



Hydrogel capsules as carriers for PGPB consortia enhance compost efficacy and nutrient uptake in Oat (*Avena sativa*) fertilization

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Abstract

Aims The incorporation of Plant Growth-Promoting Bacteria (PGPB) into compost is a promising strategy to enhance the potential of organic fertilizers. This study investigates in a pot experiment the potential of hydrogel capsules containing a PGPB consortium to improve compost efficiency, promote crop growth, and increase nutrient uptake.

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Highlights

- Increasing biomass accumulation on oat by compost and encapsulated PGPB consortium.
- Higher nutrient uptake in oat plants by application of composts and PGPB consortium.
- Enhancing photosynthetic pigment content by addition of compost fertilization and PGPB capsules prototypes.

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Methods Three PGPB strains (*Rhizobium leguminosarum* T88, *Herbaspirillum frisingense* AP21, and *Azospirillum brasilense* D7) were encapsulated in hydrogel capsules made from amidated pectin via ionic gelation. Additives like skim milk, whey protein, and Gelita® EC were included. The capsules were characterized using scanning electron microscopy (SEM), moisture content analysis, and particle size measurement. Greenhouse experiments in pots assessed the impact of PGPB-enriched compost on oat growth (*Avena sativa* var. Altoandina), nutrient uptake, and photosynthetic pigments.

Results Encapsulated PGPB prototypes P1, P2, and P3 significantly increased shoot and root biomass, compared to the organic fertilization (O.F.) + 50% mineral fertilization (M.F.) treatment with P1 improving root biomass by 74%. In contrast, non-encapsulated treatments showed no significant effects. Encapsulated P1 and P2 enhanced root biomass by 48% and 39% compared to free-living cultures. Nutrient uptake, particularly of N, Ca, and Mg, improved with PGPB encapsulation, with P3 showing the highest increases, N by 81%, Ca by 66%, Mg by 77%. Photosynthetic pigments also rose, with chlorophyll-a increasing by 21% in P3, and chlorophyll-b by 58% in P2.

Conclusions Encapsulated PGPB significantly enhances compost efficiency, boosting oat biomass, nutrient uptake, and photosynthetic pigment production, supporting sustainable agriculture.

Keywords Nutrient uptake · Encapsulation · Compost · Photosynthetic Pigments

Introduction

Current livestock systems are increasingly recognized as significant contributors to environmental pollution and soil degradation, which adversely affect both the quantity and quality of pasture production. The intensification of livestock production, particularly through concentrated animal feeding operations, has been linked to substantial environmental pollution. These systems generate large quantities of manure, which can lead to nutrient runoff and the contamination of water bodies with pollutants such as nitrogen and phosphorus, contributing to eutrophication and harmful algal blooms (Doblas-Rodrigo et al. 2023; Hoyos-Sebá et al. 2023). Furthermore, the livestock sector is a major source of greenhouse gas emissions, accounting for a significant portion of global methane and nitrous oxide emissions, which are critical contributors to climate change and environmental degradation (Herrero et al. 2013).

The integration of sustainable practices in livestock management is essential for mitigating these adverse effects. One promising approach to enhance sustainability in livestock management is the incorporation of innovative strategies that improve both the biomass quality and quantity of forage crops. This is particularly relevant in the context of integrated crop-livestock systems, which have shown potential to enhance agricultural sustainability by strategically integrating perennial vegetation. Such systems not only improve forage productivity but also contribute to carbon sequestration and reduction of greenhouse gas emissions (Conceição et al. 2017; Wiesner et al. 2020). For instance, the introduction of new cereal varieties, such as the forage oat *Avena sativa* var. Altoandina (genotype AV25 T), has shown significant potential due to its superior biomass production, enhanced nutritional quality, and increased resistance to pests like rust (Enciso et al. 2021).

The application of organic amendments, particularly compost, is increasingly recognized for its multifaceted benefits in enhancing soil properties and promoting sustainable agricultural practices. Compost serves as a vital source of organic matter, which significantly improves soil structure and its physical,

chemical, and biological properties. This enhancement is crucial for carbon sequestration, as a portion of the organic carbon applied through compost can be protected from decomposition, thereby contributing to long-term soil carbon storage (Harrison et al. 2020). The integration of compost into agricultural soils has been shown to increase the availability of essential nutrients such as nitrogen (N), phosphorus (P), and potassium (K), which are critical for optimal plant growth and development (Aguilar-Paredes et al. 2023; Cooper et al. 2020; Estrada-Bonilla et al. 2021; McClelland et al. 2022; Šimanský et al. 2021).

Recent studies underscore the role of compost not only in boosting crop yields but also in mitigating soil erosion and enhancing the soil's water-holding capacity. For instance, the addition of compost has been linked to improved soil moisture retention, which is particularly beneficial in sandy soils where water retention is typically low (El-Ngar and Abu El-Ezz 2021). Furthermore, the incorporation of compost has been shown to reduce bulk density and enhance soil aeration, thereby fostering a more conducive environment for root growth and microbial activity (Šimanský et al. 2021).

The integration of plant growth-promoting bacteria (PGPB) into compost represents an innovative approach to further enhance nutrient availability and crop resilience. PGPB can facilitate nutrient acquisition through mechanisms such as biological N fixation and phosphate solubilization, as well as the production of phytohormones that promote plant growth (Maharjan et al. 2021). These beneficial microorganisms not only improve nutrient uptake but also enhance plant tolerance to biotic and abiotic stresses, which is increasingly important in the face of climate variability (Gao et al. 2023). The synergistic effects of compost and PGPB can lead to significant improvements in crop yields and soil health, making this combination a sustainable strategy for modern agriculture (Attanayake et al. 2017).

