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RESEARCH

Digestate from psychrophilic anaerobic digestion reshapes microbial communities and facilitates the recovery of degraded acidic soils under field conditions

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Abstract

Soil degradation constitutes a primary constraint to global ecosystem services, making restoration a critical objective aligned with the United Nations Sustainable Development Goals 13 and 15. While anaerobic digestate is increasingly recognized as a promising amendment for restoring degraded soils within regenerative agriculture and circular bioeconomy frameworks, the mechanistic relationships between digestate quality and soil microbial restructuring remains insufficiently characterized. In this context, the objective of this study was to assess how psychrophilic digestate application influences soil physicochemical properties and microbial community dynamics, and to explore its role in the recovery of degraded soil under field conditions. Results revealed that alleviation of soil chemical stress, primarily associated with acidity, exchangeable Al toxicity, and nutrient imbalance was the primary regulatory factor. The input of exchangeable base cations, coming from digestate, drove a critical increase in pH, mitigating Al toxicity and unlocking P reserves. The observed soil recovery may be associated with the organic matter inputs from the digestate, which can support microbial activity and contribute to improved soil functioning. This was supported by an observed shift in the main soil microbial groups, from Gram-negative to Gram-positive bacteria, which are often associated with higher carbon use efficiency. These findings provided a detailed field-based framework supporting the use of digestate from low-tech digesters working under psychrophilic conditions as a strategic amendment for improving soil chemistry and microbial community composition, while enhancing soil organic C retention. Furthermore, it may offer a viable solution for digestate management aligned with regenerative agriculture and circular bioeconomy goals.

Keywords Biochemical soil fertility · Circular bioeconomy · Regenerative agriculture · Soil microbial community · Soil organic carbon · Soil restoration

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Introduction

Soil degradation, characterized by organic matter depletion, acidification, structural deterioration, and loss of microbial function, is a major constraint on ecosystem functioning in intensively managed or mismanaged landscapes (Ferreira et al. 2022; Sinatra et al. 2024). Declines in soil organic matter (SOM) disrupt nutrient stoichiometry, reduce microbial C-use efficiency (CUE), and limit the formation of mineral-associated organic matter (MAOM), thereby impairing long-term C sequestration and biological fertility (Lal et al. 2021). Restoring SOM quality and re-establishing a diverse, metabolically active microbial community are central goals for local rehabilitation. These efforts are also critical for achieving the United Nations Sustainable Development Goals (SDGs). In particular, they support Climate Action (SDG 13) and Land Degradation Neutrality (SDG 15) (Lal et al. 2021).

Digestate has emerged as a promising amendment within regenerative agriculture and circular bioeconomy frameworks (Schulte et al. 2022), supporting SDG 12 (Responsible Consumption and Production). It is especially relevant in rural areas where the contextual production of energy (biogas) and organic amendment improve living conditions (Ziegler-Rodriguez et al. 2025). Apart from producing valuable products, anaerobic digestion improves the sustainability of animal manure management, which in rural areas is often disposed of on soil or into surface waters, potentially leading to nutrient runoff and eutrophication, pathogen spread, and greenhouse gas (GHG) emissions (Garfí et al. 2016; Cucina et al. 2025a). The amendment value of digestate is mainly attributable to its high nutrient content, enriched concentration of partially decomposed organic matter, and its capacity to modify soil pH (Cucina 2023; Guan et al. 2024). However, its functional role in SOM dynamics remains a subject of debate. In addition to organic C inputs, digestate may also introduce exogenous microbial communities, potentially contributing to soil microbial dynamics through inoculation effects. This latter point remains debated in the literature, as field studies indicate that microorganisms originating from anaerobic digestion are unlikely to survive in soil, primarily due to the oxidative environment and competition with native soil microbial communities (Gong et al. 2023; Van Midden et al. 2023). Nonetheless, other studies have reported contrasting results, showing the transient persistence of microorganisms originating from anaerobic digestate in relation to digestate and soil characteristics, thus highlighting the need for further investigation (Goberna et al. 2011; Fongaro et al. 2017). Digestate quality strongly depends on feedstock composition and, crucially, on digestion temperature, which controls the degree of organic matter stabilization (Tambone et al. 2009; Alburquerque et al. 2012; Cucina 2023). Low-tech digesters are widely adopted in rural and tropical highland regions. Because they operate under psychrophilic conditions with limited hydrolysis and methanogenic activity, the resulting digestates retain specific characteristics. These include a high fraction of labile organic matter, partially lignified materials, and microbially processed intermediates (Garfí et al. 2016; Castro et al. 2017; Cucina et al. 2021, 2024, 2025a). These characteristics suggest a high potential for stimulating microbial activity in degraded soils; however, their biogeochemical effects under field conditions, particularly for digestates derived from low-tech psychrophilic systems, remain insufficiently characterized. Existing research largely relies on short-term incubations or mesocosm studies, which constrain redox dynamics, nutrient redistribution, and microbial succession processes (Garcia-Sánchez et al. 2015; Gurmessa et al. 2024; Pastorelli et al. 2024; Siedt et al. 2024; Ragályi et al. 2025). Moreover, few studies explicitly integrate digestate composition, particularly the relative abundance of labile and recalcitrant organic C fractions, with in-situ changes in SOM functional groups, microbial community composition, and stress physiology (Siedt et al. 2024; Vautrin et al. 2024). As a result, the mechanistic links between digestate-driven chemical amelioration, soil microbial responses, and the eventual stabilization of C in recalcitrant pools are still insufficiently understood (Sapp et al. 2015; Gómez-Brandón et al. 2016; Vautrin et al. 2024). In this context, key research gaps remain regarding the effects of psychrophilic digestate on soil chemistry and microbial community dynamics. In particular, knowledge of the temporal succession of microbial groups under field conditions remains limited. Moreover, the soil chemical drivers regulating these changes and their interactions are not yet fully understood, limiting our ability to predict soil responses to digestate application.

The gross effects of psychrophilic digestate on soil chemical fertility and SOM accumulation were demonstrated in a study conducted on a degraded acidic sandy-loam soil under long-term intensive Eucalyptus cultivation in the Colombian Andes (Cucina et al. 2025b). However, this previous work did not elucidate shifts in microbial community composition and their associated functional implication. In the present study, building on the same experimental framework and the identified knowledge gaps, we tested the following hypotheses: (H1) application of psychrophilic digestate modifies soil chemical properties, particularly pH and nutrient availability; and (H2) digestate application is associated with shifts in soil microbial community composition under field conditions. Accordingly, the objective of this study was to evaluate the effects of digestate application on soil physicochemical properties and microbial community dynamics in a degraded soil under field conditions.

Table 1 Low-tech digester description, experimental design, soil characteristics and composition of the digestate

Low-tech digester	Unit		Digestate	Unit	
Total volume	m^3	40	pH*	-	$7.8^a \pm 0$
Useful volume	m^3	28	Electrical conductivity*	mS cm^{-1}	0.42±0
Mean ambient temperature	°C	15	Total solids*	g L^{-1}	2.8±0.2
Daily feeding	m^3	3	Volatile solids*	%	61.1±0.3
Daily digestate production	m^3	2.8	Total organic C (TOC)*	%	32.9±2.2
Daily biogas production	m^3	8	Labile organic C	%TOC	68.3±1.2
Hydraulic retention time	days	14	Stable organic C	%TOC	31.7±0
			Recalcitrant organic C	%TOC	15.7±0.5
Experimental design	Unit		Water extractable organic C	%TOC	16.9±0.4
Site surface	m^2	2,700	Soluble reducing sugars	%TOC	0.21±0.02
Site altitude	m.a.s.l.	2,800	Soluble phenolic compounds	%TOC	0.28±0.0
Site annual precipitation	mm	921	Neutral detergent fibre	%	48.2±1.6
Digestate application dose	Mg d.w. ha^{-1}	40	Acid detergent fibre	%	25.8±0.4
Application dose (TOC)	Mg d.w. ha^{-1}	10	Acid detergent lignin	%	8.9±2
			Hemicellulose	%	22.4
Soil characteristics	Unit		Cellulose	%	16.9
Sand	%	54	Total N*	%	5±0.3
Silt	%	28	C/N*	-	6.6
Clay	%	18	Total P*	%	8.3±0.9
Texture	-	Sandy-loam	Ammonium-N*	%	0.63±0.15
Total carbonates	%	0	C/P	-	4
			Total K *	%	7.4±0
			C/K	-	4.4
			Total Ca*	%	5.9±0
			Total Mg*	%	8.2±0
			Total Na*	%	2.7±0
			Total Na*	%	2.7±0

*Cucina et al. 2025b.

