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Soil microbiome in long-term onion cropping systems

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The soil microbiome is essential for ecosystem functions and food production; however, it undergoes structural and functional changes due to management practices. This study describes the microbial community associated with long-term onion systems: conventional tillage (CT), no-tillage (NT), and no-tillage vegetable system (NTVS). The long-term experiment was conducted over 17 years in Ituporanga, SC, Southern Brazil. The treatments included: CT (soil turned over before planting onions, followed by corn in summer and a fallow period in winter), NT (similar to CT but with restricted soil turnover), and NTVS (similar to NT but with greater species diversity grown in a consortium during summer, including millet + velvet-bean + sunflower). The soil microbiome (16S and ITS gene) was analyzed by next-generation sequencing - NGS of soil samples collected after the onion cycle. Soil management influenced microbiome structure, with each system exhibiting distinct compositional patterns. NTVS had a higher proportion of bacteria, fewer unclassified groups, and a greater abundance of taxa linked to nutrient cycling and beneficial plant relationships. NT showed a higher relative presence of archaea, particularly nitrifying groups such as Nitrososphaeraceae. In CT, Firmicutes and Bacillaceae were more prevalent, indicating a typical response to more disturbed environments. At all taxonomic levels, NTVS reduced the occurrence of unidentified taxa, suggesting a more stable environment with clearer ecological selection. For fungi, similar trends were observed, with higher richness in NTVS and lower in CT, favoring microorganisms adapted to stressful, fast-growing conditions and readily available nutrients. Both NT and NTVS showed increased abundance of microorganisms involved in nutrient cycling, organic matter decomposition, and symbiosis, which are vital for soil health. Therefore, conservation-oriented tillage systems, especially NTVS, foster a more diverse, functional, and potentially growth-promoting microbiome in the onion, whereas CT directs the community toward opportunistic and less functional groups.

KEYWORDS
***Allium cepa*, conventional system, microorganisms, next-generation sequencing, no-tillage, no-tillage vegetable system**

1 Introduction

Soil hosts microorganisms, including bacteria, fungi, archaea, and protozoa, that comprise the soil microbiome (Pandey and Saharan, 2025). An estimated 10^9 cells and 10^6 distinct taxa are present in each Gram of soil, most of which remain unknown (Lakshmanan et al., 2014). Microorganisms are essential for ecosystem functions such as nutrient cycling, decomposition of organic matter, disease suppression, improvement of soil structure, pollutant degradation, and plant growth promotion (Chen et al., 2024). On the other hand, the microbial community and its functions are influenced by the management system used (Hermans et al., 2023), and probably the physical and chemical properties of the soil (García-Serquén et al., 2024).

Agriculture is mostly conducted under conventional tillage (CT), which relies on intensive chemical inputs, periodic soil mobilization, and dependence on external resources, thereby reducing soil diversity and biological activity (Hu et al., 2021; Domnariu et al., 2025; Loss et al., 2025; Nazarian et al., 2025). Conservation tillage systems have been developed, including the no-tillage system (NT), which uses cover crops and direct seeding with restricted soil disturbance, and the no-tillage vegetable system (NTVS), which, in addition to NT practices, includes crop rotation, greater species diversification, and permanent soil cover. Conservation tillage systems tend to support a more diverse microbiome, both taxonomically and functionally (Kumar et al., 2023; Labouyrie et al., 2023), but these relationships in vegetables, such as onions, remain poorly understood.

Onion is the third most produced vegetable in the world (Hanci, 2018), and its relationship with the soil microbiome is crucial for its healthy growth, as its reduced root system restricts nutrient and water absorption. Conservation tillage systems, especially NTVS, promote the recovery of soil properties (Vezzanini et al., 2019), improve soil structure (Loss et al., 2017), increase or maintain organic matter content and nutrient availability (Oliveira et al., 2017), and enhance biological activity (Souza et al., 2020; Bortolini et al., 2025), which are more favorable for onion production. However, we have not yet found studies that describe the effects of different management strategies on the structure of the soil microbiome, especially in long-term studies, which tend to demonstrate more significant structural and functional changes in microbial communities (Chen et al., 2020).

In addition, advances in next-generation sequencing (NGS) technologies have significantly improved our ability to characterize soil microbial communities, by enabling the detection of a much broader range of taxa, including those that cannot be cultured under laboratory conditions (Reznikova et al., 2026). In agricultural systems, NGS has proven particularly useful for assessing the impacts of different management practices on the composition and function of the soil microbiome, and provides a more detailed assessment of the ecological processes underlying soil quality and sustainability (Wang et al., 2020).

This study hypothesizes that soil conservation management in vegetable cultivation (NT and NTVS), due to lower mechanical stress and greater physical stability, the contribution of plant residues, and continuous, adequate nutrient cycling, will favor more diverse and functional microbial communities, whereas CT, with its microorganisms more adapted to disturbances and less functionally beneficial to soil health. The study aimed to characterize the soil microbiome structure (associated with onion long-term cultivation under conventional, no-tillage, and no-tillage systems.

2 Materials and methods

The experiment was conducted at the Experimental Station of the Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI) in Ituporanga, SC, Southern Brazil (27° 24′ 52″ S, 49° 36′ 9″ W, altitude 475 m). The soil is classified as Cambissolo Húmico Distrófico (EMBRAPA. Empresa Brasileira de Pesquisa Agropecuária, 2018) and Humic Dystrudept (Soil Survey Staff, 2022), with a clay loam texture (410 g kg/sand, 264 g kg/silt, and 326 g kg/clay in the 0–10 cm layer). The climate is humid subtropical (Cfa, Köppen), with an average annual temperature of 17.6 °C, rainfall of 1,400 mm, and a slope of 5%. The monthly average rainfall and temperature for 2023, along with the daily breakdown for November, are provided in Supplementary Material S1.

This study was conducted across different cropping systems and plant diversity (treatments), implemented in 2007, in a randomized block experimental design with five replications (8.7 m^2 plots) per treatment. The treatments included conventional tillage (CT), with one plowing and two harrowings before cultivating onions in spring, followed by maize (*Zea mays* L.) in summer, and fallow (weeds) in winter; no-tillage (NT), which follows a similar succession to CT but without soil turnover; and no-tillage vegetable system (NTVS), which is similar to NT but with a greater diversity of species grown in consortium in the summer, as millet (*Pennisetum glaucum* (L.) R. Br.) + velvet-bean *(Mucuna pruriens* (L.) DC) + sunflower (*Helianthus annuus* L.). In both CT and NT, during winter, the spontaneous plants are mainly from the Cyperaceae (25%) and Poaceae (20%) families.

