



Response of *Portulaca oleracea* biomass and its rhizospheric microbiome to the application of solid and liquid slumgum from beekeeping industry transformed with *Penicillium chrysogenum*

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Abstract

Background and aims Slumgum, a by-product of the beeswax-rendering process, is a nutrient-rich organic waste with potential as a sustainable fertiliser. Unlike chemical fertilisers, which can degrade soil health and contribute to environmental pollution, slumgum may improve soil structure, enhance microbial activity, and support long-term soil fertility. This study assessed, for the first time, the effects of both solid and liquid slumgum, either transformed with *Penicillium chrysogenum* and non-transformed, on purslane (*Portulaca oleracea*) biomass and soil rhizosphere microbial communities.

Methods The experiment involved applying different slumgum treatments to purslane plants, followed by the analysis of shoot biomass and foliar phosphorous content. Soil enzymatic activities related to carbon, nitrogen, and phosphorus cycles were measured. Additionally, bacterial and fungal community composition, diversity, and functional guilds in the rhizosphere were described.

Results Both transformed solid and liquid slumgum along with untransformed liquid slumgum treatments increased purslane shoot biomass (by 60–77%) and foliar phosphorous content (by 87–100%) compared to the control. Slumgum treatments modified the microbial community composition, increasing saprotrophic fungi and reducing plant pathogens. Transformed solid slumgum also enhanced enzymatic activities involved in nutrient cycling, whereas the untransformed solid slumgum treatment promoted bacterial genes associated with denitrification.

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Conclusion Our findings highlight the potential of slumgum, particularly in its transformed forms, as a sustainable alternative to chemical fertilisers. Its application may improve soil fertility and crop productivity while reducing environmental impact. Further studies should investigate its long-term effects under field conditions and evaluate its economic feasibility.

Keywords Purslane · Circular economy · Bacterial community · Fungal community · Functional genes · Sustainable agriculture

Introduction

Purslane (*Portulaca oleracea* L.) is a globally recognized wild edible plant valued for its exceptional medicinal and nutritional properties. Rich in omega-3 fatty acids and bioactive compounds, purslane has attracted increasing attention as a functional food ingredient and as a promising candidate for sustainable agricultural practices (Petropoulos et al. 2015, 2016). Its adaptability to adverse environmental conditions, such as drought, high temperatures, and moderate salinity, combined with its ability to thrive with minimal nutrient inputs, makes purslane an ideal crop for resource-limited farming systems (Jin et al. 2016; Carrascosa et al. 2023a; Chrysargyris et al. 2023).

In addition to its nutritional value, purslane plays an important ecological role, as it often grows naturally in agroecosystems with minimal human intervention (Carrascosa et al. 2023b). These characteristics highlight its potential as a sustainable crop for regions affected by soil degradation or where reducing chemical inputs is a priority, aligning with the objectives of eco-friendly and resilient agricultural models (Jang and Chen 2015; Cui et al. 2020).

These characteristics make purslane a promising crop for vulnerable areas with degraded soils or where chemical inputs must be limited to prevent contamination of aquifers and adjacent water bodies. (Jang and Chen 2015; Cui et al. 2020). In these areas, new strategies for fertilisation should be implemented using organic amendments as substitutes of synthetic fertilizers (Albuquerque et al. 2012; Reyes-Torres et al. 2018; Celestina et al. 2019). In particular, novel value-added organic amendments could broaden the

range of sustainable options under different cropping scenarios.

It is well established that organic amendments (sewage sludge, composts, farmyard manure, biochar, etc.) are a good source of plant nutrients and, if managed properly, they can improve crop production (Luo et al. 2018; Ahmad et al. 2022). Additionally, they may enhance the physical, chemical, and biological properties of soil, presenting an eco-friendly approach to maintaining and enhancing soil fertility while contributing to natural resources preservation (Diacono and Montemurro 2010; Aytenev and Bore 2020; Luis Moreno et al. 2022). Moreover, an increase of soil organic matter contributes to carbon (C) sequestration, reducing the CO₂ atmospheric level and adverse greenhouse effects, helping to mitigate the climate change (Lorenz and Lal 2023).

In the beekeeping industry, “slumgum” is generated as a solid organic waste during the beeswax-rendering process from old scraped honeycombs, which are discarded every three or four years. This waste has a dark (brown-black) colour with an intermediate appearance between animal manure and vegetable compost. It is primarily composed of brood cocoons, moths, larvae of *Galleria melonella* L., moults, dead bees, bee droppings, pollen, propolis, and small proportions of non-extractable wax, among other residues (Morales-Corts et al. 2010). Currently, slumgum has no particular use nor it is recycled (Morales-Corts et al. 2014).

The worldwide production of slumgum is estimated at 93,000 tons per year (FAOSTAT 2020). Slumgum has a highly promising nutrient profile, rich in organic matter, plant nutrients such as nitrogen, phosphorus, and potassium. Additionally, slumgum has a high content of propolis and pollen, which are rich in bioactive compounds and known for their beneficial effects on soil health and plant development (Morales-Corts et al. 2014). These beneficial components provide slumgum with a distinct ecological advantage over other organic amendments. For instance, its high organic matter content could significantly enhance soil structure, moisture retention, and carbon sequestration, while its natural nutrient profile may improve soil fertility without the need for synthetic fertilizers. Furthermore, slumgum’s production as a by-product of beekeeping makes it an environmentally sustainable alternative, contributing to waste recycling and reducing its environmental impact.

Although slumgum is known to have a promising nutritional profile, its application as an organic amendment to improve soil health and plant growth has not been extensively researched. In particular, only a few studies have examined its direct impact on soil microbial activity, nutrient cycling, or crop development (Morales-Corts et al. 2010, 2014). Additionally, even if some previous work suggests that slumgum is rich in organic matter, nutrients, and a source of beneficial compounds, its effectiveness compared to other traditional organic fertilizers has not been sufficiently evaluated (Morales-Corts et al. 2014). Therefore, the use of slumgum as a soil amendment in agricultural soils remains an under-explored research opportunity. The lack of evidence regarding its specific effects on crop productivity and soil health highlights a knowledge gap that this study aims to address.

Similar residues to slumgum, such as dry olive residues, have been successfully transformed using saprophytic fungi (*Fusarium* spp L., *Phanerochaete* spp P. Karst, *Pleurotus* spp P. Kumm, *Trametes versicolor* L. and *Penicillium chrysogenum* T., etc.) to enhance their quality as organic amendments (Sampedro et al. 2007; Reina et al. 2013; García-Sánchez et al. 2014; Siles et al. 2015). One of the methods employed for transforming such residues using these fungi is the solid-state fermentation, which is highly effective in transforming solid organic substrates, such as dry olive residue, and offers an environmentally sustainable approach to organic waste management. These processes could enhance the nutrient availability and biodegradability of raw slumgum, making it a more effective soil amendment. The solid-state fermentation breaks down complex organic matter, improving nutrient release and microbial diversity. Meanwhile, the liquid extract derived from this process enriches water-soluble compounds beneficial for soil health and plant growth (Sampedro et al. 2007). Among the saprophytic fungi, *P. chrysogenum* has the capacity to degrade complex organic materials, increasing nutrient availability, particularly nitrogen and phosphorus, by the production of enzymes capable of breaking down recalcitrant compounds. In addition, *P. chrysogenum* is also known for improving the overall quality of organic waste by making it safer and more suitable for its use as a soil amendment. This can potentially enhance soil fertility,

stimulate microbial activity, and promote plant growth (Sampedro et al. 2007). However, the effect of fungal transformation on slumgum's properties and its subsequent influence on soil and plants remain largely unexplored. Addressing these gaps is essential to determine whether slumgum could serve as sustainable bio-waste management option and a valuable tool for improving soil fertility and plant development.