[a]Mean value±SD, *n*=4.

Results are expressed on dry weight, except for Total solids, pH and electrical conductivity.

To address this, we combined (i) characterization of digestate and SOM, (ii) microbial community profiling, and (iii) multivariate statistical analyses, including principal component analysis (PCA), correlation analysis, and partial least squares (PLS) regression with variable importance in projection (VIP) scoring, to identify key factors associated with observed changes in soil properties.

Materials and methods

Study site, anaerobic digestion and experimental design

The experiment was conducted at the farm Granja Porcicola Ecocerd SAS (Floresta, Boyacá, Colombia – 5°51′42.3” N, 72°57′51.6” W), where an agricultural field subjected to intensive cultivation of Eucalyptus for 12 years was selected as the degraded soil to be restored by digestate application (Fig. S1). According to the USDA soil classification system, the soil in the study area was classified as an Inceptisol and featured a sandy-loam texture (54% sand, 28% silt, 18% clay) with no detectable carbonates. The study site is located at about 2,800 m.a.s.l., where the average temperature and precipitation are 15 °C and 921 mm, respectively. At the farm, a low-tech geomembrane digester treats pig slurry under psychrophilic temperatures (15 °C), generating biogas and 2.8 m^3 d^{-1} of digestate (Table 1; Fig. S2). Daily digestate production was applied continuously for four months over a 2,700 m² degraded site using pumps and pipes. The experimental area was divided into three homogeneous ~ 900 m² plots to serve as replicates. Homogeneity of the pedological conditions of the plots was assessed by several auger holes before the start of the experiment. Topsoil (A1 and A2 horizons, 0–25 cm depth) was sampled at four intervals: prior to digestate spreading (M1, April 2023; control), at the end of the application period (M2, August 2023), and four and eight months after the final application (M3, December 2023 and M4, April 2024, respectively). At each sampling time, 10 random subsamples (~ 1 kg each) were collected per plot and pooled into a single composite sample. This resulted in a total of 12 composite samples (3 plots × 4 sampling times) for laboratory analysis, which were carried out, if not differently specified, on air-dried samples.

According to digestate characterization, the application dose resulted in 40 Mg ha^{-1} of digestate (dry weight, d.w.). During the experimentation, the soil was not cultivated nor subjected to tillage. A detailed description of the study site, the anaerobic digestion procedures and the experimental design can be found in Cucina et al. (2025b). Digester and experimental design specifics, as well as digestate characteristics (pH, electrical conductivity - EC, total solids – TS, volatile solids – VS, total organic C – TOC, total N, P, K, Ca, Mg and Na) are reported in Table 1 (Cucina et al. 2025b).

Digestate characterization

In our previous work, digestate characterization was mainly focused on physico-chemical (i.e. nutrients and heavy metals content) and microbiological (i.e. pathogens' indicators) parameters (Cucina et al. 2025b). In this study, digestate analyses focused on the characterization of organic matter, providing evidence of the presence of easily degradable or recalcitrant organic compounds and assessing their potential effects on soil biochemical and microbiological responses. Digestate samples (1 L) were collected weekly from the digestate storage tank for one month before the beginning of the experiment ($n=4$) and freeze-dried before the analysis.

Chemical fractionation of organic C in digestate samples was carried out by adapting the methodology described by Mikutta et al. (2006) separating TOC into labile OC (LOC), stable OC and recalcitrant OC fractions. Stable organic C refers to the NaClO-resistant fraction who represents organic carbon that remains after chemical oxidation with sodium hypochlorite and is operationally defined as comprising relatively stable organic matter, including mineral-associated and chemically resistant components (Mikutta et al. 2006). LOC (labile organic C) was operationally defined as the fraction oxidizable by NaClO and calculated by difference from TOC and stable OC. Recalcitrant OC is the fraction remaining after hydrofluoric acid (HF) treatment, representing the most mineral-protected and chemically resistant organic pool (Mikutta et al. 2006). While NaClO-resistant stable OC and recalcitrant OC partially overlap conceptually with stable and recalcitrant C pools, they were used strictly as operationally defined fractions based on the applied fractionation procedure. Briefly, 1 g of the sample was treated three times with 25 mL of 6% (v/v) NaClO solution adjusted to pH 8.0, with each treatment lasting 6 h at 25 °C. Samples were then washed twice with 30 mL 1 M NaCl and then with deionized water until the solution was chloride-free (i.e. no reaction with $AgNO_3$ occurred). Between each shaking step, the samples were centrifuged (2,574xg; 5 min), and the supernatant was discarded. The samples were then dried at 60 °C and homogenized (stable OC fraction). 0.75 g of NaClO-treated dry samples were transferred into pre-weighed centrifuge bottles, shaken four times with 10 mL 10% HF for 2 h and then washed five times with 15 mL deionized water. Between each shaking step, the samples were centrifuged (2,574xg; 5 min), and the supernatant was discarded. The samples were then dried at 60 °C and homogenized (recalcitrant OC fraction). Digestate, stable OC and recalcitrant OC fractions were analyzed for the C content by an elemental analyzer (MacroCUBE CNHS, Elementar Italia, Lomazzo, Italy). LOC was then calculated by taking the difference between TOC and stable OC.

The water extractable organic C (WEOC) was analyzed according to Solé-Bundó et al. (2017). Briefly, after extraction with deionized water and successive centrifugation (12,298x*g* for 10 min) and filtration, organic C concentration in the extracts was determined by an elemental analyzer (Analytic Jena-Analyzer multi N/C 2100 S, Überlingen, Germany). Soluble phenolic compounds and soluble reducing sugars were determined in the water extracts following the Folin-Ciocalteu and phenol methods, respectively (Massaccesi et al. 2013).

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991). Hemicellulose (NDF-ADF), cellulose (ADF-ADL) and lignin (ADL) were then calculated.

The Fourier Transform InfraRed (FT-IR) spectra of digestate samples were collected in total reflectance mode (ATR) using a Shimadzu IRAffinity-1 S equipped with a Miracle Pike ATR device (Shimadzu Italia Srl, Milano, Italy) (wavenumber range of 4,000–700 cm^{-1} and resolution of 2 cm^{-1}). Shimadzu LabSolutions IR Peak Software (Shimadzu Italia srl, Milano, Italy) was used to process the collected FT-IR spectra.

Soil FT-IR spectra

To get deeper insights on SOM composition and variations following digestate application, FT-IR spectra of soil samples at different sampling times (M1 to M4) were collected in total reflectance mode (ATR) using a Shimadzu IRAffinity-1 S equipped with a Miracle Pike ATR device (Shimadzu Italia Srl, Milano, Italy) (wavenumber range of 4,000–700 cm^{-1} and resolution of 2 cm^{-1}). Shimadzu LabSolutions IR Peak Software (Shimadzu Italia srl, Milano, Italy) was used to process the collected FT-IR spectra. For this analysis, we processed soil samples and their fractions as described by Mikutta et al. (2006). The analyzed samples were processed, i.e. soil samples without treatment (TOC), soil samples following NaClO washings (stable OC), and soil samples following HF treatment (recalcitrant OC).