The coverages described were standardized in 2011. Between 2007 and 2010, the composition varied partially. CT was managed with no-tillage before 2011. The experimental area had already been under a conservationist system since 1995, when the pH was adjusted to 6.0. In 2007, all areas were sown with black oats (*Avena strigosa*), vetches (*Vicia villosa*), and turnips (*Raphanus sativus* L.). Thereafter, the succession in CT involved pig beans (*Canavalia ensiformis* L.) with millet, followed by black oats, onions, rye (*Secale cereale* L.), corn, black oats, and onions, completing the cycle from 2007 to 2010. In the NT treatment, the continuous succession of onions and corn was used and maintained after 2010. In NTVS, from 2007 to 2010, sunflower was used, followed by black oats with rye, then sunflower + velvet-bean + millet in summer, then vetch *(Vicia sativa* L.) + rye + black oats + turnip (*R. sativus* L.) in winter, followed by onion in spring.

Both treatments are desiccated at the end of winter, and the soil is furrowed, adapted to no-tillage for manual transplantation of onion seedlings (cv. 'Empasc 352' – Bola Precoce) with a spacing of 40 × 10 cm between rows and plants, respectively, and seven rows per plot. Phytosanitary control includes herbicides (ioxynil, pendimethalin, fenoxaprop-p-ethyl + clethodim), insecticides (lambdacyhalothrin and imidacloprid), and fungicides (metalaxyl + chlorothalonil, metalaxyl + mancozeb, iprodione, tebuconazole + trifloxystrobin), applied according to the recommendation of the manufacturer, in addition to occasional manual weeding.

Fertilization for onions and corn follows regional recommendations (CQFS, 2016). Until 2010, 75, 120, and 60 kg ha^{-1} of N, P_2O_5, and K_2O were applied to onions, respectively. Starting in 2010, the P_2O_5 rate was adjusted to 80 kg ha^{-1}. In maize, 90 kg ha^{-1} of N is applied annually in coverage.

The dry mass of the treatments, measured before the onion transplant in 2023, was 5, 11, and 15 Mg ha^{-1} for CT, NT, and NTVS, respectively.

The physical and chemical properties of the soil, analyzed after the 2023 onion harvest, are listed in Supplementary Material S2. These data are being used in parallel studies that examine the effect of plants specifically on these variables; however, since the objective of this study is to characterize the soil microbiome in different onion production systems, they were used in a complementary data.

2.1 Soil microbiome

After harvesting the onion (November/2023), five simple soil samples were collected, one from each replication, and combined to form a composite sample representing the three treatments (this composite sample from each treatment was sequenced). Samples were collected along the onion cultivation row within the 0–10 cm layer. The material was stored at 4 °C in a Styrofoam box and sent to the laboratory for identification by next-generation sequencing - NGS through a service provider.

Initially, total DNA was extracted from 0.25 g of soil using the DNeasy PowerSoil® kit (QIAGEN) following the recommendations of the manufacturer. For the bacterial community, sequencing targeted the V4 region of the 16S gene using the primers 515F (5′ GTG CCA GCM GCC GCG GTA A 3′)/806R (5′ GGA CTA CHV GGG TWT CTA AT 3′) (Caporaso et al., 2011). The reaction mixture contained 1 µL of DNA + 0.2 µM of each primer +10.0 µL of 2× PCRBio Ultra Mix (PCRBiosystems), and ultrapure water to reach a final volume of 25 µL. Amplification was performed in a thermocycler at 94 °C for 3 min, followed by 35 cycles of 45 s, 1 min at 50 °C, 90 s at 72 °C, and a final extension of 10 min at 72 °C (Walters et al., 2016).

For the fungal community, the ITS region was amplified using primers ITS1 (5′ CTT GGT CAT TTA GAG GAA GTA A 3′) (Gardens and Bruns, 1993)/ITS2 (5′ GCT GCG TTC TTC ATC GAT GC 3′) (White et al., 1990). The reaction mixture included 1 µL of DNA +0.2 µM of each primer +10.0 µL of 2x PCRBio Ultra Mix (PCR Biosystems), and ultrapure water to reach a final volume of 25 µL. The PCR protocol consisted of an initial denaturation at 95 °C for 3 minutes, followed by 30 cycles of 30 s at 95 °C for denaturation, 30 s at 55 °C for annealing, and 45 s at 72 °C for extension, and finally a step of 10 min at 72 °C for final extension (Morgan and Egertonwarburton, 2017).

The microbiome was characterized on the Illumina MiSeq® sequencer using two 250 bp runs, yielding an average coverage of 30,000 reads. The depth of coverage was reached at approximately 10,000 reads (Supplementary Material S3).

For bioinformatics analysis, data were processed in QIIME 2 v.2025.04. Initially, sequences were demultiplexed and primers removed (trim-paired cutadapt). Then, the reads were processed through the DADA2 pipeline (dada2denoise-paired) for error correction, read merging (or stitching), removal of chimeric sequences, and generation of Amplicon Sequence Variants (ASVs). The sequencing depth was assessed with rarefaction curves generated in QIIME 2 (diversity alpha-rarefaction).

The ASVs were compared with the Silva database version 138.2 (https://www.arb-silva.de/) for bacteria and with the UNITE database version 10.0 (https://unite.ut.ee/) for fungi. The sequences have been deposited with NCBI (6141016 – 16S and 16141055 – ITS).

The microbiome description and interpretation used qualitative statistics with a descriptive approach, employing stacked bar graphs (relative abundance).

3 Results

3.1 16S gene

351, 363, and 366 ASV of Bacteria and Archaea (16S gene) were found in the NTVS, CT, and NT systems, respectively. The kingdom Bacteria was predominant across all management systems, accounting for 93.1% (NTVS), 88% (CT), and 79.4% (NT) of the sequences. Archaea accounted for 6.9%, 12%, and 20.6% in the NTVS, CT, and NT systems, respectively (Figure 1a).

