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RECEIVED 16 June 2026

REVISED 25 July 2026

ACCEPTED 29 July 2026

PUBLISHED 27 August 2026

CITATION

Ureta Sierra C, Goicochea Silva P,
Chávez-Collantes A, Velarde-Apaza LD,
Campos-Ruiz J, Cuevas-Giménez JP,
Ramírez Maguiña HA and Solórzano R
(2026) Microbial inoculant-mediated
transformation of *Jarava ichu* biomass:
effects on nutrient availability and heavy
metal concentrations.
Front. Soil Sci. 6:1911253.
doi: 10.3389/fsoil.2026.1911253

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Microbial inoculant-mediated transformation of *Jarava ichu* biomass: effects on nutrient availability and heavy metal concentrations

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Introduction: *Jarava ichu* is an abundant grass species in high-Andean ecosystems but remains underutilised because its slow natural decomposition limits its potential as an organic soil amendment. Its accumulation may also contribute to the retention of heavy metals in soils, potentially affecting soil quality and agricultural productivity. This study evaluated the effects of microbial inoculants on the physicochemical and mineral composition of *J. ichu* biomass and their potential to reduce heavy-metal concentrations.

Methods: A 2 × 2 × 2 factorial experiment was conducted under a randomised complete block design (RCBD), comprising eight treatments, including a non-inoculated control. The microbial inoculants evaluated were *Beauveria bassiana*, *Trichoderma viride*, and EM-Compost. After 180 days of incubation, pH, electrical conductivity (EC), organic matter (OM), macronutrients (N, P, K, Ca, and Mg), and heavy metals (Ni, Cr, and Pb) were determined. Treatment and interaction effects were evaluated to identify changes in biomass composition and contaminant concentrations.

Results: *T. viride* and EM-Compost significantly increased EC to 53.57 and 56.83 mS·m⁻¹, respectively, and enhanced macronutrient availability, particularly P (4,455.24 and 4,462.60 mg·kg⁻¹, respectively) and Mg (3,630.33 and 3,706.39 mg·kg⁻¹, respectively). *T. viride* reduced Ni and Cr concentrations by 15.53% and 19.75%, respectively, whereas EM-Compost reduced Pb concentration by 43.98%. Among the interaction effects, the combination of *B. bassiana* and EM-Compost produced the highest K concentration (2,659.45 mg·kg⁻¹).

Discussion and conclusion: Microbial inoculation promoted substantial changes in the chemical composition of *J. ichu* biomass after 180 days, particularly by increasing nutrient availability and reducing selected heavy-metal concentrations. *T. viride* and EM-Compost showed the greatest potential for enhancing nutrient release and reducing contaminants, suggesting that microbial treatment may

improve the suitability of *J. ichu* biomass for subsequent valorisation as an organic soil amendment. Further studies should directly quantify decomposition rates, nutrient mineralisation, and the agronomic effects of the resulting biomass to confirm its potential for soil management.

KEYWORDS

biomass transformation, bioremediation, heavy metals, microbial processing, organic matter dynamics

1 Introduction

Jarava ichu is a dominant perennial grass native to high-Andean ecosystems (2,800–4,200 m.a.s.l.) that plays a crucial ecological role in soil conservation, water regulation, and the restoration of degraded lands (1, 2). Although historically utilized for livestock fodder, adobe bricks, and roofing, it has become an underutilized biomass with high potential for sustainable exploitation (3). Consequently, this species represents an abundant, underexploited natural resource, particularly in high-Andean regions where its wide availability remains untapped.

Furthermore, *J. ichu* populations often thrive in areas affected by informal mining, where soils accumulate heavy metals such as Pb, Ni, and Cr to levels above permissible limits (4). This contamination poses severe environmental risks and limits the direct reuse of its abundant biomass, prompting research into alternative value-added applications such as supplementary cementitious materials (5) or cultivation substrates for edible mushrooms of the genus *Pleurotus* (6, 7).

However, the potential of *J. ichu* as an organic soil amendment is severely restricted by its recalcitrant lignocellulosic composition, containing approximately 34% cellulose and 9.58% lignin (7). The highly crystalline structure and extensive hydrogen bonding within cellulose microfibrils impart considerable mechanical resistance and chemical stability, leading to slow natural decomposition and representing a major challenge for biological composting (8, 9).

Efficient degradation requires the coordinated action of diverse hydrolytic enzymes, including endoglucanases, exoglucanases, β -glucosidases, and xylanases (10). While single microbial strains often exhibit unstable performance due to limited survival, adaptation, and colonization capacities during organic matter (OM) decomposition (11), microbial consortia offer superior degradation efficiency through synergistic interactions that enhance overall enzymatic activity and substrate utilization rates (12, 13).

Among candidate decomposers, *Trichoderma viride* stands out for its robust secretion of hydrolytic enzymes—particularly endo- and exo- β -1,4-glucanases—that cleave complex polysaccharides into soluble molecules while suppressing competing microorganisms (14–16). Crucially, *Trichoderma* spp. possess advanced biosorption and intracellular accumulation mechanisms, enabling them to tolerate, immobilize, and remove multi-metal contaminants like Cr(VI), Cu, and Ni from degraded substrates (17–23).

At the same time, the fungus *Beauveria bassiana*, widely recognized for establishing endophytic and plant growth-promoting associations (24, 25), secretes a diverse array of industrial biocatalysts including chitinases (26), lipases (27), and proteases

(28). Furthermore, *B. bassiana* can transition into a facultative saprophyte following host senescence, colonizing dry plant residues by expressing carbohydrate-active enzymes (CAZymes), amylases, β -glucosidases, cellulases, and xylanases to utilize lignocellulosic carbon sources (29–32). Additionally, the application of Effective Microorganisms combined with compost (EM-Compost) accelerates the decomposition of OM by stimulating polymer-degrading enzymes while improving nutrient cycling and ecosystem resilience (33, 34).

Despite the substantial nutrient reserves of *J. ichu*, studies investigating its controlled biomass transformation—and the concurrent effects on nutrient dynamics and heavy metal concentrations—remain scarce (7). Although *J. ichu* is highly in high-Andean ecosystems, its application as an organic soil amendment is currently constrained by recalcitrant nature of its lignocellulosic structure, which severely limits natural decomposition and practical exploitation.