Utilizing consortia of plant growth-promoting bacteria (PGPB) enhances inoculation efficiency across various crops and environmental conditions. A consortium comprising *Rhizobium leguminosarum* T88, *Herbaspirillum frisingense* AP21, and *Azospirillum brasilense* D7 has been developed, demonstrating efficacy under drought conditions and low P and N levels in field trials with *Kikuyu* grass (*Cenchrus clandestinus* [Hochst. ex Chiov])

Morrone), cabbage (*Brassica oleracea* var. capitata L.), and oat (*Avena sativa* L.) (Bejarano-Herrera et al. 2024; Torres-Cuesta et al. 2023; Villagran et al. 2024). However, the effectiveness of PGPB is contingent upon complex soil–plant interactions and varying environmental conditions, which can complicate their application in diverse agricultural systems (Heydarian et al. 2016). Recent advancements in microbial biotechnology have led to the formulation of commercial products that utilize mixed bacterial consortia, combining various PGPB strains to optimize their synergistic effects on plant growth (Olanrewaju and Babalola 2019).

Currently, efforts are focused on developing a solid formulation to enhance its effectiveness. Encapsulation techniques have been developed, which serve to protect and sustain the viability and biological activity of PGPB. Among these techniques, the use of hydrogel capsules has emerged as a particularly effective method for ensuring the controlled release of beneficial microorganisms into the soil environment (Cesari et al. 2020; Cruz Barrera et al. 2020; Mendoza-Labrador et al. 2021b; Pour et al. 2021). Hydrogel encapsulation not only enhances the survival rates of PGPB but also facilitates their gradual colonization of plant roots, thereby maximizing their beneficial effects on plant growth. Moreover, the incorporation of biodegradable supports such as alginate, pectin, starch, or cellulose into the encapsulation process has been shown to further improve the efficacy of compost enrichment systems. These biodegradable materials not only provide a protective matrix for the PGPB but also contribute to soil health by enhancing soil structure and nutrient availability (Cruz Barrera et al. 2020; Mendoza-Labrador et al. 2021a). The synergistic effects of encapsulation and the use of biodegradable supports ultimately lead to improved survival and colonization rates of PGPB in the soil, fostering a more sustainable agricultural practice.

We hypothesize that encapsulating a previously selected PGPB consortium enhances its efficacy when applied with organic fertilizer (Compost), thereby improving oat plant development and nutrient absorption while reducing the need for soluble fertilizers. Therefore, this study aimed to evaluate the effect of inoculating encapsulated prototypes of a PGPB consortium (*H. frisingense* AP21, *R.*

leguminosarum T88, and *A. brasilense* D7) combined with compost on oat plant development, nutrient uptake, and photosynthetic pigment levels.

Material and methods

Bacterial strains and inoculants

The PGPB consortium, consisting of *R. leguminosarum* strain T88 (SAMN15498640), *H. frisingense* strain AP21 (SAMN15498633), and *A. brasilense* strain D7 (SAMN16830199), was provided by the Microorganisms Germplasm Collection of the Colombian Corporation for Agricultural Research (AGROSAVIA). These strains were previously identified by whole genome sequencing and were characterized as facultative endophytes (Cortes-Patino et al. 2021). In addition, the compatibility test of these three strains was performed using the methodology reported by (Loaces et al. 2011), and no inhibitory effect was observed in the solid medium. For all the experiments, the strains *H. frisingense* AP21 and *A. brasilense* D7 were cultivated in a solid ABRA culture medium (g/L: Monosodium glutamate 28.33, Yeast extract 2.92, Dipotassium phosphate dihydrate 1.34, Magnesium sulfate heptahydrate 0.5, Ferric chloride 0.02.) were grown in the ABRA culture medium (Moreno-Galván et al. 2012) and incubated at 30 °C for 48 h, and the strain *R. leguminosarum* T88 were cultivated in the solid RHIZO culture medium: (g L⁻¹): glutamate (13.67), yeast extract (0.73), H₂PO₄ (0.48), MgSO₄ 7H₂O (0.2), NaCl (0.1) and CaCl₂ (0.1) agar (20), incubated at 30 °C for 72 h. For the greenhouse experiment, each bacterial suspension was standardized to optical density (OD)_{600 nm} = 0.5. [$\sim 10^8$ colony forming units (CFU) mL⁻¹].

Formulation hydrogel capsules

The prototype based in the consortium bacteria was carried out using the ionic gelation method with the biopolymer Amidated pectin (GRINDSTED®, Denmark LA 410, esterification degree 31%, amidation degree 19%) using the method described by Cruz et al. (2020) (Cruz Barrera et al. 2020). Briefly, 100 g of amidated pectin was used at a concentration of 4.0%, w/w which was dissolved in sterile water maintaining

constant agitation at 600 rpm and 75 ± 5 °C using a stir plate (DLAB Ms-H340-S4). The bacterial suspensions (30 mL) of the consortium AP21, D7 and T88 were mixed with the biopolymer Amidated pectin and homogenized in an orbital shaker at 120 rpm (Unimax 1010, Heidolph®, Schwabach, Germany) at a room temperature for 30 min. The additives tested as drying protectors in the prototypes were skim milk [Slight, Colanta] (P1), whey protein (P2) [Cat: 91,082–88 -1, McKenna Group S.A.S], and Gelita® EC [GELITA, Enteric capsule release] (P3). The substances were included at 1% w/w within the polymeric matrix a stirring plate at 300 rpm for 10 min. Subsequently, the prepared suspension was dripped using a syringe of 20 mL and dripped through a cannula (diameter 2.1×0.8 mm, Sterican, B. Braun Melsungen AG, Melsungen, Germany) into the stirred crosslinking agent solution of Calcium gluconate (Cimpa S.A.S. Bogotá, Colombia) at concentration of 0.1 M. Then, the hydrogel capsules formulated with amidated pectin were left in contact with the calcium gluconate solution for 10 min. Finally, the capsules were dried in a drying oven at a temperature of 25 ± 2 °C for 72 h.

