Comprehensive analysis of 37 composts: microbial indicators for soilborne disease suppression in three plant-pathogen systems

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Abstract

Soilborne diseases pose a significant threat to crop production and their control remains a major challenge. Compost is a valuable soil amendment when it comes to suppressing soilborne pathogens, but the effectiveness of different composts varies and cannot yet be predicted. Microbial communities in compost play a key role in disease suppression, and therefore microbial taxa may serve as indicators for suppressive composts. In this study, we investigated 37 composts from seven commercial compost producers to analyze the association of their bacterial and fungal communities with disease-suppressive activity across three plant-pathogen systems: cress-*Globisporanigum ultimum*, cucumber-*Globisporanigum ultimum* and cucumber-*Rhizoctonia solani*. Our results underscore that compost suppressiveness is primarily pathogen-specific and, to a smaller extent, host-plant-specific. Suppressiveness was not found to be correlated to physicochemical properties or microbial activity, alpha- and beta-diversity of the composts. However, specific bacterial and a few fungal taxa were identified as potential indicators of suppression for each plant-pathogen system. Notably, for *G. ultimum*-suppression, bacteria from the genera *Luteimonas*, *Sphingopyxis*, and *Algoriphagus* emerged as promising candidates. These findings provide a foundation for targeted microbial isolation, aiming to elucidate

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the role of microorganisms in compost-mediated disease suppression, and to predict and optimize suppressive activity.
Keywords: Soil amendments, *Globisporanigum ultimum*, *Pythium ultimum*, *Rhizoctonia solani*, Biocontrol, Biofertilizer

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Acronyms

$\mathbf{C_{org}/N_{tot}}$ carbon-nitrogen ratio.

G. ultimum *Globisporanigum ultimum.*

R. solani *Rhizoctonia solani.*

ASV amplicon sequence variant.

$\mathbf{C_{org}}$ total organic carbon.

$\mathbf{CaCl_2}$ calcium chlorid.

DS dry substance.

FDA Fluorescein diacetate.

$\mathbf{N_{min}}$ total mineral nitrogen.

$\mathbf{N_{tot}}$ total nitrogen.

$\mathbf{NH_4^+}$ ammonium.

$\mathbf{NO_2^-}$ nitrite.

$\mathbf{NO_3^-}$ nitrate.

$\mathbf{NO_3^-\text{-}N/N_{min}}$ nitrification index.

$\mathbf{OD_{550}}$ soluble humic substances.

PCA Principle Component Analysis.

$\mathbf{PO_4^{3-}}$ phosphate.

WHC water holding capacity.

1. Introduction

Soilborne diseases are a major yield-limiting factor in agricultural crop production and are difficult to control. They are still primarily managed through soil fumigation with chemicals or heat. In particular, chemical soil treatments, e.g., with methyl bromide, are facing increasing criticism and legal restrictions due to the negative impact on the environment and non-target soil organisms including the soil microbiome (European Union, 2015). A functional soil microbiome is crucial for plant health and crop productivity (Banerjee and Van Der Heijden, 2023) and increases the resilience of a soil to pathogen infestation (Wang and Li, 2019). Thus, control measures that suppress soilborne diseases while preserving or even enhancing the functionality of the soil microbiome are urgently needed (Gullino et al., 2022). A desirable approach to managing soilborne diseases is to establish disease-suppressive soils (Sagova-Mareckova et al., 2023), which are soils where pathogens are naturally reduced or cause minimal to no damage (Baker and Cook, 1974). The application of compost to soil or potting substrate can enhance suppressivness and improve plant health (Hoitink and Kuter, 1986; Noble and Coventry, 2005; Termorshuizen et al., 2006; Edlinger et al., 2025) and represents a promising strategy to increase natural soil suppressivness (De Corato, 2020).

Compost is a product of the controlled aerobic decomposition of organic materials or feedstock by microorganisms (Oshins et al., 2022). The composting process begins with a mesophilic phase, during which the substrate temperature rises, followed by a thermophilic phase. As easily degradable organic materials become depleted, the temperature drops, signaling the start of the cooling phase. During this phase, mesophilic microorganisms recolonize, initiating humification processes that lead to the maturation phase of the composting process (Oshins et al., 2022).

Suppressive activity of composts has been reported against fungal pathogens and oomycetes, as well as bacterial pathogens, nematodes and viruses (Neher et al., 2022; Lutz et al., 2020). However, the efficacy varies strongly among composts. Studies have identified various factors that can influence the disease-suppressive activity of a compost, such as the feedstock composition, the maturation stage of the compost, the target pathogen, and the microbial communities in the compost (Scheuerell and Mahaffee, 2005; Termor-

shuizen et al., 2006; Zmora-Nahum et al., 2008). However, despite decades of compost research, we still lack a fundamental understanding on the key factors and their interplay in disease suppression. As a result, it is not yet possible to predict whether a particular compost will successfully control a specific disease (Bonanomi et al., 2010; Neher et al., 2022). For the targeted use and commercialization of suppressive composts in crop protection, we need a better understanding of the underlying mechanisms and diagnostic tools to predict the potential of individual composts (Hadar and Papadopoulou, 2012).

It is widely acknowledged that microorganisms play a crucial role in disease suppression by composts (Hoitink and Kuter, 1986; Bonanomi et al., 2010; Mehta et al., 2014; De Corato, 2020). This is supported by the findings that most composts lose their disease-suppressive activity when subjected to heat or gamma irradiation (Noble and Coventry, 2005; De Corato et al., 2019), and that disease-suppressive activity of sterilized composts can be restored through reintroduction of compost microorganisms (Nelson et al., 1983; Cao et al., 2018). Further, it has been shown that microbial recolonization of microorganisms during the cooling phase is necessary for the development of disease-suppressive activity (Scheuerell and Mahaffee, 2005).

Several mechanisms by which individual compost microorganisms can suppress soil-borne diseases have been identified. These mechanisms include direct effects such as predation, parasitism, and the production of antibiotic substances, as well as competition for nutrients (Mehta et al., 2014). Moreover, compost may act indirectly by activating the plant immune response or, more generally, by enhancing plant vigor through improved nutrient availability. In the context of suppression mechanisms, the terms general and specific suppression are often used. General disease suppression is described as a substrate's ability to sustain a competitive and active microbial community that can compete with competition-sensitive pathogens such as the oomycetes *Globisporanigum spp.* and *Phythopthora spp.* (Van Bruggen and Semenov, 2000; Weller et al., 2002). In contrast, specific suppression relies on the biocontrol activity of one or several microorganisms. Specific suppression can be transferred by adding small amounts of a suppressive substrate to a conducive one (Hoitink and Boehm, 1999; De Corato et al., 2018). It is often reported to work also against highly competitive pathogens, such as the fungus *Rhizoc-*

tonia solani (Papavizas, 1970; Wibberg et al., 2014). Mechanisms of action for disease suppression have only been studied for a few genera of compost microorganisms (Lutz et al., 2020), while for the vast majority of the diverse bacteria and fungi present in composts, the effects on plant pathogens and crop health, as well as their mechanisms of action, remain unknown.

We proposed an integrated approach to identify beneficial microorganisms and key microbial consortia in disease-suppressive composts (Lutz et al., 2020). This approach includes assessing disease-suppressive activity through standardized bioassays, characterizing compost microbial communities using metagenomics (e. g., amplicon or shotgun sequencing), isolating relevant compost microorganisms, and investigating their role in disease suppression. Studies following a similar approach have identified bacterial and fungal groups associated with disease-suppressive composts (Yu et al., 2015; De Corato et al., 2018, 2019; Scotti et al., 2020; Hernández-Lara et al., 2022). However, most of these studies were based on a small number of compost samples (n <10). Notably, bacterial communities among composts exhibit high taxonomic variability (Wang et al., 2020), highlighting the need for larger datasets to identify robust indicators of disease-suppressive activity across diverse composts. Moreover, to define isolation targets later in the process, it is crucial to identify indicators at finer taxonomic levels (e.g., genera, species, or strains).

In a previous study (Mayerhofer et al., 2021a), we initiated the implementation of the integrated approach by investigating a set of 17 composts. We evaluated the disease-suppressive activity against *Globisporanigum ultimum* in the model plant cress, while also characterizing the composts' bacterial communities using amplicon sequencing of the 16S rRNA (V3-V4) gene. This revealed a significant association between the bacterial community structure and disease-suppressive activity, and identified 75 bacterial amplicon sequence variants (ASVs) as indicative of the most suppressive composts.

Building on this foundation, we aimed to validate the robustness of these bacterial indicators, and expand upon the scope of previous work by identifying fungal indicators, utilizing an unprecedented dataset of 37 compost samples. Disease suppression is of-

ten specific to plant-pathogen interactions (Termorshuizen et al., 2006; Bonanomi et al., 2010). Thus, we evaluated the suppressive activity of these composts not only in the cress-*G. ultimum* model system but also in two additional plant-pathogen systems. To compare suppression between host plants, we tested *G. ultimum*-mediated suppression in cucumber as a host plant. Additionally, to compare suppression across pathogens, we evaluated the suppression of the fungal pathogen *Rhizoctonia solani* in cucumber. Finally, we constructed an extensive dataset by integrating bacterial and fungal community profiling with assessments of 14 compost properties. This integration enabled us to comprehensively explore the relationships between compost properties, microbial communities, and disease-suppressive activity across multiple plant-pathogen systems, ultimately identifying indicators of disease-suppressive composts.

2. Materials and Methods

2.1. Selection and collection of composts

Recognizing that composts derived from different feedstock, such as manure, green waste, and sludge, contain distinct microbial communities (Estrella-González et al., 2020; Hernández-Lara et al., 2022), we focused our study on composts primarily based on green waste. Composts were collected from seven large-scale compost producers/sites (sites A-G) in Switzerland in four sets (set I-IV) of nine to eleven composts in May, June, and September 2022 and April 2023. We aimed to collect two composts of different maturation stages from sites A to E at each collection time point. However, since two composting piles were not always available, further composts were collected from composting sites F and G. The composting sites A, B and F composted organic material in tabular windrows on paved ground (up to 3.5 m height, width not limited). Composting sites C, D and G produced compost in triangular windrows on paved ground (up to 2 m height, 3-4 m width). The site E also composted in triangular windrows, but along agricultural fields and therefore directly on the soil. Composting process and feedstock for all sites and specific for each compost are specified in Tables S1 and S2, respectively. Three composts (referred to as Ex 1-3) reduced the cress shoot weight in the pots without pathogen application compared to the no-compost control (Figure S1) and were therefore excluded, which resulted in a final data set of 37 composts.

Three to five subsamples of each compost pile were pooled and sieved through a 10 mm mesh for bioassays and physicochemical analyses. For the assessment of maximum water holding capacity (WHC), microbial activity and microbial communities, composts were further sieved through a 2 mm mesh. Most analyses were initiated within one week after compost collection. For microbial activity analysis and the *R. solani* bioassays, composts were stored at 5°C for two weeks due to logistical reasons.

2.2. Physicochemical properties and microbial activity of composts

Dry substance (DS) was determined by quantifying water loss after heating of 200 g of compost at 105°C for 24 h. To measure total nitrogen (N_{tot}) and total organic carbon (C_{org}), dried compost samples were milled with an impact rotor mill (Retsch, Germany)

and sieved through a 1 mm mesh,. Then, 500 mg each were analyzed using an elemental analyzer (Vario Max Cube, Elementar, Langenselbold, Germany) as described by Krause et al. (2022). The maximum WHC was assessed via capillary binding capacity using the reference method of Agroscope (2020). Nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+), phosphate (PO_4^{3-}) and pH were measured in a calcium chlorid ($CaCl_2$)-extract, while salinity and the content of soluble humic substances (OD_{550}) were measured in a water-extract of the compost. Microbial activity was assessed using fluorescein diacetate (FDA) hydrolysis and basal respiration (Ntougias et al., 2006; Alef and Nannipieri, 1995). Details on the methods are provided in the Supplementary Methods A and B. The total mineral nitrogen (N_{min}) was calculated as the sum of NO_2^-, NO_3^- and NH_4^+; the nitrification index (NO_3^--N/N_{min}) as NO_3^- divided by N_{min}; and the carbon-nitrogen ratio (C_{org}/N_{tot}) as C_{org} divided by N_{tot}.