Composition of main soil microbial groups

The composition of main soil microbial groups was analyzed by Ester linked-Fatty Acid Methyl Ester (El-FAME) extraction of soil samples (5 g), including the following steps (Li et al. 2020; Marinari et al. 2021; Massaccesi et al. 2024): (1) release and methylation of ester-linked fatty acids at 37 °C with 15 ml of 0.2 M KOH in methanol for 60 min; (2) neutralization with 3 ml of 1.0 M acetic acid; (3) extraction of the El-FAMEs with 10 ml of hexane followed by drying under N_2 flow; and (4) resuspension in 200 µl hexane. The El-FAMEs were analyzed using a Varian CP3800 gas chromatograph, equipped with a flame ionization detector (GC-FID), and a ZB-WAX capillary column (60 m × 0.25 mm i.d., 0.25 µm film thickness) made of polyethylene glycol (Phenomenex, Torrance, CA, USA). Helium was used as carrier gas, with a column flow rate of 1.5 mL min^{-1} and a split ratio of 1:100. The oven temperature program was as follows: the temperature was initially held at 140 °C for 2 min, then increased from 140 °C to 240 °C at a rate of 4 °C min^{-1}, followed by a 15 min hold at 240 °C, and a final 42 min period at 240 °C. We identified El-FAMEs by comparing their retention times with those of the Supelco 37 Component FAME Mix (Merck KGaA, Darmstadt, Germany). El-FAMEs were quantified and converted to µmol g^{-1} using peak areas from internal standard (methylnonadecanoate, C19:0, Merck KGaA, Darmstadt, Germany) used at known concentrations. We identified 22 El-FAME biomarkers as Gram-positive bacteria (GPos) (i14:0, i15:0, a15:0, i16:0, i17:0, a17:0), Gram-negative (GNeg) (16:1ω7c, cy17:0, cy19:0, 18:1ω9t, 18:1ω7c), general bacteria (14:0, 15:0, 16:0, 17:0, 18:0, 20:00, 22:00), total fungi (18:3ω6c, 18:1ω9c), saprophytic fungi (18:2ω6,9), and protozoa (20:4ω6) following the procedure described by Marinari et al. (2021).

The total fungal-to-total bacteria and saprophytic fungi-to-total bacteria ratios were calculated and expressed as % to serve as indices of changes in the composition of main soil microbial groups (Massaccesi et al. 2024). Ratios of Gram-positive to Gram-negative bacteria (GPos/GNeg) and monounsaturated to polyunsaturated fatty acids (MUFA/PUFA) are often used as proxies for shifts in microbial community composition and environmental conditions, although their interpretation as stress indicators remains context-dependent (Fanin et al. 2019; Li et al. 2020).

Statistical analysis

A comprehensive suite of univariate and multivariate statistical techniques was employed to analyze the temporal dynamics and the relationships existing among soil chemical, biochemical, and microbiological properties following digestate application (Table S1). Data for chemical (pH, electrical conductivity - EC, cation exchange capacity - CEC, TOC, TN, C/N, total and available P, total and exchangeable K, Ca, Mg, Fe, Al, Na, WEOC, soluble sugars and phenols, Specific Ultraviolet Absorbance - SUVA at 254 nm) and biochemical (microbial biomass C, N, and C/N, respiration rate, microbial and metabolic quotients, fluoresceine diacetate - FDA hydrolysis activity, biological fertility index - BFI) soil analysis were those reported in Cucina et al. (2025b). All analyses were conducted using XLSTAT version 2023.2 (Addinsoft, Paris, France).

We performed a one-way Analysis of Variance (ANOVA) to determine whether significant differences in microbiological parameters and stress indicators occurred over time after digestate application. Prior to analysis, the assumptions of normality and homogeneity of variances were assessed using the Shapiro–Wilk test and Levene's test, respectively. When data did not meet these assumptions, appropriate transformations (i.e. log or square root) were applied. Upon confirmation of assumptions, the ANOVA model was fitted, considering sampling time as the fixed factor. Differences among time points were considered statistically significant at $p < 0.05$. Where significant effects were detected, Tukey's Honest Significant Difference (HSD) post hoc test was applied for pairwise comparisons to identify specific temporal changes in microbiological parameters.

We applied z-score standardization and PCA to a matrix of soil variables (Siedt et al. 2024), to reduce dataset complexity and reveal underlying temporal patterns. We analyzed datasets using the covariance matrix method. We retained principal components with eigenvalues greater than 1 based on the Kaiser criterion (Nizamutdinov et al. 2025). The PCA model provided a spatial representation of sample distribution across sampling times, enabling the identification of temporal clustering patterns. The contribution of individual variables to each component was evaluated through loading values, and the most influential parameters were identified based on the magnitude of their standardized loadings. We grouped parameters into three functional categories, i.e. chemical, biochemical, and microbiological, to interpret their functionality (Table S1). For each category, the average loading score was calculated to determine its overall importance in explaining variance among the soil samples. The top 10 discriminating parameters were selected based on the highest absolute standardized loadings and used to construct cluster profiles across sampling points. We compared standardized values of these variables across time to assess their role in differentiating sample groups and to visualize shifts in soil condition over the experimental timeline. In addition, the temporal evolution of the top five most discriminating parameters was analyzed, providing insight into the response trajectories of key indicators following digestate application.

We conducted a Pearson correlation analysis on the dataset to assess relationships among variables. Prior to the Pearson correlation analysis, we inspected the data for normality using the Shapiro–Wilk test. When necessary, we log- (WEOC) or square-root (EC, TOC, TotK, TotMg, ExchMg, TotNa, SUVA254) transformed the data to meet normality assumptions. In addition, we standardized all variables, which further contributed to reducing skewness and ensuring comparability among variables. To account for multiple comparisons, *p*-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction. Furthermore, to reduce redundancy and avoid multicollinearity, variables derived as ratios were excluded prior to the analysis. Only statistically significant correlations (adjusted $p < 0.001$) were considered. Correlation matrices were visualized using the "corrplot" package in R (R Core Team 2020).

PLS regression (PLSR) was applied to specifically quantify the influence of soil chemical properties on biochemical and microbiological responses (Stellaci et al. 2021). The analysis used chemical parameters as predictor variables (X matrix) and biochemical and microbiological parameters as response variables (Y matrix). Both matrices were standardized (mean-centered and scaled to unit variance) prior to modeling to ensure comparability and eliminate scale bias. Model robustness and predictive power were evaluated using leave-one-out cross-validation (LOOCV). The number of latent variables retained was selected based on the minimum predicted residual sum of squares (PRESS) criterion to avoid overfitting. We calculated the VIP scores for each chemical predictor variable to quantify their relative contribution to explain variance in the response dataset. Variables with VIP scores exceeding 1.0 were considered influential drivers of biochemical and microbiological soil properties (Rocher et al. 2017). Model fit was assessed through explained variance metrics (R^2 for calibration and Q^2 for cross-validation), with satisfactory values indicating reliable predictive relationships.

Results

Digestate characterization

The digestate applied in the field trial was characterized by a near-neutral pH (7.8 ± 0.1) and an electrical conductivity of 0.42 mS cm^{-1} (Table 1). The total N (TN) content reached 5 ± 0.3%, with a low C/N ratio of 6.6. Elemental analysis showed high concentrations of total P (8.3 ± 0.9%), K (7.4%), Ca (5.9%), Mg (8.2%), and Na (2.7%). Regarding C pools, the total organic C (TOC) content was 32.9 ± 2.2%, with labile organic C accounting for 68.3 ± 1.2% of TOC and stable organic C for 31.7% of TOC. Recalcitrant OC and water-extractable organic C (WEOC) represented 15.7 ± 0.5% and 16.9 ± 0.2% of TOC, respectively. Soluble fractions, including reducing sugars and phenolic compounds, contributed 0.21 ± 0.02% and 0.28% to the TOC. Fiber analysis revealed the dominance of NDF (48.2 ± 1.6%), followed by ADF (25.8 ± 0.4%) and ADL (8.9 ± 2.2%), with calculated hemicellulose and cellulose contents of 22.4% and 16.9% of the organic matter. The FT-IR spectrum (Fig. S3) exhibited major absorption bands typical of complex organic matter (Deng et al. 2025). Key signals included a broad band at ~3,300 cm^{-1} (O–H stretching in carbohydrates and phenols), aliphatic C–H stretching at ~2,920 and ~2,850 cm^{-1}, and C=O stretching of carboxylic acids/esters near 1,700 cm^{-1}. A secondary signal at ~1,600 cm^{-1} (aromatic C=C or amide I) and bands at ~1,500–1,240 cm^{-1} (amide II and lignin-associated C–O stretching) confirmed the presence of stabilized proteins and aromatic polysaccharides. Intense bands at ~1,030–1,050 cm^{-1} highlighted the polysaccharide and cellulose backbone.