Therefore, there is an urgent need to identify and evaluate microbial strategies that promote the transformation of lignocellulosic materials, as indicated by changes in nutrient availability and reductions in contaminant concentrations. While microbial inoculation has been used to accelerate organic waste degradation, the comparative and interactive effects of fungal strains and microbial consortia on both nutrient release and heavy metal dynamics of this specific high-altitude grass have not yet been established.

To address this gap, this study evaluated the individual and combined effects of *B. bassiana*, *T. viride*, and EM-Compost on *J. ichu* degradation. It is hypothesized that the application of these microbial inoculants alters the transformation dynamics of *J. ichu* biomass, with individual treatments differentially enhancing macronutrient availability and heavy metal reduction, while their combinatorial interactions exert distinct influences on specific physicochemical parameters, depending on microbial compatibility.

2 Materials and methods

2.1 Experimental design

The experiment was conducted using a randomized complete block design (RCBD) with a full $2 \times 2 \times 2$ factorial arrangement. The three experimental factors corresponded to the microbial inoculants *B. bassiana*, *T. viride*, and EM-Compost, each evaluated at two levels: inoculated and non-inoculated (Figure 1). The experimental design comprised two blocks and three replicates per treatment, resulting in a total of 48 experimental units.

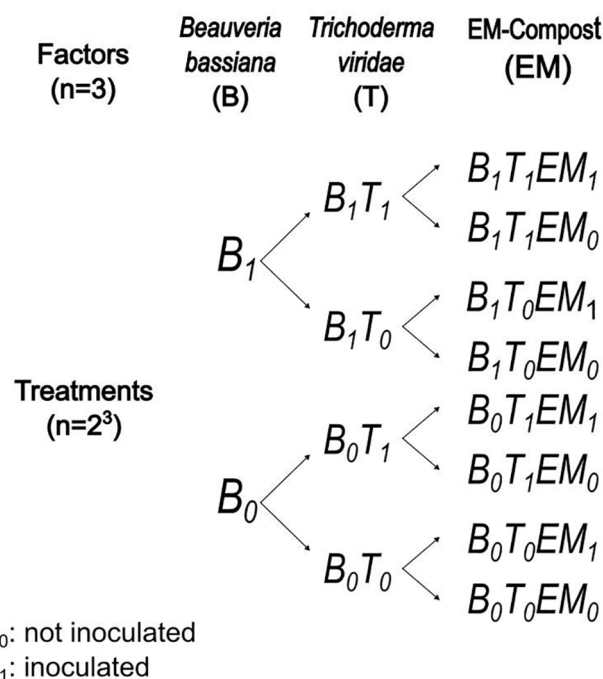


FIGURE 1

Factorial arrangement of the experimental factors and treatment combinations evaluated in the study.

Each experimental unit consisted of an independent composting pile. At the end of the 180-day incubation period, five 300 g subsamples were collected from different locations within each pile and thoroughly homogenized to obtain a single composite sample representative of that experimental unit. All physicochemical analyses were subsequently performed on these composite samples, with each composite sample corresponding to one independent experimental replicate.

2.2 Biological materials and experimental preparation

2.2.1 Biomass material

J. ichu plants were collected from the village of Porcón, located in the Cajamarca region of Peru. The aerial parts of the plants were harvested, cut into approximately 10cm segments, air-dried at room temperature ($25 \pm 2^\circ\text{C}$), and homogenized prior to use in the experiment. A voucher specimen of the collected material was deposited in the Truxillense Herbarium (HUT) of the National University of Trujillo under accession number 66115.

A total of 1,104 kg of *J. ichu* biomass was collected, with 23 kg allocated to each of the 48 experimental units to establish the composting piles. Seven days prior to inoculation, the piles were moistened to field capacity through flooding to facilitate microbial establishment and activity.

2.2.2 Inoculants

The volume of activated inoculum applied to each experimental unit was determined according to the experimental design and

treatment structure. Activated inoculant solutions were prepared from stock formulations following the manufacturers' technical specifications as follows: EM-Compost (1 L EM-Compost + 1 kg molasses + 10 L water), *T. viride* (800 g *T. viride* + 1 kg molasses + 10 L water), and *B. bassiana* (800 g *B. bassiana* + 1 kg molasses + 10 L water). The inoculation treatments were applied at the following rates per experimental unit: B₁T₀EM₀, 4 L of *B. bassiana*; B₀T₁EM₀, 4 L of *T. viride*; B₀T₀EM₁, 4 L of EM-Compost; B₁T₁EM₀, 2 L of *B. bassiana* + 2 L of *T. viride*; B₁T₀EM₁, 2 L of *B. bassiana* + 2 L of EM-Compost; B₀T₁EM₁, 2 L of *T. viride* + 2 L of EM-Compost; and B₁T₁EM₁, 1.3 L each of *B. bassiana*, *T. viride*, and EM-Compost. The control treatment (B₀T₀EM₀) received 4 L of water without microbial inoculation.

The microbial inoculants used in this study were *B. bassiana* and *T. viride*, applied as the commercial products BEAUVESOL[®] and TRICOMAX[®] (SOLUCIONES AGROSOSTENIBLES S.A.C.), respectively, each containing 1×10^{12} conidia·kg⁻¹. BEAUVESOL[®] is recognized for its effectiveness as an entomopathogenic agent and its potential as a plant growth-promoting microorganism, whereas TRICOMAX[®] is widely used for its antagonistic activity against soil-borne pathogens and its bio stimulant properties.

The inocula were prepared following the same spore-washing procedure. For each 800-g package of product, a solution containing 1 mL of agricultural surfactant per liter of water was prepared, after which the entire contents of the package were added and thoroughly agitated to detach the conidia from the maize substrate used as an inert carrier material.

The resulting spore suspension was filtered through a fine mesh to remove maize particles and prevent clogging of the backpack sprayer tank. Water was then added to adjust the final volume to 20 L, and the suspension was continuously stirred during both

preparation and application to ensure a homogeneous distribution of conidia.