2.3. Pot assays to assess disease suppression

A total of 37 composts were tested for their disease-suppressive activity in three plant-pathogen systems: cress-*Globisporanigum ultimum*, cucumber-*G. ultimum*, and *Rhizoctonia solani*-cucumber. Peat was chosen as the substrate for standardized bioassays because, unlike soil, which may naturally suppress or already contain pathogens (Bongiorno et al., 2019), peat is generally considered conducive to pathogens (Hoitink and Boehm, 1999). To this end, the composts were mixed in a ratio of 1:4 (v/v) with a peat-substrate (Einheitserde Typ 0, Einheitserdewerke Werkverband e.V., Sinntal-Altengronau, Germany), moistened with water (20%, v/v), fertilized with horn meal (2.30 g L^{-1} substrate, Biorga Hornmehl, Hauert, Grossaffoltern, Switzerland) and incubated at room temperature for one week. The mixes of compost with peat-substrate are called compost-substrates hereafter. Peat-substrate served as a control in all experiments and will be referred to as no-compost control hereafter. It which was fertilized with 2.30 g L^{-1} horn meal, 1.94 g L^{-1} Granuphos (Landor, Muttenz, Switzerland), and 1.33 g L^{-1} Biorganic Kali-Magnesia (Hauert, Grossaffoltern, Switzerland) to compensate for input of nutrients by compost application,

For each bioassay, *G. ultimum* and *R. solani* were freshly cultured from long-term stocks on Potato Dextrose Agar (PDA; Carl Roth, Karlsruhe, Germany) at 20°C and

24°C, respectively. Long term stocks consisted of mycelium-agar plugs in sterilized ultra pure water (ELGA) stored at 20°C for *G. ultimum* and in 10% glycerin + 5% trehalose at -80°C for *R. solani*. For *G. ultimum* bioassays, three 5 mm-diameter agar plugs of a seven-day-old culture on PDA were transferred to a Petri dish filled with 20 g sterilized organic millet seeds soaked in 10 mL autoclaved water and were incubated in the dark at 20°C for seven days. The colonized millet was chopped, mixed with sand, and gently homogenized with a mortar. Immediately thereafter, 20 g of the pathogen-millet-sand mix, containing 0.45 g or 1.35 g inoculum (low or high pathogen concentration), was added per L of compost- or peat-substrate. Control treatments received sand without pathogen inoculum. The substrates with and without pathogen were filled into pots with 6 or 9 cm diameter for cress and cucumber assays, respectively.

For *R. solani* bioassays, the pathogen was cultured on PDA for three days, and three agar plugs with a diameter of 0.8 or 1.4 cm for sets I-III and of 0.6 and 0.8 for compost set VI were transferred into pots pre-filled to one-third with substrate. The pathogen plugs were covered with substrate and the pot filled to the brim. Different pathogen concentrations were tested, because pathogen virulence can vary across experiments. In the cress bioassay, six replicate pots per treatment were sown with 0.6 g of organic cress seeds (approximately 260 seeds, *Lepidium sativum*, Bigler Samen AG, Steffisburg, Switzerland). Seeds were moistened with a hand sprayer, and the pots were placed on a plastic tray (Licefa, Germany), which was covered with a plastic bag to maintain high humidity. For the cucumber bioassays, eight replicate pots per treatment were planted with five sterilized, pre-germinated cucumber seeds (*Cucumis sativus*), cultivar "Chinese snake", Bigler Samen, AG, Steffisberg, Switzerland) at a depth of 2 cm. Pots were placed in a tray and covered with a transparent lid (Anzuchtsbox M, Romberg, Germany). For the pre-germination, cucumber seeds were sterilized in 0.1% (v/v) potassium hydrochloride solution for 10 min, rinsed six times with sterile water, placed on 1.5% water agar, and incubated for 46-52 hours at 24°C in the dark.

All bioassays were performed in climate chambers (GroBanks, CLF Plant Climatics, Wertingen, Germany) at a day/night regime of 16 h/8 h and a light intensity of 130 μmol m^{-2} s^{-1}. The air humidity was maintained at 70% and the temperatures set to 20°C

day/18°C night for *G. ultimum* and 23°C day/21°C night for *R. solani*. The plastic bag for the cress and the transparent lid for the cucumber were removed after three days. Following this, the cress pots were watered daily. The cucumber pots were watered every two to four days. Shoot biomass was harvested after 6, 11 and 13 days in the cress-*G. ultimum*, cucumber-*G. ultimum* and cucumber-*R. solani* system, respectively.

Disease suppression for each substrate was calculated as relative biomass by dividing the shoot weight of plants grown with pathogen application by the mean shoot weight of plants grown without. Additionally, disease symptoms were scored, and the results were found to correlate with relative biomass across all three plant-pathogen systems (Figure S2). Consequently, only relative biomass was used for further analyses. To compare disease suppression by composts across test sets, one pathogen level was selected based on the relative biomass observed in the no-compost control (Figure S3). We aimed at a reduction of at least 50% in plant biomass due to pathogen inoculation in the no-compost control and no significant difference among the four sets. This was achieved by combining a pathogen concentration of 0.45 g *G. ultimum*-millet mix L^{-1} substrate for cress-*G. ultimum*, 0.45 g L^{-1} for sets I and II, and 1.35 g L^{-1} for sets III and IV in cucumber-*G. ultimum*. For cucumber-*R. solani*, a plug size of 1.4 cm was used for set I, and 0.8 cm for sets II, III, and IV.

2.4. 16S rRNA gene V3–V4 and ITS2 amplicon sequencing

For DNA extraction, 200 mg of 2 mm-sieved compost or peat samples were combined with 1 mL of NucleoMag lysis buffer and 1.5 g of beads (SiLibeads Type ZY S 0.4-0.6 mm, Sigmund Lindner, Germany) in 2 mL screw-cap tubes. The samples, prepared in four replicates, were stored at -20°C until use. DNA was extracted using the "NucleoMag DNA Microbiome for DNA purification from soil, stool and biofilm" DNA extraction kit (Macherey Nagel, Düren, Germany) following the manufacturer's instructions. All samples were homogenized using a TissueLyser (Qiagen, Netherlands) at 30 Hz for 4 min. Prior to elution of the DNA in 0.1 x TE buffer (1 mM Tris, 0.1 mM Na_2EDTA, pH 8.0), the magnetic beads, were dried at 70°C for 30 min to remove leftover ethanol. DNA extracts were stored at -20°C. DNA was quantified using Quant-iT PicoGreen dsDNA Assay kit (Invitrogen, Waltham, MA, USA) with a Cary Eclipse fluorescence spectrophotometer

(Varian, Palo Alto, CA, USA). For PCR amplification DNA extracts were diluted to 5 ng μL^{-1} using deionized and autoclaved H_2O.

For the bacteria, the variable regions 3 and 4 of the 16S rRNA gene marker were amplified using the primers 341F (5'-CCTAYGGGDBGCWSCAG-3') and 806R (5'-GG ACTACNVGGGTHTCTAAT-3'). For the fungi, the internal transcribed spacer region 2 (ITS2) was amplified using the primers ITS3 (5'-CANCGATGAAGAACGYRG-3') and ITS4 (5'-CCTSCSCTTANTDATATGC-3') modified by Tedersoo et al. (2015). PCR reactions were performed as described in Mayerhofer et al. (2021a) with the following modifications: 1 μM of each primer was used, the PCR included 30 and 33 cycles for the 16S V3-V4 and ITS2 primer pair, respectively, and the annealing temperature was set to 56°C. A no-template control was included for each 96-well plate. PCR reactions were run in quadruplets and pooled following quality control on an Agarose gel. DNA concentration of PCR products was quantified as described for genomic DNA and diluted to 20 ng mL^{-1}. Library preparation included tagging with TruSeq indices, normalizing read counts from an Illumina MiniSeq run, and sequencing on the Illumina NextSeq2000 platform. Paired-end sequencing was performed with 300 bp long reads (Illumina Inc., San Diego, CA, USA) at the Functional Genomics Center Zurich, University of Zurich / ETH Zurich (Switzerland).

2.5. Bioinformatics and quality control

Quality control, ASV inference and taxonomic classification of sequences was performed using a pipeline adapted from Frey et al. (2016) and Mayerhofer et al. (2021b). Briefly, raw sequences were trimmed by removing primer sequences using cutadapt (v.3.5, Martin (2011), one mismatch allowed). Implemented in vsearch (v.2.27.0, Rognes et al. (2016)), sequences were merged, quality filtered and dereplicated as described by Frey et al. (2016). To obtain amplicon sequence variant (ASV), sequences were de-noised with the UNOISE algorithm (Edgar and Flyvbjerg, 2015). Chimera removal was done with UCHIME (Edgar et al., 2011). Checking for the presence of ribosomal signatures was performed using Metaxa2 (v.2.2.3, Bengtsson-Palme et al. (2015)) for the bacteria and ITSx (v.1.1.3, Bengtsson-Palme et al. (2013)) for the fungi. All sequences without ribosomal signature were discarded. Finally, reads were mapped against the final set of identified ASVs using the algorithm "usearch_global". Taxonomic classification of ASVs

was performed with the command "classify.seqs" in MOTHUR (v.1.47, Schloss et al. (2009)) using a modified 16S version (Mayerhofer et al. in preperation) of the Genome Taxonomy DataBase (GTDB, release SSU 214, Parks et al. (2021)) for the bacterial sequences. The UNITE was used to classify the fungal sequneces (version 9.0, Abarenkov et al. (2024)). Raw sequences are deposited at the NCBI SRA database (bacteria: PRJNA1200637, fungi: PRJNA1201072).

Non-bacterial sequences (e.g., archaea, chloroplasts, and mitochondria) and non-fungal sequences (e.g., protists and plants) were removed. For the fungi three samples were excluded as outliers since they had less than than 10,000 fungal sequences. To standardize read counts, all samples were rarefied to the lowest counts (35,928 bacterial; 10,211 fungal sequences) with 100 iterations using "rrarefy" from the *vegan* package. Analyses were performed on the 100 times subsampled ASV tables or their median counts. Number of sequences and ASVs for the different filtering steps are indicated in Table S4.

2.6. Statistical analyses

All analyses and visualizations were performed in R (v.4.3.1, R Core Team (2023)) using Rstudio (v.2023.06) and the *tidyverse* packages (Wickham et al., 2019). Statistical significance was set at p <0.05, with p-values adjusted for multiple testing using the Benjamini-Hochberg correction (Benjamini and Hochberg, 1995). Between-group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (Tukey HSD) post hoc test (Lenth, 2024). Normality was assessed using model residual plots. When normality was violated, the Kruskal-Wallis test followed by pairwise Wilcoxon tests was applied. Correlations were evaluated with Pearson or Spearman correlation based on Shapiro-Wilk normality results. Only correlations with $|coefficient|$ >0.4 were considered significant. Principle Component Analysis (PCA) of compost properties was performed using Euclidean distance of scaled and centered values. Strongly co-correlated compost properties ($|\rho| > 0.8$, Fig. S7) and properties with missing data (basal respiration, compost age) were removed. Microbial community analyses were performed with the *vegan* package (Oksanen et al., 2022). Alpha diversity measures of microbial communities were calculated using the "*specnumber*" and "*diversity*" functions on 100 times subsampled ASV-tables and then averaged. Bray-

Curtis dissimilarities were computed also based on the 100 times subsampled ASV-tables with "*vegdist*". Compositional differences in physicochemical properties or microbial communities were tested with multivariate permutation analysis of variance (PERMANOVA, Anderson et al. (2006)) using the "*adonis2*" function. Community structures were visualized using nonmetric multidimensional scaling (NMDS) using the "*metaMDS*" function.