SOM characterization

FT-IR spectra of bulk soil (TOC), NaClO-treated (stable OC), and HF-treated (recalcitrant OC) fractions showed relative increases in the intensity of bands associated with organic functional groups from M1 (degraded pre-amendment baseline) to M4 (12 months post-amendment) (Fig. S4). In TOC and stable OC fractions, the most pronounced increases were observed in the bands at ~3,300 cm^{-1} (hydroxyl groups), ~1,700 cm^{-1} (carboxyl/ester groups), ~1,600 cm^{-1} (aromatic C=C/amide I), and ~1,030 cm^{-1} (polysaccharides) (Lima et al. 2009). The stable OC spectra, in particular, highlighted the persistence of stable organic residues after the oxidative removal of labile C. In contrast, recalcitrant OC spectra exhibited minor variations between M1 and M2 but developed a distinct aromatic C=C signal at ~1,600 cm^{-1} from M3 onwards, which persisted through M4, indicating the durable accumulation of lignin-derived aromatic structures.

Dynamics of main soil microbial groups following digestate application

The composition of main soil microbial groups of the digestate-treated soil exhibited clear temporal variations during and after the amendment period (Fig. 1). At the initial sampling (M1), GPos bacterial biomarkers were approximately 40 nmol El-FAME g^{-1}, while GNeg biomarkers were below 15 nmol El-FAME g^{-1}. At the end of the digestate amendment (M2), GNeg biomarkers increased with respect to M1, reaching approximately 20 nmol g^{-1} ($p < 0.05$), while GPos biomarkers did not show significant variations with respect to M1. Conversely, in the following sampling times (M3 and M4), both GPos and GNeg showed a significant increase with respect to M1 ($p < 0.05$), reaching 171 nmol g^{-1} and 37 nmol g^{-1} at M4, respectively. General bacterial biomarkers increased progressively from about 80 nmol

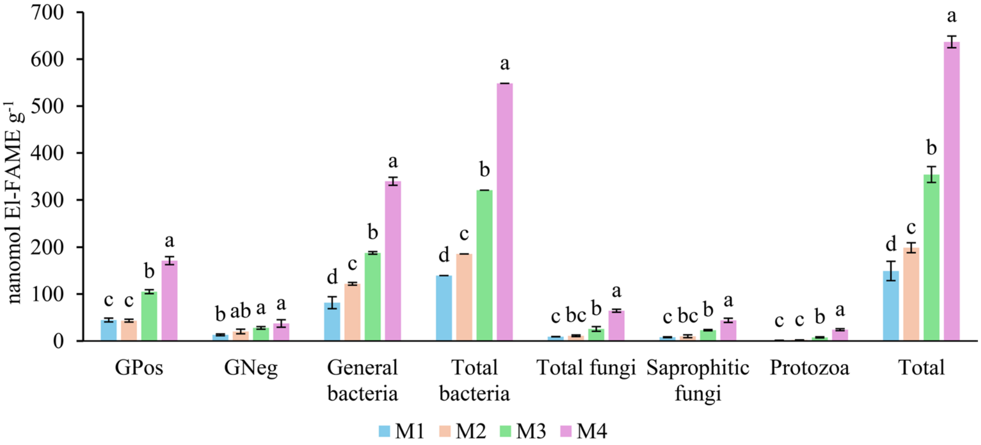


Fig. 1 Composition of main soil microbial community groups in digestate-treated soil at different sampling times (M1-M4). M1: before the amendment; M2: end of the amendment; M3: 4 months after the end of the amendment; M4: 8 months after the end of the amendment. Error bars represent the standard deviation ($n = 3$). Different letters indicate significant differences among each sampling time (HSD test, $p < 0.05$)

g^{-1} at M1 to more than 300 nmol g^{-1} at M4. Total bacterial biomarkers rose from approximately 140 nmol g^{-1} at M1 to over 600 nmol g^{-1} at M4, with significant differences among sampling times ($p < 0.05$). Fungal biomarkers were comparable between M1 and M2 (ranging from 9 to 11 nmol g^{-1}) and then increased at M3 (about 26 nmol g^{-1}) and M4 (about 64 nmol g^{-1}), with values at M3 and M4 significantly higher than at M1–M2 ($p < 0.05$). Saprophytic fungi showed a similar temporal pattern, with significantly higher abundance at M4 compared with earlier sampling times ($p < 0.05$). Protozoa biomarkers did not vary during the first two samplings (2 nmol g^{-1}), increased to about 8 nmol g^{-1} at M3 and reached about 24 nmol g^{-1} at M4. Overall, total microbial biomass increased continuously from 150 nmol g^{-1} at M1 to more than 630 nmol g^{-1} at M4, with statistically significant differences among the sampling times ($p < 0.05$).

Microbial community composition indicators based on EL-FAME analysis showed distinct temporal dynamics (Fig. 2). The GPos/GNeg ratio decreased significantly at the end of the amendment (M2) compared to pre-amendment (M1), from approximately 3.4 at M1 to 2.1 at M2 ($p < 0.05$). After the amendment ended, the ratio increased at M3 (3.8) and further at M4 (4.6); the value at M4 was significantly higher than those at M1 and M2 ($p < 0.05$). The MUFA/PUFA ratio declined after the amendment, with values at M3 and M4 lower than at M1 ($p < 0.05$). The total fungi/total bacteria ratio was relatively stable at M1 and M2 (6–7%) but increased at M3 (10%) and M4 (> 12%), with M3 and M4 being significantly different from M1–M2 ($p < 0.05$). The saprophytic fungi/total bacteria ratio followed the same trend, showing significantly higher values at M3 and M4 compared with M1 and M2 ($p < 0.05$).

Dynamics and relationships among soil properties following digestate application

PCA scoring plot identified two axes that explained about 64% (PC1) and 23% (PC2) of the variation, respectively (Fig. 3A and B). Using an absolute loading threshold of 0.15, PC1 was mainly driven by strong positive loadings for indicators of microbial activity and microbial community size, including FDA hydrolytic activity, microbial biomass C and N, microbial quotient, biological fertility index, total bacteria, total fungi, GPos and GNeg bacteria, saprophytic fungi, and protozoa, together with positive loadings for total and available P, pH, TOC, total base cations (Ca, Mg, Na, K), exchangeable Mg and Fe, and soluble phenols. Negative loadings on PC1 were associated with the metabolic quotient, water-extractable C, and exchangeable Al. PC2 showed positive loadings for EC, CEC, exchangeable cations and Al, total N, TOC, microbial biomass C/N ratio, and respiration rate, while negative loadings were observed for total Fe, total Al, and bulk C/N ratio, as well as GPos to GNeg ratio. PCA revealed a clear temporal shifting of soil samples (Fig. 3A), with a directional progression along PC1 observed from M1 to M4. Cluster profiles of the top discriminating parameters (Figs. 3 and 4) and their temporal evolution (Fig. 4C) showed low standardized values of microbial indicators at M1 and M2, followed by higher values at M3 and maximum values at M4.

Pearson correlation analysis (Fig. 5) revealed a prominent cluster of positive correlations between available P (AvP) and the entire suite of microbiological parameters. Of ecological significance were the strong positive correlations between AvP and the microbial quotient (qMic) ($r = 0.989$) and soil pH and exchangeable bases (Fig. 5; Fig. S5). Strong positive associations were also found between contrasting microbial guilds, such as total bacteria vs. saprophytic fungi ($r = 0.992$) and GPos bacteria vs. total fungi ($r = 0.991$, Fig. S5).