Characterization of the hydrogel capsules

Dried hydrogel capsules (0.2 g) were dissolved in 10 mL of a sterile solution containing citric acid (5.76 g/L) and sodium carbonate (5.30 g/L), with a pH of 7 ± 2 . The mixture was placed in a 50 mL Falcon tube and agitated at $300 \times g$ for 20 min using a Multi Reax 1010 shaker (Heidolph®, Schwabach, Germany). After complete dissolution, serial dilutions were plated onto a solid culture medium optimized for each PGPB and incubated at 30 °C for 48 h. Moisture content was determined using a KERN brand moisture determining halogen balance (MLS 50–3), particle size (Digimizer Image, MedCalc Software, Ostend, Belgium) and capsule shape (Santos-Díaz et al. 2022).

Scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) was used to visualize immobilized strains within the polymeric matrix. The prototypes formulated were fixed by immersion in 2% (v v⁻¹) glutaraldehyde for 10 min and then washed with ethanol gradients (50, 80 and 99% -v v⁻¹) for 15 min. Subsequently, were

dried in an extraction cabinet to remove the solvent and placed in a desiccator with silica gel for 48 h as an additional dehydration step. The dry samples were placed on a sample holder and covered with 30 nm of gold–palladium at a ratio of 60:40 using a metallizer (Q150R ES, Quorumtech, United Kingdom) at sputter current 60 mA, 40 s, tooling factor 2.30. Observations were made using a Quanta 200 microscope with Everhart Thornley-SE and Solid state-BSE detectors (SEMTECH Solutions Inc, Massachusetts, USA) (Chaparro-Rodríguez et al. 2023).

Effects of inoculation of enriched compost with PGPB consortium in forage oat plants var. Altoandina (genotype AV25 T)

The experiment was conducted under semi-controlled greenhouse conditions at AGROSAVIA's Tibaitatá Research Center in Mosquera, Cundinamarca, Colombia ($4^{\circ}41'43.6''$ N, $74^{\circ}12'19.9''$ W; 2600 m above sea level). Throughout the study, environmental parameters were monitored using a HOBO U23 -001 data logger (Onset Computer Corporation, Bourne, USA), which recorded temperature ranges from 12.5 °C to 24.6 °C and relative humidity levels between 46 and 83%. A completely randomized design was employed, comprising eight treatments, each with three replicates (Table 1). We used a substrate composed by a mixture of vermiculite:sand in a ratio of 2:1 non-sterile. Throughout the experiment, the substrate was maintained at 80% of its field capacity. To verify the repeatability of the experiment, two consecutive assays were conducted.

Seeds of forage oat *var.* Altoandina (genotype AV25 T) were surface-sterilized in 3% (v v⁻¹) hypochlorite and 70% (v v⁻¹) ethanol. Subsequently, the seeds were transplanted into pots, each pot containing 2 kg of a substrate non-sterile. In the compost treatments (O.F.) from (treatments of 3 to 8), 92 g of compost were added and homogenized in each pot. The compost used has the following characteristics in dry base: organic matter (48.96 g kg⁻¹); organic carbon (22.55 g kg⁻¹); N (2.30 mg kg⁻¹); P (1.80 mg kg⁻¹); K (3.70 mg kg⁻¹); calcium (Ca) (1,603 mg kg⁻¹); magnesium (Mg) (167.7 mg kg⁻¹); sodium (Na) (58.2 mg kg⁻¹). The inoculation was performed during sowing. The treatment number 5 was inoculated with 5 mL of cell broth bacterial strains (D7, AP21 and T88)

Table 1 Treatments evaluated in plants of oat plants *var.* Altoandina (genotype AV25 T)

Treatments	Compost (g pot ⁻¹)	Hoagland 50% (mL pot ⁻¹)	Hoagland 100%* (mL pot ⁻¹)	Inoculum PGPB Type
1. N.F.*	-	-	-	Without
2. 100% M.F	-	-	15	Without
3. O.F. + 100% M.F	92	-	15	Without
4. O.F. + 50% M.F	92	15	-	Without
5. Cell-Broth + O.F. + 50% M.F	92	15	-	Cell-Broth
6. P1 + O.F. + 50% M.F	92	15	-	Encapsulated
7. P2 + O.F. + 50% M.F	92	15	-	Encapsulated
8. P3 + O.F. + 50% M.F	92	15	-	Encapsulated

*N.F: Non fertilized. O.F: Organic fertilizer (compost) M.F: Mineral fertilizer. P1, P2, P3: Encapsulated prototypes. Solution of Hoagland 100%: K: 1.25×10^{-4} mol/L.; NO₃: 1.50×10^{-2} mol/L.; CaCl₂ • 2H₂O: 1.25×10^{-3} mol/L.; NH₄Cl: 2.50×10^{-4} mol/L.; MgSO₄ • 7H₂O: 2.50×10^{-4} mol/L.; Fe EDTA: 2.02×10^{-8} mol/L.; H₃BO₃: 1.12×10^{-5} mol/L.; MnCl₂ • 4H₂O: 2.50×10^{-6} mol/L.; ZnSO₄ • 7H₂O: 1.90×10^{-7} mol/L.; CuSO₄ • 5H₂O: 5.99×10^{-8} mol/L.; Na₂MoO₄ • 2H₂O: 1.30×10^{-7} mol/L and KH₂PO₄: 2.50×10^{-3} mol/L

at a concentration of 1×10^8 CFU mL⁻¹ each one. The treatments 6–8 were inoculated with 1.2 g of pectin capsules amidated with the immobilized bacterial consortium (D7, AP21 and T88) at a concentration of $> 6.0 \times 10^8$ CFU g⁻¹. Subsequently, after seed emergence and inoculation of all treatments, three thinning were carried out with a frequency of 8 days, leaving one plant per pot with experimental unit per treatment. Then, the plants were irrigated to 60% of the field capacity (188 mL) with Hoagland solution varying its percentage (100% or 50%) depending on the treatment, this irrigation was performed every 4 days. In total, the experiment and its replication lasted 45 days each. Then, destructive sampling was carried out and dry weight values were taken and the biomass production of the aerial part and roots of the plants per treatment was calculated.