Soil microbial communities play a crucial role in the functioning of soil ecosystems since microorganisms are key in processes of organic matter decomposition, nutrient cycling, and plant productivity (Saleem et al. 2019; Xia et al. 2020; Naylor et al. 2022). Moreover, soil microorganisms rapidly respond to environmental shifts and are considered to be good indicators of soil quality. In a recently meta-analysis conducted by Shu et al. (2022), it was reported that the use of organic amendments enhances the soil microbial diversity and functionality. However, the response of microbial communities to the addition of organic residues can be affected by diverse factors including soil properties, experimental and environmental conditions, the residue type, or dose, the crop species etc. (Ning et al. 2020; Li et al. 2021; Shu et al. 2022; Chen et al. 2023). Therefore, a detailed understanding of microbial composition, diversity and function as well as soil properties linked to soil fertility and plant growth in soils amended with slumgum is essential in order to understand the patterns and relationship between soil functioning and processes mediated by microorganisms in agroecosystems. This knowledge may have important implications for promoting more sustainable agricultural management practices and conserving soil biodiversity in a climate changing environment.

The present study aimed to evaluate the potential of slumgum as an organic amendment in agricultural soils. Specifically, we investigate the short-term effects of solid and liquid extracts of slumgum both non-transformed and fungal-transformed (using *Penicillium chrysogenum*) on purslane biomass, soil properties and microbial communities. Our objectives were to: (1) Assess the nutritional composition and enzymatic activities in the rhizosphere soil related to P, N and C cycles; (2) characterize the composition, diversity and functionality of fungal and bacterial communities in the rhizosphere; and (3) determine whether fungal transformation enhances slumgum's

effectiveness as a soil amendment. These insights aim to advance in sustainable agricultural practices, improving soil health and plant productivity.

Materials and methods

Design of the experiment and sampling

The soil used in the experiment was collected from the CEBAS-CSIC Experimental Farm (Santomera, Murcia, Spain). The baseline properties of the soil were as follows: pH 7.8, electrical conductivity (EC, measured at 25 °C) 1000 $\mu\text{S cm}^{-1}$, total organic carbon content 7.3 g kg^{-1} , total nitrogen 5.7 g kg^{-1} , available phosphorus content 6.0 mg kg^{-1} , available potassium content 33.9 mg kg^{-1} and CaCO_3 3 g kg^{-1} . The soil has a clay-loam texture. These properties indicate a moderately fertile soil, suitable for testing the effects of different organic amendments. For further details, see Carrascosa-Robles et al. (2024).

The organic beekeeping raw residue called “slumgum” was collected from the “Apizafra S.L.” beekeeping company (Albudeite, Murcia, Spain). The untransformed solid slumgum (S) was obtained by directly autoclaving the raw slumgum. The untransformed liquid slumgum extract (L) was obtained by mixing the slumgum with distilled water 1:3 (w/v ratio), followed by agitation for 24 h, filtering the mixture through a mesh and then sterilised by autoclaving. The mycotransformation of the solid slumgum was carried out by solid-state fermentation as described by Reina et al. (2013) with some modifications using the saprophytic fungi, *Penicillium chrysogenum* (EEZ-28). Firstly, fungi were inoculated into flasks with 18 g of barley and 30 mL of sterile water

for one week. After this, 10 g of previously sterilised solid slumgum (S) and 2.5 mL of water were added and incubated at 28 °C for 4 weeks. The resulting biotransformed solid slumgum (TS) was then homogenised and sterilised by autoclaving. The initial step for the biotransformation of liquid untransformed slumgum (L) involved the growth of the same strain of *P. chrysogenum* (EEZ-28), in a modified Czapek-Dox medium (MB) composed of (g/L): glucose 10.0, ammonium tartrate 2.0, KH_2PO_4 1.0, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, KCl 0.5, yeast extract 1.0, peptone 1.0, CuSO_4 0.125, and 1 mL/L of a mineral solution of (mg/L): $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 5.0, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.6, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 1.4, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 2.0, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ 2.0, $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ 0.4, for 7 days at 80 rpm and 28 °C. Following this incubation period, the untransformed slumgum liquid extract was added to this medium in a 1:1 v/v ratio and the mycotransformation was carried out under the conditions previously described. After a 14-day incubation period, the biotransformed liquid extract was obtained, filtered through a mesh, diluted to half (1:2 v:v ratio) with distilled water (TL) and sterilised by autoclaving. A brief description of the treatments (TS, TL, S and L) and control can be found in Table 1. The Fig. S1 represents the flowchart of the biotransformed products preparation steps. Both TS and TL were stored at 4 °C until use, while the raw materials before biotransformation (S and L) were autoclaved and then stored at 4 °C. The main chemical characteristics of the studied materials are shown in Table 2.

In May 2022, seeds of purslane (*Portulaca oleracea* L.) provided by the Department of Agriculture Crop Production and Rural Environment (University of Thessaly, Greece) were sown under nursery conditions, in peat substrate trays for 3 weeks prior the

Table 1 Description of the treatment and control groups in the experiment

Treatment	Description	Material Type	Application Method
TS	Biotransformed slumgum by <i>Penicillium chrysogenum</i>	Biotransformed	Mixed with the soil:sand mixture (5%) at the beginning of the experiment
TL	Biotransformed liquid slumgum extract by <i>Penicillium chrysogenum</i>	Biotransformed	Added through irrigation water (5%) one week after transplantation
S	Non-biotransformed slumgum	Non-biotransformed	Mixed with the soil:sand mixture (5%) at the beginning of the experiment
L	Non-biotransformed liquid slumgum extract	Non-biotransformed	Added through irrigation water (5%) one week after transplantation
Control	No slumgum treatment	-	No slumgum added (no treatment)

Table 2 Chemical and nutrient composition of the different types of slumgum used in this experiment