PLSR models yielded robust predictions for biochemical ($R^2 = 0.741$) and microbiological ($R^2 = 0.806$) responses (Fig. 6). For biochemical parameters (Fig. 6, left panel), the highest VIP scores were attributed to total Mg (VIP = 1.33), soluble phenols (VIP = 1.28), AvP (VIP = 1.26), WEOC (VIP = 1.25), total Na (VIP = 1.20), total Ca (VIP = 1.20),

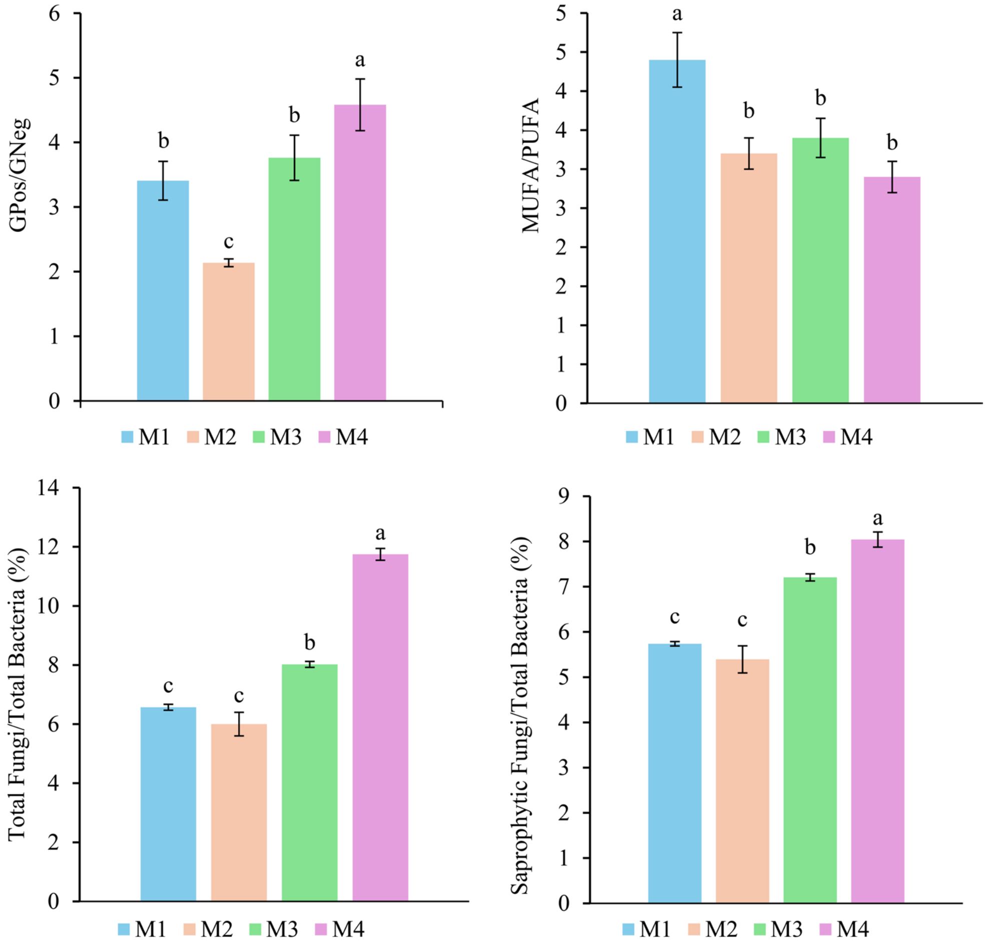


Fig. 2 Microbial groups indicators according to El-FAME analysis in digestate-treated soil at different sampling times (M1-M4): GPos/GNeg, Monounsaturated/Polyunstaturated (MUFA/PUFA) fatty acids ratio, Total Fungi/Total Bacteria (%), and Saprophytic fungi/Total Bacteria (%) ratio. M1: before the amendment; M2: end of amendment; M3: 4 months after the end of the amendment; M4: 8 months after the end of the amendment. Error bars represent the standard deviation ($n=3$). Different letters indicate significant differences among each sampling time (HSD test, $p<0.05$)

and pH (VIP = 1.19). For the microbiological model (Fig. 6, right panel), AvP was the primary predictor (VIP = 1.44), followed by exchangeable Al (VIP = 1.40) and Specific Ultraviolet Absorbance SUVA254 (VIP = 1.35).

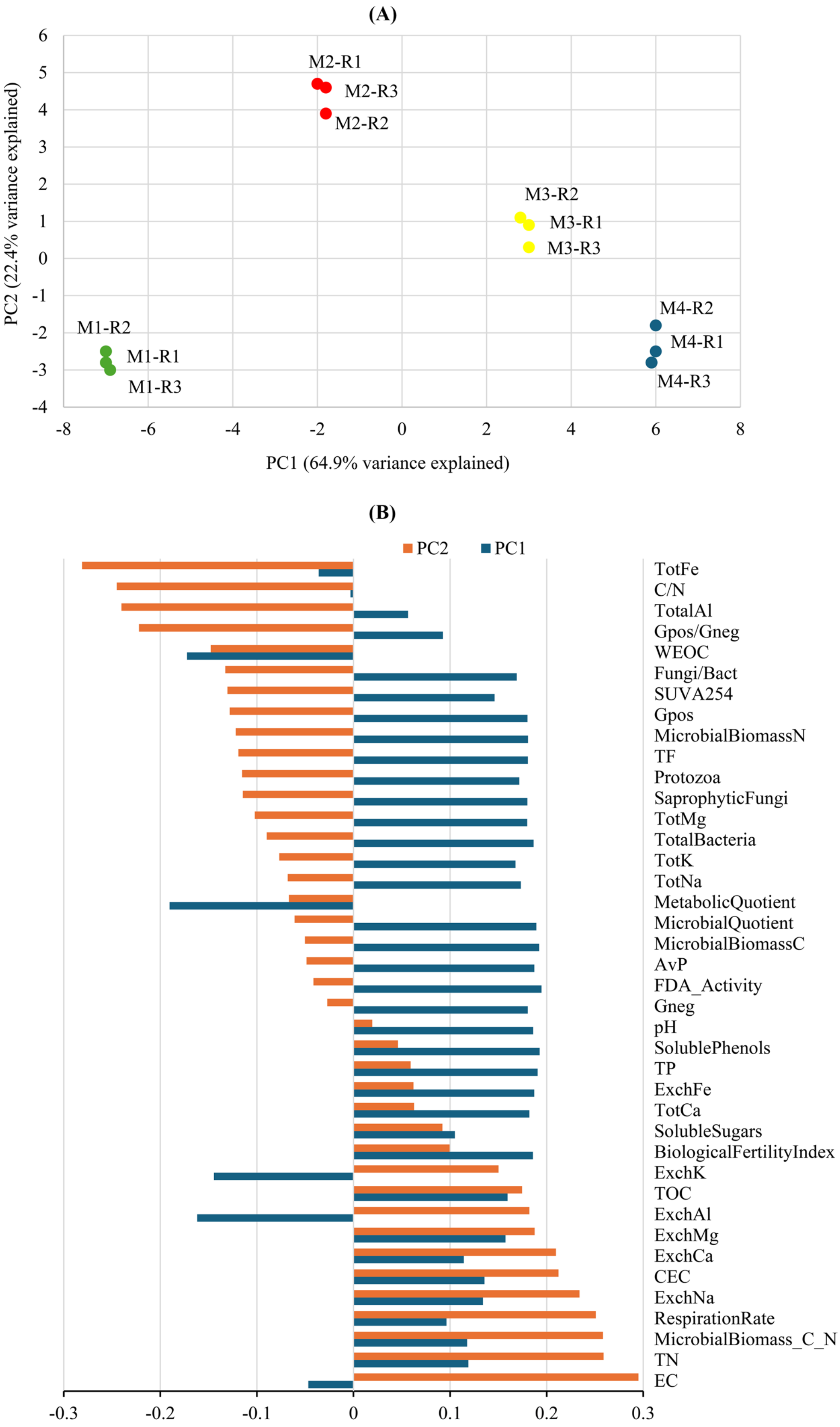
Discussion

Linking digestate composition to soil organic C and main soil microbial groups dynamics

The characterization of the digestate used in this study revealed a composition rich in both labile (e.g., carbohydrates and water-soluble compounds) and recalcitrant (e.g., lignin-rich fibers) organic fractions (Table 1). This heterogeneous nature confirmed its potential to supply rapidly available substrates while simultaneously contributing stable organic matter to the soil, consistent with literature identifying digestate as a source of both easily biodegradable and persistent organic compounds (Tambone et al. 2013; Greenberg et al. 2019; Fernández-Domínguez et al. 2022). While the proportion of labile organic matter is often reduced in thermophilic digestion due to higher biogas conversion (Ossa-Arias and González-Martínez 2024), the psychrophilic digestate employed here maintained a heterogeneous organic nature that may be particularly effective for restoring degraded soils.