To ensure sufficient shoot dry biomass for nutrient concentration analysis, we combined the biomass from both assays. The N, P, K, Ca and Mg was performed using the (NIRS) technique, according to the equation generated for forage oat via the FOSS analytics (model DS2500, Denmark) (Balehegn et al. 2022). The quantification of chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), and total carotenoid content ($x + c$) (mg cm⁻²), was performed on three-leaf disks per plant in dimethyl sulfoxide (DMSO). The leaf disks were placed in 1 mL of DMSO and heated in a water bath at 95 °C for 1 h. The extract

was then read at 480, 649, and 665 nm using a microplate reader. The pigment content was determined using DMSO equations established by Wellburn (1994). The photosynthetic pigment data were analyzed using measurements from both assays.

Statistical analysis

The data was analyzed using the SPSS Statistics v.27 software (SPSS, Chicago, IL) and is presented as the mean values and standard deviation. The statistical differences between treatments were analyzed using one-way ANOVA and Duncan post-hoc tests ($p < 0.05$); data was checked for normality and homogeneity of variance using Shapiro-Wilks and Bartlett tests.

Results

Capsules characterization

The microbiological composition obtained showed that the concentration of each strain in the three prototypes (P1, P2, and P3) remained at approximately 50% of the cells for D7, 40% for the T88, and 2.5% for AP21 strain. The concentrations of D7 and T88 were 10^8 CFU g⁻¹, while AP21 was in 10^7 CFU g⁻¹. The total cell counts for each prototype were $> 6.0 \times 10^8$ CFU g⁻¹. The results at the physicochemical

Table 2 Physicochemical and microbiological characterization of the three formulation prototypes

Strain/characteristic	P1	P2	P3
Active ingredient (CFU/g)	$7,41 \times 10^8$	$8,62 \times 10^8$	$6,24 \times 10^8$
<i>Azospirillum brasilense</i> D7 (%)	50,5	55,0	56,2
<i>Rhizobium leguminosarum</i> T88 (%)	46,7	42,5	40,4
<i>Herbaspirillum frisingense</i> AP21 (%)	2,8	2,6	3,4
Moisture content (%)	$3,11 \pm 0,09$	$3,23 \pm 0,15$	$2,26 \pm 0,20$
Water activity (a_w)	$0,62 \pm 0,01$	$0,61 \pm 0,005$	$0,62 \pm 0,01$
Particle size (mm)	$2,55 \pm 0,28$	$2,30 \pm 0,26$	$2,43 \pm 0,24$

level for the prototypes showed that the humidity of the three was less than 3.2%, the water activity (a_w) for the three prototypes was less than 0.62 and the particle size was in a range between 2.30 mm and 2.50 mm and spherical factor was less than 0.05 (Table 2).

Shoot and root dry biomass accumulation.

To assess the repeatability of the experiment, we conducted it twice consecutively. While the greenhouse environment allowed for control over temperature and humidity, it did not regulate light

exposure. Consequently, variations in biomass accumulation between the two experiments are attributed to fluctuations in solar radiation during each period. Despite these differences, the treatment trends remained consistent across both trials. Figure 1 illustrates the evaluation of shoot and root dry biomass accumulation in oat plants in both trials. Notably, all treatments exhibited statistical differences when compared to both N.F. and 100% M.F. without compost and inoculant addition ($p < 0.05$). The application of organic fertilizer (compost) had significant increment compared to the treatments that received mineral fertilization alone (100%

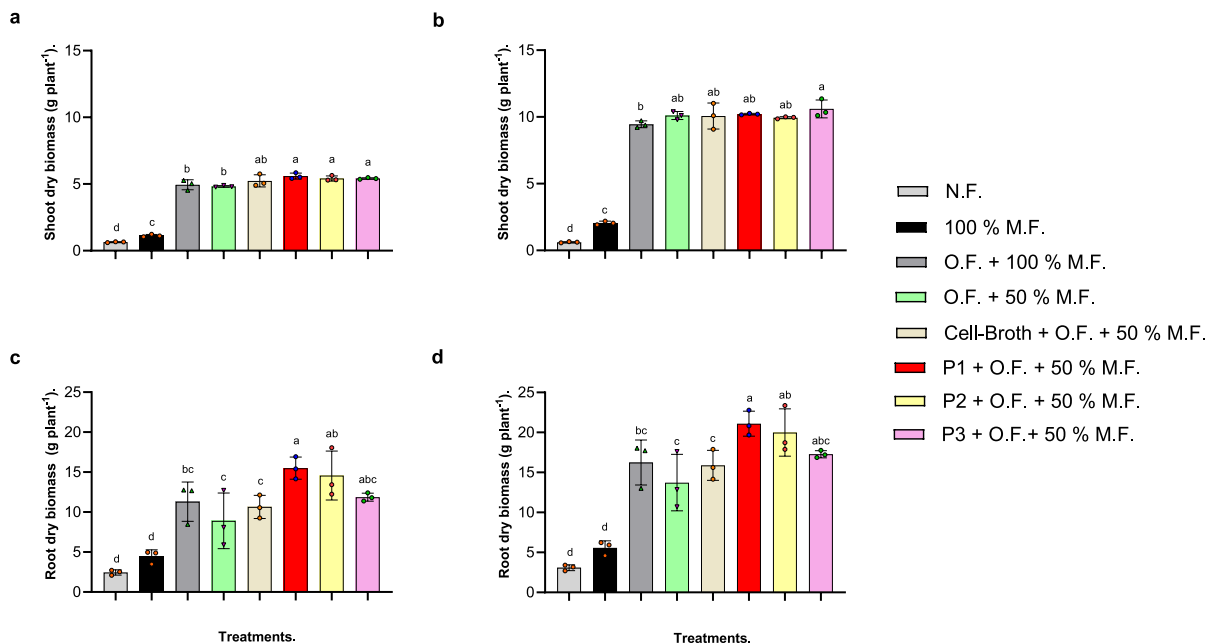


Fig. 1 Accumulation of dry biomass in oat plants. Shoot dry biomass accumulation: (a) Experiment 1 and (b) Experiment 2. Root dry biomass accumulation: (c) Experiment 1 and (d)