Four statistical methods—Point Biserial Correlation and Indicator Species Analysis (Cáceres and Legendre, 2009), MaAsLin2 (Mallick et al., 2021), and ALDEx2 (Fernandes et al., 2014)—were used to identify ASV associations with the nine most suppressive composts for each plant-pathogen system. A detailed description is provided in Supplementary Methods C. ASVs were included if their mean abundance was at least one read in six or more of the 18 selected composts. A consensus approach was applied, considering ASVs as "indicative" if they were significantly associated in at least three methods, following the approach of Nearing et al. (2022). A phylogenetic tree of sequences of indicative ASVs was constructed using a distance matrix based on the Jukes-Cantor model and visualized with *ggtree* (Yu et al., 2017) and *phytools* (Revell, 2024). The heatmap was constructed and visualized using *ComplexHeatmap* (Gu et al., 2016)). R code and session info are available on GitHub (`https://github.com/logoa/Bioassay_Bacteria_paper_1.git`, public once accepted for publication). ChatGPT version 4o was used for code debugging.

3. Results

3.1. The disease-suppressive activity of composts depended on the plant-pathogen system

To unveil the factors that shape disease suppression by composts, we tested 37 composts in three plant-pathogen systems: cress-*G. ultimum*, cucumber-*G. ultimum* and cucumber-*R. solani*. Thirty-three were significantly disease-suppressive in at least one of the systems (Fig. 1A-C), but only three composts in all systems, namely compost 24, 34 and 35. Four composts (9, 10, 11, 12) did not show disease-suppressive activity at all. The highest number of suppressive composts was observed in the cress-*G. ultimum*-system with 28 protecting the plants (Fig. 1D). In comparison, in the cucumber-*G. ultimum*-system, fewer composts (17) exhibited disease-suppressive activity with an overlap of 14 suppressive composts with the cress-*G. ultimum*-system. Suppression of *G. ultimum* in cress and cucumber was correlated ($r = 0.63$, $p < 0.001$, Fig. S4). In the cucumber-*R. solani*-system, nine composts suppressed the pathogen compared to the peat control. No correlation was found between suppression in the *R. solani*-system and in the two *G. ultimum*-systems (cress: $r = -0.07$, $p = 0.662$; cucumber: $r = 0.03$, $p = 0.543$).

The composts were produced at seven different composting sites (A–G, Table S1). At least one compost from each site suppressed disease in at least one plant-pathogen system. Overall, suppression did not significantly differ between composting sites, with two exceptions: composts from site D showed, on average, higher suppression in the cucumber-*G. ultimum* system compared to those from site C, and composts from site E performed better in the cucumber-*R. solani* system than those from site B (Fig. S5). Thus, disease suppression could not be attributed to specific composting sites.

The 37 composts were collected and tested in sets of eight to ten composts at four different time points. Within each set, both disease-suppressive and non-suppressive composts were identified. The mean suppressive activity observed for composts of set I, III, and IV was similar. However, set II exhibited significantly reduced suppression in both *G. ultimum* systems (Fig. S5).

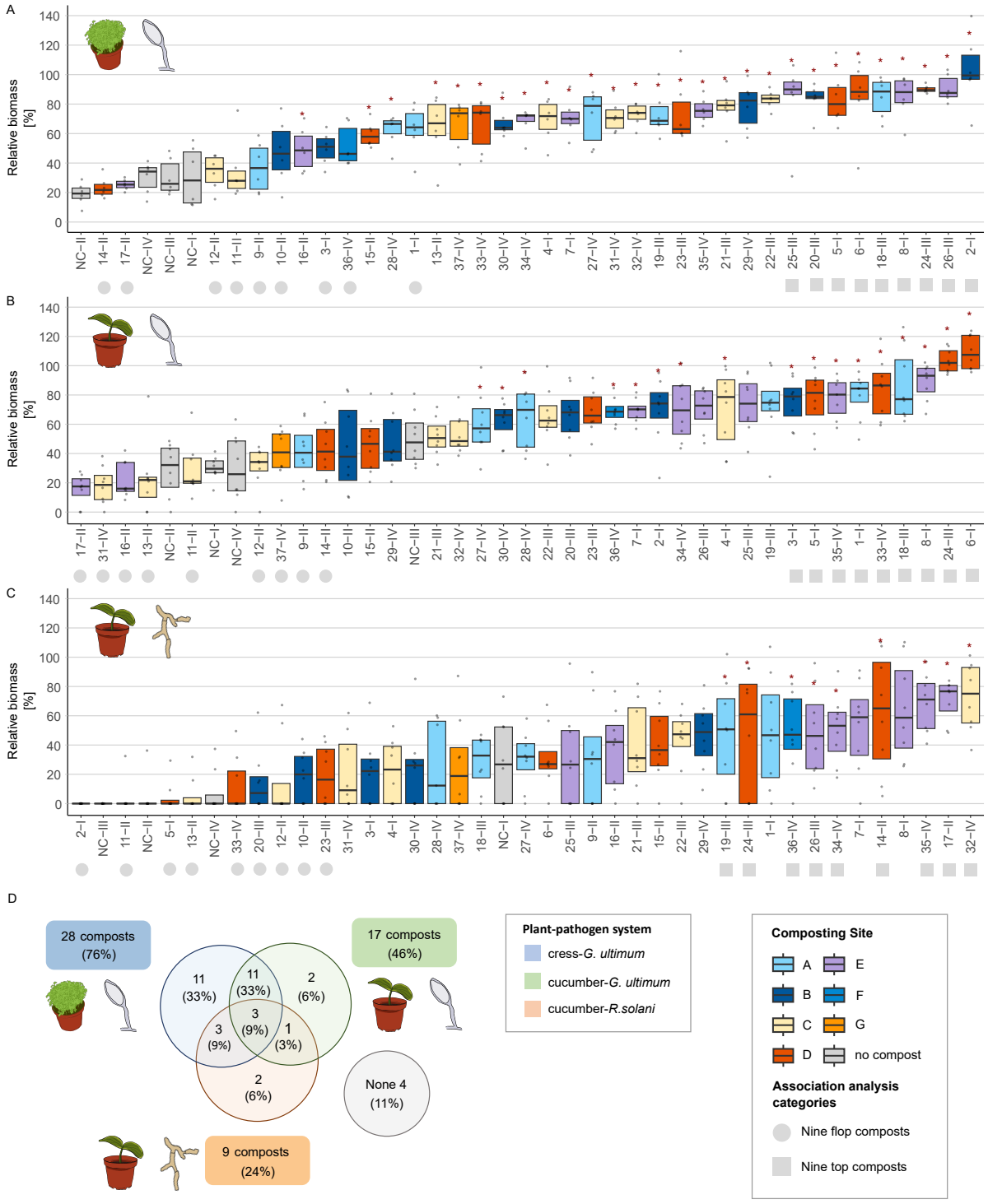

Figure 1: Caption next page

Figure 1: **Disease suppression of composts differed among plant-pathogen systems.** Thirty-seven composts were tested in three plant-pathogen systems: (A) cress-*Globisporanigum ultimum*, (B) cucumber-*G. ultimum* and (C) cucumber-*Rhizoctonia solani*. Disease suppression is expressed as relative biomass, calculated by dividing the shoot biomass with pathogen application by the average shoot biomass without pathogen application for the same treatment. The relative biomass of each replicate pot (n = 6 for cress and n = 8 for cucumber) is shown as a grey point; boxes represent the interquartile range, and dashes denote the median. Composts are identified by numbers followed by Roman numerals, which represent their respective compost sets (I-IV; I = May 2022, II = July 22, III = September 22, IV = May 2023). No-compost controls are labeled with "NC". Substrate treatments are ordered by increasing means. Red asterisks indicate compost-substrates with significantly higher relative biomass compared to the no-compost control in the respective set (Tukey HSD test for parametric or pairwise Wilcoxon test for non-parametric data, p <0.05, ANOVA or Kruskal-Wallis test results in Table S5). The shape of the symbol following the substrate name highlights the nine most (circle) and least (square) suppressive composts in each system, used for associating single ASVs with the most suppressive composts. (D) The Venn diagram depicts the number of suppressive composts shared across plant-pathogen systems.

3.2. Physicochemical properties and microbial activities of composts were primarily associated with composting site and compost maturation

Mean disease suppression of the 37 composts in the three plant-pathogens systems did not correlate with any of the 14 physicochemical compost properties, basal respiration, FDA hydrolysis or compost age (Spearman's Rank sum test, $|\rho| > 0.4$, p-adj < 0.05, Table 1 and Fig. S8, S9, S10). We hypothesized that a combination of properties, rather than individual ones, may be linked to disease suppression. However, a Principle Component Analysis (PCA) based on nine compost properties (selection criteria in caption Table 1) did not reveal differences in disease suppression in any of the three plant-pathogen systems (Table 1, Fig. S6). Nevertheless, the PCA revealed a clear separation of composts along the first principal component (PC) based on their composting site (PERMANOVA, $R^2 = 42.4\%$, Pseudo-F = 3.68, p = 0.001, Fig. 2A). Less pronounced but still significant, the factor compost age explained 8.3% of the variation (Pseudo-F = 2.99, p = 0.014), separating the composts along the second PC (Fig. 2B). The loading vectors of the PCA are illustrated in Fig. 2B and show that the compost properties C_{org}, FDA, PO_4^{3-} and salinity contributed strongest to the first PC and are therefore separating composting sites. The loading vectors of the compost properties pH, NH_4^+ and NO_3^- contributed most to the second PC which, as mentioned above, was associated with compost age. Thus,

the assessed compost properties were primarily associated with the composting site and the stage of compost maturation, but could not explain differences in disease suppression.

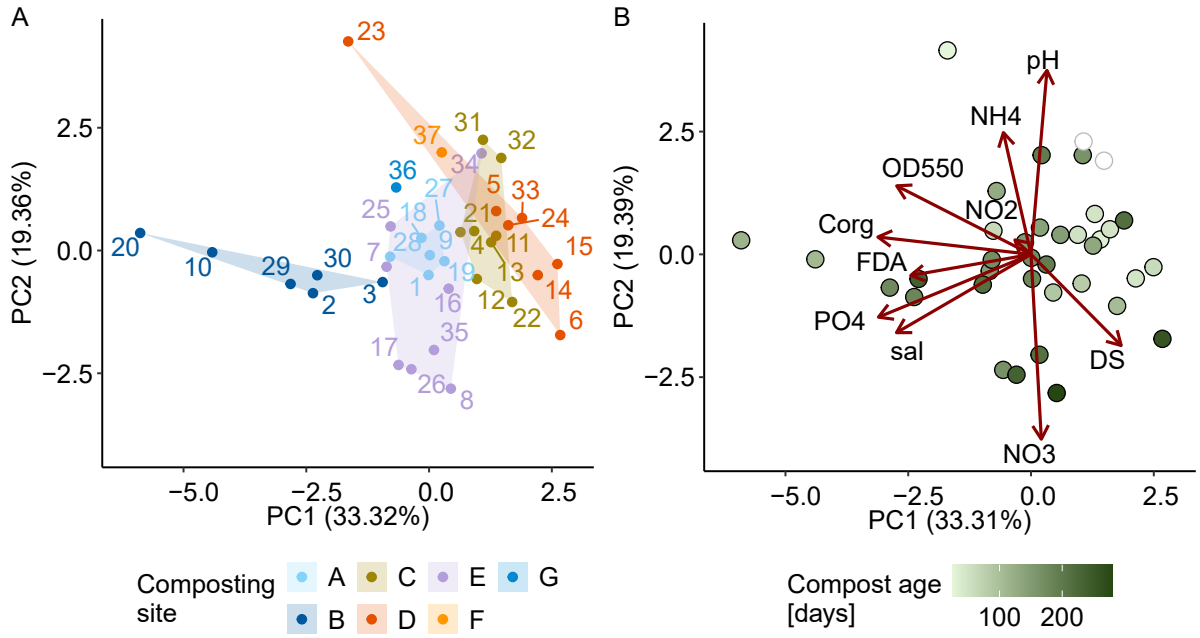

Figure 2: **Compost properties were associated with the composting site and compost age.** The Euclidean distance between composts was calculated based on nine selected compost properties (indicated with an asterisk in Table 1). The plots display the first and second principal component. Each compost is represented by a dot colored according to either its (A) site of origin or (B) composting age. Two composts (31, 32) with unknown compost age are displayed in white. In A, the compost IDs (1-37) are indicated, and in B, the loading vectors for all nine compost properties are shown in red.