EM•COMPOST®-BIOEM is a commercial inoculant composed of a consortium of effective microorganisms, including lactic acid bacteria (10^4 CFU·mL⁻¹), photosynthetic bacteria (10^3 CFU·mL⁻¹), yeasts (10^3 CFU·mL⁻¹), and various enzymatic compounds. The product was diluted 1:20 and is widely used as a soil bioactivator (35). Prior to application, the product was activated according to the manufacturer's recommendations. Briefly, 1 L of EM•COMPOST®-BIOEM was diluted in chlorine-free water to a final volume of 20 L and transferred to an airtight container. The container was sealed and stored in a cool, dry place for 7 days to allow microbial activation. Following the activation period, the resulting solution was applied to the *J. ichu* biomass.

2.3 Experimental conditions

The experiment was conducted under controlled conditions at the Pampa Grande Research Station of the National Institute of Agrarian Innovation (INIA), located in the province of Cajabamba, Cajamarca Region, Peru (8°23'38.89" S, 78°57'59.3" W; 2,635 m.a.s.l.). Each *J. ichu* pile was covered with a black polypropylene sheet and maintained at an average temperature of 20 ± 3 °C, a relative humidity of 65–70%, and in darkness throughout the experimental period. The experimental units consisted of pits measuring 1.0 m × 1.2 m and 0.5 m in depth. Chopped *J. ichu* biomass was placed in homogeneous layers within each pit, and the microbial inoculants were applied according to the established experimental design.

2.4 Application of experimental treatments

For each experimental unit, 11.5 kg of moistened *J. ichu* biomass was arranged in successive layers approximately 10 cm thick. The corresponding inoculum was applied uniformly to each layer to ensure adequate distribution of the microorganisms throughout the biomass. This procedure was repeated until all the plant material had been incorporated into the pile. Subsequently, the piles were covered with black polyethylene sheeting to maintain optimal temperature and moisture conditions and to protect the inoculated microorganisms from direct solar radiation, which may negatively affect their viability and activity (36). The control treatment did not receive any microbial inoculation.

Monitoring of the *J. ichu* biomass began one week after inoculation to ensure that optimal conditions for microbial activity and substrate decomposition were maintained. This process included weekly assessments of substrate moisture content and regular turning of the piles to promote adequate aeration. Moisture content was evaluated using the manual “fist test,” which involves squeezing a handful of the material to assess its moisture content. The appearance of water droplets upon compression indicates excessive moisture, whereas the inability of the material to retain its shape after the hand is opened indicates insufficient moisture content (37). These procedures were performed throughout the seven-month experiment to ensure optimal substrate moisture conditions were maintained.

Although direct measurements of biodegradation kinetics (e.g., mass loss, CO₂ evolution, or enzymatic activity) were not conducted, the stabilization of internal temperature near ambient levels and the observed changes in physicochemical properties were used as indirect indicators of OM transformation.

Upon completion of the transformation process, as indicated by the stabilization of the internal biomass temperature at values closer to ambient temperature (approximately 22 °C), samples were collected for microbiological analysis. A total of 500 g of biomass was obtained from each treatment by randomly sampling the three replicates. Samples were collected from the surface to a depth of 10 cm to ensure representative sampling of the biodegraded material.

2.5 Measured variables and methods of analysis

2.5.1 Physico-chemical analysis

Substrate pH was measured potentiometrically in a 1:2.5 substrate-to-water suspension following EPA Method 9045. EC was determined in the corresponding extract. OM content was quantified using the Walkley–Black method and total N was determined by the Kjeldahl method. Available P was measured using the Bray and Kurtz method.

2.5.2 Exchangeable bases

Exchangeable K, Ca and Mg were determined by ammonium acetate extraction. These analyses followed the procedures established in the Mexican Official Standard NOM-021-REC/NAT-2000 (38).

2.5.3 Heavy metal analysis

Ni, Cr, and Pb concentrations were determined by atomic absorption spectrometry (AAS) following acid digestion with HNO₃ and HCl, in accordance with EPA Method 3050B (39).

2.6 Statistical analysis

Data were analyzed using R software v4.5.1 within the RStudio environment. The assumptions of normality and homogeneity of variances were evaluated using the Shapiro–Wilk and Levene tests, respectively. A factorial analysis of variance (ANOVA) was then performed to assess the main effects of the inoculants and their interactions. When significant differences were detected, means were compared using the Tukey test. All statistical analyses were conducted using the *agricolae* package (40) and base R functions.

3 Results and discussion

3.1 Chemical properties and OM

Factorial analysis of variance revealed that *B. bassiana* exerted significant main effects on substrate pH ($p < 0.01$) and electrical conductivity (EC; $p < 0.05$), resulting in slight increases in both

TABLE 1 Analysis of variance of the chemical properties and OM content of *J. ichu* biomass following transformation by microbial inoculants.

Factors	pH	EC	OM
Main effects			
<i>B. bassiana</i> (B)	***	*	n. s.
<i>T. viride</i> (T)	n. s.	n. s.	n. s.
EM-Compost (EM)	n. s.	n. s.	n. s.
Interaction effects			
B*T	n. s.	n. s.	n. s.
T*EM	n. s.	n. s.	n. s.
B*EM	n. s.	***	**
B*T*EM	n. s.	***	**

EC, electrical conductivity; OM, organic matter. *n.s.*, not significant. Significance levels: ****p* < 0.001; ***p* < 0.01; and **p* < 0.05.

parameters. Likewise, *T. viride* showed a significant individual effect on EC. Significant interaction effects were also detected for several properties. The interaction between *B. bassiana* and EM-Compost significantly affected EC (*p* < 0.01) and organic matter (OM) content (*p* < 0.05), while the three-way interaction among *B. bassiana*, *T. viride*, and EM-Compost likewise influenced EC (*p* < 0.01) and OM (*p* < 0.05) but had no significant effect on pH. These findings indicate that *B. bassiana* was the primary driver of pH changes, whereas its interactions with the other inoculants, particularly EM-Compost, contributed to changes in EC and organic matter content during the processing of *J. ichu* biomass (Table 1). Regarding OM, significant differences were observed among treatments relative to the control. The highest OM contents were recorded in the treatments inoculated solely with *T. viride* and EM-Compost (Figure 2, Table 2). The effect of *T. viride* is likely attributable to its ability to produce enzymes that degrade cellulose and lignin, as suggested by Ghorbani et al. (41). In contrast, treatments involving *B. bassiana*, either alone or in combination with the other inoculants, did not differ significantly from the control. These results suggest that *B. bassiana* had a limited effect on OM degradation under the conditions evaluated