Nine bacterial phyla (Proteobacteria, Actinobacteria, Acidobacteriota, Chloroflexi, Firmicutes, Verrucomicrobiota, Myxococcota, Bacteroidota, Latescibacterota) were detected in the evaluated systems, with Proteobacteria predominating at 32%, 29%, and 38% in NT, CT, and NTVS, respectively (Figure 1b). The second most frequent phylum was Actinobacteria. CT showed higher proportions of Firmicutes (4.8%), Verrucomicrobiota (2.3%), and Myxococcota (3.7%) than the other systems. In turn, NTVS had a lower prevalence of unclassified phyla (16.7%) than CT (19.4%) and NT (25.3%).

Nineteen classes were identified across the systems, including Alphaproteobacteria, Actinobacteria, Nitrososphaeria, Acidobacteriia, Gammaproteobacteria, Acidimicrobiia, Chloroflexia, Thermoleophilia, Bacilli, Blastocatellia, KD4-96, Verrucomicrobiae, Polyangia, Bacteriudia, Clostridia, BD2-1 terrestrial_group, Latescibacterota, Gitt-GS-136, and Coriobacteriia. Alphaproteobacteria predominated, representing 24.3%, 26%, and 33.2% in CT, NT, and NTVS, respectively (Figure 1c). The class Actinobacteria accounted for 19.7% in NT and NTVS systems, compared to 15.2% in the CT, as the second most prevalent class in both NTVS and CT. Nitrososphaeria was the second most abundant in NT, at 20.6%. Acidobacteriia, Thermoleophilia, Bacilli, and Polyangia were more abundant in CT than in the other evaluated systems. Gitt-GS-136 and Coriobacteriia were not detected in NTVS. A higher proportion of unidentified classes was observed in NTVS (9.2%) than in CT (7.3%) and NT (4.6%).

Forty-one orders were identified in the evaluated systems, including Rhizobiales, Nitrososphaerales, Streptosporangiales, Gaiellales, Burkholderiales, Propionibacteriales, Acidobacteriales, Bacillales, Streptomycetales, IMCC26256, Chloroflexales, KD4-96, Blastocatellales, Thermomicrobiales, Azospirillales, Solibacterales, Microtrichales, Micrococcales, Pedosphaerales, Subgroup_2, Nitrosotaleales, Acetobacterales, Micromonosporales, Polyangiales, Corynebacteriales, Bryobacterales, Chitinophagales, BD2-11_terrestrial_group, Pseudonocardiales, Latescibacterota,

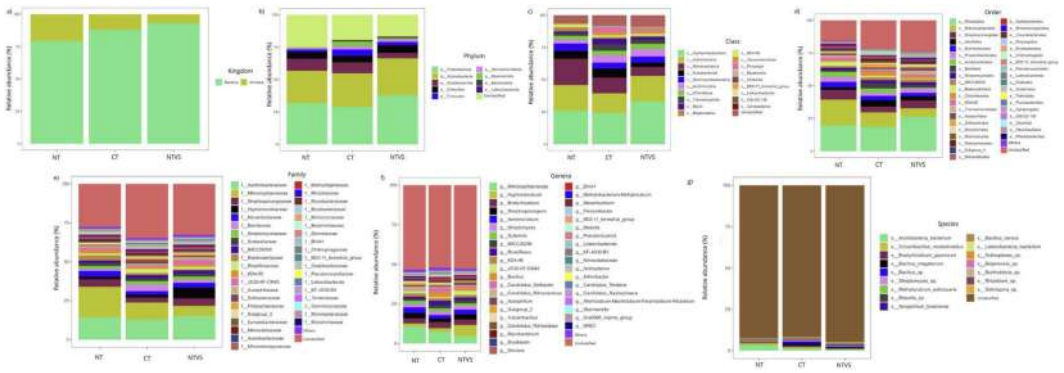

FIGURE 1
Bacterial community (16S) presents in the soil of different onion cropping systems: **(a)** kingdom, **(b)** phylum, **(c)** class, **(d)** order, **(e)** family, **(f)** genera, and **(g)** species.

Elsterales, Tistrellales, Tenderiales, Flavobacteriales, Cytophagales, Clostridia, Paenibacillales, Gitt-GS-136, Rhodobacterales, Enterobacterales, and Coriobacteriales (Figure 1d). Rhizobiales was the most common order in NT (19.8%) and NTVS (26.1%), whereas unclassified groups were more prevalent in CT (22.1%). However, in NTVS, unclassified groups accounted for 23.6%, compared with 15.8% in NT.

The CT showed 40 orders different from those in the system, except for Flavobacteriales. In contrast, NT and NTVS had 37 different orders, with Flavobacteriales, Cytophagales, Clostridia, and Paenibacillales absent in NT. Meanwhile, Clostridia, Gitt-GS-136, Enterobacteriales, and Coriobacteriales were missing in NTVS (Figure 1d). The order Nitrososphaerales accounted for 19.6% of the relative abundance in NT and decreased in the other systems, to 11.1% in CT and 6.5% in NTVS.

Fifty-one families were identified across the systems: Xanthobacteraceae, Nitrososphaeraceae, Streptosporangiaceae, Hyphomicrobiaceae, Nocardioidaceae, Bacillaceae, Streptomycetaceae, Sutterellaceae, IMCC26256, Roseiflexaceae, KD4-96, Blastocatellaceae, JG30-KF-CM45, Solibacteraceae, Azospirillaceae, Pedosphaeraceae, Subgroup2, Ilumatobacteraceae, Nitrosotaleaceae, Acetobacteraceae, Methyloligellaceae, Micromonosporaceae, Rhizobiaceae, Mycobacteriaceae, Micrococcaceae, Bryobacteraceae, Beijerinckiaceae, Devosiaceae, BIrii41, Chitinophagaceae, BD2-11_terrestrialgroup, Oxalobacteraceae, Pseudonocardiaceae, Latescibacterota, KF-JG30-B3, Geminicoccaceae, Tenderiaceae, Microbacteriaceae, Microtrichaceae, Flavobacteriaceae, Microscillaceae, Rhodanobacteraceae, Hungateiclostridiaceae, Acidobacteriaceae (Subgroup1), Paenibacillaceae, Gitt-GS-136, Rhodobacteraceae, Burkholderiaceae, Propionibacteriaceae, Eggerthellaceae, and Nocardiaceae (Figure 1e). Unclassified families predominated in all systems (27.7%–34.8%), followed by Xanthobacteraceae in the CT (12.7%) and NTVS (15.2%) systems. In NT, Nitrososphaeraceae represented 19.6%, and this family accounted for 11.1% and 6.5% in CT and NTVS, respectively.