Table 1: **Correlation analyses of disease-suppressive activity of composts with physicochemical properties, microbial activity, and alpha- and beta-diversity measures.** For univariate analysis, Spearman's Rank sum correlation was used. For multivariate analysis, PERMANOVA was calculated based on Euclidean distance of compost properties and the Bray Curtis dissimilarity of bacterial and fungal ASV abundances. Compost properties included in the PCA in Figure 2 are marked with an asterisk. *P*-values for Spearman's correlation were adjusted for multiple testing using the Benjamini-Hochberg correction for compost properties and microbial activities (n = 17) and alpha diversity metrics (n = 3).

		cress-*G. ultimum*			**cucumber-*G. ultimum***			**cucumber-*R. solani***		
		ρ	***p***	***p*-adj**	ρ	***p***	***p*-adj**	ρ	***p***	***p*-adj**
Compost properties	Compost age [days]	0.21	ns	ns	0.37	*	ns	0.18	ns	ns
	DS* [%]	-0.08	ns	ns	0.04	ns	ns	0.32	.	ns
	Max. WHC [a]	0.01	ns	ns	-0.18	ns	ns	-0.27	.	ns
	pH*	-0.03	ns	ns	-0.20	ns	ns	-0.17	ns	ns
	Salinity* [b]	0.11	ns	ns	0.14	ns	ns	0.11	ns	ns
	OD_{550}*	0.12	ns	ns	0.05	ns	ns	-0.19	ns	ns
	NO_2^--N [c]	-0.28	.	ns	-0.24	ns	ns	0.14	ns	ns
	NO_3^--N* [c]	0.10	ns	ns	0.12	ns	ns	0.30	.	ns
	NH_4^+-N* [c]	-0.22	ns	ns	-0.30	.	ns	-0.10	ns	ns
	N_{min}[c]	0.06	ns	ns	0.06	ns	ns	0.23	ns	ns
	NO_3^--N/N_{min}	0.20	ns	ns	0.37	*	ns	0.27	ns	ns
	PO_4^{3-}-N* [c]	0.09	ns	ns	0.06	ns	ns	0.05	ns	ns
	N_{tot} [%]	0.11	ns	ns	0.06	ns	ns	0.06	ns	ns
	C_{org}* [%]	-0.03	ns	ns	-0.07	ns	ns	-0.10	ns	ns
	C_{org}/N_{tot}	-0.27	ns	ns	-0.26	ns	ns	-0.38	*	ns
Microbial activity	Basal respiration [d]	0.05	ns	ns	0.04	ns	ns	-0.30	.	ns
	FDA hydrolysis* [e]	-0.16	ns	ns	-0.45	**	ns	-0.01	ns	ns
Alpha diversity	Bacterial richness	0.49	**	**	0.31	.	ns	0.29	.	ns
	Bacterial Shannon evenness	0.45	**	**	0.16	ns	ns	0.04	ns	ns
	Bacterial Shannon diversity	0.55	***	**	0.24	ns	ns	0.18	ns	ns
	Fungal richness	0.25	ns	ns	0.13	ns	ns	0.14	ns	ns
	Fungal Shannon evenness	0.35	*	.	0.18	ns	ns	0.14	ns	ns
	Fungal Shannon diversity	0.36	*	.	0.16	ns	ns	0.16	ns	ns
		F (R^2)	***p***	***p*-adj**	**F (R^2)**	***p***	***p*-adj**	**F (R^2)**	***p***	***p*-adj**
Multivariate analysis	PCA compost properties	0.82 (2.3%)	ns	-	1.01 (2.8%)	ns	-	1.54 (4.0%)	ns	-
	Bacterial community structure	1.48 (4.0%)	ns	-	0.92 (2.6%)	ns	-	1.38 (3.8%)	ns	-
	Fungal community structure	1.36 (3.9%)	ns	-	1.10 (3.1%)	ns	-	1.16 (3.2%)	ns	-

ns = not significant, "." p <0.1, "*" p <0.05, "**" p <0.01, "***" p <0.001,

[a] [g H_20 (g DS)$^{-1}$] , [b] [g KCl_{eq} (kg DS)$^{-1}$], KCl_{eq} = Potassium chloride-equivalent, [c] [mg (kg DS)$^{-1}$] , [d] [mg CO_2-C h^{-1}], [e] [μg FDA (min g DS)$^{-1}$]

3.3. Bacterial and fungal communities were linked with compost properties but not with disease-suppressive activity

We determined the bacterial and fungal communities of the 37 composts and four no-compost controls using 16S (V3-V4) and ITS2 amplicon sequencing. In total, we identified 19,129 bacterial and 2,309 fungal ASVs. Non-metric multidimensional scaling (NMDS) ordination of the bacterial and fungal community structures showed clear clustering of the technical replicates of each compost (Fig. S11). Statistical analysis revealed strong differences among the 37 composts for the bacterial (PERMANOVA, $R^2 = 96.1\%$, Pseudo-F = 76.01, p = 0.001) and fungal community structure ($R^2 = 96.2\%$ Pseudo-F = 76.14, p = 0.001). The distinctness of the microbial communities of the composts is further supported by the low number of bacterial ASVs (155, 0.8% of all ASVs) and fungal ASVs (7, 0.3%) shared among all composts. Composts with similar bacterial communities also tended to have similar fungal communities, and vice versa (Mantel test, Pearson's r = 0.62, p = 0.001).

Although the number of ASVs shared across all composts was generally low, the most abundant classifications at the phylum, family and genus level for both bacteria and fungi were common to most composts (Fig. S12 and S13). While compost bacterial communities included a diverse range of genera, fungal communities in most composts were dominated by one or a few genera. For both bacteria and fungi, the number of different taxonomic classifications, as well as the most common phyla, families and genera across all 37 composts are presented in Table S6.

For both bacteria and fungi, all three assesed alpha diversity measures—ASV richness, Shannon evenness, and Shannon diversity—differed significantly across the 37 compost samples (Fig. 3, Fig. S14, ANOVA results in Table S7). Bacterial richness ranged from 955 to 5,021 ASVs (mean: 2,691, SD: 936), while fungal richness ranged from 35 to 401 ASVs (mean: 177.5, SD: 96.9). All three alpha diversity measures were significantly correlated between bacteria and fungi (richness: $\rho = 0.74$; evenness: $\rho = 0.66$; diversity: $\rho = 0.72$; $p < 0.001$).

Bacterial richness, evenness, and diversity had a positive correlation with *G. ulti-*

mum suppression in cress (Fig. 3C, Table 1). A similar, though non-significant, trend was observed for fungal evenness and diversity. No correlation was found between alpha diversity measures and disease suppression in the cucumber systems. Further analysis revealed that the positive correlation between bacterial diversity measures and *G. ultimum*-suppression in cress was driven by composts from set II, which had particularly low richness and low disease-suppressive activity (Fig. 3D and E). In contrast, set III, despite low diversity, showed consistently high suppressive activity. Excluding set II from the analysis eliminated the positive correlation for bacterial diversity and *G. ultimum* suppression in cress, as well as the positive trend for fungal evenness and diversity ($p > 0.05$).

To better understand which factors might affect the bacterial and fungal alpha diversity in composts, we assessed their associations with other compost properties (Table S8). Bacterial richness significantly increased with compost age and the nitrate-N/N_{min}, which are both properties associated with compost maturation (Table S8). Fungal diversity measures did not correlate with any compost properties.

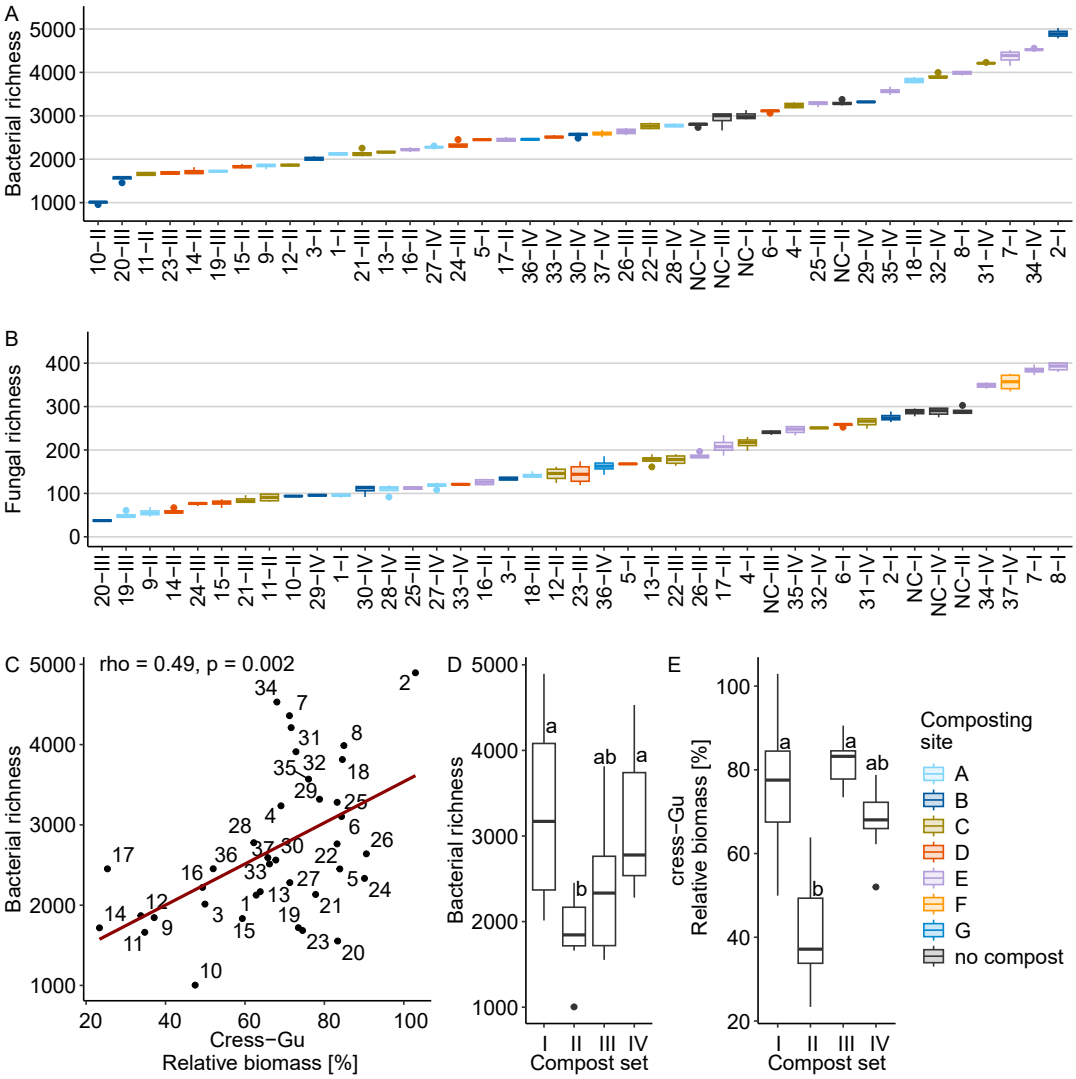

Figure 3: **Bacterial and fungal diversity differed among composts and sets but did not explain differences in disease suppression**. Boxplots display (A) bacterial and (B) fungal richness for the 37 composts (1-37) and the no-compost control (NC) of the four compost sets (I-IV) ordered by their means (n =4). (C) The scatter plot shows the correlation between bacterial richness and disease suppression in the cress-*G. ultimum*-system. The linear trend line is depicted in red. Boxplots show differences among compost sets for (D) bacterial richness and (E) disease suppression in the cress-*G. ultimum*-system. For all boxplots the boxes represent the interquartile range (IQR, 25th to 75th percentile), the horizontal line within the box indicates the median, the whiskers extend to 1.5 x IQR, and outliers (>1.5 x IQR) are shown as points.