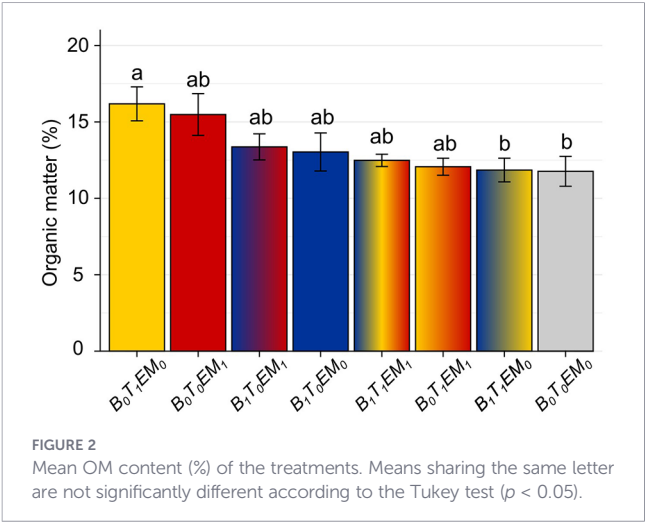
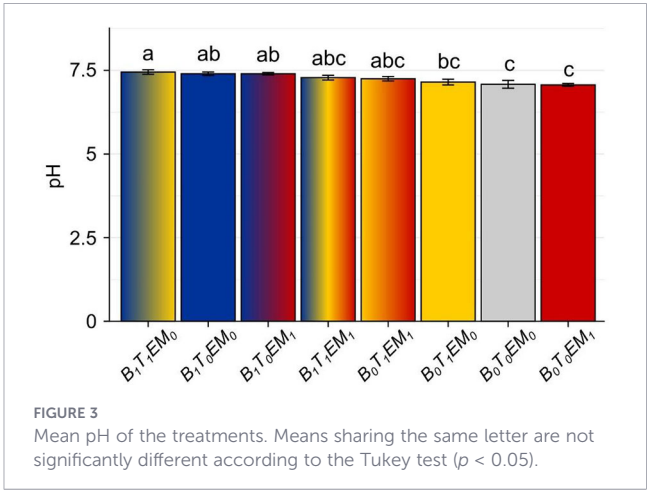


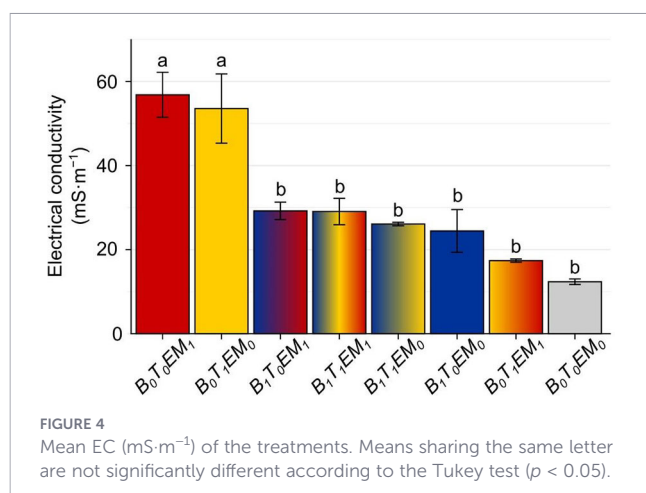
TABLE 2 Chemical properties and OM content of *J. ichu* biomass following transformation by microbial inoculants.

Treatments	pH	EC (mS·m ⁻¹)	OM (%)
B ₁ T ₀ EM ₀	7.4 ab	24.45 b	13.03 ab
B ₀ T ₁ EM ₀	7.15 bc	53.57 a	16.18 a
B ₀ T ₀ EM ₁	7.07 c	56.83 a	15.48 ab
B ₁ T ₁ EM ₀	7.45 a	26.08 b	11.85 b
B ₀ T ₁ EM ₁	7.4 ab	29.22 b	12.07 ab
B ₁ T ₀ EM ₁	7.25 ab	17.4 b	13.37 ab
B ₁ T ₁ EM ₁	7.28 abc	29.07 b	12.48 ab
B ₀ T ₀ EM ₀ (Control)	7.08 c	12.37 b	11.77 b

EC, electrical conductivity; OM, organic matter. Treatment codes indicate the presence (1) or absence (0) of each inoculant: B, *Beauveria bassiana*; T, *Trichoderma viride*; EM, EM-Compost. Means followed by the same lowercase letter within a column are not significantly different according to Tukey's honestly significant difference (HSD) test (*p* < 0.05).

and may have attenuated the decomposition process when combined with other microbial inoculants. However, it should be noted that higher residual OM content does not necessarily indicate greater decomposition; rather, it may reflect differences in the rate of OM mineralization or the preservation of recalcitrant fractions. Future studies should include direct measurements of mass loss and enzymatic activity to confirm degradation rates. With respect to pH, significant differences relative to the control were observed only in treatments inoculated with *B. bassiana*, *T. viride*, or their combination (Figure 3). Among these treatments, the interaction between *B. bassiana* and *T. viride* produced the greatest change in pH. The relatively small pH variations observed in treatments inoculated with *T. viride* may be explained by the slow degradation of recalcitrant organic compounds. Changes in pH are closely influenced by physiological adaptations involved in the decomposition of specific fractions of OM; therefore, in substrates rich in lignocellulosic compounds, biodegradation and the accumulation of degradation by-products occur gradually, resulting in only minor pH fluctuations over time (42). According to Solórzano et al. (7), high-Andean *J. ichu* contains approximately 34% cellulose and 9.58% lignin, which likely





contributed to the limited pH changes observed during biodegradation. Furthermore, the results suggest that the degradation of these compounds was not constrained by pH conditions, as *Trichoderma* spp. have been shown to degrade lignin effectively across a broad pH range (4.0–10.0) (43, 44).