	Solid Slumgum (S)	Solid Slumgum Transformed (TS)	Liquid extract of Slumgum (L)	Liquid extract of Slumgum Transformed (TL)
Moisture (%)	23.52±0.41	23.54±0.42		
pH	4.93±0.62	8.47±0.52	6.25±0.33	6.70±0.28
Electric conductivity (EC) (dS m ⁻¹)	1.74±0.42	2.60±0.50	3.71±0.11	3.10±0.15
Total carbon (TC) (%)	55.60±1.73	48.33±1.52	1.27±0.51	0.37±0.60
Total organic carbon (TOC) (%)	53.49±1.25	48.26±1.30		
Total nitrogen (TN) (%)	3.61±0.08	3.63±0.05	0.12±0.05	0.06±0.01
Relation TOC/TN	14.82±0.46	13.27±2.50		
Total P (mg kg ⁻¹)	3500±39	3700±40	500±29	346±19
Total K (mg kg ⁻¹)	3900±40	5500±49	830±28	745±25
Total Ca (mg kg ⁻¹)	3600±87	2250±79	189±19	57±9
Total Mg (mg kg ⁻¹)	1000±22	1100±26	110±12	67±9
Total Na (mg kg ⁻¹)	190±3	240±5	35±3	46±4
Total Fe (mg kg ⁻¹)	499±8	330±7	10.46±0.22	5.74±0.24
Total Cu (mg kg ⁻¹)	14.46±1.11	11.93±1.05	0.19±0.02	15.87±2.05
Total Mn (mg kg ⁻¹)	29.76±1.03	25.72±1.08	2.85±0.12	1.66±0.14
Total Al (mg kg ⁻¹)	257±5	209±4	8.09±0.20	1.58±0.11
Total Zn (mg kg ⁻¹)	255±3	176±2	11.63±1.04	9.59±1.13
Total Pb (mg kg ⁻¹)	3.74±1.11	3.17±1.17	<0.01	<0.01
Total Ni (mg kg ⁻¹)	1.86±0.51	1.15±0.32	0.09±0.01	0.06±0.01
Total Cd (mg kg ⁻¹)	<0.025	<0.025	<0.010	<0.010
Total Co (mg kg ⁻¹)	0.57±0.01	0.21±0.01	<0.01	<0.01
Total Li (mg kg ⁻¹)	0.91±0.02	1.08±0.01	0.09±0.01	0.12±0.02
Total Se (mg kg ⁻¹)	<0.01	1.14±0.01	<0.01	<0.01

transplanting to 0.3 L pots filled with a mixture of soil:sand (1:1 w:w). One plant was transplanted into each of them. The experiment consisted in a randomised factorial design, comprising five treatment groups: two biotransformed treatments, solid transformed slumgum (TS) and liquid transformed (TL); two non-transformed treatments, solid untransformed slumgum (S) and liquid untransformed slumgum (L); and a control treatment without slumgum. Five replicates were used for each treatment ($n=5$; 25 pots in total). For soil amendment, both solid treatments (TS and S) were mixed with the soil:sand mixture at a rate of 5% (17.5 g) at the beginning of the experiment. Similarly, both liquid treatments (TL and L) were added at a rate of 5% (17.5 mL) to the pots through the irrigation water one week after transplantation. This experimental design allows a clear comparison of the effects of the biotransformed and non-transformed slumgum

treatments on plant growth and soil microbial communities. The experiment was carried out for six weeks in total without additional fertilization under greenhouse conditions at CEBAS-CSIC Experimental Farm. Soil moisture content of the pots was maintained near field capacity (0.03 MPa), which was monitored by weighing the pots and adjusting irrigation as needed to maintain consistent moisture levels. Day/night temperature was 30 °C/25 °C and the average relative humidity at 60%.

At the end of the six-week experiment period, all plants were harvested, and soil samples were taken from the pots. Each root system was put in a polyethylene bag and shaken to collect the rhizospheric soil. One aliquot of the rhizospheric soil was kept in a freezer at -20 °C for molecular analysis and the other was sieved through 2-mm mesh and stored at 4 °C for carrying out the chemical and biochemical analysis. For plant analyses, all replicates were processed

($n=5$) and for soil analyses, four replicates ($n=4$) were processed.

Analyses of plant tissue

Fresh and dry (at 105 °C for 5 h) shoot biomass were measured. Then, dried shoots were ground with a mill before nutrient analysis. Shoot total N was determined using a TruSpec CN Analyzer (LECO, St. Joseph, MI, USA). Shoot total P, K, Ca, Mg and Fe contents were analysed by ICP-OES (ICAP 6500 DUO, Thermo Fisher Scientific, Hayward, California, USA).

Chemical, biochemical, and biological analyses of rhizosphere soil

All soil determinations were performed through a randomised complete block design using the air-dried soil to avoid differences among samples. Soil pH and electrical conductivity were determined at 1:2.5 and 1:5 soil:water ratios, respectively. Available P was extracted according to Olsen et al. (1954) method and determined according to Murphy and Riley (1962). Organic C (%) was quantified according to Walkley and Black (1934). Organic C values were transformed into organic matter by multiplication using an empirical factor (1.724). Total nitrogen (TN) and total carbon (TC) were determined using an elemental CHNS-O analyzer (EA- 1108, Carlo Erba, Barcelona, Spain); total phosphorus (TP) and total potassium (TK) were assessed using ICP-OES (ICAP 6500 DUO, Thermo Fisher Scientific, Hayward, California, USA). Dehydrogenase activity (DHA) was determined by measuring the amount of triphenyl formazan (TPF) released by incubating the soil with 2,3,5 triphenyltetrazolium chloride according to Trevors et al. (1982) and modified by García et al. (1993). Urease activity was determined as described by Kandeler et al. (1999), using urea as substrate (Nannipieri et al. 1980); β -glucosidase activity (β -Glu) was assessed by using 25 mmol L⁻¹ p-nitrophenyl- β -D-glucopyranoside as substrate according to Eivazi and Tabatabai (1988), and alkaline phosphomonoesterase activities were assessed by using p-nitrophenyl phosphate disodium as substrate and then determined colorimetrically by measuring the amount of p-nitrophenol (PNF) released at pH 8 (Naseby and Lynch 1997).

DNA extraction and Illumina sequencing

DNA was extracted from 0.25 g of rhizosphere soil using the DNeasy PowerSoil DNA Isolation kit (Qia-gen), following the manufacturer's protocol. DNA yield and quality were checked both by electrophoresis in 0.8% (w/v) agarose gels stained with GelRed and visualized under UV light, and by using a Qubit 3.0 fluorometer (Life Technologies, Grand Island, NY). DNA from each sample was sequenced using the Illumina MiSeq platform at the genomics service of the Institute of Parasitology and Biomedicine "López Neyra" (CSIC), Granada, Spain.

Prokaryotic libraries were constructed by amplifying the hyper-variable V3–V4 regions of the 16S rRNA gene using the primer pair 341F (5'-CCTACGGGNBGCASCAG-3') and 806R (5'-GACTACNVGGGTATCTAATCC-3') according to Takahashi et al. (2014).

These amplicons were tagged to be attached to PNA PCR clamps to reduce plastid and mitochondrial DNA amplification (Lundberg et al. 2013).

Fungal libraries were constructed by amplifying the ITS2 region using the primer pair ITS4 (5'-TCC TCCGCTTATTGATATGC-3') (White et al. 1990) and fITS7 (5'-GTGARTCATCGAATCTTTG-3') (Ihrmark et al. 2012). Both runs were sequenced using a paired-end 2 × 300 bp (PE 300) strategy.