Bacterial and fungal community structure, based on the mean ASV abundance of technical replicates, was not significantly associated with disease-suppressive activity in any of the three plant-pathogen systems (PERMANOVA, Table 1, Fig. S15). However, both bacterial and fungal communities were significantly associated with the composting site (Fig. 4A and B, Table S9), the compost age (Fig. 4C and D) and with most compost properties (Table S8). Bacterial community structures of the composts was primarily associated with OD_{550}, PO_4^{3-} and FDA hydrolysis, while fungal community structure with N_{tot}, salinity and NO_3^- (Fig. 4 C and D). Thus, composting site and compost properties determined the fungal and bacterial community structure, but no association between the community structure of the compost and their disease-suppressive activity was found.

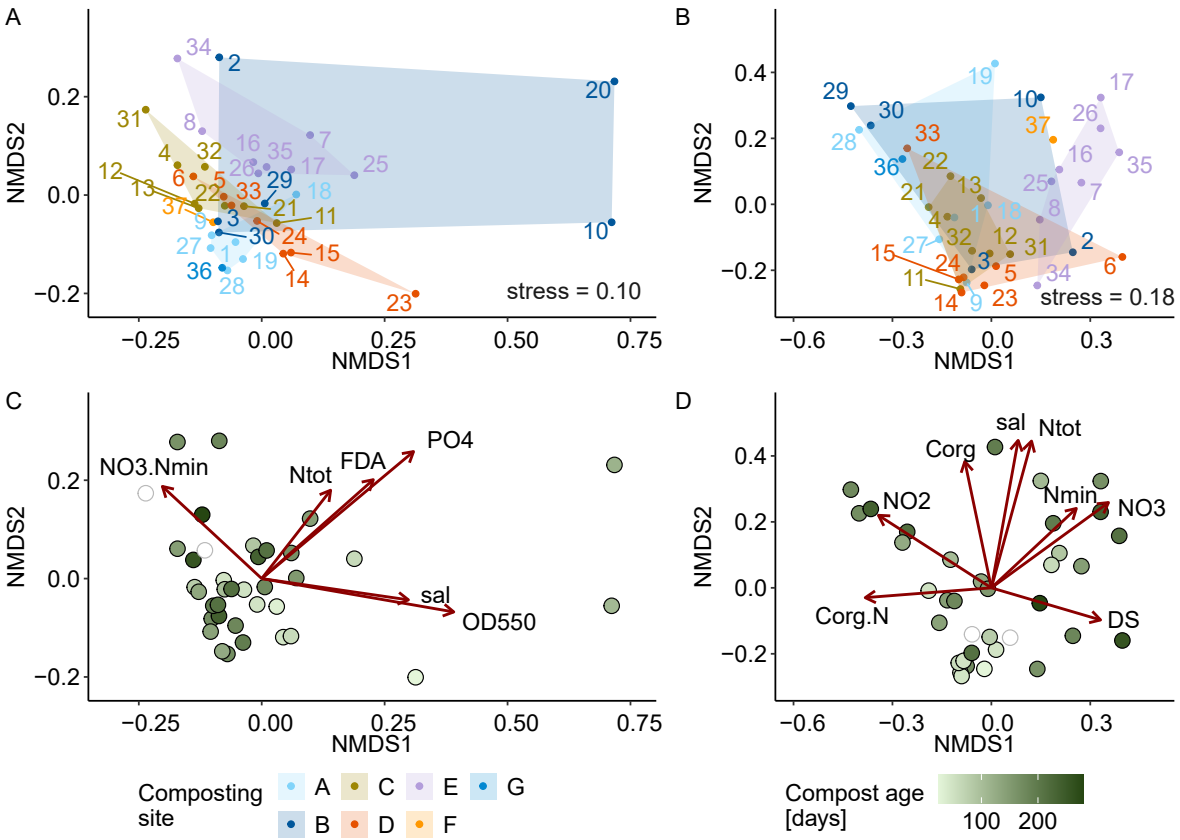

Figure 4: **Bacterial and fungal community structure were linked to composting site, compost age, and compost properties.** Bray-Curtis dissimilarities were calculated based on the mean ASV abundance of the four replicates. A NMDS plot is shown for (A, C) bacterial community structure and (B, D) fungal community structure colored by the (A, B) composting site and (C, D) composting age. The red arrows indicate compost properties significantly associated with community structure along the first two NMDS axes (full PERMANOVA results are provided in Tables S8 and S9). Compost 20, which exhibited a distinct community structure, was considered an outlier and excluded from the fungal ordination (a plot including compost 20 is provided in Fig. S11).

Table 2: **Top 20 indicative ASVs for all three plant-pathogen systems based on the cumulative rank of the effect size of all four statistical methods.**

System	ASV	n	rho	p	Phylum	Family	Genus/Species
cress-G. ultimum	618	32	0.48	**	*Planctomycetota*	*Lalipirellulaceae*	NA
	594	29	0.45	**	*Planctomycetota*	*Tepidisphaeraceae*	NA
	242	30	0.50	**	*Acidobacteriota*	NA	NA
	622	28	0.55	***	*Gemmatimonadota*	GWC2-71-9	Palsa-1233
	198	29	0.45	**	NA	NA	NA
	543	22	0.54	**	NA	NA	NA
	135	30	0.47	**	*Acidobacteriota*	*Pyrinomonadaceae*	OLB17
	922	23	0.42	**	*Pseudomonadota*	*Sphingobacteriaceae*	*Sphingomicrobium*
	1257	20	0.58	***	*Planctomycetota*	NA	NA
	94	33	0.47	**	*Chloroflexota*	*Phototrophicaceae*	NA
	1927	28	0.50	**	*Pseudomonadota*	*Caulobacteraceae*	*Brevundimonas basaltis*
	87	36	0.38	*	*Pseudomonadota*	*Steroidobacteraceae*	NA
	2750	25	0.50	**	NA	NA	NA
	174	29	0.41	*	NA	NA	NA
	824	31	0.53	**	*Pseudomonadota*	*Rhizobiaceae*	NA
	702	32	0.37	*	*Bacteroidota*	*Cyclobacteriaceae*	NA
	698	32	0.47	**	*Planctomycetota*	NA	NA
	1379	31	0.43	**	*Pseudomonadota*	*Beijerinckiaceae*	*Microvirga*
	214	29	0.54	**	*Patescibacteria*	JACDBH01	JACDBH01
	689	30	0.44	**	*Chloroflexota*	*Caldilineaceae*	*Caldilineaceae*
cucumber-*G. ultimum*	124	34	0.43	**	*Bacteroidota*	*Cyclobacteriaceae*	UBA2336
	424	32	0.55	***	*Pseudomonadota*	*Sphingomonadaceae*	NA
	661	30	0.62	***	*Pseudomonadota*	*Burkholderiaceae* C	NA
	1339	21	0.61	***	*Bacteroidota*	*Sphingobacteriaceae*	*Parapedobacter*
	146	35	0.50	**	*Pseudomonadota*	*Sphingomonadaceae*	*Sphingopyxis*
	308	33	0.55	***	*Pseudomonadota*	*Xanthomonadaceae*	NA
	1513	26	0.65	***	*Pseudomonadota*	*Rhizobiaceae*	*Sinorhizobium fredii*
	840	29	0.54	**	*Pseudomonadota*	*Erythrobacteraceae*	*Croceibacterium*
	184	32	0.47	**	*Bacteroidota*	*Flavobacteriaceae*	NA
	1432	17	0.50	**	*Planctomycetota*	*Planctomycetaceae*	NA
	500	34	0.47	**	*Pseudomonadota*	*Nitrosomonadaceae*	*Nitrosomonas nitrosa*
	58	37	0.45	**	*Pseudomonadota*	*Xanthomonadaceae*	*Luteimonas D*
	567	32	0.48	**	*Bacteroidota*	*Chitinophagaceae*	*Flavipsychrobacter*
	384	32	0.33	*	*Sumerlaeota*	JAHLLQ01	JAHLLQ01
	124	34	0.39	*	*Bacteroidota*	*Cyclobacteriaceae*	UBA2336
	3130	24	0.52	**	*Planctomycetota*	NA	NA
	2100	24	0.46	**	*Chloroflexota*	NA	NA
	1830	34	0.54	**	*Gemmatimonadota*	UBA6960	NA
	252	28	0.31	.	*Bacteroidota*	*Chitinophagaceae*	*Edaphocola*
	2767	22	0.49	**	*Pseudomonadota*	*Xanthomonadaceae*	*Luteimonas D* colneyensis

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Table 2 **Top 20 indicative ASVs (continued)**

System	ASV	n	ρ	p	Phylum	Family	Genus/Species
cucumber-*R. solani*	243	37	0.56	***	*Actinomycetota*	*Mycobacteriaceae*	*Mycobacterium hassiacum*
	434	33	0.34	*	*Pseudomonadota*	*Rhodomicrobiaceae*	*Rhodomicrobium*
	423	36	0.40	*	*Planctomycetota*	*Tepidisphaeraceae*	UBA2421
	144	35	0.24		*Pseudomonadota*	*Steroidobacteraceae*	NA
	178	34	0.51	**	*Actinomycetota*	*Streptosporangiaceae*	*Nonomuraea*
	13	37	0.45	**	*Gemmatimonadota*	NA	NA
	2784	30	0.57	***	*Actinomycetota*	NA	NA
	803	30	0.45	**	*Chloroflexota*	UBA6265	NA
	394	34	0.45	**	*Pseudomonadota*	NA	NA
	921	31	0.37	*	NA	NA	NA
	1101	35	0.33	*	*Pseudomonadota*	*Steroidobacteraceae*	NA
	114	37	0.42	*	*Gemmatimonadota*	NA	NA
	379	35	0.34	*	*Pseudomonadota*	HTCC2089	NA
	45	36	0.56	***	*Gemmatimonadota*	NA	NA
	483	35	0.50	**	*Actinomycetota*	*Ilumatobacteraceae*	NA
	595	26	0.37	*	*Actinomycetota*	*Micromonosporaceae*	NA
	609	32	0.35	*	*Actinomycetota*	*Streptosporangiaceae*	*Actinocorallia*
	642	33	0.42	**	*Pseudomonadota*	*Rariloculaceae*	ZC4RG20
	301	31	0.41	*	*Actinomycetota*	NA	NA
	10	37	0.52	**	*Chloroflexota*	*Thermomicrobiaceae*	*Sphaerobacter thermophilus*

"NA" indicates unclassified. Column "n" indicates the number of composts the ASV was found in. Significance levels for Spearman's Rank Sum test are denoted as follows: ns = not significant, "." p <0.1, * p <0.05, ** p <0.01, *** p <0.001.

3.4. Potential indicator taxa for disease-suppressive composts were mostly bacteria and specific to the plant-pathogen systems

Bacterial and fungal community structures did not explain differences in disease suppression. However, specific taxa may still be associated with suppressive composts. To investigate this, we conducted an association study comparing the abundance of individual bacterial and fungal ASVs between the nine most suppressive and nine least suppressive composts for each plant-pathogen system (selected composts shown in Fig. 1A–C). This analysis identified 317 bacterial and 9 fungal ASVs as indicative of disease suppression in at least one plant-pathogen system. ASVs were considered indicative if they were statistically significant in at least three of the four methods used. The number of indicative ASVs identified by each method and their overlap is shown in Fig. S16.