Experiment 2. N.F.: Non-fertilized. O.F.: Organic fertilizer (compost) M.F.: Mineral fertilizer. P1, P2, P3: Encapsulated prototypes

M.F.) ($p < 0.05$). The effect of organic fertilization was further enhanced by inoculating the formulated PGPB consortium, particularly in root dry biomass accumulation. Compared to the O.F. + 50% M.F. treatment, the encapsulated prototypes P1, P2, and P3 increased shoot dry biomass by 16.1%, 12.4%, and 12.3%, respectively, and root dry biomass by 74%, 63.6%, and 33.2%, respectively (Fig. 1a, Fig. 1b).

Inoculation with the consortium cell broth (without encapsulation) showed no significant difference compared to the treatment without inoculation (O.F. + 50% M.F.). However, the encapsulated prototypes P1 and P2 significantly increased root dry biomass by 47.9% and 39%, respectively, compared to treatments with free-living culture broth cells (Fig. 1c, Fig. 1d). Additionally, capsules formulated with inoculum exhibited a statistically significant increase in dry shoot and root biomass compared to capsules used alone (Table 1S, 2S, 3S, 4S). Hydrogel capsules after the experiment were not observed, indicating a total degradation. These results highlight the critical role of bacteria in promoting plant growth when incorporated into a solid carrier.

Nutrients uptake

Figure 2 shows the nutrient uptake across the assessed encapsulated prototypes, broth cells, and treatments without inoculation. The positive trend observed in dry biomass accumulation was similarly observed in treatments receiving organic fertilization in terms of nutrient uptake by oat plants. Compared to mineral fertilization (100% M.F.), treatments with compost fertilization, reducing 50% MF (O.F. + 50% M.F.), increased the uptake of N, P, Ca, and Mg by 312%, 70%, 247% and 223% respectively.

Inoculated treatments with bacteria consortium, regardless of the formulation, showed a significant increase in the uptake of N (43%), Ca (50.4%), and Mg (19.4%) compared to the same fertilized treatment without inoculation (O.F. + 50% M.F.) (Fig. 2a, Fig. 2d, Fig. 2e). Prototype P1 increased N, Ca, and Mg uptake by 28.8%, 42.2%, and 45.9%, respectively, compared to complete mineral fertilization (O.F. + 50% M.F.). Prototype P2 improved N, Ca, and Mg uptake by 56.2%, 41.4%, and 29.1%, respectively, while P3 showed the highest increases,

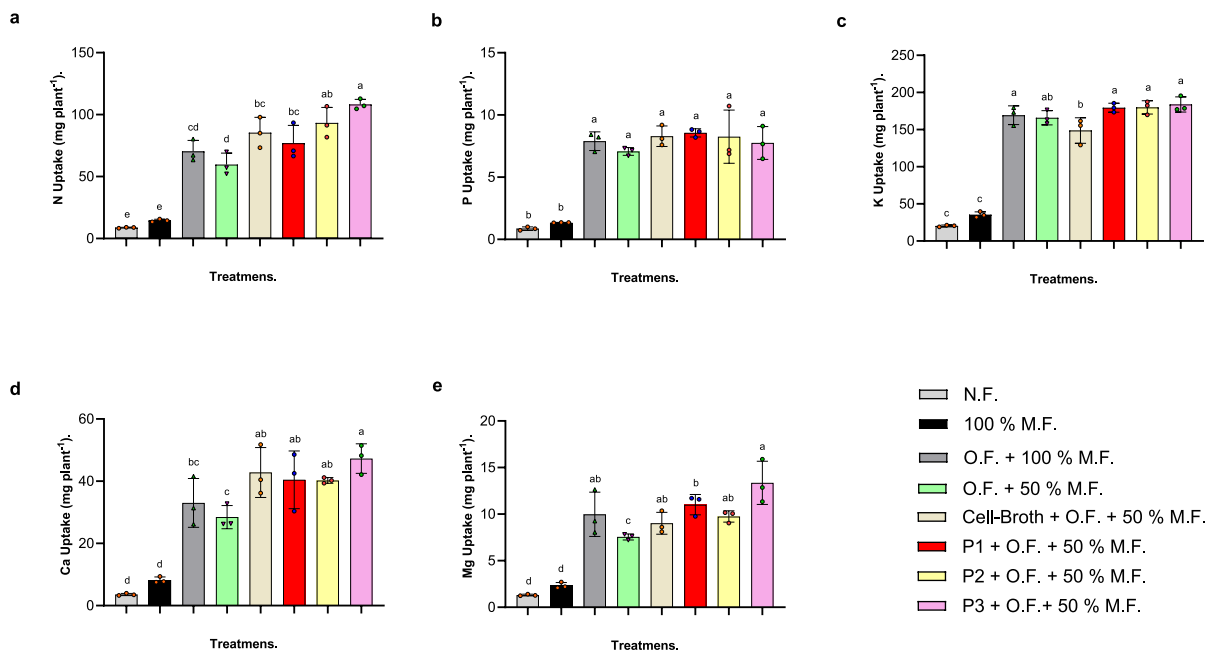


Fig. 2 Nutrients uptake in oat plants; (a) nitrogen (N), (b) Phosphorus (P), (c) Potassium (K), (d) Calcium (Ca) and (e) Magnesium (Mg). N.F.: Non-fertilized. O.F.: Organic fertilizer (compost) M.F.: Mineral fertilizer. P1, P2, P3: Encapsulated prototypes

with N, Ca, and Mg uptake rising by 81%, 66%, and 77%, respectively, compared to complete mineral fertilization (O.F. + 50% M.F.). In general, no significant differences were observed in P and K content across treatments and prototypes (Fig. 2b, Fig. 2c).