The NT system included 48 families, except Flavobacteriaceae, Microscillaceae, Hungateiclostridiaceae, and Paenibacillaceae, which were absent (Figure 1e). In turn, CT hosts 49 families, except for Flavobacteriaceae and Nocardiaceae; while NTVS hosts 45 families, excluding KF-JG30-B3, Rhodanobacteraceae, Hungateiclostridiaceae, Acidobacteriaceae (Subgroup 1), Gitt-GS-136, and Eggerthellaceae. The Hyphomicrobiaceae family accounted for 7.1% of the relative abundance of NTVS, while only 2.1% in CT and 1.8% in NT (Figure 1e). In turn, Bacillaceae accounted for 4.0% of the abundance in CT and 1.9% and 1.2% in NTVS and NT, respectively.

Fifty-four genera were identified across the systems, including Nitrososphaeraceae, *Hyphomicrobium*, *Bradyrhizobium*, *Streptosporangium*, *Aeromicrobium*, *Streptomyces*, *Sutterella*, *IMCC26256*, *Roseiflexus*, *KD4-96*, *JG30-KF-CM45*, *Bacillus*, *Candidatus_Solibacter*, *Candidatus_Nitrocosmicus*, *Azospirillum Subgroup_2*, *Vulcanibacillus*, *Candidatus_Nitrosotalea*, *Mycobacterium*, *Bryobacter*, *Devosia*, *BIrii41*, *Mesorhizobium*, *Methylobacterium-Methylorubrum*, *Flavisolibacter*, *BD2-11_terrestrial_group*, *Massilia*, *Pseudonocardia*, *Latescibacterota*, *KF-JG30-B3*, *Arthrobacter*, *Nitrosotaleaceae*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Candidatus_Alysiosphaera*, *Candidatus_Tenderia*, *Skermanella*, *Actinoplanes*, *Sva0996_marine_group*, *MND1*, *Flavobacterium*, *Nocardioides*, *Micromonospora*, *Dyella*, *Candidatus_Nitrososphaera*, *Hungateiclostridium*, *Paenibacillus*, *Gitt-GS-136*, *Beijerinckia*, *Burkholderia-Caballeronia-Paraburkholderia*, *Acidiphilium*, *Marinilutecoccus*, *Salinispora*, *Slackia,* and *Nocardia* (Figure 1f). The majority of genera in both systems were unclassified (52%–53.3%). *Nitrososphaeraceae* was the second most prevalent in NT (10.9%) and CT (7.6%). In NTVS, *Hyphomicrobium* was dominant, accounting for 7.1%.

The NT treatment presented 50 genera, except for *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Flavobacterium*, *Hungateiclostridium,* and *Paenibacillus* (Figure 1f). CT presented 51 genera, with *Flavobacterium*, *Salinispora,* and *Nocardia* absent, and NTVS presented 46 genera, with the exception of KF-JG30-B3, *Actinoplanes*, *Dyella*, *Candidatus_Nitrososphaera*, *Hungateiclostridium*, *Gitt-GS-136*, *Beijerinckia,* and *Slackia*. The genus *Flavobacterium* was found exclusively in NTVS, whereas *Hungateiclostridium* was observed only in CT.

The genus *Bacillus* represented 3.1% of the relative abundance in CT; however, this value was 1.7% in NTVS and 0.1% in NT

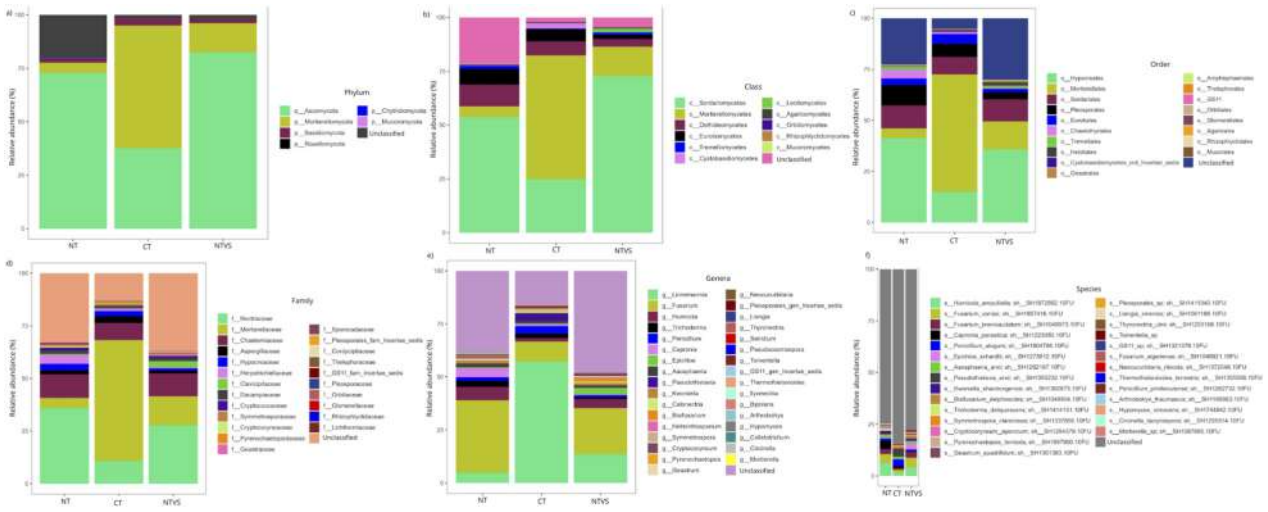

FIGURE 2
Fungi community (ITS) presents in the soil of different onion cropping systems: **(a)** phylum, **(b)** class, **(c)** order, **(d)** family, **(e)** genera, and **(f)** species.

(Figure 1f). *Candidatus_solibacter* accounted for 2.6% of the abundance in CT, 1% in NTVS, and 0.5% in NT.