Sequencing data processing

Raw sequence data from the sequencer were processed by QIIME v2022.2 (Caporaso et al. 2010). Before the analysis, raw sequences were checked using the fastqQC program (Andrews 2023). After that, sequences were uploaded to QIIME and primers were removed using cutadapt (Martin 2011), removing sequences with less than 250 pb. For denoising the readings, the DADA2 algorithm was used (Callahan et al. 2016) and sequences were truncated to an average length of $Q > 30$.

The resulting amplicon sequence variants (ASVs) were classified using UNITE v9 released in October 2022 or SILVA v138 released in November 2020 in the fungal and bacterial community, respectively. Fungal functional guilds were determined using the genus-guild database Fungal Traits (Pölme et al. 2020). Functional analysis of the bacterial community was performed using the PICRUSt2 algorithm

(Douglas et al. 2020), predicting the potential bacterial genes related to N, C, and P soil cycles. These genes were chosen based on their ecological relevance and their availability in the PICRUSt2 reference database.

For the nitrogen cycle, we included genes related to biological nitrogen fixation (*nifH*, *nifD*, *nifK*), denitrification (*narG*, *narH*, *narI*, *nirK*, *norB*, *norC*, *nosZ*), and nitrification (*amoA*, *amoB*, *amoC*, *hao*), all of which play central roles in nitrogen transformations in microbial ecosystems.

For the carbon cycle, we selected genes such as *malZ*, *bglX*, *FUCA*, *ppc*, *Gpx*, and *CAT*, which are involved in carbohydrate degradation, carbon metabolism, and oxidative stress response.

In the case of the phosphorus cycle, we considered *phnX*, associated with the utilization of organic phosphonates, and *ppk*, involved in intracellular polyphosphate accumulation. These genes are widely recognized as functional markers of microbial phosphorus cycling.

Statistical analyses

The normality and homogeneity of the variance of the environmental variables and microbial taxa and predicted functionality were checked using the Kolmogorov–Smirnov ($p < 0.05$) and Levene's tests ($p < 0.05$), respectively. When the data did not fit the assumptions, it was normalized using a $\log_{10}$ transformation. A two-way ANOVA was performed to determine the influences of the slumgum type and transformation with *P. chrysogenum*, as well as their interactions on the measured variables, and differences among means were assessed according to Tukey's HSD test ($p < 0.05$). Correlation analyses were performed using Pearson's method. For statistical analyses, IBM-SPSS v.29 was utilized.

Concerning microbial communities' datasets, the ASVs per sample were rarefied at the minimum number of sequences detected among samples (91,201 and 270,840 reads per sample for fungal and bacterial datasets respectively) to avoid bias in further analyses due to differences in sequencing effort between samples.

Non-metric multidimensional scaling (NMDS), a distance-based ordination technique that is robust to the nonlinearity of the data, based on the non-Euclidean Bray–Curtis distance was performed to visually

compare the qualitative relationships (rather than the exact dissimilarities) of taxonomic composition of the fungal and bacterial communities independently, using the “metaMDS” function implemented in the “vegan” package of R (Oksanen et al. 2019) using 2 dimensions ($k=2$) and establishing a seed using set.seed(14). To test the effect of the two experimental factors – slumgum type and transformation with *P. chrysogenum*– on the rhizosphere fungal and bacterial taxa, a two-way ANOVA was performed. Also, for fungal and bacterial communities' composition and structure, a permutational multivariate analysis of variance (PERMANOVA) using 999 permutations was conducted on the same Bray–Curtis matrix with the “adonis” function in “vegan” package (Oksanen et al. 2019). Heat maps were also constructed using the “vegan” package. For further details about statistical analyses, please check Borcard et al. (2018).

Data availability

The sequences of this study were submitted to the NCBI Sequence Read Archive repository and are accessible in the BioProject PRJNA1051377.

Results

Effects of the experimental factors on plant characteristics

Shoot dry biomass was significantly affected by slumgum type (solid or liquid), transformation by *P. chrysogenum*, and the interaction between both factors (Fig. 1). TS, L and TL treatments resulted in significantly higher values of dry purslane biomass than control (increasing between 60–77%); however, the application of solid slumgum without fungal transformation (S) significantly reduced dry mass compared to the control (69%).

The type of slumgum had a significant effect on shoot nutrient content, especially influencing the levels of P, K, Ca and Mg. Fungal transformation notably affected only the P content (Table S1). The interaction between slumgum type and fungal transformation significantly impacted the levels of N, P, Ca and Mg. Compared to the control, all slumgum treatments caused a significant decrease in K and Ca levels. Mg and Fe levels were reduced in the S,

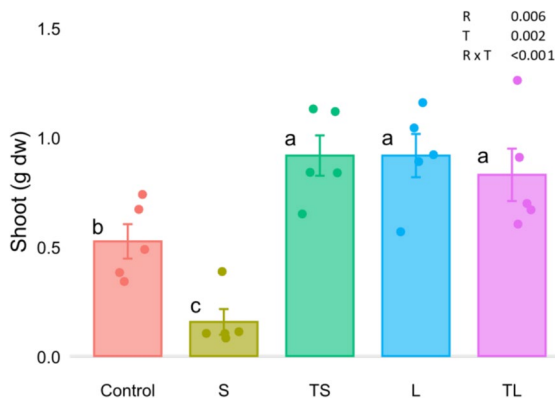


Fig. 1 Shoot dry weight in grams (g dw) of purslane (*Portulaca oleracea*) plants grown under control and four different slumgum treatments (S=solid slumgum; TS=solid slumgum transformed with *Penicillium chrysogenum*; L=liquid slumgum; TL=liquid slumgum transformed with *P. chrysogenum*) ($n=5$). Significance of effects of slumgum type (R), transformation with *P. chrysogenum* (T) and their interaction (R x T) on the measured variable is also shown (p-values). Groups labelled with different letters indicate statistically significant differences according to Tukey HSD test ($p < 0.05$)

L, and TL treatments (Mg) and in the TS, L, and TL treatments (Fe). In contrast, P levels increased significantly in the TS, L, and TL treatments, with the TS treatment also showing the highest N content.