Regarding EC, the highest values were observed in the treatments inoculated solely with EM-Compost and *T. viride*, both of which differed significantly from the remaining treatments, including the control (Figure 4). Moreover, none of the inoculation treatments reduced EC relative to the control. The increase in EC is likely associated with the release of soluble ions during the decomposition of OM and the mineralization of nutrients.

These effects may have been favored by the relatively stable pH, which remained near neutral throughout the transformation of *J. ichu* biomass, thereby promoting microbial activity and nutrient transformation.

3.2 Macronutrient content

The initial chemical composition of the *J. ichu* biomass includes the following values: C $1997.695\text{mg}\cdot\text{g}^{-1}$; S $0.17\text{mg}\cdot\text{g}^{-1}$; B $7.32\text{mg}\cdot\text{g}^{-1}$; Ca $0.127\text{mg}\cdot\text{g}^{-1}$; P $0.194\text{mg}\cdot\text{g}^{-1}$; Fe $39.03\text{mg}\cdot\text{g}^{-1}$; Mg $0.059\text{mg}\cdot\text{g}^{-1}$; Mn $253.16\text{mg}\cdot\text{g}^{-1}$; K $0.587\text{mg}\cdot\text{g}^{-1}$; Na $65.82\text{mg}\cdot\text{g}^{-1}$; Zn $9.79\text{mg}\cdot\text{g}^{-1}$; Total N $0.69\text{mg}\cdot\text{g}^{-1}$.

Factorial analysis of variance revealed that *B. bassiana* exerted significant main effects on macronutrient concentrations, resulting in higher P ($p < 0.05$), K ($p < 0.01$), and Ca ($p < 0.01$) contents, while having no significant effect on N or Mg. *T. viride* significantly affected only K concentrations ($p < 0.05$), whereas EM-Compost showed a pronounced effect on K release ($p < 0.01$). Several interaction effects were also significant.

In particular, the interaction between *B. bassiana* and EM-Compost significantly influenced all macronutrients ($p < 0.01$), indicating a suppressive effect on nutrient accumulation. Likewise, the three-way interaction among *B. bassiana*, *T. viride*, and EM-Compost had significant effects on macronutrient concentrations ($p < 0.01$).

These findings suggest that although individual inoculants enhanced the availability of specific nutrients, certain microbial combinations may have reduced nutrient accumulation through

TABLE 3 Analysis of variance of macronutrient concentrations in *J. ichu* biomass following transformation by microbial inoculants.

Factors	N	P	K	Ca	Mg
Main effects					
<i>B. bassiana</i> (B)	n. s.	*	***	**	n. s.
<i>T. viride</i> (T)	n. s.	n. s.	*	n. s.	n. s.
EM-Compost (EM)	n. s.	n. s.	***	n. s.	n. s.
Interaction effects					
B*T	n. s.	n. s.	*	n. s.	n. s.
T*EM	n. s.	n. s.	***	n. s.	n. s.
B*EM	***	***	***	***	***
B*T*EM	***	***	***	***	***

N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium; n.s., not significant. Asterisks indicate the level of statistical significance: *** $p < 0.001$; ** $p < 0.01$; and * $p < 0.05$.

antagonistic interactions during the transformation of *J. ichu* biomass (Table 3).

The Tukey *post hoc* test indicated that microbial inoculation generally enhanced macronutrient concentrations compared with the uninoculated control, particularly for N and K. Nevertheless, the magnitude of the response varied among treatments, and only specific microorganisms and their combinations resulted in significantly higher nutrient concentrations (Table 3).

Although *T. viride* and EM-Compost did not exhibit significant main effects on most macronutrients, with the exception of K, the *post hoc* analysis revealed that treatments B₀T₁EM₀ and B₀T₀EM₁ (corresponding to the pure inoculations of *T. viride* and EM-Compost, respectively) were associated with the concentrations of most macronutrients and produced the highest mean values. In particular, these treatments resulted in significantly higher P and Mg concentrations than the other treatment combinations. In contrast, the B₁T₀EM₁ treatment (*B. bassiana* + EM-Compost) did not achieve a similar response and was statistically inferior to most other inoculated treatments. This combination produced the second-lowest macronutrient concentrations, outperforming only the uninoculated control (Table 4).

Regarding the individual factor, *B. bassiana* had significant main effects on most macronutrients, except for N and Mg. The *post hoc* analysis further revealed that inoculation with *B. bassiana* alone resulted in higher macronutrient concentrations in the biofertilizer derived from *J. ichu* than in the uninoculated control. However, its effectiveness was lower than that observed for the pure *T. viride* and EM-Compost treatments. Likewise, when combined with the other microorganisms, *B. bassiana* tended to attenuate the positive effects of these microorganisms on nutrient accumulation. For example, the B₀T₀EM₁ and B₀T₁EM₀ treatments yielded P concentrations of 4462.6 and 4455.25 $\text{mg}\cdot\text{kg}^{-1}$, respectively. In contrast, the addition of *B. bassiana* to these treatments (B₁T₀EM₁ and B₀T₁EM₁) resulted in significantly lower P concentrations of 2047.67 and 2416.24 $\text{mg}\cdot\text{kg}^{-1}$, respectively. These values were statistically lower than those obtained with the corresponding single-inoculant treatments, suggesting that the presence of *B. bassiana* may have exerted an antagonistic effect on nutrient release during transformation of *J. ichu* biomass.

TABLE 4 Macronutrient concentrations in *J. ichu* biomass following transformation by microbial inoculants.