All indicative fungal ASVs were specific to the plant-pathogen-system (Fig. 5A). The three fungal ASVs indicative of *G. ultimum*-suppression in cress and the single ASV indicative of *R. solani*-suppression in cucumber belonged to the phylum *Ascomycota* but could not be classified at genus level (Table S14). In the cucumber-*G. ultimum*-system, three indicative fungal ASVs were identified as *Ascomycota*, including one identified as *Kernia* sp. and one as *Scedosporium* sp.. Additionally, one ASV was classified to the phylum *Basidiomycota* (genus *Coperinellus*), and one to the phylum *Mortierollomycota* (genus *Mortierella*). The number of indicative ASVs was lower for fungi than for bacteria, and their association with suppressive composts was weaker, as evidenced by smaller effect sizes in the statistical tests (Table S14). Consequently, bacterial taxa appear more relevant for indicating disease suppression by composts, and further analyses focused on bacterial indicators.

The majority of the bacterial ASVs was specific to the plant-pathogen system with more ASVs being indicative for suppression of *G. ultimum* in cress and cucumber than for suppression of *R. solani* in cucumber (Fig. 5B). Only one bacterial ASV (ASV2887 of the order Longimicrobiales) was indicative for the most suppressive composts in all three plant-pathogen systems. The indicative bacterial ASVs showed considerable taxonomic diversity, with classifications encompassing 18 different phyla. However, for all three systems, the most common phylum of the indicative ASVs was *Pseudomonadota* (Table S10), which was also the most common phylum for ASVs (Table S6). While more than two thirds of the indicative ASVs were assigned to a family, substantially fewer could be assigned to a genus—specifically, 23%, 37% and 35% in the cress-*G. ultimum*-system, cucumber-*G. ultimum*-system and cucumber-*R. solani*-system, respectively. At family and genus level the indicative ASVs showed high phylogenetic diversity with only few ASVs being assigned to the same taxonomic classification (Table S10).

To identify the statistically most relevant bacterial indicators for each plant-pathogen system, we ranked the bacterial indicators based on effect size for each statistical method and calculated the rank sum across methods. The top 20 for each plant-pathogen-system are shown in Table 2. For the cress-*G. ultimum*-system, three of the top 20 indicative ASVs could be assigned to a described genus i.e., to *Sphingomicrobium*, *Brevundimonas*

and *Microvirga*. In the cucumber-*G. ultimum*-system, ten of the top 20 indicative ASVs were assigned to a described genus, i. e., to *Parapedobacter*, *Sphingopyxis*, *Sinorhizobium*, *Croceibacterium*, *Nitrosomonas*, *Luteimonas*, *Flavipsychrobacter*, *Enhygromyxa* and *Flavobacterium*. For suppression of *R. solani* in cucumber, five of the top 20 indicative ASVs could be assigned to described genera, i.e., to *Mycobacterium*, *Rhodomicrobium*, *Nonomuraea*, *Actinocorallia* and *Sphaerobacter* (Table 2).

Although most indicative ASVs were specific to the plant-pathogen system, a considerable overlap, comprising 40 ASVs, was observed between the two *G. ultimum*-systems (Fig. 5B). These 40 shared indicative ASVs are of particular interest as they may serve as more general indicators of suppression of *G. ultimum*. Phylogenetic analysis revealed a high diversity of bacteria, i.e., including only few ASVs with the same taxonomic classification (Fig. 5C). Eleven of the forty indicative ASVs were assigned to described genus, i.e., to *Algoriphagus*, *Flavobacterium*, *Lacipirellula*, *Synoicihabitans*, *Sinorhizobium*, *Brevundimonas*, *Croceibacterium*, *Cellvibrio*, *Luteimonas* and *Nitrosomonas*.

We conducted a literature search for the 62 bacterial and four fungal genera to which indicative ASVs were assigned to. We found that 44 bacterial and all fungal genera have been previously reported in composts (Table S13). For 28 bacterial and one fungal genus we found reported evidence for their involvement in disease suppression (Table 3). Most of these 28 bacterial genera were associated with ASVs indicative of *G. ultimum*-suppression. Only five genera were assigned to ASVs indicative of *R. solani*-suppression. Two of those five genera, *Streptomyces* and *Mycobacterium*, belong to the phylum Actinomycetota, which comprised one-fourth of all 80 indicative ASVs indicative in the cucumber-*R. solani*-system (Table S10).

In a next step, we performed a BLAST search to compare the 317 indicative ASVs identified in this study with the 75 ASVs associated with suppression of *G. ultimum* in cress in our previous work (Mayerhofer et al., 2021a). Matches with >99% sequence identity included six ASVs specific to the cress-*G. ultimum*-system, four to the cucumber-*G. ultimum*-system, one shared between both *G. ultimum*-systems, and one in the cucumber-*R. solani* system (Table S11). Notable matches included ASV467 (*Algoriphagus* sp.),

indicative for both *G. ultimum*-systems, and ASV87 (family *Steroidobacteraceae*) and ASV146 (*Sphingopyxis* sp.), which ranked among the top 20 indicators for *G. ultimum* suppression in cress and cucumber, respectively.

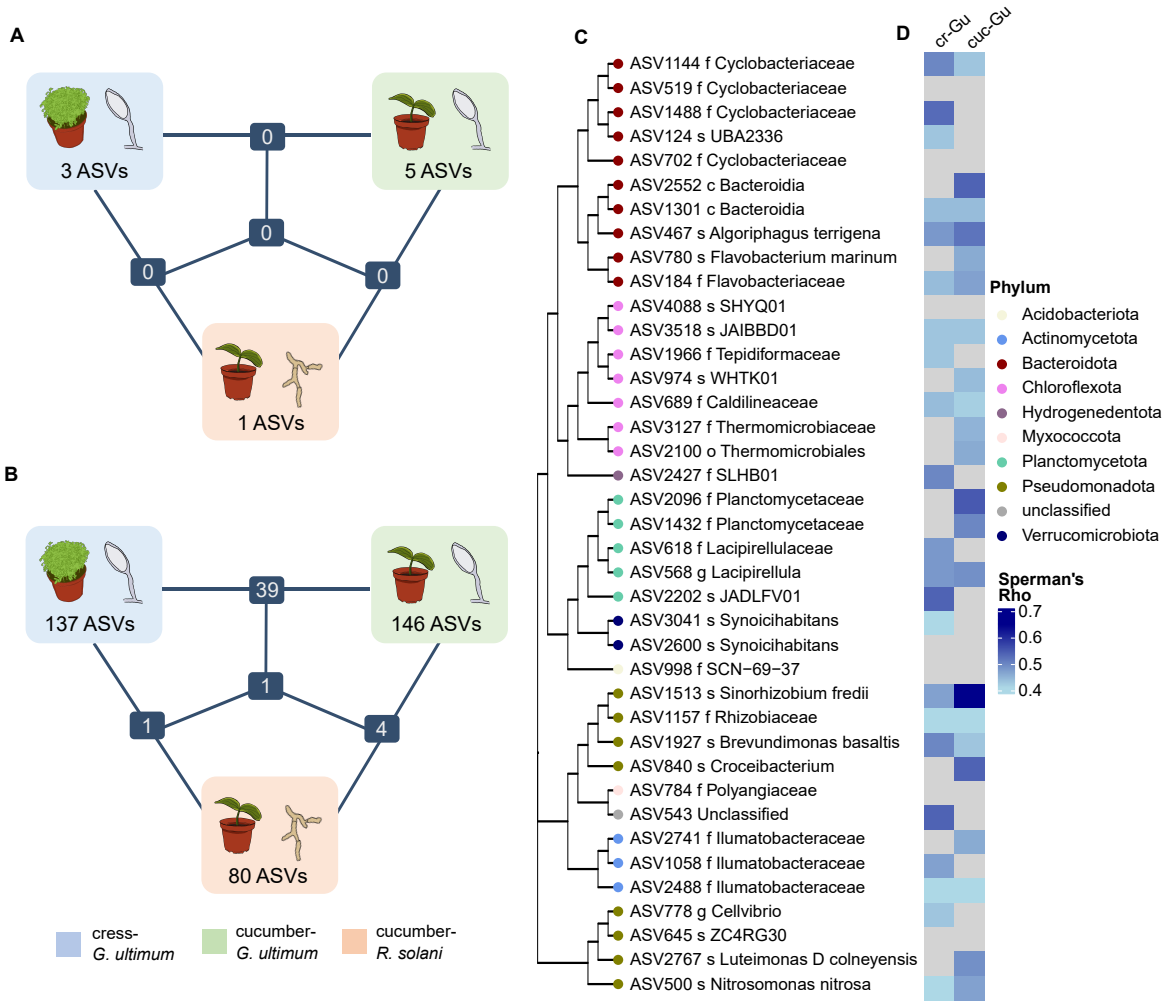

Figure 5: **ASVs indicative of the most suppressive composts were predominantly bacteria and specific to a plant-pathogen system.** Number of indicative ASVs in the three plant-pathogen systems, cress-*G. ultimum*, cucumber-*G. ultimum* and cucumber-*R. solani*, and the number shared among the systems are shown for (A) fungi and (B) bacteria. (C) A phylogenetic tree based on ASV sequences depicts the 40 indicative bacterial ASVs shared between the two *G. ultimum*-systems. For all ASVs the lowest taxonomic classification based on the Genome Taxonomy Database (GTDB) is indicated (s = species, g = genus, f = family, o = order, c = class). ASVs that were classified to a described genus are marked with a black square. (D) A heat map shows the correlation between their abundance and the disease-suppressive activity against *G. ultimum* in cress and cucumber across all 37 composts. Spearman Rank sum correlation was used and p-values were adjusted for multiple testing (Benjamini & Hochberg). Non-significant correlations (p-adj <0.05) are indicated as gray boxes. cr-Gu: cress-*G. ultimum*, cuc-Gu: cucumber-*G. ultimum*

Table 3: **Bacterial genera and a fungal genus that were assigned to indicative ASVs, and which were previously reported in the context of disease suppression.**

cr-Gu	cuc-Gu	cuc-Rs	Genus	Literature search results
	ASV864		*Advenella*	Low abundant member of suppressive syncom against root rot (Li et al., 2021)
ASV3229			*Agrobacterium*	Control of crown call (Ryder et al., 1991), disease-suppressive compost isolate (Charest et al., 2005)
			Algoriphagus	Enriched in rhizosphere suppressive of bacterial wilt (Zheng et al., 2024)
	ASV90		*Arenimonas*	Indicator for suppressive soils (Ou et al., 2019)
ASV1927	ASV1927		*Brevundimonas*	Control of *Fusarium redolens* in chickpea (Bekkar and Zaim, 2024)
ASV231 ASV778	ASV778		*Cellvibrio*	Associated with bacterial wilt decrease (Messiha et al., 2023)
ASV427			*Chthoniobacter*	Consortia suppressive of *Fusarium oxysporum* (Kudjordjie et al., 2023)
ASV325 ASV399 ASV652	ASV2820		*Devosia*	Responsible for suppressive conditions in rhizosphere (Liu et al., 2024)
ASV780 ASV975	ASV65 ASV561 ASV780		*Flavobacterium*	Part of consortium suppression of *R. solani* (Carrión et al., 2019)
ASV739			*Glutamicibacter*	Isolate from compost with plant beneficial properties (Borker et al., 2021)
	ASV1137		*Glycomyces*	Antifungal properties (Ting et al., 2010)
		ASV1249	*Hyphomicrobium*	Enriched under suppressive conditions (Cao et al., 2024)
ASV2767 ASV903	ASV58 ASV849 ASV2767		*Luteimonas*	Enriched in suppressive composts (Scotti et al., 2020; Hernández-Lara et al., 2022)
ASV1379 ASV1408			*Microvirga*	Enriched in *Fusarium* wilt suppressive soils (Siegel-Hertz et al., 2018)
	ASV122		*Mortierella* (Fungi)	Associated with soils suppressive to *Fusarium* wilt (Khatri et al., 2023; De Corato et al., 2019; Xiong et al., 2017), enriched in soil suppressive of *G. ultimum* (Kurm et al., 2023)
		ASV243	*Mycobacterium*	Enriched in community suppressive of *Globisporangium aphanidermatum* in cucumber (Postma et al., 2005), production of siderophores (Meena et al., 2017)
ASV500	ASV500		*Nitrosomonas*	Enriched under suppressive conditions (Shen et al., 2019)
	ASV3060		*Novosphingobium*	Enriched under suppressive conditions (Deng et al., 2022)
ASV1781			*Phenylobacterium*	Associated with bacterial wilt control (Ding et al., 2023)
ASV1113		ASV794	*Povalibacter*	Enriched in *Fusarium oxysporum* suppressive soils (Ou et al., 2019)
	ASV327		*Pseudoxanthomonas*	Significantly associated with *Fusarium* wilt incidence (Wen et al., 2023)