Effects of the experimental factors on rhizospheric soil parameters

The soil electrical conductivity was only affected by slumgum type, where TS treatment recorded the significantly highest value (Table S2). On the other hand, the dehydrogenase activity was affected by the two experimental factors and their interaction, with TS treatment showing significantly higher dehydrogenase activity than the rest of the treatments and the control (Fig. 2A). The same treatment (TS) beneficially affected the activity of β -glucosidase without being significantly different from the control and L treatment (Fig. 2B). Concerning alkaline phosphatase and urease activities, both were significantly influenced by the slumgum type. The solid slumgum treatments (S and TS) were more efficient in significantly

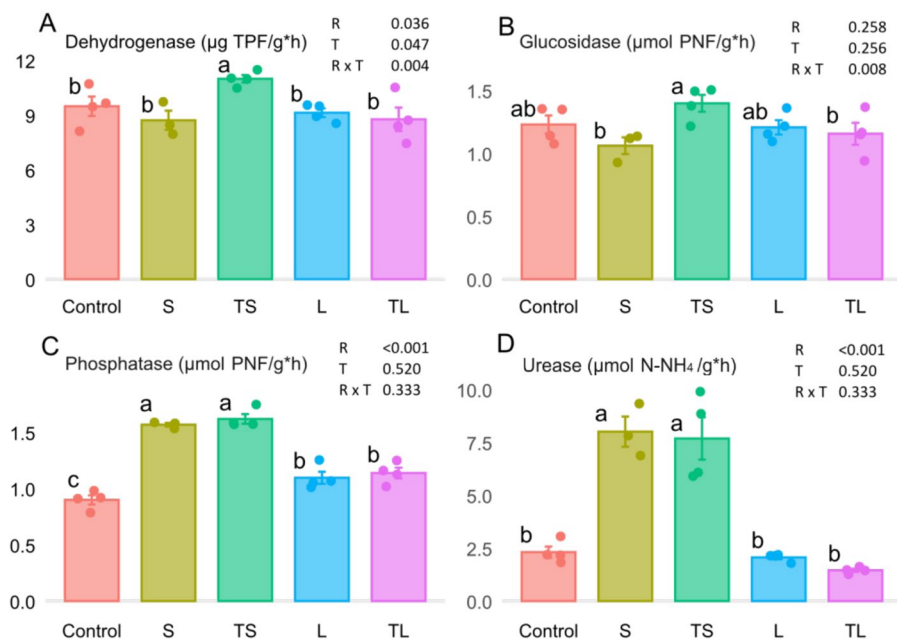


Fig. 2 Soil enzymatic activity of dehydrogenase (TPF=triphényl formazan) (A), β -glucosidase (B), alkaline phosphatase (PNF=p-nitrophenol) (C) and urease (D) under control and four different slumgum treatments (S=solid slumgum; TS=solid slumgum transformed with *Penicillium chrysogenum*; L=liquid slumgum; TL=liquid slumgum trans-

formed with *P. chrysogenum*) ($n=4$). Significance of effects of slumgum type (R), transformation with *P. chrysogenum* (T) and their interaction (R x T) on the measured variables is also shown (p-values). Groups labelled with different letters indicate statistically significant differences according to Tukey HSD test ($p < 0.05$)

increasing these enzymatic activities compared to both the control and the liquid slumgum treatments (L and TL; Fig. 2C, D).

Effects of the experimental factors on the fungal community composition and functionality of rhizospheric soil

Sequencing of the fungal ITS region yielded a total of 7,171,200 high-quality sequences clustered into 2,260 fungal ASVs before rarefaction at 91,201 reads per sample. The ANOVA showed that all experimental factors and their interactions, except the transformation for fungal richness, had a significant effect on α -diversity (measured by fungal richness and Shannon diversity); the S, TS and TL treatments significantly decreased both fungal richness and Shannon diversity index (Fig. S2A and B). The rhizosphere fungal communities differed significantly among slumgum types and the transformation process (Table S3), and an interaction between both factors was recorded with the PERMANOVA analysis. The NMDS ordination revealed distinct clusters corresponding to each treatment, indicating that the fungal community structure is strongly influenced by the slumgum type and transformation process (Fig. 3A). Specifically, fungal communities in the L and TL treatments were more

similar to those in the control treatment, suggesting a lesser impact of these treatments compared to S and TS treatments, which exhibited distinct and separate clustering patterns. This highlights that S and TS treatments induced more pronounced changes in the fungal community composition.

The fungal ASVs were assigned to 13 phyla. The most abundant phylum was Ascomycota (82.7% of the total relative abundance), followed by Mortierellomycota (10.5%), Basidiomycota (3%), Chytridiomycota (2.3%) and Mucoromycota (1.3%) (Table S4, Fig. S3A). The remaining identified phyla accounted for less than 0.1% of the fungal sequences. Ascomycota, Mortierellomycota and Mucoromycota were significantly affected by the slumgum type, whereas Basidiomycota, were affected by the slumgum type, the transformation and their interaction. Ascomycota and Basidiomycota significantly decreased under the S and TS treatments compared to the control soil and the rest of the treatments (L and TL). In contrast, Mortierellomycota and Mucoromycota showed opposite trends, increasing significantly under the S and TS treatments.

At the genus level, *Humicola* was the most dominant fungal genus out of a total of 303 different genera, with a mean relative abundance of 15.4% across samples, which was primarily influenced by the

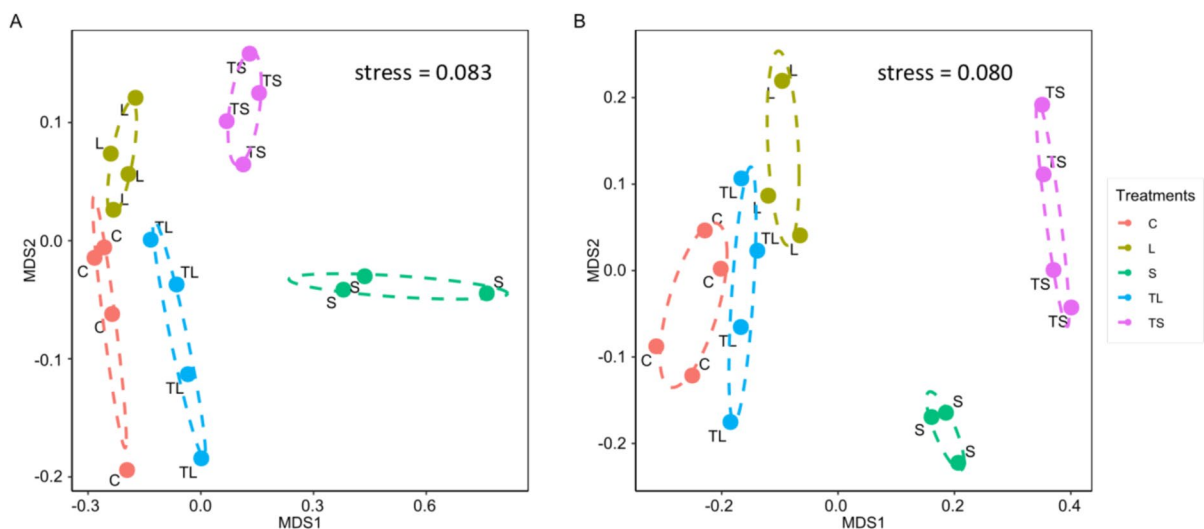


Fig. 3 Non-metric multidimensional scaling (NMDS) analysis on a Bray–Curtis dissimilarity matrix based on the ASVs dataset retrieved from the fungal (A) and bacterial (B) communities of the rhizospheric soil of purslane (*Portulaca oleracea*)

grown under control and four different slumgum treatments (S=solid slumgum; TS=solid slumgum transformed with *Penicillium chrysogenum*; L=liquid slumgum; TL=liquid slumgum transformed with *P. chrysogenum*) ($n = 5$)

transformation process, significantly increased after the transformation of slumgum of both types, especially in the TL treatment, covering up to the 32% (Table S5, Fig. S3B). *Mortierella* and *Mucor* were primarily affected by the slumgum type, with relative abundances that increased in solid slumgum (S and TS). Conversely, *Preussia*, *Hormiactis* and *Myceliophthora* were also predominantly influenced by the slumgum type and both factors interaction, showing significantly decreased under the same treatments (S and TS). *Alternaria*, *Canaryomices* and *Ochroconis* were impacted by both experimental factors and their interaction, showing the highest relative abundance under the S treatment.