Regarding the identified species, 91.8%–94.9% were not classified by the evaluated systems (Figure 1g). Among those classified, 16 distinct species were found: *Acidobacteria bacterium*, *Vulcanibacillus modesticaldus*, *Bradyrhizobium japonicum*, *Bacillus megaterium*, *Bacillus* sp., *Streptomyces* sp., Methylorubrum *extorquens*, *Massilia* sp., *Bacillus cereus*, *Azospirillum brasilense*, *Latescibacteria bacterium*, *Actinoplanes* sp., *Beijerinckia* sp., *Rhizobium* sp., *Burkholderia* sp., and *Salinispora* sp. Among the classified species, *A. bacterium* predominated across treatments, at 3.3% in NT, 1.2% in CT, and 0.7% in NTVS.

The NT system showed 13 distinct species, except for *Bacillus* sp., *Bacillus cereus,* and *Rhizobium* sp. (Figure 1g). CT presented 15 species, except for *Salinispora* sp. While NTVS hosted 14 species, except for *Actinoplanes* sp. and *Beijerinckia* sp. The species *V. modesticaldus* accounted for 1.2% of the abundance in NT, 0.9% in CT, and only 0.2% in NTVS. In turn, *A. brasilense* represented 0.5% in NTVS and 0.2% in both NT and CT.

3.2 ITS gene

Regarding fungi (ITS gene), 149, 146, and 115 ASV were found in the NTVS, NT, and CT systems, respectively. Six phyla were identified across the evaluated systems: Ascomycota, Mortierellomycota, Basidiomycota, Rozellomycota, Chytridiomycota, and Mucoromycota (Figure 2a). The phylum Ascomycota predominated in NT (72.8%) and NTVS (82.4%). In CT, Mortierellomycota accounted for 57.5%, while Ascomycota represented 37.6%. 20.3% of the phyla were not found in NT. Chytridiomycota was present only in NT, and Mucoromycota was absent in CT.

Eleven classes of fungi were identified across the systems: Sordariomycetes, Mortierellomycetes, Dothideomycetes, Eurotiomycetes, Tremellomycetes, Cystobasidiomycetes, Leotiomycetes, Agaricomycetes, Orbiliomycetes, Rhizophlyctidomycetes, and Mucoromycetes (Figure 2b). Sordariomycetes predominated in NT (53.8%) and NTVS (72.7%), and accounted for 24.9% in CT, where Mortierellomycetes also dominated (57.4%). In NT, 21.6% of the classes were unclassified. All 11 classes were present in NT (Figure 2b), while eight were found in NTVS—excluding Cystobasidiomycetes, Orbiliomycetes, and Rhizophlyctidomycetes—and seven in CT, missing Leotiomycetes, Orbiliomycetes, Rhizophlyctidomycetes, and Mucoromycetes. The classes Orbiliomycetes and Rhizophlyctidomycetes were present only in NT.

Eighteen orders were identified among the systems: Hypocreales, Mortierellales, Sordariales, Pleosporales, Eurotiales, Chaetothyriales, Tremellales, Helotiales, Cystobasidiomycetes_ord_Incertae_sedis, Geastrales, Amphisphaeriales, Thelephorales, GS11, Orbiliales, Agaricales, Glomerellales, Rhizophlyctidales, and Mucorales (Figure 2c). Hypocreales dominated in NT (41.2%) and NTVS (37.8%), whereas Mortierellales was most common in CT (57.4%). Approximately 22.7% and 30.1% of sequences in NT and NTVS, respectively, were not classified at the order level. The Eurotiales order represented 4.7% in CT but decreased in NT (3.2%) and NTVS (1.6%).

Among the 18 orders identified, 16 were present in NT, except for Geastrales and Agaricales (Figure 2c). NTVS hosts 14 orders, excluding Cystobasidiomycetes_ord_Incertae_sedis, Orbiliales, Agaricales, and Rhizophlyctidales; CT holds 13 orders, except for Helotiales, Geastrales, Orbiliales, Rhizophlyctidales, and Mucorales. The order Geastrales was present only in NTVS, Agaricales only in CT, and Orbiliales and Rhizophlyctidales only in NT.

A total of 23 families were identified across the systems, including Mortierellaceae, Nectriaceae, Chaetomiaceae, Aspergillaceae, Hypocreaceae, Herpotrichiellaceae, Dacampiaceae, Clavicipitaceae, Cryptococcaceae, Symmetrosporaceae, Cryptocoryneaceae, Pyrenochaetopsidaceae, Geastraceae, Pleosporales_fam_Incertae_sedis, Sporocadaceae, Cordycipitaceae,

Thelephoraceae, GS11_fam_Incertae_sedis, Orbiliaceae, Pleosporaceae, Glomerellaceae, Rhizophlyctidaceae, and Lichtheimiaceae (Figure 2d). The Mortierellaceae family predominated in CT (57.4%), while Nectriaceae predominated in NT (35.9%) and NTVS (27.9%). Nectriaceae accounted for 35.9% in NT, 27.9% in NTVS, and 10.8% in CT. Additionally, 33% of sequences from NT and 37.2% from NTVS could not be classified at the family level, compared with 13.3% in CT. Notably, the Chaetomiaceae family accounted for 11.1%, 10.8%, and 8.0% of relative abundance in NT, NTVS, and CT, respectively (Figure 2d).

Among the identified families, 21 were present in NT, except for Geastraceae and Cordycipitaceae (Figure 2d). In NTVS, 20 families were found, excluding Symmetrosporaceae, Orbiliaceae, and Rhizophlyctidaceae. In CT, 19 families were present, except for Geastraceae, Orbiliaceae, Rhizophlyctidaceae, and Lichtheimiaceae. Geastraceae was present only in NTVS, and Orbiliaceae and Rhizophlyctidaceae only in NT.

Thirty-three distinct genera were identified across the systems: *Linnemannia*, *Fusarium*, *Humicola*, *Trichoderma*, *Penicillium*, *Capronia*, *Pseudothielavia*, *Aaosphaeria*, *Epichloe*, *Kwoniella*, *Calonectria*, *Bisifusarium*, *Helminthosporium*, *Symmetrospora*, *Cryptocoryneum*, *Pyrenochaetopsis*, *Neocucurbitaria*, *Geastrum*, *Pleosporales_gen_Incertae_sedis*, *Liangia*, *Thyronectria*, *Tomentella*, *Seiridium*, *Pseudocosmospora*, *GS11_gen_Incertae_sedis*, *Thermothielavioides*, *Ilyonectria*, *Arthrobotrys*, *Bipolaris*, *Hypomyces*, *Colletotrichum,* Circinella, and *Mortierella* (Figure 2e). *Linnemannia* predominated in CT (57.3%), whereas *Fusarium* predominated in NT (34.2%) and NTVS (21.6%). Approximately 38.2% and 47.7% of the sequences from NT and NTVS, respectively, were not classified at the genus level, whereas only 16.2% were unclassified in CT.