Photosynthetic pigments

Photosynthetic pigments are vital components of the photosynthetic apparatus in plants. In our study, we observed a significant increase in pigment concentrations in forage oat when compost was enriched with formulated PGPB, compared to the control (O.F. + 50% M.F.). Specifically, Chlorophyll-a content increased by 9.2% with prototype P2 and 6.2% with prototype P3. For Chlorophyll-b, increases of 4.4% were observed with P1, 4.6% with P2, and 23% with P3. Additionally, carotenoid content rose by 12.1% in the P2 treatment. These results are based on a comparative analysis with the O.F. + 50% mineral fertilization treatment (Table 3).

Scanning electron microscopy (SEM)

In the SEM micrographs, at a magnification of 50x, we observed that the three capsule prototypes tend to have a spherical shape, although some capsules of prototype P2 exhibit an ovoid shape (Fig. 3a, Fig. 3c, and Fig. 3e). Regarding the surface at a magnification of 5000x, some cracks and

depressions are visible in all three systems (Fig. 3b, Fig. 3d, Fig. 3f). At 5000 × magnification, it can be observed that the surfaces are smooth, and the presence of immobilized bacteria in the matrix of each prototype can be observed. In all prototypes, a high density of immobilized bacterial cells is evident.

Discussion

The application of compost as a fertilizer has garnered significant attention due to its multifaceted benefits for soil health and crop productivity. In the present study fertilization with compost significantly enhanced biomass accumulation, with shoot dry weight reaching 7.2 g plant⁻¹ and root dry weight at 13.8 g plant⁻¹. In contrast, treatments using only mineral fertilizers resulted in lower biomass accumulation, averaging 1.6 g plant⁻¹ for shoots and 5.01 g plant⁻¹ for root dry weight. Interestingly, the nutrient levels where applied compost closely correlated with dry biomass accumulation. Compost, like fertilizer, has the potential to provide vital macronutrients such as N, P, and K, which are critical for plant development (Essel et al. 2023). Furthermore, similar results have demonstrated that the integration of compost with mineral fertilizers has been shown to significantly enhance crop yields and soil fertility compared to the application of either amendment alone (Essel et al. 2023; Sultana et al. 2020). Additionally, the compost applied could be linked to improved physical properties of the soil or substrate, such as increased

Table 3 Photosynthetic pigment content of forage oat plants var. Altoandina (genotype AV25 T)

Treatments	Photosynthetic Pigments (mg cm ²)		
	Chlorophyll—a	Chlorophyll—b	Carotenoids
1. N.F	4,782 b* (± 1,01)	1,478 b* (± 0.14)	1,492 cd (± 0.27)
2. 100% M.F	6,568 a (± 0.53)	1,938 ab (± 0.16)	1,360 d (± 0.34)
3. O.F. + 100% M.F	7,755 a (± 1,11)	2,339 a (± 0.40)	2,138 a (± 0.21)
4. O.F. + 50% M.F	6,502 a (± 0.65)	2,010 a (± 0.22)	1,726 bcd (± 0.18)
5. Cell-Broth + O.F. + 50% M.F	6,549 a (± 0.29)	2,249 a (± 0.45)	2,00 ab (± 0.11)
6. P1 + O.F. + 50% M.F	7,089 a (± 0,66)	2,098 a (± 0.15)	1,820 abc (± 0.04)
7. P2 + O.F. + 50% M.F	7,098 a (± 0.65)	2,102 a (± 0,15)	1,929 ab (± 0.19)
8. P3 + O.F. + 50% M.F	6,902 a (± 0.42)	2,066 a (± 0.18)	1,77 abc (± 0.09)

* According to the post hoc Duncan test, values denoted by a different small letter differ significantly ($P < 0.05$) in one-way ANOVA (median ± standard desviation). N.F.: Non-fertilized. O.F.: Organic fertilizer (compost) M.F.: Mineral fertilizer. P1, P2, P3: Encapsulated prototypes

water retention and reduced bulk density, which are vital for supporting healthy root systems and enhancing plant resilience to drought conditions (Strachel et al. 2017; Sultana et al. 2020).