The fungal functional guilds analysis assigned 1224 ASVs (54% of the total ASVs) to the six main functional guilds groups: plant pathogens, parasites, lichenized, symbiotrophs, endophytes and saprotrophs (Fig. 4; Table S6). The plant pathogens and saprotrophs were significantly affected by the slumgum type, the transformation process and the interaction between both factors, suggesting that both fungal guilds showed opposite trends under the TS, L and TL treatments (when saprotrophs increased, the plant pathogens decreased). The parasites were significantly decreased by the treatments with slumgum transformation (TS and TL), as shown also with the

ANOVA. Related to the endophytes and symbiotrophs, the most influential factor was slumgum type, showing the lowest relative abundance in the treatments with solid slumgum (S and TS).

Effects of the experimental factors on the bacterial community composition and functionality of rhizospheric soil

A total of 5,452,140 reads with 5,567 different ASVs were obtained before rarefaction at 270,840 reads per sample. Bacterial richness and Shannon diversity index were significantly affected by both experimental factors and their interactions (Fig. S2C, D). The richness was significantly higher in the S and TL treatments than the rest of the treatments, while the significantly lowest values were recorded for the TS treatment. The PERMANOVA analysis showed that both factors, slumgum type and transformation, as well as their interaction (S x T) significantly influenced the composition and structure of the rhizospheric bacterial communities (Table S3B). The NMDS ordination of soil bacterial communities was clustered by slumgum treatment, showing the same pattern as fungal communities (Fig. 3B). The bacterial community consisted of 17 bacterial phyla with Actinomycetota and Pseudomonadota being the most dominant, representing 32% and 26% of

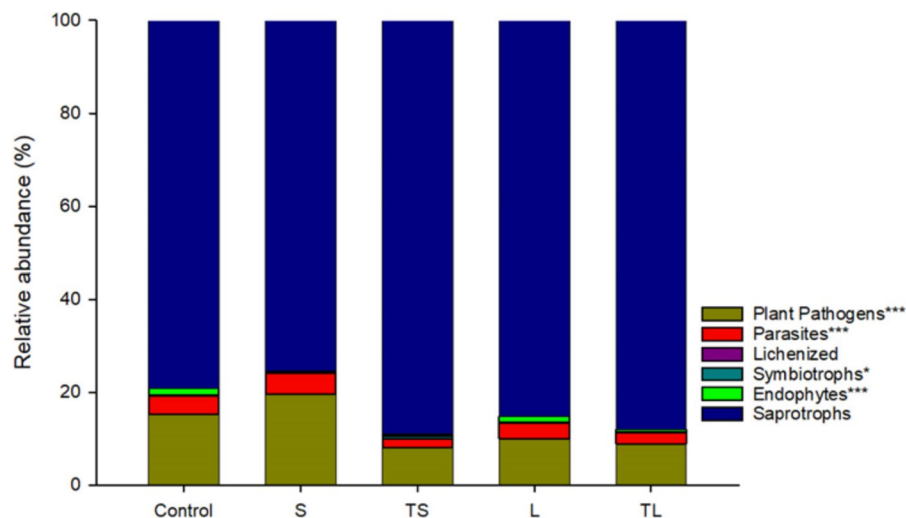


Fig. 4 Mean relative abundance of fungal ASVs belonging to six major functional guilds in rhizospheric soil under control and four different slumgum treatments (S=solid slumgum; TS=solid slumgum transformed with *Penicillium chrysogenum*; L=liquid slumgum; TL=liquid slumgum transformed

with *P. chrysogenum*). ($n=4$). For each functional guilds, significant differences were assessed by the Tukey's post-hoc test calculated at $p < 0.05$ and indicated by an asterisk, * $p < 0.05$; *** $p < 0.001$

total relative abundance, respectively and were affected by both experimental factors and their interaction (Fig. S3C, Table S7). The TS and S treatments were the most efficient to significantly increase Actinomycetota and Pseudomonadota, respectively. The Bacillota phylum abundance (24%) was significantly decreased by both factors, being the solid slumgum treatments (S and TS) those with the lowest values. Myxococcota phyla abundance, although accounted for only 1.43% of the bacterial sequences, was strongly affected by the two experimental factors with significant increase under TS, S and L treatments.

With respect to bacterial taxonomy at the genus level, we identified 114 distinct genera. The most significant factor was the slumgum type (Table S8). Genera such as *Microbacterium*, *Virgibacillus*, *Ilumatobacter* and IMCC26256 decreased their relative abundance under the treatments with solid slumgum addition (S and TS). In contrast, TM7a and unknown bacterial genus significantly increased their abundances under the solid treatment (S and TS; Fig. S3D).

The relative abundance of predicted genes was used to infer bacterial potential functionality involved in nitrification and nitrogen fixation process of N cycling, were not significantly affected by the slumgum addition (Table S9, Fig. 5). However, the relative abundance of genes related to denitrification like nitrate reductase genes (*narG*, *narH*), and nitric oxide reductase (*norB*, *norC*, *nirK*) were significantly influenced by both experimental factors, especially in the S and TS treatments, were these genes increased compared to the control. However, the TS treatment only significantly increased genes related to nitrate reductase (except the *nirK* gen). Similarly, the relative abundance of C-cycling genes participating in organic matter decomposition (cellulose (*bglX*) and hemicellulose (*FUCA*) biodegradation or C fixation (*ppc*)) showed significantly higher values in the S treatment compared to the other treatments. Finally, regarding the P turnover pathway genes, they were not significantly affected by any of the studied treatments.

Correlations between the soil properties, plant biomass and the fungal and bacterial communities of the rhizospheric soil

Correlation heat maps were carried out to explore the relationships between the fungal and bacterial communities with respect to the plant biomass and the

soil chemical and biological properties (Fig. S4A and B). In general, fungi and bacteria showed stronger correlations with the soil biological properties than its chemical properties. With respect to the fungal community, it was observed that Mortierellomycota and Mucoromycota phyla and their respective genera, *Mortierella* and *Mucor* were the ones that showed the highest significant correlation with urease and alkaline phosphatase activities, as well as with EC. The *Ochroconis* and *Alternaria* genera also revealed significant positive correlations with the same enzymatic activities, but with lower significance levels. On the other hand, only *Geomyces* genus revealed a significant positive correlation with β -Glucosidase. The dehydrogenase activity was only significantly positively correlated by the *Geomyces* and *Mortierella* genus. The majority of fungal phyla and genera showed negative correlations with alkaline phosphatase and urease activities and EC.

