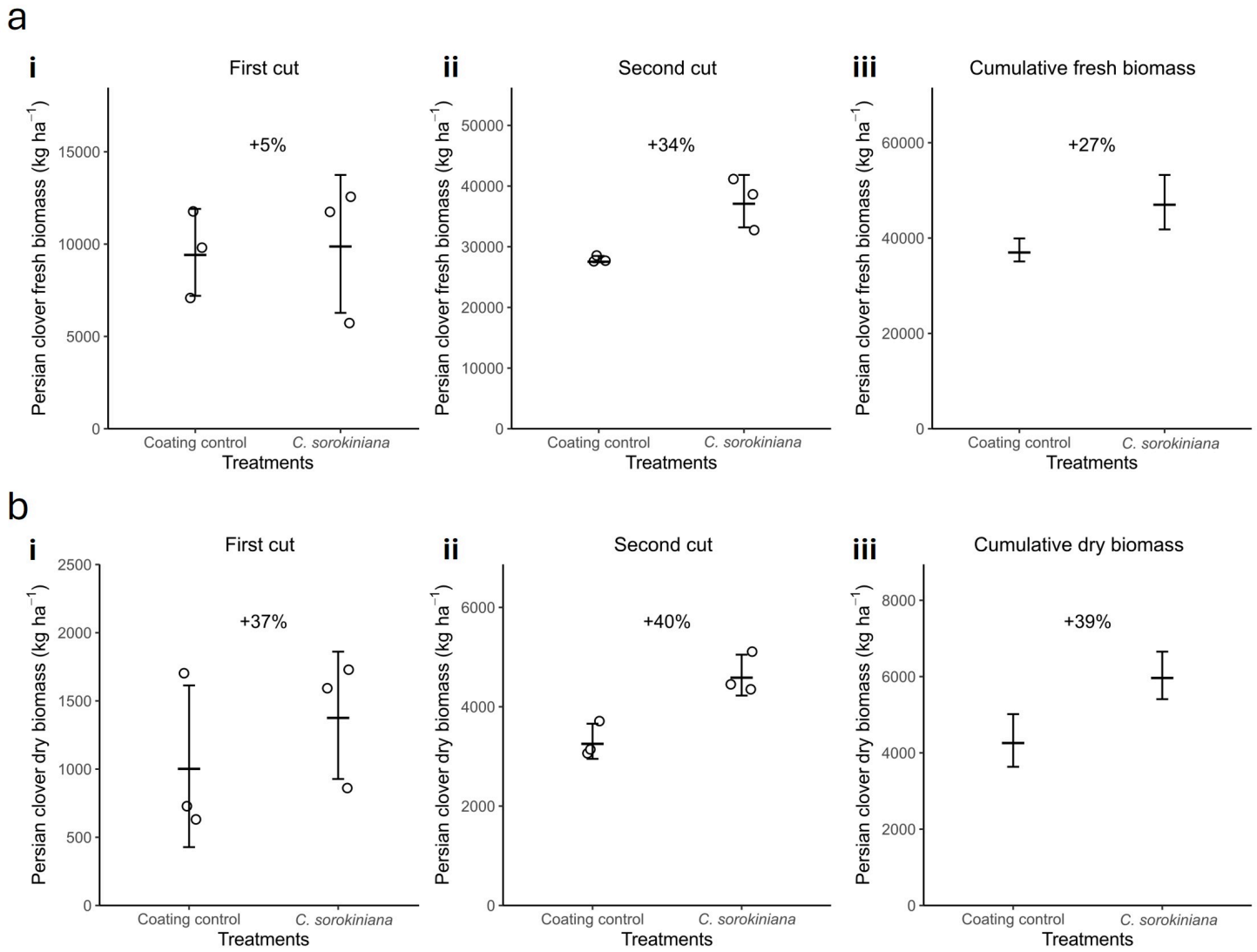


Fig. 4

Figure 4

Field-scale validation of the *Chlorella sorokiniana*–based seed coating in Persian clover. (a) Fresh biomass at: (i) the first cut, (ii) the second cut following regrowth and (iii) cumulative biomass across both cuts. (b) Dry biomass at: (i) the first cut, (ii) the second cut following regrowth and (iii) cumulative biomass across both cuts. For individual cuts, open circles represent 1 m² spatial subsamples (n = 3 per

treatment area and cut), with horizontal bars and error bars indicating the mean ± SD. Cumulative fresh and dry biomass were calculated as the sum of the treatment-area means from the two independently sampled cuts; horizontal bars indicate the cumulative estimates, and error bars represent propagated SD calculated from the SDs of the two cuts. These propagated SDs represent uncertainty associated with the cumulative estimates and do not represent variability among independent cumulative field replicates. Percentages indicate descriptive differences in the *C. sorokiniana*–coated treatment relative to the coating control. Because each treatment was established in a single field area and the 1 m² sampling areas represented spatial subsamples rather than independent field replicates, treatment comparisons were descriptive, and no inferential statistical tests were performed.

Supplementary Files

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- ESM1.pdf