Treatments	N (mg·g ⁻¹)	P (mg·kg ⁻¹)	K (mg·kg ⁻¹)	Ca (mg·kg ⁻¹)	Mg (mg·kg ⁻¹)
B ₁ T ₀ EM ₀	8.45 ab	2407.97 b	2146.39 d	12321.98 bc	2205.18 b
B ₀ T ₁ EM ₀	11.10 a	4455.24 a	2489.30 bc	14371.79 a	3630.33 a
B ₀ T ₀ EM ₁	11.23 a	4462.60 a	2876.40 a	14125.20 ab	3706.39 a
B ₁ T ₁ EM ₀	8.95 ab	2047.67 bc	2485.37 bc	13019.01 abc	2439.99 b
B ₀ T ₁ EM ₁	7.58 bc	1569.14 bc	2115.24 d	9555.72 d	1595.74 bc
B ₁ T ₀ EM ₁	8.00 bc	2416.24 b	2659.45 ab	12180.45 c	2341.00 b
B ₁ T ₁ EM ₁	9.08 ab	2118.36 bc	2313.91 cd	12679.45 abc	2242.69 b
B ₀ T ₀ EM ₀ (Control)	5.40 c	670.81 c	1292.89 e	8827.18 d	1171.11 c

N, nitrogen; P, phosphorus; K, potassium; Ca, calcium; Mg, magnesium. Treatment codes indicate the presence (1) or absence (0) of each inoculant: B, *Beauveria bassiana*; T, *Trichoderma viride*; EM, EM-Compost. Means followed by the same lowercase letter within a column are not significantly different according to Tukey's honestly significant difference (HSD) test ($p < 0.05$).

An exception to this trend was observed for K concentrations. The B₁T₀EM₁ treatment (*B. bassiana* + EM-Compost) resulted in a K concentration of 2,659.45 mg·kg⁻¹, which was comparable to that achieved by the EM-Compost treatment alone (2,876.40 mg·kg⁻¹). Both treatments produced the highest K concentrations among all treatments evaluated and did not differ significantly from one another. These findings suggest that, unlike its generally suppressive effect on the accumulation of other macronutrients, the presence of *B. bassiana* did not negatively affect K accumulation when combined with EM-Compost.

Furthermore, significant interaction effects on all macronutrients were detected for the *B. bassiana* × EM-Compost combination (B₁T₀EM₁) and for the three-way interaction among *B. bassiana*, *T. viride*, and EM-Compost ($p < 0.01$). For K concentrations, all microbial interactions had significant effects. The B₁T₀EM₁ treatment exhibited a suppressive effect on macronutrient accumulation relative to the EM-Compost treatment alone. Although EM-Compost as a single inoculant produced the highest concentrations of N (11.23 mg·kg⁻¹), P (4,462.60 mg·kg⁻¹), K (2,876.40 mg·kg⁻¹), Ca (14,125.20 mg·kg⁻¹), and Mg (3,706.39 mg·kg⁻¹), the addition of *B. bassiana* (B₁T₀EM₁) significantly reduced the concentrations of these nutrients to levels statistically comparable to those observed in the *B. bassiana*-only treatment (B₁T₀EM₀). Nevertheless, both treatments maintained significantly higher nutrient concentrations than the uninoculated control, indicating that, despite its attenuating effect, *B. bassiana* still contributed to greater nutrient accumulation than no inoculation.

Regarding the combination of all three microorganisms, no synergistic effect was observed. The three-way inoculation treatment (B₁T₁EM₁) did not outperform the other treatments and was significantly inferior to the single-inoculant treatments of EM-Compost and *T. viride* in P and Mg concentrations. Moreover, for the remaining macronutrients, the three-way treatment consistently produced lower mean values than those obtained with either EM-Compost or *T. viride* alone. These results indicate that combining all three microorganisms did not enhance nutrient accumulation and may have generated antagonistic interactions that limited the positive effects observed in the single-inoculant treatments.

Several studies have reported the capacity of *Trichoderma* spp. to enhance nutrient concentrations in solid-state fermentation systems. For instance, Pertiwinigrum et al. (45) observed higher

N, P, and K concentrations following inoculation with *Trichoderma harzianum*, with the most pronounced effect in phosphorus, which was higher in the inoculated treatment (1.1%) than in the control (0.1%).

Similarly, the results of the present study demonstrate *T. viride*'s ability to effectively degrade *J. ichu*, despite its high lignin content and elevated C/N ratio. Previous research has shown that the final nutrient concentrations of solid fermentates produced by *Trichoderma* spp. are strongly influenced by substrate composition. For example, limited colonization has been reported in wood-sawdust-based substrates (46), while low concentrations of N, P, K, and Ca have been observed in fermentates derived from cocoa husks without nutrient supplementation (47).

The present study provides novel insights into the potential role of *B. bassiana* in biofertilizer production. Although this fungus is widely recognized as a biological control agent against arthropod pests in agricultural systems, most research has focused on conidial production (48, 49) and the characterization of secondary metabolites with insecticidal and fungicidal properties (50, 51).

In contrast, its lignocellulolytic capacity remains relatively unexplored. Nevertheless, previous studies have demonstrated that *B. bassiana* can colonize and grow on substrates with high C/N ratios, such as rice husks (52), suggesting a potential role in the biodegradation of lignocellulosic residues. Further research is therefore required to optimize the growth conditions of *B. bassiana*, elucidate the mechanisms underlying its role in OM degradation, and identify strains with enhanced potential for biofertilizer production. These strains may also exhibit biopesticidal activity, offering the potential to develop integrated products that combine nutrient enhancement with biological pest control.

Furthermore, the effective microorganisms (EM-Compost) demonstrated a strong capacity to produce high-quality biofertilizer. These findings are consistent with those of Yanqoritha (53), who reported that the combined activity of lactic acid bacteria and yeasts significantly increased the N and P contents of compost derived from plant residues. However, unlike the results obtained in the present study, inoculation with *Trichoderma* in that investigation resulted in lower nutrient concentrations than those observed in the uninoculated control.

Similarly, Nair and Okamitsu (54) concluded that the concentrations of nitrate, nitrite, and orthophosphate in compost are not

necessarily enhanced by the addition of effective microorganisms alone; rather, nutrient dynamics are influenced by multiple factors, including composting method, temperature, substrate composition, and microbial interactions. The high macronutrient concentrations observed in the EM-Compost treatment in the present study therefore suggest that the effectiveness of EM formulations is highly dependent on substrate characteristics and the environmental conditions under which *J. ichu* biomass is transformed.

In the present study, combining microbial inoculants did not necessarily show higher macronutrient concentrations. This lack of synergy suggests microbial antagonism and competitive exclusion within the substrate matrix, which strongly aligns with the baseline mechanisms of interspecific competition documented in the literature. In ecological consortia, when distinct fungal strains (such as *T. viride* and *B. bassiana*) are introduced alongside complex bacterial-fungal mixtures like EM-Compost, they often compete aggressively for limited spatial niches and readily available carbon sources. This aggressive behavior is triggered by mixed cultures, which inevitably disrupt the microenvironment's metabolic equilibrium (55).