Among the bacterial phyla, Pseudomonadota showed a significant positive correlation with alkaline phosphatase, urease, EC and Total N. Actinomycetota was also positively correlated, specifically with β -glucosidase and dehydrogenase activities (Fig. S4C, D). In contrast, Bacillota exhibited a stronger negative correlation with these same soil parameters than Pseudomonadota, while Chloroflexota showed significant negative correlations with all measured enzymatic activities.

At the genus level, positive correlations were observed between the *TM7a*, *Rhizobium* and *Flavitalea* genera and the variables alkaline phosphatase, urease, and EC. *Myxococcus* was the only genus that showed significant positive correlations with soil chemical properties, specifically TC, TK and TP. The remaining bacterial genera exhibited negative correlations with alkaline phosphatase and urease activities and EC.

Discussion

Sustainable agricultural systems increasingly require alternative crops that are both resource-efficient and resilient to environmental stressors such as drought and soil degradation. Purslane (*Portulaca oleracea* L.) has emerged as a promising candidate crop in this context due to its rich nutritional profile, interesting for human consumption;

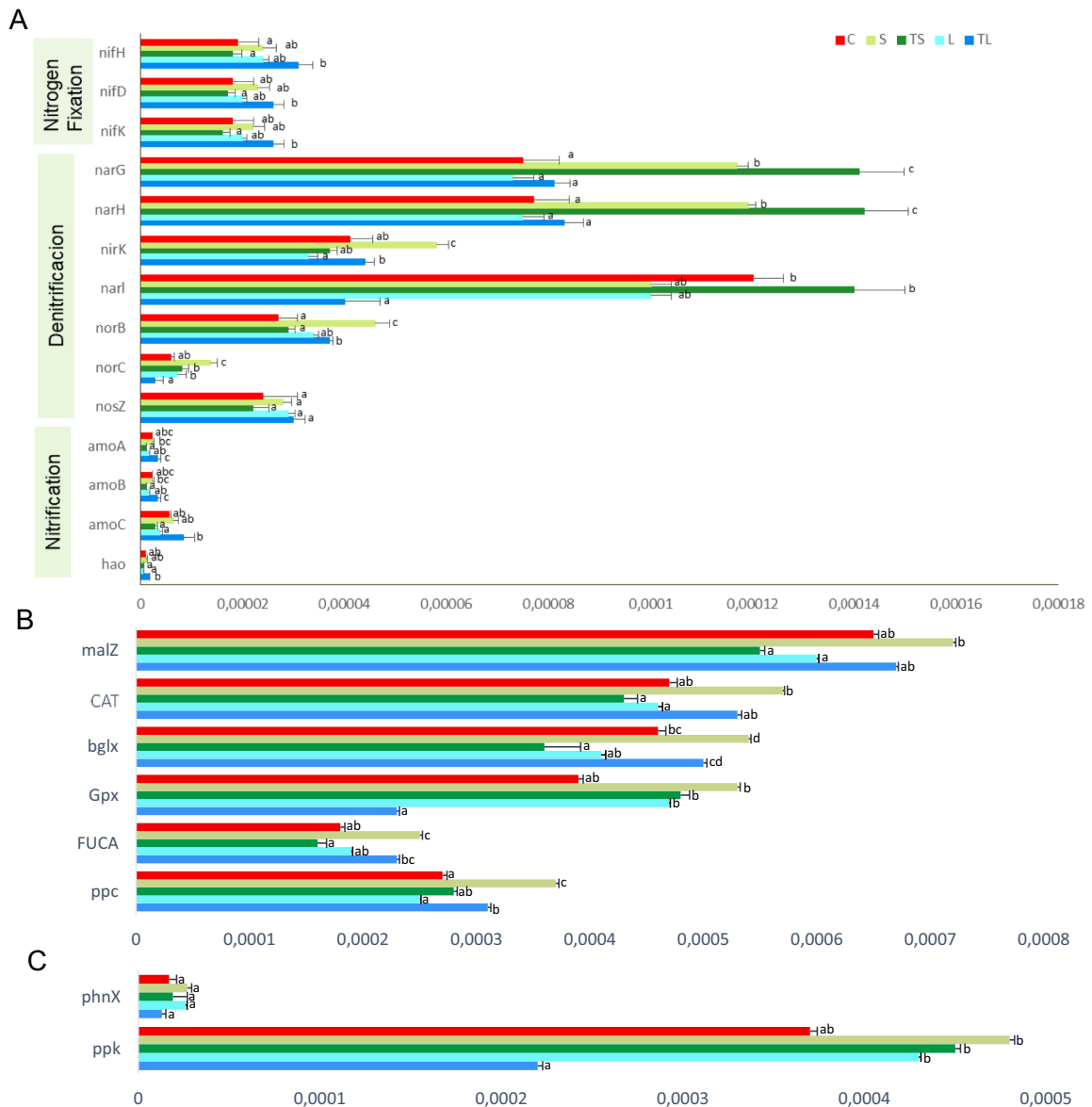


Fig. 5 Relative abundance of functional predicted genes related to nitrogen (A), carbon (B) and phosphorous (C) cycling in rhizospheric soil under different slumgum treatments (S=solid slumgum; TS=solid slumgum transformed with *Penicillium chrysogenum*; L=liquid slumgum; TL=liquid slumgum transformed with *P. chrysogenum*) $n=4$. For

each gene, means and standard error are represented. When significant differences among groups were detected by ANOVA, a Tukey HSD post hoc test was performed. Groups labelled with different letters indicate statistically significant differences ($p < 0.05$)

tolerance to drought and salinity; and ability to grow in marginal soils with minimal inputs (Jacobsen et al. 2012; Montoya-García et al. 2023; Carrascosa et al. 2023b). Its inclusion in low-input agroecosystems could promote food security while

enhancing soil health, especially under sustainable practices and combined with organic amendments capable of closing nutrient cycles (Yang et al. 2020; Carrascosa-Robles et al. 2024).

Numerous studies over the years have documented how the use of organic residues, such as compost, manure, bone meal, poultry litter slurry, straw, wood residues, among others, can exert suppressive effects on certain phytopathogenic microorganisms by modifying indigenous microbial communities and stimulating microorganisms antagonistic to soil-borne pathogens (Reardon and Wuest 2016; Tao et al. 2020; Shu et al. 2022; Chen et al. 2023). However, in our study, we evaluated for the first time the effects of raw and transformed slumgum—an organic residue from the beekeeping industry—on purslane performance, soil biochemical properties, and the composition and function of rhizosphere microbial communities. Slumgum is rich in carbon, nitrogen, and phosphorus, but its raw form may contain hydrophobic compounds and residual contaminants such as beeswax or aluminium that limit its agronomic utility. Biotransformation of slumgum using *Penicillium chrysogenum* aimed to improve its bioavailability and reduce potential phytotoxicity (García-Sánchez et al. 2019).