The genus *Humicola* accounted for 6.2%, 4.3%, and 1.6% in NTVS, NT, and CT, respectively (Figure 2e). *Trichoderma* accounted for 2.9%, 2.3%, and 0.9% of the relative abundance in NT, CT, and NTVS, respectively. In turn, *Penicillium* accounted for 3.4%, 1.6%, and 0.9% of the genera identified in CT, NT, and NTVS, respectively. In NT, *Capronia* accounted for 4.2% of the relative abundance.

Among the identified genera, NT presented 30, except for *Geastrum*, *Liangia*, and *Thyronectria* (Figure 2e). 28 genera were identified in CT, except for *Geastrum*, *Thyronectria*, *Ilyonectria*, *Arthrobotrys,* and *Circinella.* NTVS also presented 28 genera, except for *Pseudothielavia*, *Symmetrospora*, *Arthrobotrys*, *Hypomyces*, and *Mortierella*. The genera *Geastrum* and *Thyronectria* were present only in NTVS, and *Arthrobotrys* only in NT.

Among the identified species, 74.8%–84.4% were not classified (Figure 2f). Of those classified, we found 28 distinct species: *Humicola ampulliella*, *Fusarium variasi*, *Fusarium brevicaudatum*, *Capronia parasitica*, *Penicillium algum*, *Pseudothielavia arxii*, *Aaosphaeria arxii*, *Epichloe schardlii*, *Kwoniella shandongensis*, *Bisifusarium delphinoides*, *Trichoderma deliquescens*, *Symmetrospora clarorosea*, *Cryptocoryneum japonicum*, *Pyrenochaetopsis terrícola*, *Geastrum quadrifidum*, *Pleosporales* sp., Liangia *sinensis*, *Thyronectria ulmi,* Tomentella sp., GS11 sp., *Fusarium algeriense*, *Thermothielavioides terrestris*, *Neocucurbitaria ribicola*, *Penicillium pimiteouiense*, *Arthrobotrys thaumasius*, *Hypomyces virescens,* Circinella lacrymispora, and *Mortierella* sp. The species *H. ampulliella*, *Fusarium variasi,* and *Fusarium brevicaudatum* accounted for 6.2%, 4.3%, and 1.6%; 4.3%, 4.6%, and 1.4%; and 2.4%, 2.8%, and 0.9%, respectively, across the NT, NTVS, and CT systems. *Capronia parasitica* represented 4.2% in NT. *Trichoderma deliquescens* composed 1.1% and 0.2% of NT and NTVS, respectively.

Among the species found, NT hosts 24, except for *G. quadrifidum*, *Liangia sinensis*, *T. ulmi,* and *P. pimiteouiense.* CT hosts 25 species, except for *G. quadrifidum*, *T. ulmi*, *P. pimiteouiense*, *A. thaumasius,* and *Circinella lacrymispora.* In turn, NTVS presents 24 species, except *for Pseudothielavia arxii*, *S. clarorosea*, *Fusarium algeriense*, *A. thaumasius*, *H. virescens,* and *Mortierella* sp. Notably, only NTVS hosts *G. quadrifidum*, *T. ulmi,* and *P. pimiteouiense*, while only NT hosts *A. thaumasius*.

4 Discussion

Soil conservation practices in onion farming affected microbial diversity and structure, supporting the hypothesis of this study. The dominance of the Bacteria kingdom (79.4%–93.1%) across all systems and the smaller share of Archaea (6.9%) in NTVS (Figure 1a) suggest that this more complex system provides a more structured environment; it likely has increased rhizospheric activity and oxygen availability, which promote bacterial growth (Tripathi et al., 2019). About chemical and physical properties (Supplementary Material S2), the NTVS exhibited higher organic matter (4.6%), total organic carbon (3.1%), and total nitrogen (0.3%) compared to NT and CT, as well as greater availability of phosphorus (84 mg dm^{-3}) and potassium (379 mg dm^{-3}), which may contribute to a richer and more diverse microbial community (Cui et al., 2023). However, CT, characterized by higher pH (6.4), greater calcium and magnesium, 11.7 and 3.2 $cmol_c$ dm^{-3} in order content, and higher soil density (1.32 g cm^{-3}) and penetration resistance (1.3 MPa), appears to favor a more restricted microbial environment, with lower bacterial diversity.

The NT system, in general, showed intermediate values for both soil properties and microbial composition, reflecting a transitional state between the conventional and more complex no-tillage vegetable system. These observations indicate that soil physicochemical conditions, influenced by management long-term practices, are consistent with the observed patterns in microbial structure and diversity.

The predominance of the Proteobacteria and Actinobacteria phyla, especially in NTVS (Figure 1b), may indicate high metabolic capacity, particularly in carbon and nitrogen cycling (Boubekri et al., 2022). The higher percentage of Proteobacteria (38%) in NTVS indicates the positive influence of greater vegetation cover and the diversity of cover crops, which provide a variety of root exudates and organic substrates, thereby stimulating populations associated with the dynamics of organic substance transformation in the soil (Yang et al., 2021). The NTVS system produced three times more dry matter (15 Mg ha^{-1}) than CT (5 Mg ha^{-1}) before onion transplant, which, as it decomposed, likely led to greater microbial diversity and activity due to the availability of food for microorganisms (Chinthalapudi et al., 2026) and, this pattern is also related to the improved soil chemical conditions in this system, as mentioned above, which promote greater diversity of microorganisms involved

in mineralization and nutrient cycling (Castellano-Hinojosa and Strauss, 2020). On the other hand, the CT showed a higher proportion of phyla such as Firmicutes, Verrucomicrobiota, and Myxococcota, which are often associated with stressful chemical, physical, or climatic conditions (Liu C. et al., 2025). This is in line with the physicochemical characteristics of CT soil mentioned above, suggesting that more compacted and less organically enriched soils may favor microbial taxa adapted to these conditions (Xu et al., 2021).