Furthermore, the results confirm that *Trichoderma* spp. are well known for their rapid growth rates and high efficiency in producing cellulolytic enzymes, which may have led to the competitive suppression of *B. bassiana* and other microorganisms within the EM consortium. This enzymatic supremacy and swift hyphal extension allow *Trichoderma* to monopolize space and carbon, effectively arresting the functional and metabolic activities of weaker or slower-growing co-inhabitants within the substrate (56, 57).

Likewise, *B. bassiana* has been reported to exhibit antagonistic activity against a wide range of plant pathogens (58, 59), suggesting that it may also inhibit the activity of other beneficial microorganisms. These interactions could explain the reduced nutrient accumulation observed in several combined inoculation treatments. Therefore, evaluating the compatibility of microbial strains prior to their use as biodegradation or fermentation accelerators is essential.

Indeed, although numerous studies have shown that microbial consortia often outperform single inoculants in the degradation of lignocellulosic materials (13), the integration of multiple aggressive strains in this specific matrix induced critical metabolic friction rather than collaborative synergy, ultimately reducing the overall bioremediative and mineralization efficiency of the combined bioprocess. The present findings highlight the importance of prior characterization in identifying and avoiding antagonistic interactions that may compromise process efficiency.

3.3 Heavy metal content

Factorial analysis of variance revealed that *T. viride* had significant main effects on Ni ($p < 0.01$) and Cr ($p < 0.01$) concentrations, whereas EM-Compost had significant effects on Cr ($p < 0.05$) and Pb ($p < 0.05$) levels. Notably, Ni was one of the few variables evaluated, together with pH, for which a significant response was attributable solely to the main effect of a single inoculant. In this case, only *T. viride* significantly influenced Ni

concentrations, while neither the other inoculants nor their interactions produced significant effects. These results highlight the specific capacity of *T. viride* to influence nickel dynamics during the transformation of *J. ichu* biomass.

Regarding the other factor, *B. bassiana* did not exert significant main effects on any of the heavy metals evaluated. However, the interaction between *B. bassiana* and EM-Compost had highly significant effects on the reduction of Cr ($p < 0.01$) and Pb ($p < 0.01$), suggesting a synergistic interaction between these inoculants related to heavy metal remediation. In contrast, the remaining interactions, including the three-way combination of *B. bassiana*, *T. viride*, and EM-Compost, did not significantly affect heavy metal concentrations. These findings highlight the potential of *T. viride* and EM-Compost as bioremediation agents for heavy metal-contaminated soils.

Both inoculants were effective when applied individually, and EM-Compost also showed enhanced performance when combined with *B. bassiana*, particularly in reducing Cr and Pb concentrations (Table 5).

Some microbial inoculants applied to *J. ichu* reduced measurable heavy metal concentrations (Table 6). In particular, treatments inoculated with *T. viride* were associated with lower measurable concentrations of Ni and Cr, reducing Ni by 15.53% (6.89 to 5.82 mg·kg⁻¹, $p < 0.01$) and Cr by 19.75% (20.51 to 16.46 mg·kg⁻¹, $p < 0.01$), on average. Likewise, EM-Compost was associated with a 43.98% reduction in the measured Pb concentration (from 6.39 to 3.58 mg·kg⁻¹, $p < 0.05$).

These reductions in measurable heavy metal concentrations may be attributed to the diverse metal sequestration mechanisms exhibited by microorganisms, including biosorption and bioaccumulation (60, 61). However, the effectiveness of these remediation processes likely depends on several environmental factors, including pH, temperature, contact time, and ionic strength (62).

In the present study, the degraded *J. ichu* biomass contained measurable concentrations of Ni, Cr, and Pb. The presence of these metals can be attributed to the species' ability to grow in contaminated environments and its recognized potential for

TABLE 5 Analysis of variance of heavy metal concentrations in *J. ichu* biomass following transformation by microbial inoculants.

Factor	Ni	Cr	Pb
Main effects			
<i>B. bassiana</i> (B)	n. s.	n. s.	n. s.
<i>T. viride</i> (T)	**	**	n. s.
EM-Compost (EM)	n. s.	*	*
Interaction effects			
B*T	n. s.	n. s.	n. s.
T*EM	n. s.	n. s.	n. s.
B*EM	n. s.	**	**
B*T*EM	n. s.	n. s.	n. s.

Ni, nickel; Cr, chromium; Pb, lead; n.s., not significant. Asterisks indicate the level of statistical significance: *** $p < 0.001$; ** $p < 0.01$; and * $p < 0.05$.

TABLE 6 Heavy metal concentrations in *J. ichu* biomass following transformation by microbial inoculants.

Treatment	Ni (mg·kg ⁻¹)	Cr (mg·kg ⁻¹)	Pb (mg·kg ⁻¹)
B ₁ T ₀ EM ₀	7.15	20.95 ab	7.15 a
B ₀ T ₁ EM ₀	5.82	16.46 b	4.49 ab
B ₀ T ₀ EM ₁	7.24	23.04 a	3.58 b
B ₁ T ₁ EM ₀	5.16	19.81 ab	4.50 ab
B ₀ T ₁ EM ₁	6.82	19.29 ab	4.66 ab
B ₁ T ₀ EM ₁	5.16	24.60 a	4.49 ab
B ₁ T ₁ EM ₁	5.74	20.46 ab	5.24 ab
B ₀ T ₀ EM ₀ (Control)	6.89	20.51 ab	6.39 ab

Ni, nickel; Cr, chromium; Pb, lead. Treatment codes indicate the presence (1) or absence (0) of each inoculant: B, *Beauveria bassiana*; T, *Trichoderma viride*; EM, EM-Compost. Means followed by the same lowercase letter within a column are not significantly different according to Tukey's honestly significant difference (HSD) test ($p < 0.05$).

phytoremediation. *J. ichu* is well adapted to high-Andean ecosystems and exhibits tolerance to the adverse effects of heavy metal contamination, enabling it to accumulate metals from the soil in its tissues (63). Consequently, biomass harvested from contaminated areas may contain elevated concentrations of heavy metals, which can subsequently be detected during biomass transformation.