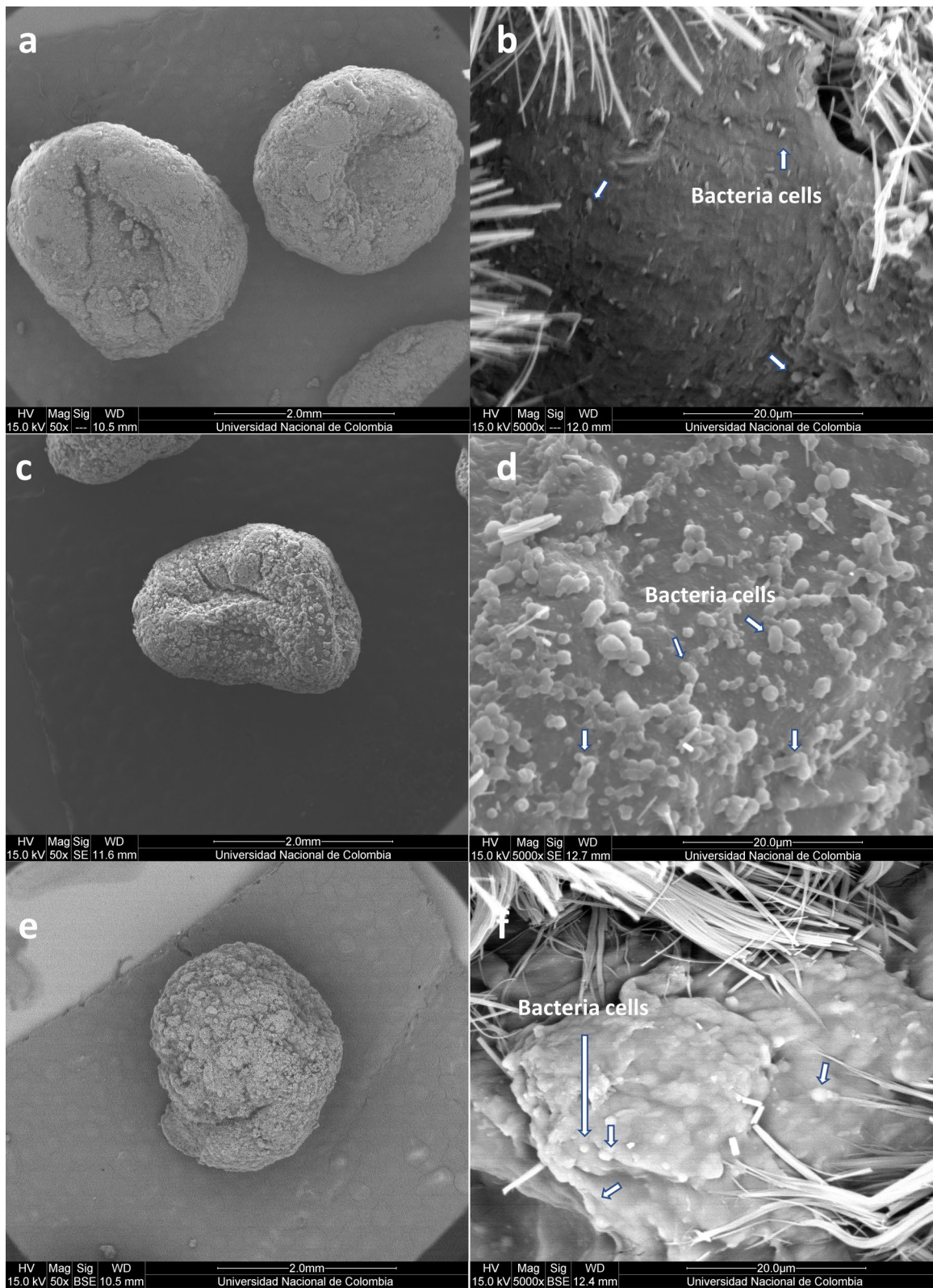
Reducing the reliance on synthetic fertilizers while maintaining or enhancing crop yields can significantly contribute to environmental sustainability by minimizing chemical runoff (Sultana et al. 2020). The amendment with organic compost, particularly when combined with encapsulated PGPB consortium, showed promise in enhancing the nutritional quality of forage crops, such as oats. This enhancement is attributed to the synergistic effects of microbial consortium on plant growth and soil health, which can be more effective than traditional soluble chemical fertilizers (Imran et al. 2022). The immobilization of PGPB in hydrogel capsules has been reported to improve their viability and activity, leading to better compost performance and nutrient availability in the soil (Cruz Barrera et al. 2020; Imran et al. 2022). This novel encapsulation approach enhances the stability and effectiveness of these microbial agents, ensuring that beneficial microbes remain active and effective in promoting plant health, even under abiotic stresses.

This study demonstrated that prototypes (P1, P2, and P3) containing encapsulated PGPB consortia improved compost performance and increased root dry and shoot biomass. The type of fertilization—organic, inorganic, biological, or mixed—also influenced forage production. Similar results were seen in *Avena sativa* L., where plants treated with a combination of organic and inorganic fertilizers showed higher dry matter production and root exudate response (Morales et al. 2023). This enhanced effect, likely due to the efficient combined use of fertilizers, resulted in a 15% increase in oat crop yield and improved microbial C-use efficiency. The study further highlighted that hydrogel-encapsulated PGPB was more effective than free cells. Prototypes increased dry biomass by 20.6%, 8.1%, and 24.7% for P1, P2, and P3, respectively, mirroring results from Mendoza-Labrador et al. (2021), where encapsulated *Bacillus* sp. strains increased Guinea grass biomass by 22.4%. Encapsulation offers advantages such as protecting cells from stress, maintaining viability, and allowing slow release of bacteria into the soil (Berninger et al. 2018; Mendoza-Labrador et al. 2021a). The observed increase in root biomass in the encapsulated prototypes can be largely attributed to the presence of *A. brasilense* D7, which is known for its

ability to synthesize plant growth regulators like auxins (Pardo-Diaz et al. 2021). These substances are crucial for promoting lateral root development and enhancing root hair formation, thereby improving nutrient absorption (Amogou et al. 2019; Tufail et al. 2022). The role of diazotrophic bacteria, particularly *Azospirillum* sp. and *Herbaspirillum* sp., as key players in nitrogen fixation and nutrient availability in crops has been well documented (Cortes-Patino et al. 2021; Estrada et al. 2013). Besides, *Rhizobium* sp. enhances plant growth via nitrogen fixation and co-inoculation with *Azospirillum*, improving nodulation and yield in soybeans and maize (Furtak et al. 2020).

The comparison of encapsulated prototypes with the control treatment of 100% Hoagland fertilization reveals a promising potential for reducing fertilization levels to 50% when utilizing a compost and PGPB consortium. This reduction is significant as it suggests a more sustainable approach to fertilization without compromising plant health and productivity. The encapsulated prototypes demonstrated enhanced nutrient uptake and increased plant biomass relative to the compost-only treatment. This improvement can be attributed to the hydrogels' capacity to absorb water and subsequently release the encapsulated bacteria (D7, T88, and AP21), thereby facilitating a gradual inoculation of beneficial microorganisms into the soil, which aligns with findings from previous studies highlighting the efficacy of biofertilizers in promoting plant growth through enhanced microbial activity (Pardo-Diaz et al. 2021; Woźniak et al. 2019).