The Alphaproteobacteria class was prevalent across all systems, particularly in NTVS (33.2%) (Figure 1c), indicating a more biologically active and healthier environment, as this class includes genera such as Bradyrhizobium, *Rhizobium*, and *Azospirillum*, which are known for fixing atmospheric nitrogen (Gazdag et al., 2018).

The higher relative abundance of Nitrososphaeria (ammonium-oxidizing archaea) in NT (20.6%) (Figure 1c) indicates increased nitrification activity in this system. In contrast, in NTVS, Nitrososphaeria accounts for 6.9% of the abundance, suggesting greater ammonium accumulation (Könneke et al., 2005). Generally, these bacterial classes are found in environments with greater soil disturbance or low to moderate levels of organic matter (Bahram et al., 2026), as observed in CT (3.7%) and NT (3.9%), compared to NTVS (4.6%) in this study (Supplementary Material S2).

The predominance of Rhizobiales in NT and NTVS reinforces the role of NTVS in promoting mutualistic microorganisms and producers of beneficial secondary metabolites in conservation tillage systems (Mendes et al., 2011). In turn, the higher occurrence of Bacillales and Clostridia in CT, spore-forming orders under stressful conditions, indicates a community more adapted to disturbances, such as intensive soil movement, compacted soil, and lower quality of chemical properties (Paredes-Sabja et al., 2011).

The Xanthobacteraceae family, dominant in NTVS and CT (Figure 1e), comprises bacteria that fix nitrogen and degrade complex organic compounds, contributing to carbon and nitrogen cycling (Brescia et al., 2023). This character in NTVS is also favored by the better physical and chemical conditions (Supplementary Material S2), diversified cover crops and reduced soil disturbance, may contribute to the preservation of symbiotic and rhizosphere-associated microorganisms. But, in CT, this may relate to the rapid degradation of organic matter and the presence of labile carbon during soil movement.

Meanwhile, Nitrososphaeraceae—a group of ammonium-oxidizing archaea more abundant in NT—is associated with the Nitrososphaeria class (Figure 1c), indicating nitrification activity in this environment and pointing to increased oxidation likely caused by ammonium buildup from the mineralization of plant debris on the soil surface (Melcher et al., 2023). NT exhibited physicochemical characteristics intermediate between CT and NTVS (Supplementary Material S2), indicating that it is still developing and is likely fostering better conditions for the nutrient cycle.

Regarding the identified genera, the systems presented *Nitrososphaeraceae*, *Bradyrhizobium*, *Streptomyces*, *Azospirillum,* and *Mesorhizobium* (Figure 1f), known for their beneficial roles in agricultural systems (Benedetto et al., 2017; Khmelevtsova et al., 2022). Low species-level classification (Figure 1g) was expected given the limited available database. *Hyphomicrobium* dominant genus in NTVS (7.1%) was obligate methylotrophic bacteria associated with denitrification and carbon cycling (Martineau et al., 2015), thus, its predominance in NTVS suggests an active role in these cycling, potentially reflecting enhanced microbial activity and more dynamic biogeochemical processes in this organically enriched and structurally complex system.

The results for the bacterial microbiome (Figure 1) highlight differences among the evaluated systems, even in the absence of major compositional changes. NT and NTVS favored oligotrophic groups associated with soil stability (e.g., Acidobacteria) and increased total microbial biomass due to less physical disturbance and greater contributions to and retention of organic matter (Li et al., 2020). By contrast, CT tends to favor copiotrophic groups that benefit from more labile organic matter fractions and greater aeration, with a relative increase in Actinobacteria and Firmicutes (Zhang et al., 2019). These results are reinforced by the physical and chemical characteristics of the soil and cover crops' dry mass production.

The fungal community (ITS gene) showed greater richness in more conservationist systems (146-149 ASV for NT and NTVS, respectively) than in CT (115), indicating that restricted soil turnover, increased vegetation cover, and better physical and chemical soil conditions (Supplementary Material S2) tend to preserve or increase fungal diversity, as observed by Detheridge et al. (2016) and Gao et al. (2022), by preserving mycelia and fungal spores within the soil structure.

The predominance of the phylum Ascomycota in NT (72.8%) and NTVS (82.4%), and of Mortierellomycota (57.5%) in CT (Figure 2a), suggests functional differentiation between the systems. Ascomycota is a diverse phylum with numerous functions, including saprophytic (nutrient decomposition and cycling), mutualistic, and biological control roles; they are slower-growing fungi that prefer more stable environments with low disturbance of structure and temperature (Korniłłowicz-Kowalska et al., 2022), which is expected in conservationist environments (NT and NTVS). And, the higher abundance of this phylum in these systems, in particular NTVS, may be related to the greater availability of organic substrates, this system presented levels of organic matter (4.6%), total organic carbon (3.1%), and total nitrogen (0.3%), as well as greater phosphorus and potassium availability (Supplementary Material S2), conditions that favor microbial activity and the development of fungal decomposers (Mayer et al., 2021). In turn, the Mortierellomycota phylum is commonly associated with the cycling of labile carbon in soil, as observed in CT, which had the lowest total organic carbon content between the systems, has a high turnover rate, and is therefore better adapted to stressful environments (e.g., higher soil density and penetration resistance) with soil turnover (Bai et al., 2024).

The dominance of the Sordariomycetes class in conservation tillage systems (NT and NTVS) and of Mortierellomycetes in CT (Figure 2b) is also linked to the growth strategies and environmental adaptations of these fungi. The Sordariomycetes class includes fungi with a greater capacity to decompose plant residues, cycle nutrients, and form symbioses; they also grow more slowly, use and transform resources more efficiently, and persist in the environment (Wang et al., 2023), conditions favored by the continuous input of organic matter from the NT and NTVS systems, and the resulting improvements in the physicochemical properties of these systems. In turn, the Mortierellomycetes class is associated with

more disturbed environments and rapid mineralization of organic matter. These fast-growing organisms degrade readily available substrates (Rakotonindrina et al., 2025).