The soil FT-IR spectra mirrored this chemical complexity, showing relative increases in band intensity, indicative of changes in SOM functional composition and the emergence of aromatic structures (Fig. S3). These shifts in SOM quality are intrinsically linked to the microbial successions observed throughout the experiment. Initially, the influx of easily degradable compounds supported the rapid proliferation of GNeg bacteria which thrive under high resource availability and could be ascribed to copiotrophic, r-strategist microorganisms (Veum et al. 2021; Massaccesi et al. 2024). This was reflected in the lower GPos/GNeg ratio observed at M2 compared to the pre-application baseline. Fast-growing Gram-negative bacteria are known to respond rapidly to short-term pulses of labile substrates. This interpretation is supported by the high availability of water-extractable organic C and soluble compounds at this stage (Table 1), suggesting that the observed pattern may represent a transient physiological response rather than a stable community restructuring. As expected, as labile pools were depleted

Fig. 3 (**A**) PCA visualization of soil sample clusters; (**B**) PCA variables contribution. M1: before the amendment; M2: end of the amendment; M3: 4 months after the end of the amendment; M4: 8 months after the end of the amendment. R: replicate



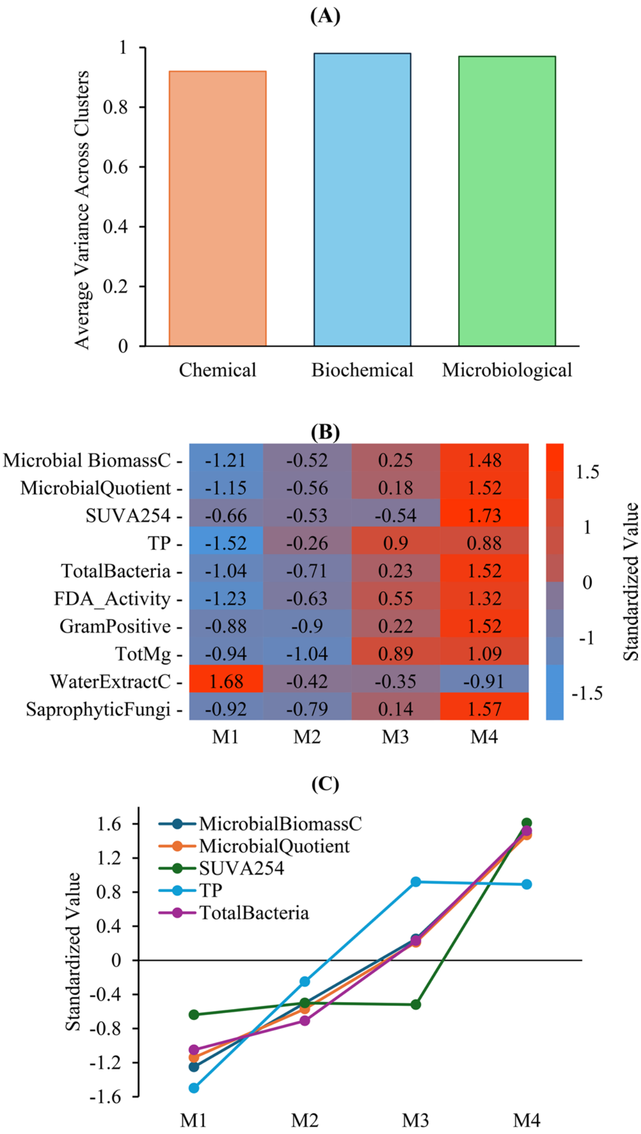


Fig. 4 (**A**) Parameter category importance; (**B**) Top 10 parameters; (**C**) Evolution of Top 5 discriminating parameters. M1: before the amendment; M2: end of the amendment; M3: 4 months after the end of the amendment; M4: 8 months after the end of the amendment

over time, the increasing contribution of stable C and lignin-like components favored the establishment of GPos bacteria and fungi which are better adapted to metabolizing complex substrates. GPos bacteria are also usually ascribed as oligotrophic and K-strategist (Massaccesi et al. 2024; Uwituze et al. 2025). This shift toward a fungal-dominated microbial consortium is further corroborated by the rise in the Total Fungi/Total Bacteria ratio and the decrease in the MUFA/PUFA ratio (Fig. 2). In this context, the continuous increase of the soil microbial community abundance over 12 months suggests endogenous succession rather than transient inoculation from digestate, in accordance with literature (Gong et al. 2023; Van Midden et al. 2023). Furthermore, our findings are consistent with the context-dependent framework proposed by Vautrin et al. (2024), who showed that the response of soil microbial communities to digestate application depends on both digestate characteristics and soil type.

The observed increase in SOM suggests that multiple processes may have contributed to C accumulation rather than a single dominant pathway. Changes in the relative abundance of more resistant organic fractions and the appearance of aromatic signals in the recalcitrant OC and stable OC pools may indicate the accumulation of chemically complex organic compounds during digestate decomposition (Cotrufo et al. 2019). These observations are consistent with conceptual frameworks such as the SOM continuum concept (Lehmann and Kleber 2015), although direct evidence for specific stabilization mechanisms was not assessed in this study. In parallel, increases in microbial biomass and enzymatic activity indicate enhanced microbial processing of organic inputs following digestate application. Such responses may contribute to transformations of organic matter and influence its persistence in soil (Cotrufo et al. 2013, 2019; Veum et al. 2021); however, the formation of specific stabilized pools, including mineral-associated organic matter, cannot be directly inferred from the present data.

Overall, these processes may jointly contribute to improvements in soil biochemical properties and carbon retention, although their relative importance likely depends on soil characteristics and management context (Sinatra et al. 2024; Ragályi et al. 2025). In this study, shifts in microbial communities were likely driven by the addition of organic substrates from the digestate. Given the low total solids content of the digestate (2.8 g L^{-1}), direct microbial inputs from the amendment were likely limited (Gong et al. 2023; Van Midden et al. 2023). Furthermore, due to the use of EL-FAME analysis, which provides broad functional group resolution, it was not possible to distinguish between stimulation of indigenous microbial communities and contributions from introduced microorganisms. Future studies integrating high-resolution molecular approaches would help to better resolve these mechanisms.