Continued on next page

Table 3 **Bacterial genera and a fungal genus (continued)**

cressGu	**cucGu**	**cucRs**	**Genus**	**Disease Suppression**
ASV1700			*Rhizomicrobium*	Enriched in soil suppressive of *Fusarium* wilt (Lv et al., 2023)
ASV1678			*Shinella*	Enriched by biochar application leading to *Fusarium* suppression (Jaiswal et al., 2017)
ASV1513	ASV1513	ASV3191	*Sinorhizobium*	Enriched under suppressive conditions (Xue et al., 2015)
	ASV280		*Sphingobacterium*	Suppression of fungal pathogens (Xu et al., 2020), suppression of *Fusarium* (Matsuda et al., 2001)
	ASV1563		*Sphingobium*	Associated with bacterial wilt control (Ding et al., 2023)
	ASV146		*Sphingopyxis*	Antagonist of *Verticillium* pathogen (Snelders et al., 2020), part of a suppressive consortium (Fujiwara et al., 2016), enriched on healthy plants (Gu et al., 2022)
	ASV452 ASV737 ASV1749	ASV791	*Streptomyces*	Suppresses tomato wilt (Shen et al., 2021; Salamoni et al., 2010), antimicrobial properties (Kinkel et al., 2012), suppression of *R. solani* in pepper plants (Wang et al., 2015)
ASV2839			*Telluria*	Potential root-knot nematode control (Oka, 2010)

cr-Gu = cress-*G. ultimum*, cuc-Gu = *G. ultimum*-cucumber, cuc-Rs = cucumber-*R. solani*

3.5. Abundance of the indicative bacterial ASVs could explain differences in disease suppression across all 37 composts

The indicative bacterial ASVs were identified by comparing the nine most and nine least suppressive composts for each plant-pathogen system. However, do these ASVs also explain differences in disease suppression across all composts? We found that most of the 317 indicative ASVs were present in more than two-thirds of the 37 composts (Fig. S17A). Their mean relative abundance in the suppressive composts for the system they were indicative of ranged from 0.002 to 2.53% (median: 0.016%), compared to 0.0002 to 1.53% (median: 0.004%) in the non-suppressive composts (Fig. S17B and C). Overall, the indicative ASVs were widely distributed across composts, with most showing intermediate abundance (0.1–0.01%) in suppressive composts and low abundance (<0.01%) in non-suppressive composts.

Eighty ASVs indicative of the cress-*G. ultimum*-system (60.6%), 76 of the cucumber-*G. ultimum*-system (52.1%), and 45 of the cucumber-*R. solani*-system (56.3%) were significantly and positively correlated with disease suppression in their respective systems (Table S15, S16, S17; $|\rho|$ >0.4, p <0.05). Among these, 18, 20, and 13 ASVs from the cress-*G. ultimum*, cucumber-*G. ultimum*, and cucumber-*R. solani*-systems, respectively, were part of the top 20 indicative ASVs (Table 2). Of the 40 indicative ASVs shared between the two *G. ultimum*-systems, twelve were positively correlated with disease suppression in both host plants, five of which were identified at the genus level (Fig. 5D).

To determine whether the indicative ASVs reflect the disease-suppressive activity of specific composts, we summed the scaled abundances (range: 0 to 1) of all indicative ASVs for each compost and plant-pathogen system. The summed ASV abundances were then plotted against the disease-suppressive activity of the respective compost (Fig. 6). As expected, the nine least suppressive composts ("flop" category) exhibited low summed ASV abundances, while the nine most suppressive composts ("top" category) had high abundances. Notably, the 19 composts with intermediate suppression ("intermediate" category), which were excluded from the association analysis, also showed an increase in summed ASV abundance with increasing disease suppression. When including all composts, summed ASV abundance was positively correlated with disease suppression across

all three plant-pathogen systems (cress-*G. ultimum*: $\rho = 0.61$; cucumber-*G. ultimum*: $\rho = 0.62$; cucumber-*R. solani*: $\rho = 0.71$; all $p < 0.001$).

The summed abundance of the indicative ASVs represented the disease-suppressive activity of the composts best in the cucumber-*R. solani* (Fig 6C). In contrast, for the cress-*G. ultimum*-system a group of significantly disease-suppressive composts, most of them not among the nine most suppressive composts and therefore not considered in the association analysis, showed a similar low abundance of the indicative ASVs as the non-suppressive composts (Fig. 6A). Moreover, some non-suppressive composts, composts 15, 25, 29 and 32 in the cucumber-*G. ultimum*-system showed high abundance of the indicative ASVs (Fig. 6B). These results indicate that at an intermediate suppression level reliable distinction between significantly suppressive composts and non-suppressive composts is difficult based on the indicative ASVs. Overall, however, the indicative ASVs reflect the trend in disease-suppressive activity, not only among the 18 composts included in the association analysis but also across all 37 composts for all three plant-pathogen systems.

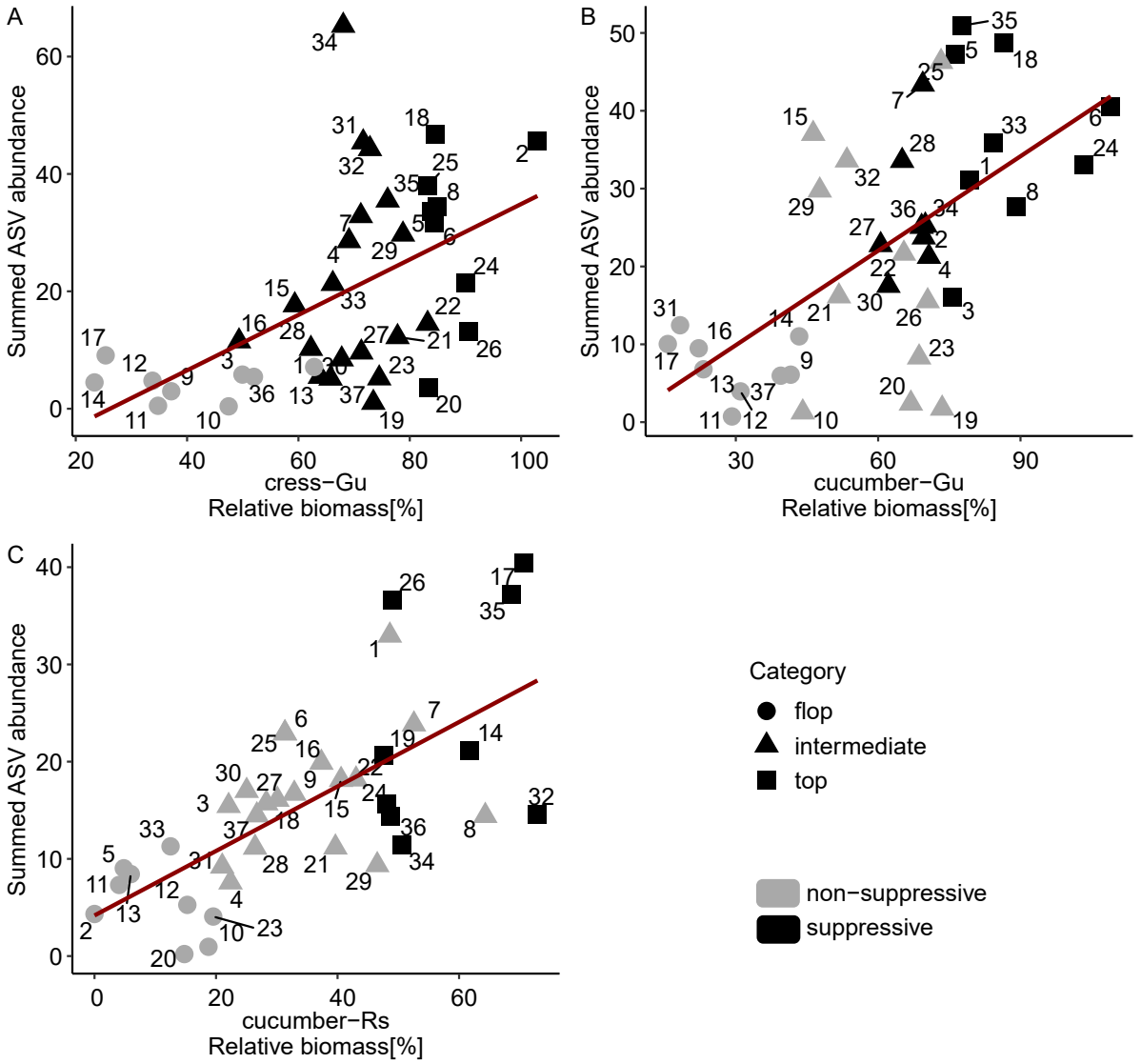

Figure 6: **Summed abundance of indicative ASVs was correlated with disease suppression across all 37 composts**. Correlation of summed ASV abundance with disease-suppressive activity across all 37 composts for (A) the cress-*G. ultimum*-system (137 ASVs), (B) the cucumber-*G. ultimum*-system (147 ASVs) and (C) the cucumber-*R. solani*-system (80 ASVs). To ensure equal weighting of each ASV, its abundance was scaled to a range of 0 to 1 prior to summation. The shape of the points represents the suppression category, with "top" and "flop" used to identify indicative ASVs during the association analysis. Shading indicates whether a compost was significantly more disease-suppressive compared to the no-compost control (Fig. 1). The linear trend line is shown in dark red.

4. Discussion

4.1. Disease-suppressive activity of compost was specific to the plant-pathogen system

In this work, of the 37 compost tested, 33 were fount to demonstrate disease-suppressive activity in at least one plant-pathogen system. Three composts were fount to be effective in all three systems (Fig. 1). Disease-suppressive activity of *G. ultimum* in cress and cucumber was positively correlated, whereas no correlation was observed with suppression of *R. solani* in cucumber. These findings align with previous studies (Scheuerell and Mahaffee, 2005; Termorshuizen et al., 2006; Bonanomi et al., 2018; De Corato et al., 2019), reinforcing the notion that disease suppression by composts is often pathogen-specific. More, and different composts were effective against *G. ultimum* than against *R. solani*, which indicates that different mechanism of disease suppression may be primarily involved in the control of the two pathogens. *G. ultimum* is often reported to be controlled by general suppression because it competes poorly with saprophytic microorganisms in nutrient-rich environments (Scheuerell and Mahaffee, 2005), whereas *R. solani* is suggested to be suppressed by specific biocontrol microorganisms (Hoitink and Boehm, 1999). However, for each compost-pathogen combination multiple mechanisms likely contribute to disease-suppressive activity (Hadar and Papadopoulou, 2012), and disentangling them *in situ* is experimentally challenging.

The largest differences were observed between systems with different pathogens, however, the host plant— cress or cucumber—also affected the disease-suppressive activity of composts. Plants can actively recruit disease-suppressive microorganisms to the rhizosphere through root exudates, as demonstrated in a study on suppressive soils (Mendes et al., 2011). Similarly, the plant-induced assembly of beneficial compost microorganisms has been proposed as an underlying mechanism for the suppressive effects of compost (De Corato, 2020) and has been shown to play an important role in the suppression of vascular wilt in tomato plants by composts (Antoniou et al., 2017; Ding et al., 2023). Microbial community assembly in the rhizosphere differs between plant species (Matthews et al., 2019), which may explain the observed differences in *G. ultimum*-suppression between cress and cucumber.