The greater presence of the order Hypocreales in NT and NTVS (35.8%–41.2%), including genera such as *Fusarium* and *Trichoderma* (Figure 2c), may indicate greater beneficial fungal activity in these systems. In turn, the predominance of Mortierellales in CT indicates fungi with rapid growth and high activity in decomposing readily available carbon (Orrù et al., 2021). This pattern is also evident in the predominance of the Nectriaceae family in the conservation tillage systems (NT and NTVS) and of Mortierellaceae in the system with revolving soil practices (CT) (Figure 2d), as previously discussed.

The genus *Fusarium*, predominant in conservation tillage systems (NT and NTVS) (Figure 2e), is known to represent pathigenic diseases (rot), but it also comprises decomposing species of organic matter, which probably predominate in this environment because rot was not observed in the bulbs (except under favorable climatic conditions such as high precipitation and temperature), which did not occur in the year of the study (Supplementary Material S1). *Fusarium* spp. are depending on climatic conditions can act as pathogens or saprotrophs (Nikitin et al., 2023), in this study, probably their primary role is likely saprotrophic due to the high levels of organic matter and the climatic conditions already mentioned. Nevertheless, it is important to note that metabarcoding does not allow precise functional assignment at the species or guild level, and approaches such as ecological annotation tools (e.g., FUNGuild) or complementary assays would be necessary to confirm the functional roles of these taxa (Nguyen et al., 2016). In turn, the predominance of Linnemannia in CT (57.3%) is related to fungi that grow and reproduce faster and require labile carbon for their cycle.

The high proportion of unclassified sequences, especially at the species level (Figure 2f), underscores the importance of studies of this nature in strengthening the available database.

In addition, the absence of arbuscular mychorrizal fungi (AMF) (members of Glomeromycota) in our data should not be interpreted as their absence in the soil, but rather as a limitation of the sequencing approach employed. Which is likely related to primers used (ITS1/ITS2) are not optimal for arbuscular mychorrizal fungi (AMF) detection, therefore, primers specific for AMF should also be used, such as NS31/AML2 (Morgan and Egerton-Warburton, 2017).

The results of the fungal microbiome indicate that the NT and NTVS systems have more stable soils and a higher abundance of decomposing and biocontrol species (Figure 2). In contrast, CT shows a greater abundance of opportunistic and pathogenic fungi (Leff et al., 2015; Tiemann et al., 2015; Schmidt et al., 2019). In addition, these results are also related to the management practices used, soil disturbance, and the diversity of species, as well as soil organic matter levels, nutrient availability, and soil physical structure, factors that determine the ecological niches available for fungal decomposers and other functional groups in agricultural soils (Xu et al., 2025).

In other studies, in this same area, carbon and nitrogen stocks, soil density, and soil fertility (0–10 cm and 0–30 cm) (Nazarian et al., 2025), light organic matter (LOM) (Giumbelli et al., 2021), and the distribution of aggregate classes (Giumbelli et al., 2020) have already been evaluated. The results of these studies showed that the NTVS system has the highest potential for C and N storage, nutrient cycling, higher C and N content in LOM, and a predominance of stable macroaggregates. These results are likely related to the microbial community, since NT and NTVS host more diverse, functional, and beneficial communities, with a greater presence of Rhizobiales, Mortierellaceae, and Chaetomiaceae (Figures 1, 2), whereas CT favored opportunistic and pathogenic groups, such as Nectriaceae and *Fusarium*.

In addition, NTVS also favored higher onion productivity, with 41 Mg ha^{-1} in 2023, compared to 35 and 34 Mg ha^{-1} in NT and CT, respectively (Nazarian et al., 2025).

Overall, the findings indicate that conservation tillage systems (NTVS and NT) promote a better balance between microbial diversity and functionality, associated with the continuous presence of plant residues and lack of soil disturbance. Long-term research confirms that soil conservation enhances biomass, microbial stability, and often enhances soil quality indicators (Liu J. et al., 2025). Conversely, CT exhibited a notably less diverse microbiota that, while more resilient to disturbances, had reduced ecological and functional capacities.

In addition, the present study does not include robust statistical comparisons among treatments, which can be considered a restriction, due to the use of composite samples for microbiome sequencing, the results still provide meaningful insights into the relationships between soil management, physicochemical properties, and microbial structure. The use of composite samples allowed the integration of multiple subsamples from each system, capturing the overall microbial profile associated with each management strategy and reducing local spatial variability within the fields. The microbial patterns were consistent with the measured soil physicochemical attributes (Supplementary Material S2). The concordance between microbial composition and soil chemical and physical characteristics supports the ecological plausibility of the observed patterns, even in the absence of formal statistical inference. Similar descriptive approaches have been used in exploratory soil microbiome studies aimed at identifying ecological trends and generating hypotheses about the influence of management practices on microbial communities (Prosser, 2015).

5 Conclusion

Soil management alters the composition of the soil microbiome in long-term onion farming.

Conventional tillage (CT) with annual soil turnover favors microorganisms adapted to stressful, fast-growing conditions and readily available food, including the bacterial orders Bacillales and Clostridia and the fungal order Mortierellales. Furthermore, these results are inextricably linked to physicochemical and productive characteristics, given that this system exhibited higher alkalinity, lower levels of soil organic matter, total organic carbon, phosphorus, potassium, sulfur, cover crops dry mass yield, and higher soil density.

Conservation tillage systems such as no-tillage (NT) and the no-tillage vegetable system (NTVS), due to greater plant complexity, absence of soil turnover, higher dry mass production, and improved

physicochemical properties, support a higher abundance of microorganisms involved in nutrient cycling, decomposition of organic matter, and symbioses, including the bacterial order Rhizobiales and the fungal order Hypocreales. This reinforces the importance of conservation management as a promising strategy to sustainable productivity.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LG: Conceptualization, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review and editing. LR: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review and editing. EM: Conceptualization, Formal Analysis, Methodology, Writing – original draft, Writing – review and editing. CdA: Conceptualization, Data curation, Formal Analysis, Software, Writing – original draft, Writing – review and editing. CK: Conceptualization, Formal Analysis, Funding acquisition, Writing – review and editing. JC: Conceptualization, Funding acquisition, Investigation, Writing – original draft, Writing – review and editing. PL: Conceptualization, Funding acquisition, Investigation, Validation, Writing – original draft, Writing – review and editing. AL: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

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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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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2026.1772808/full#supplementary-material

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