Furthermore, the nutritional analysis of plants treated with encapsulated prototypes containing PGPB demonstrated significant increases in N and K levels compared to the control treatment. This enhancement in nutritional content is particularly beneficial for forage plants (Victoria Arellano et al. 2021), as it contributes to improved livestock nutrition and overall agricultural efficiency (Zhu and She 2018). Similar results have been observed in studies comparing synthetic N sources with bacterial suspensions, reinforcing the notion that PGPB can effectively supplement traditional fertilization methods (Amogou et al. 2019; Noirot-Gros et al. 2018). Additionally, alginate microbeads containing *Bacillus* spp. have been shown to increase N and P availability in the rhizosphere, further supporting the role of microbial inoculants in enhancing soil nutrient profiles (Zhang et al. 2023; Zheng et al. 2022). Despite the



◀**Fig. 3** SEM Micrographs of capsules with immobilized bacteria consortium at 50x, 5000x. (a, b) skim milk (P1), (c, d) whey protein (P2), and (e, f) Gelita® EC (P3)

observed increases in P levels with encapsulated formulations, no significant differences were noted compared to bacterial suspensions and mineral controls. This may be attributed to the developmental stage of the plants at the time of measurement, as younger plants often exhibit variable P uptake patterns, a phenomenon documented in other trials involving forage species (da Silveira et al. 2019). This suggests that further research is needed to optimize the timing and application methods of PGPB to maximize nutrient uptake and plant growth.

The success of solid prototypes could be related for the microbiological, chemical and physical characteristics. The microbiological composition analysis reveals significant insights into the dynamics of bacterial strains within the three prototypes (P1, P2, and P3). The dominant strains, D7 and T88, maintained concentrations around 50% and 40%, respectively, while the AP21 strain was considerably less prevalent at 2.5%. The observed concentrations of D7 and T88 at 10^8 CFU g⁻¹, compared to AP21 at 10^7 CFU g⁻¹, suggest a robust interaction between the dominant strains and the environment, potentially influenced by their metabolic activities and ecological niches. The total cell counts exceeding 6.00×10^8 CFU g⁻¹ across all prototypes indicate a thriving microbial community, which is crucial for the stability and functionality of the prototypes, as microbial diversity often correlates with enhanced resilience and nutrient cycling in ecosystems (Fang et al. 2022; Torres-Cuesta et al. 2023). At the physicochemical level, the prototypes exhibited low humidity levels (< 3.2%) and water activity (a_w < 0.62), which are critical factors for microbial stability and preservation of food products. Low water activity is known to inhibit microbial growth, thereby extending shelf life and maintaining quality (Mohibbullah et al. 2018). The particle size range of 2.30 mm to 2.50 mm, along with a spherical factor of less than 0.05, suggests that the prototypes possess favorable physical characteristics for both microbial activity and consumer acceptance, as particle size can influence packing.

The response of oat plants to the inoculation with consortium and compost has been characterized by a notable increase in the concentration of photosynthetic

pigments. This enhancement is particularly relevant in the context of current agricultural practices that aim to manipulate photosynthetic processes to improve plant productivity. The application of biostimulants, including bacterial inoculants, has garnered significant attention for their potential to enhance photosynthetic efficiency and overall plant growth (Rossi et al. 2021; Smith et al. 2023). The augmentation of photosynthetic pigments in PGPR-inoculated plants suggests that these inoculants can mitigate the adverse effects of nutritional stress by improving the activities of electron transporters and the biosynthesis of proteins and enzymes related to pigment stabilization (Alto-baishi et al. 2023). The presence of these pigments is critical, as they not only reflect the efficiency of the photosynthetic system but are also directly linked to biomass production (Abd El-Mageed et al. 2022). In addition to bacterial inoculation, the application of compost, particularly when combined with fungal inoculation, has been shown to significantly improve photosynthetic activity (Zhu et al. 2020).

Our research corroborates these findings, demonstrating that the inoculation of PGPB in prototypes P1 and P2 positively influences the production of photosynthetic pigments, further supporting the beneficial role of bacterial inoculants in enhancing plant productivity. Regarding the effect of formulation carriers, our results indicated no significant impact on the increase in dry biomass or nutritional enhancement in plants, specifically concerning N, P, and K levels. This suggests that the observed growth-promoting effects in plants were primarily due to the presence of viable encapsulated bacteria, highlighting the potential of the three prototypes to enrich compost (Abdelkhalik et al. 2023). The superior performance of prototypes P1 and P2, which exhibited the best results in yield and nutritional enhancement of oat plants, can also be attributed to the incorporation of various substances into the gels that assist bacterial activity or enhance growth-promoting effects (Xing et al. 2022). Notably, the entrapment of protein derivatives and clay within these gels has been reported to enhance microbial metabolic activities and improve the production of compatible solutes such as proline, which plays a crucial role in plant stress responses (Tirry et al. 2021).

To conclude, this study highlights the effectiveness of encapsulated PGPB in enhancing compost performance and improving plant development and nutrient

absorption, particularly in forage oats (*Avena sativa* var. Altoandina genotype AV25 T). The prototypes (P1, P2, and P3) containing encapsulated PGPB consortium significantly increased root and shoot dry biomass, demonstrating the synergistic benefits of combining biological inoculants with compost. When used the middle of fertilization, hydrogel encapsulation protected the bacterial consortium, enabling a slow release of viable microbes, which enhanced nutrient uptake and overall plant growth compared to free-cell formulations or complete fertilization. Integrating compost with encapsulated PGPB offers a sustainable approach to agriculture by potentially reducing the reliance on synthetic N, P, and K inputs. To fully understand its impact on crop productivity, soil fertility, and overall soil health, further field studies under diverse environmental conditions are essential.

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Authors' contributions Mauricio Cruz, German Estrada and Martha Chaparro planned and designed the manuscript. All authors wrote and edited the manuscript and made figure and tables. German Estrada and Martha Gómez provided financial support and edited the manuscript. Daniel Torres and Jonathan Labrador organize and analyzed the data. All authors contributed to carry out the experiments, data analysis, and writing the manuscript. All authors read and approved the manuscript.

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Data availability All datasets generated for this study can be provided upon request.

Declarations

Human and animal rights and informed consent This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the publication of the paper.

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