Several fungal species have been used in mycoremediation, potentially because they produce a wide range of enzymes, including laccases, peroxidases, and hydrolases, which enable the degradation of complex organic compounds, xenobiotics, and, in some cases, facilitate the transformation or immobilization of heavy metals into less harmful forms (64).

Among these fungi, *Trichoderma* spp. have shown considerable potential for heavy-metal bioremediation, likely due to their ability to secrete organic acids that promote metal chelation and the formation of metal oxalates or phosphates, thereby reducing metal mobility and bioavailability (65). These organic acids include citric and oxalic acids. Citric acid, in particular, can form stable complexes with metals such as Pb, Cd, and Al, thereby decreasing their bioavailability and toxicity (66).

Additionally, these microorganisms potentially possess the ability to bind heavy metals to their cell walls through ion exchange, complex formation, and chelation, owing to the presence of functional groups such as carboxyl, amino, and hydroxyl groups (65–67). They are also known to take up and internalize metal ions into their cells, where the metals may be sequestered within vacuoles or bound to intracellular proteins. For example, *Trichoderma* spp. have been reported to bioaccumulate substantial amounts of Cu and Zn, thereby contributing to the removal or immobilization of these metals in contaminated soils (65). These mechanisms may explain the significant reductions in Ni and Cr concentrations observed in the *T. viride* treatments in the present study.

Another mechanism by which fungi may contribute to the transformation and detoxification of contaminants is through the well-known mediation of redox reactions. These processes involve the transfer of electrons between fungal cells and contaminant molecules, resulting in the oxidation or reduction of the pollutants.

In the case of heavy metals, such transformations can substantially reduce toxicity and environmental mobility.

For example, fungi have been shown to reduce hexavalent Cr (VI), a highly toxic and mobile form of Cr, to trivalent Cr(III), which is considerably less toxic and more readily removed from the environment (66). Such redox-mediated transformations represent an important mechanism of fungal bioremediation and may have contributed to the reductions in Cr concentrations observed in the present study. Nevertheless, further research is required to elucidate the specific mechanisms employed by *T. viride*, *B. bassiana*, and EM-Compost to transform *J. ichu* and remediate heavy metals.

Although the present study demonstrated significant effects of microbial inoculation on nutrient availability and heavy metal dynamics after 180 days of *J. ichu* biomass transformation, the experimental design did not include temporal monitoring of inoculant establishment, survival, colonization, or population dynamics throughout the composting process. Consequently, the observed responses cannot be attributed exclusively to the inoculated microorganisms, as they may also have been influenced by the natural succession of indigenous microbial communities and by environmental factors operating during transformation.

Therefore, the results should be interpreted as evidence of changes in substrate composition following microbial inoculation rather than as definitive proof of enhanced transformation rates. Future studies should integrate microbiological monitoring approaches, including culture-dependent methods, quantitative PCR, metabarcoding, or metagenomic analyses, together with repeated temporal sampling, to verify inoculant persistence, characterize microbial succession, and establish stronger mechanistic links between microbial community dynamics and the physicochemical transformations observed during the transformation of lignocellulosic biomass.

4 Conclusions

The findings of this study indicate that *B. bassiana*, *T. viride*, and EM-Compost are associated with significant compositional changes in *J. ichu* biomass, including increased macronutrient concentrations and reduced heavy metal levels. Under the specific experimental parameters evaluated, EM-Compost and *T. viride* appeared to be the most effective treatments for increasing the concentrations of essential macronutrients (N, P, K, Ca, and Mg), whereas *B. bassiana* had a minor effect, primarily altering the substrate pH.

Regarding bioremediation potential, *T. viride* demonstrated high efficacy in reducing Ni and Cr concentrations, while EM-Compost showed the greatest capacity to decrease Pb levels. Furthermore, combinations involving *B. bassiana* generally attenuated the positive effects observed in single-inoculant treatments, resulting in lower macronutrient concentrations, OM content, and EC, which highlights the critical importance of evaluating microbial compatibility and antagonistic interactions prior to consortium formulation.

While these results suggest that specific microbial inoculants—particularly *T. viride* and EM-Compost—show potential for heavy-

metal immobilization and nutrient enhancement in lignocellulosic residues, they must be interpreted within the limitations of the current experimental design. Because the persistence and temporal establishment of the inoculated strains were not monitored longitudinally, the underlying biochemical mechanisms cannot be unequivocally attributed solely to the introduced microorganisms, as interactions with the baseline native microbiota may have occurred.

Consequently, further field-based research and detailed microbiological analyses are required to validate the stability, long-term agronomic performance, and ecological impacts of these inoculants on native soil communities under diverse agricultural conditions. Ultimately, while this study provides a baseline for the potential valorization of heavy-metal-exposed *J. ichu* biomass, scaling these strategies requires careful validation to confirm their viability and safety in high-Andean agroecosystems.

While the observed changes in physicochemical properties suggest microbial-mediated transformation of the biomass, direct measurements of biodegradation kinetics (e.g., mass loss, carbon mineralization, or enzymatic activities) are needed to definitively confirm accelerated decomposition. The results presented here should therefore be interpreted as evidence of treatment-associated compositional changes rather than unequivocal proof of enhanced biodegradation rates.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

CU: Investigation, Writing – original draft, Writing – review & editing. PG: Investigation, Writing – original draft. AC-C: Conceptualization, Methodology, Supervision, Writing – review & editing. LV-A: Investigation, Supervision, Writing – review & editing. JC: Writing – original draft. JC-G: Writing – review & editing. HR: Data curation, Formal Analysis, Writing – review & editing. RS: Conceptualization, Formal Analysis, Methodology, Writing – review & editing.

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Funding

The author(s) declared that financial support was received for this work and/or its publication. The research was funded by the INIA Project “Mejoramiento de los servicios de investigación y transferencia tecnológica en el manejo y recuperación de suelos agrícolas degradados y aguas para riego en la pequeña y mediana agricultura en los departamentos de Lima, Áncash, San Martín, Cajamarca, Lambayeque, Junín, Ayacucho, Arequipa, Puno, y Ucayali” CUI 2487112.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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