Biomass production of purslane was significantly enhanced by the transformed slumgum treatments, solid (TS) and liquid (TL) forms and the untransformed liquid extraction (L) (Fig. 1). These treatments also increased phosphorus concentration in plant tissues, suggesting improved P availability and uptake. The enhanced effect of the solid formulation may relate to its slower nutrient release and its contribution to soil structure and microbial habitat (Bonanomi et al. 2018; Margalef et al. 2021). In contrast, raw solid slumgum (S) reduced biomass (Fig. 1), possibly due to the presence of undecomposed hydrophobic substances or aluminium residues (Table 2) that interfere with nutrient absorption or root function (Cozzolino et al. 2016).

Interestingly, slumgum treatments reduced concentrations of magnesium, potassium, calcium, and iron in purslane tissues. This trend, especially marked in the transformed treatments, may reflect nutrient competition or reduced solubility of these elements due to the precipitation of phosphates in alkaline conditions (Hinsinger 2001; Kruse et al. 2015). Unlike mineral phosphate fertilizers such as struvite, which contain high levels of magnesium and typically promote Mg uptake (González-Ponce et al. 2009; Bastida et al. 2019), slumgum appears to lack sufficient Mg or contains antagonistic elements that inhibit its uptake.

Soil enzyme activities—key indicators of microbial function and nutrient cycling—(Naseby and Lynch 1997; Rao et al. 2014) responded differentially to the slumgum amendments (Fig. 2). Urease activity, involved in N mineralization, was significantly enhanced by the solid formulations (S and TS), likely due to their higher organic nitrogen content and sustained microbial stimulation (Klose and Tabatabai 2000; Siles et al. 2014a). Alkaline phosphatase activity increased across all treatments, with the highest values under TS, reflecting increased P turnover and microbial adaptation to complex organic substrates (Nannipieri et al. 2012; Suman et al. 2022). The presence of recalcitrant carbon fractions in the solid slumgum may have promoted phosphatase-producing microbial groups capable of accessing organically bound phosphorus (van der Wal et al. 2013; Bonanomi et al. 2018).

Dehydrogenase activity, a proxy for total microbial oxidative metabolism and biomass, only increased under TS (Fig. 2A), suggesting a unique stimulation of microbial activity by the transformed solid material. This may be due to enhanced biodegradability and a more balanced C:N:P ratio resulting from fungal pre-treatment, particularly with *P. chrysogenum*, known for producing organic acids and enzymes that break down complex substrates (Macci et al. 2012; García-Sánchez et al. 2019).

The composition of rhizosphere microbial communities was significantly influenced by both the nature and form of slumgum applied. Fungal diversity generally declined under slumgum amendments, except under the liquid transformed treatment (TL), possibly due to increased competition or the inhibitory effects of specific compounds present in the raw material (Siles et al. 2014b). However, the relative abundance of beneficial saprotrophic fungi increased under transformed treatments, particularly TS, suggesting a selective enrichment of fungi capable of decomposing the organic residue and contributing to soil nutrient cycling (Xiong et al. 2017; Kepler et al. 2017).

Conversely, the raw solid slumgum (S) reduced the abundance of saprotrophs and favoured pathogenic taxa such as *Alternaria* (Fig. 4), indicating a potential phytotoxic or suppressive effect on beneficial microbial guilds (Thomma 2003). This highlights the importance of pre-treatment or bio-transformation to detoxify the organic residue and

support beneficial microbial communities (Clocchiatti et al. 2020; Liu et al. 2021).

Fungal phyla such as Mortierellomycota — strongly associated with improved nutrient cycling and plant health— (Ozimek and Hanaka 2020) were enriched in the TS treatment and positively correlated with enzyme activities (urease and phosphatase) (Fig. S3A, Fig. S4A) reinforcing their role as indicators of functional microbial enhancement (Clocchiatti et al. 2020). Among bacterial groups, Actinomycetota and Pseudomonadota increased in abundance in the TS treatment (Fig S3 B), both known for their capacity to degrade complex organic compounds and promote plant growth (Kramer et al. 2016; Johnston et al. 2016).

Root exudates may also have shaped microbial communities indirectly. Increased plant biomass and root development likely resulted in greater rhizodeposition, which favours saprotrophic and rhizosphere-competent microbes (de Vries and Caruso 2016; Zhelnina et al. 2018). Correlation analyses revealed that the abundance of saprotrophs was positively related to biomass production, whereas plant pathogens were negatively correlated, suggesting that microbial composition may directly affect plant performance through both nutrient mobilization and disease suppression (Bonanomi et al. 2018; Carrascosa-Robles et al. 2024).

PICRUSt2-based functional predictions indicated that slumgum treatments, particularly the raw solid (S), increased the abundance of genes involved in denitrification and carbon degradation. This may reflect microbial strategies to cope with the chemically complex and nutrient-rich nature of the raw residue (Jiménez et al. 2014; Krause et al. 2017). However, only TS significantly increased plant nitrogen content, and this was associated with greater abundance of *Rhizobium*, a genus with both nitrogen-fixing and plant growth-promoting properties (Adesemoye et al. 2010). This suggests that bio-transformed slumgum not only supports microbial activity but also promotes more efficient nitrogen use by the plant.

We acknowledge that this study's short duration (6 weeks) carried out in controlled conditions limits the direct application of our results to long-term field conditions (Bai et al. 2018). Therefore, further long-term trials are essential to validate our findings

to better understand the impacts of slumgum products on crop production and soil health.

However, slumgum, as a low-cost by-product of beekeeping, promises to be an affordable and sustainable alternative to synthetic fertilizers. Its transformation through microbial processes or liquid extraction could reduce input costs and improve soil health, potentially lowering farmers' reliance on agrochemicals.

Additionally, its viability as a commercial product needs to be further explored. Food production strongly relies on farmers inputs cost-effectiveness to be profitable enough (Qu et al. 2024; Alharbi and Ghonimy 2025). Therefore, an in-depth analysis of the production costs and scalability, fully nutritional composition, application systems costs, comparative benefits with other sustainable practices and market opportunities are needed (Qu et al. 2024; Shawky et al. 2025). By exploring these challenges, slumgum could be indeed proposed as a new alternative for sustainable agriculture.

Conclusions

To the best of our knowledge, this study highlights the potential of slumgum, a beekeeping by-product, either in its solid form or as a liquid extractor transformed with *Penicillium chrysogenum* as an effective organic soil amendment. Slumgum processed treatments (L, TS and TL) significantly enhanced purslane (*Portulaca oleracea*) biomass, foliar phosphorous content and overall stimulated soil enzymatic activities. Also, these treatments promoted beneficial shifts in the rhizosphere microbiota, increasing saprotrophic fungi or reducing pathogens, even withing a short 6-week period. In contrast, the untransformed solid slumgum (S) showed an overall limited performance, especially decreasing plant biomass.

Overall, the transformed slumgum products — especially TS— emerge as promising sustainable alternatives to synthetic fertilizers, with the added advantage of recycling bio-waste within a circular economy framework. However, these results are based on a short-term greenhouse trial. Long-term field studies across different crops and soils are needed to assess their broader agronomic and environmental impact, including effects on nutrient

cycling, microbial ecology. Economic analyses comparing slumgum-based amendments with conventional fertilizers would further clarify their viability as a cost-effective solution for sustainable agriculture.

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Data availability Data will be made available on request.

Declarations

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

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