Multivariate analysis of temporal soil evolution and drivers of recovery

PCA highlighted a progressive shift in the drivers of soil differentiation over time following digestate application (Fig. 3). While samples from M1 and M2 clustered closely along PC1, they were separated primarily along PC2, indicating that early changes induced by the amendment were mainly associated with soil chemical properties, particularly those related to ionic strength, exchangeable cations, and C and N pools. This pattern suggests that the immediate effects of digestate application were dominated by

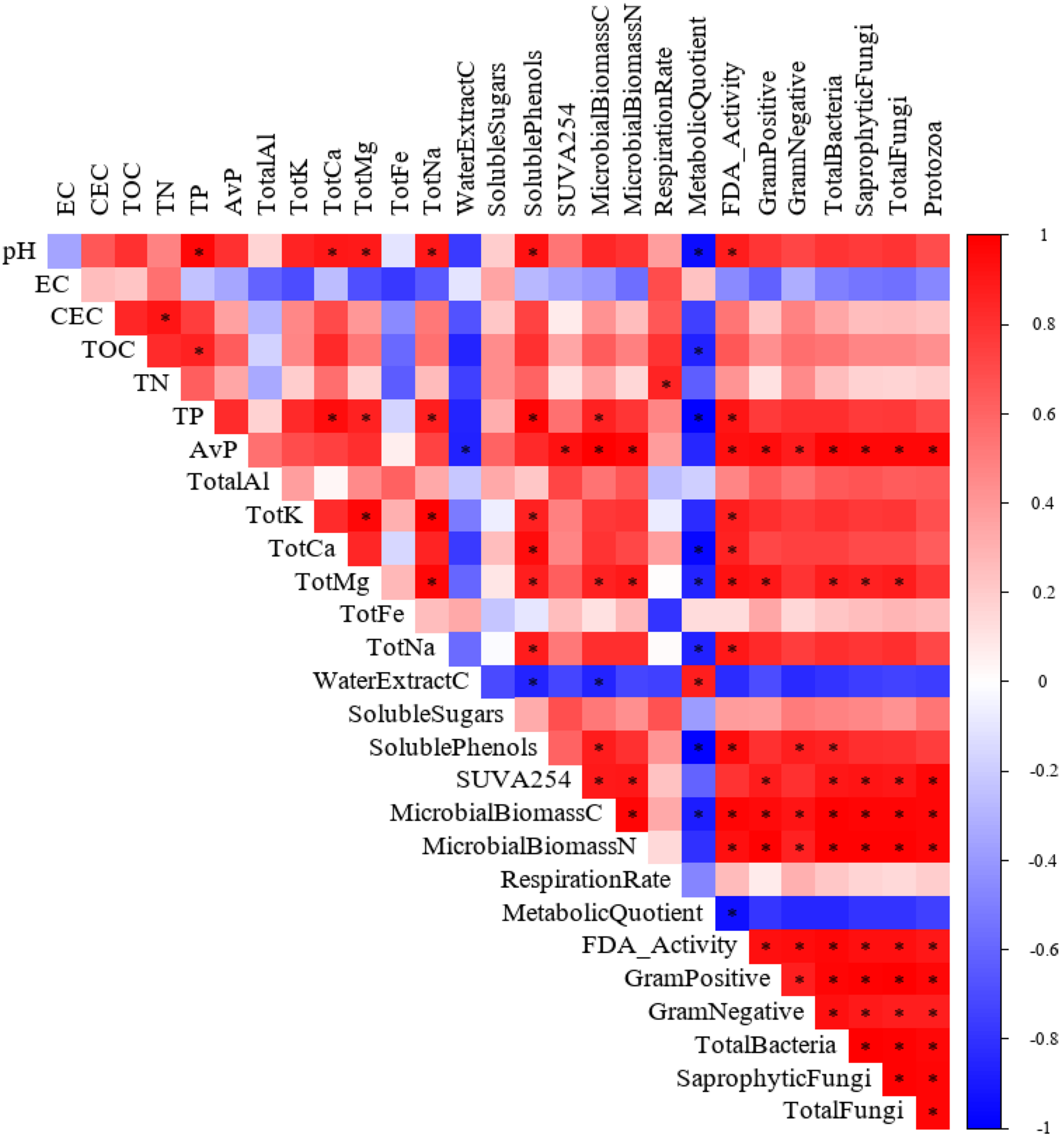


Fig. 5 Pearson correlation matrix among selected chemical, biochemical, and microbiological parameters of soils. Significance markers (*) are shown ($p < 0.001$)

alterations in the soil exchange complex and nutrient availability, reflecting the direct chemical input of the amendment rather than a rapid microbial response. Such chemically driven early shifts are consistent with the well-documented short-term effects of organic amendments on soil EC, CEC, and exchangeable nutrient pools (Bouajila et al. 2023). In contrast, the separation between M2 and the post-amendment sampling points (M3 and M4) occurred predominantly along PC1, indicating that biochemical and microbiological parameters became the primary drivers of soil differentiation at later stages (Figs. 3 and 4). This transition reflects a temporal decoupling between initial chemical perturbation

Fig. 6 Variable Importance in Projection (VIP) scores in soils: most influential chemical variables affecting (**A**) biochemical and (**B**) microbiological parameters. The red dashed line indicates the VIP Score threshold equal to 1

and subsequent microbiological reorganization, whereby microbial communities progressively responded to the altered soil environment and to the availability of digestate-derived substrates. The increasing weight of PC1 in distinguishing M2, M3, and M4 underscores the emergence of microbial biomass, enzymatic activity, and microbiological fertility indicators as dominant structuring forces of soil functioning.

The strong separation between M1 and M4 almost exclusively along PC1 further reinforces this interpretation, demonstrating that, with time, differences between pre-amendment and restored soil states were governed mainly by biochemical and microbial properties rather than by purely chemical factors (Francioli et al. 2016; van der Bom et al. 2018). The marked increase in microbial biomass C, microbial quotient, and total bacterial abundance at M3 and M4 indicates that digestate application triggered a sustained enhancement of microbial activity and efficiency, consistent with improved C use efficiency and ecosystem functioning, in accordance with García-Sánchez et al. (2015) and Gurmessa et al. (2024). Together, these axis-dependent patterns suggest a two-stage response of soil to digestate application: an early, chemically driven phase characterized by modifications of the exchange complex and nutrient pools, followed by a microbially driven phase marked by enhanced microbial activity, organic matter processing and content increase (Fig. 7).

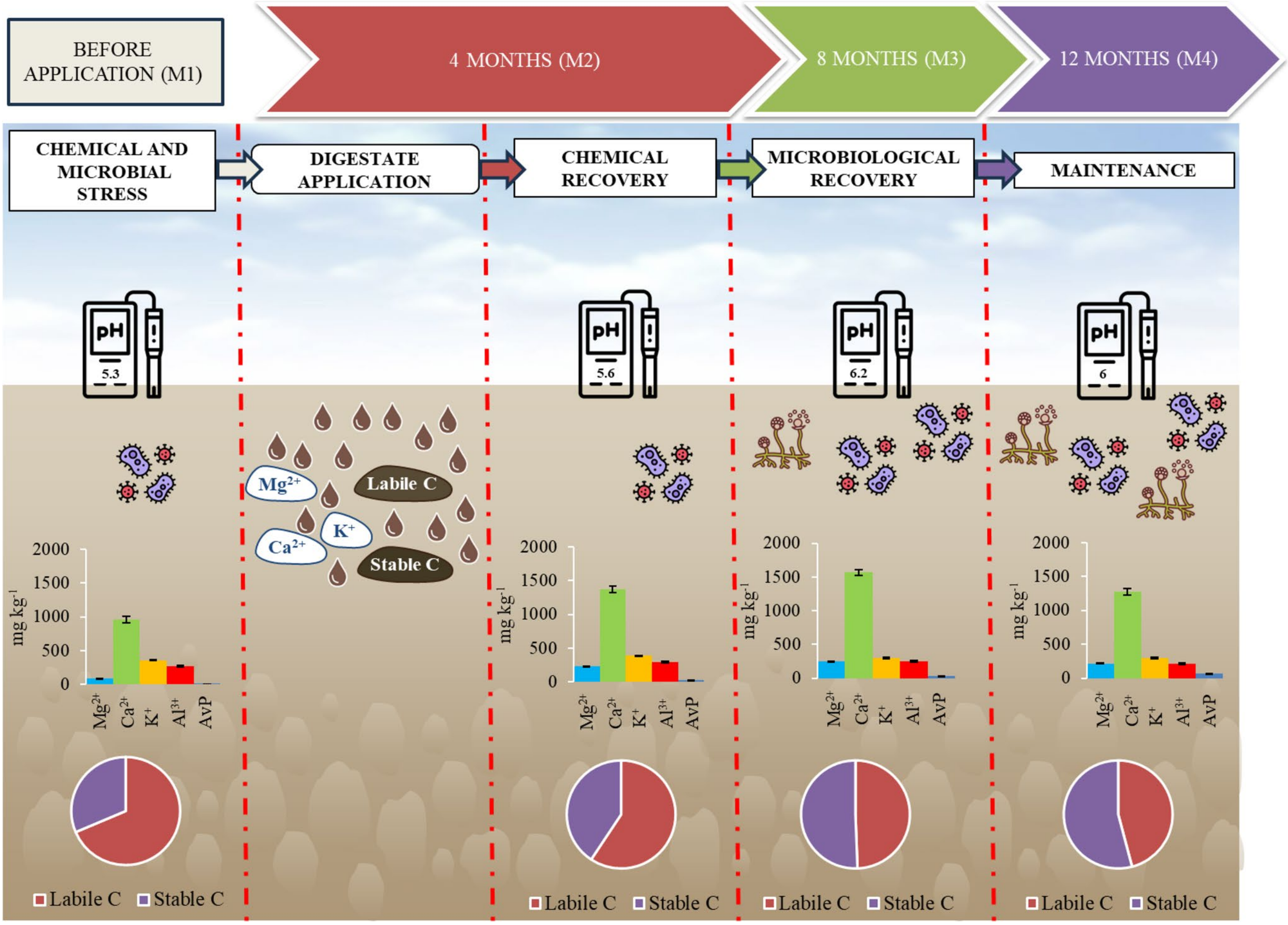


Fig. 7 Conceptual framework illustrating the mechanism of soil restoration induced by digestate application

Linking soil chemical parameters to soil biochemical and microbiological functioning

The correlation analysis aimed to assess how shifts in soil chemistry induced by digestate application were related to changes in the main soil microbial groups, thereby highlighting general relationships before applying more targeted regression models (i.e., PLSR).

The positive correlations between AvP, qMic, soil pH, and exchangeable bases are of ecological relevance, since in many degraded systems, P availability is the primary limiting factor for microbial growth and C assimilation (Cao et al. 2016). Crucially, the rise in bioavailable P might be attributed not only to a direct input of the element from digestate, but rather to a pH-mediated solubilization process (Pérez-Esteban et al. 2014). This improved stoichiometry allowed the microbial community to efficiently convert organic C into biomass. Furthermore, strong positive correlations between contrasting microbial guilds (e.g., total bacteria vs. saprophytic fungi) indicate co-occurrence patterns within the microbial community.

However, simple bivariate correlations are insufficient to capture the complexity of interaction within the soil system or account for collinearity among nutrient pools. Therefore, to move beyond simple association and quantitatively rank the specific chemical drivers responsible for triggering these microbial responses, a PLSR was applied, as detailed in the following section.

Hierarchical drivers of restoration: from chemical alleviation to microbial maintenance

PLSR allowed for a quantitative ranking of the abiotic drivers governing the ecosystem recovery, together with the VIP score analysis that revealed distinct patterns of influence that corroborate a sequential restoration mechanism, conceptualized in Fig. 7. For biochemical parameters, the prominence of labile C sources and readily available P confirms that the reactivation of soil microbial activity was primarily fueled by the supply of easily degradable substrates provided by the amendment. This corresponds to the initial transition from stress to recovery (M1 to M2 in Fig. 7). For microbiological parameters, the hierarchy of drivers shifted, highlighting a deeper structural change. While AvP remains the top predictor, confirming the P-limitation relief hypothesis, exchangeable Al emerges as a critical determinant, indicating that the suppression of Al toxicity emerged as a statistically important predictor, with a contribution comparable to nutrient-related variables within the model.

Based on these statistical weights, we proposed a mechanistic model (Fig. 7) describing the cascading effect of digestate application:

- Chemical recovery (M1 → M2): the process was initiated by the substantial input of exchangeable bases from the digestate (Table 1). The VIP analysis strongly identifies these cations as the primary agents of soil buffering, in accordance with literature highlighting the liming potential of organic amendments (Naramabuye and Haynes 2006). The accumulation of these bases directly drove the pH rise, which in turn caused the precipitation of exchangeable Al and created a hospitable environment for microbial growth.
- Microbial recovery (M2 → M3): the fraction of labile organic C stimulated the microbial community and, in concurrence with the pH rise, facilitated the solubilization of P, removing the stoichiometric bottleneck for growth.
- Microbiological Maintenance (M3 → M4): as the labile substrates were consumed, the microbial community sustained its activity by shifting utilization towards more complex C forms. This is evidenced by the high VIP score of SUVA254 in the microbiological model, suggesting that the stable, aromatic C fraction provided by the digestate served as a slow-release energy reserve, ensuring the persistence of the restored ecosystem functions over time.

Management implications for soil restoration in the regenerative agriculture and circular bioeconomy frameworks

The results reported in this study potentially provide a framework for optimizing the use of psychrophilic digestates in land restoration. Beyond nutrient supply, digestate application promoted soil recovery through coupled chemical and microbiological improvements, offering several advantages for sustainable soil management. It is important to underline that:

(i) the efficacy of the psychrophilic digestate highlights the importance of anaerobic digestion temperature in determining the chemical fingerprint of the resulting amendment. While thermophilic processes maximize energy recovery, the preservation of a balanced ratio between labile and recalcitrant organic C in psychrophilic conditions may represent a promising option for treating degraded soils under similar pedoclimatic and management conditions;

(ii) the observed hierarchical recovery demonstrates that digestate application can function as a high-efficiency alternative to conventional liming. This suggests that for acidic or industrially degraded soils, digestate-based restoration can achieve a more rapid shift toward microbiological fertility compared to other interventions;

(iii) the strong linkage between P availability and microbial activity suggests that digestate can improve P use efficiency, reducing dependence on mineral fertilizers and associated environmental impacts, particularly in P-limited systems;

(iv) from a C management perspective, digestate supports both short-term microbial activity and C stabilization, reinforcing its potential role in carbon farming and soil C sequestration strategies;

(v) the temporal decoupling between chemical and microbial responses indicates that restoration outcomes may be underestimated by short-term assessments. Monitoring should therefore extend over at least one year to capture the full development of soil microbiological recovery. In addition, the inclusion of untreated control treatments throughout the experimental period would allow a clearer disentangling of the relative contributions of digestate application and environmental or pedoclimatic drivers to soil recovery. Although the baseline condition (M1) provided a reference point in this study, the absence of a parallel untreated control limits the attribution of observed changes exclusively to the amendment. Nevertheless, the magnitude and consistency of the observed differences across multiple indicators suggest a predominant treatment effect.

Conclusions

In line with our hypotheses, this study indicated that digestate application effectively reversed soil degradation, suggesting soil chemical amelioration and shifts of the microbial communities that may underline gradual functional changes. Anaerobic digestion under low-temperature conditions is often regarded as producing digestates of limited agronomic value. However, psychrophilic digestate offers important advantages, as it retains a heterogeneous fraction of organic matter with high potential bioavailability, which can be effectively exploited for soil restoration. Multivariate analysis identified the alleviation of acidity and

Al toxicity, mediated by the substantial input of exchangeable bases (Ca, Mg, Na), as the primary trigger that potentially unlocked native and added P reserves. Crucially, this chemical amelioration was complemented by the heterogeneous quality of the exogenous organic matter: while the labile fraction acted as an immediate metabolic primer to kick-start microbial activity, the recalcitrant pools provided a persistent energy reserve, sustaining a mature community over the long term. This geochemical and organic rebalancing fostered synergistic microbial growth, characterized by a progressive shift towards a stable, K-strategist population. These findings support the use of digestate as a strategic amendment for the rehabilitation of marginal or stressed agroecosystems, offering a viable solution aligned with regenerative agriculture and circular bioeconomy goals. However, acknowledging that ecosystem trajectories are modulated by site-specific pedoclimatic factors, further large-scale and long-term case studies across diverse environmental conditions are essential to generalize these mechanisms and fully validate digestate-based strategies for broad-scale degraded soil regeneration.

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Author contributions **Mirko Cucina: ** conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft; **Luisa Massaccesi: ** methodology, investigation, writing –review & editing; **Marianna Garfí:** writing –review & editing, project administration, funding acquisition; **Humberto Escalante: ** supervision, funding acquisition; **Liliana Castro: ** writing –review & editing, project administration, funding acquisition.

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Data availability The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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