In summary, we found that most composts showed activity in specific plant-pathogen

systems, but still a few specific composts exhibited broad-spectrum efficacy. This suggests, that identifying pathogen-specific and potentially even pathogen- and plant-specific indicators of disease suppression is crucial to predict compost activity for practical applications in the future. The demonstration of broad-spectrum efficacy is rare, and motivates further work to unravel the underlying mechanisms for disease suppression in the different systems, and by different composts.

4.2. Bacterial and fungal communities in composts correlated with compost properties but not with disease-suppressive activity

We assessed the relationship between disease-suppressive activity and 14 physicochemical properties, microbial activity, compost age, and bacterial and fungal alpha- and beta-diversity of the composts. The only significant correlation was a positive association between bacterial diversity measures and disease suppression in the cucumber-*G. ultimum*-system (Fig. 3C, Table 1). However, this correlation was primarily driven by composts belonging to set II, which was characterized by a generally low bacterial diversity and weak *G. ultimum*-suppressive activity (Fig. 3D and E). In contrast, composts from set III also showed low bacterial diversity, but, on average, demonstrated high disease-suppressive activity. Thus, low bacterial diversity alone did not explain the lack of disease suppression in set II. Composts from set II were produced during a spring drought in 2022 (MeteoSchweiz, 2022). We suspect that this diminished the disease-suppressive activity against *G. ultimum*, which could be associated with the absence of specific microbes instead of only overall diversity.

Our results confirm previous research that indicates compost disease suppression cannot be reliably explained by physicochemical properties and microbial activity of the compost (Bonanomi et al., 2010; Yu et al., 2015). We extend this finding to the fungal and bacterial community structures and alpha diversity of composts. In our previous work with 17 composts (Mayerhofer et al., 2021a), bacterial richness did not correlate with suppression of *G. ultimum*, corroborating what was found in other studies, e.g., for *G. ultimum* in cucumber (Yu et al., 2015) or *Phytophthora nicotianae* in pepper plants (Blaya et al., 2016). However, bacterial community structure was significantly associated with disease suppression in the cress-*G. ultimum*-system (Mayerhofer et al., 2021a). No-

tably, disease suppression in these composts was negatively correlated with compost age, whereas no such correlation was observed in the 37 composts analyzed in this study. Further analysis of the 17 composts (Mayerhofer et al., 2021a) revealed that, when compost age was included as a covariant, the association between bacterial community structure and disease suppression was no longer significant (Table S12). This suggests that the previously observed link was likely indirect, mediated by the correlation with compost age.

We found that the physicochemical properties and the fungal and bacterial community structures of the composts were associated with the composting site and with each other (Fig. 2 and 4, Table S8 and S9). Moreover, the bacterial richness had a positive correlation with compost age and the nitrification index, both of which increase with compost maturation (Kong et al., 2024). This suggests that bacterial richness increases as compost matures, a trend also reported by other studies (Estrella-González et al., 2020; Hernández-Lara et al., 2022). The composting site reflects various factors, including feedstock composition, composting method, site-specific microbiota, and environmental conditions. Previous studies suggest that feedstock composition particularly influences both the physicochemical properties of compost (reviewed by Kong et al. (2024)) , its bacterial and fungal communities (Estrella-González et al., 2020), and determines the microbial dynamics during the maturation phase of composting (Villar et al., 2016). However, a study of 116 composts found that pH, moisture content, and total nitrogen—rather than feedstock—best explained variations in bacterial communities (Wang et al., 2020). Thus, it remains unclear whether the compost properties themselves or other site-specific factors are the strongest determinants of microbial community composition in composts.

In summary, our findings highlight that disease suppression by composts is not simply explained by physicochemical properties, overall microbial diversity or community structure. Instead, microbial communities in composts are shaped by site-specific composting conditions and the maturation process, which are not necessarily related to the disease-suppressive activity. These findings emphasize the need for more nuanced approaches to identify compost traits that promote disease suppression, particularly by investigating specific microbial taxa that may serve as indicators of disease-suppressive composts.

4.3. Bacterial ASVs comprised as promising indicators for disease-suppressive composts

The community structure is mainly shaped by abundant taxa. However, disease suppression can also be linked to less abundant taxa (Wei et al., 2019). In the present study, we identified 145, 137 and 80 bacterial ASVs and 3, 5 and 1 fungal ASVs significantly associated with the nine most suppressive composts in the three plant-pathogen systems cucumber-*G. ultimum*, cucumber-*G. ultimum* and cucumber-*R. solani*, respectively (Table S15, S16 and S17). Most bacterial and all fungal ASVs were unique to a single plant-pathogen system (Fig. 5A and B). However, 45 bacterial ASVs were shared across two or three systems, with 39 specifically occurring in both *G. ultimum* systems (Fig. 5C). Notably, the greatest overlap was observed in systems involving the same pathogen.

One of the few fungal ASVs indicative of *G. ultimum* suppression in cucumber was assigned to the genus *Mortierella*, recently identified as a key indicator of soil suppressiveness against *G. ultimum* (Kurm et al., 2023). Except for this interesting finding, only a few additional fungal ASVs were indicative of disease suppression. Unlike bacterial communities, fungal communities were not only ten times less diverse in our study, but were also often dominated by few taxa, with most taxa sparsely distributed across composts (Fig. S12 and S13). Similar patterns have also been reported in other compost studies (Hernández-Lara et al., 2022; De Corato et al., 2019; Galitskaya et al., 2017). Consequently, only 70, 85 and 88 fungal ASVs met all requirements to be included in the association analysis, compared to 1,738, 1,827 and 1,664 bacterial ASVs, for the cress-*G. ultimum*, cucumber-*G. ultimum* and cucumber-*R. solani* system, respectively. Additionally, sequence replicates for fungi showed greater variability than for bacteria (Fig. S11), suggesting that fungal communities in composts are more heterogeneously distributed.

For organically fertilized soils, it has been demonstrated that cellulolytic fungi—one of the dominant fungal groups in composts (Langarica-Fuentes et al., 2014)—were associated with particles with lower degree of humification (Zhang et al., 2017). In this study, the composts were sieved to 10 mm particle size for the bioassays, thereby also including larger less-degraded wood particles. On the other hand, DNA for metabarcoding was extracted from 2mm-sieved samples, which contained only the finer particles. Thus,

refining the sampling strategy and DNA extraction methods to include all particle sizes could improve the coverage of fungal communities and increase the likelihood of identifying fungi associated with suppressive composts. Nonetheless, our findings suggest that bacteria are more promising indicators of disease-suppressive composts than fungi.

For all three plant-pathogen systems, the identified bacterial indicators were phylogenically diverse, with no single taxonomic group being particularly dominant. For ASVs indicative of *G. ultimum*-suppression in cress and/or cucumber, most genera associated with disease suppression were reported in soils suppressive to *Fusarium* or bacterial wilt. Only a few genera, such as *Luteimonas*, *Streptomyces*, *Glutamicibacter*, and *Agrobacterium*, were specifically linked to disease suppression by composts (Table 3). Particularly the genus *Luteimonas* has been reported as an indicator of composts suppressive of *Globisporanigum irregulare* in lettuce (Hernández-Lara et al., 2022) and *Rhizoctonia solani* and *Sclerotinia minor* in cress (Scotti et al., 2020). Indicative ASVs assigned to this genus were not only found among the top 20 for suppression of *G. ultimum* in cucumber (Table 2), but also among the 40 ASVs indicative of *G. ultimum* independently of the host plant (Fig. 5C). Additionally, ASVs indicative of *G. ultimum*-suppression identified in our previous study (Mayerhofer et al., 2021a), which could be confirmed in this study and thus robustly indicate disease suppression, were classified to the genera *Algoriphagus*, *Devosia*, *Povalibacter* and *Sphingopyxis* (Table S11). Furthermore, many of the genera assigned to ASVs indicative of *G. ultimum*-suppression (Table 3), such as *Sphingobium*, *Devosia*, *Flavobacterium*, *Chthoniobacter*, *Cellvibrio*, *Brevundimonas*, *Shinella*, *Microvirga*, *Sphingopyxis* and *Novosphingobium*, were found to be enriched together in the tomato rhizosphere under *Fusarium*-suppressive soil conditions (Jaiswal et al., 2017). This suggests that the identified bacteria may contribute to *G. ultimum*-suppression together as part of a consortium (Sarma et al., 2015).

For suppression of *R. solani* one of the top 20 indicative ASVs could be assigned to a genus previously reported in the context of disease suppression, i.e., the genus *Mycobacterium* belonging to the phylum Actinomycetota (Table 2 and 3). However, overall, a fourth of all ASVs indicative of suppression of *R. solani* was assigned to the phylum Actinomycetota, which is generally known for containing disease-suppressive strains

(Palaniyandi et al., 2013) and has been previously associated with compost and soil suppressiveness against *R. solani* (Díaz-Díaz et al., 2022; Mendes et al., 2011; Cordovez et al., 2015).

In summary, the association analysis identified bacterial ASVs, along with a few fungal ASVs, as indicative of the most suppressive composts. While the majority of bacterial ASVs were specific to a plant-pathogen system, a notable overlap was observed between the two *G. ultimum*-systems. Especially for *G. ultimum*-suppression, several indicative bacterial ASVs were classified into genera previously associated with disease suppression. In particular, bacteria belonging to the genera *Sphingopyxis*, *Algoriphagus*, and *Luteimonas* appear to be promising candidates for further exploration. For the first two genera, the same ASVs were also identified as indicative of *G. ultimum*-suppression in our earlier work (Mayerhofer et al., 2021a). The latter contained several indicative ASVs and has also been previously reported as an indicator for disease-suppressive composts (Scotti et al., 2020; Hernández-Lara et al., 2022). That said, the value of indicative ASVs that could not be classified at the genus level should not be overlooked. They may be identified in the future with the discovery and description of novel bacterial taxa. Moreover, these ASVs hold promise for meta-studies or could serve as genetic markers for diagnosing disease-suppressive composts (Freeland, 2017).

4.4. Towards general indicators for disease-suppressive composts and the use of compost microorganisms for crop protection

For many indicative bacterial ASVs, their individual and summed abundance explained differences in disease suppression also across all 37 composts (Fig. 5D and Fig. 6). However, at intermediate suppression levels, the abundance of these ASVs did not reliably distinguish between suppressive and non-suppressive composts, particularly in the *G. ultimum*-systems. This indicates that the identified indicators may not capture the full spectrum of disease-suppressive composts, possibly due to varying suppression mechanisms. Given the complex nature of compost and that multiple factors influence disease-suppressive activity of composts (Hadar and Papadopoulou, 2012; Lutz et al., 2020), this is not unexpected. Ideally, models would be established that consider both microbiome data and other compost properties together. An approach that we have at-

tempted, but which was not successful. Modeling was hampered by two main factors: the strong composting site effect and the limited number of observations compared to the large number of possible predictors. This is despite the fact that, to our knowledge, our dataset includes the largest number of composts tested for disease suppression and sequenced for bacterial and fungal communities to date. These limitations could be addressed by either narrowing down the number of predictors by testing specific hypothesis-driven models or expanding data sets via meta-studies. For suppressive soils, meta studies successfully identified and suggested more general predictors (Zhang et al., 2022; Sagova-Mareckova et al., 2023; Wang and Zou, 2024). For disease-suppressive composts, however, meta-studies are constrained by the limited number of comparable studies and additional challenges, such as the need to test composts as an amendment to other soil substrates, which introduces an additional variable.

Moreover, even with extensive and reliable compost datasets, the high variability in taxonomic composition among composts likely limits the identification of universal indicators for disease suppression based on microbial taxonomy only. Similar challenges in identifying taxonomy-based indicators have been reported in a study on compost maturation (Estrella-González et al., 2020). For soil microbial communities, it has been demonstrated that despite the high taxonomic diversity across different soils, microbial functional attributes tend to be redundant (Chen et al., 2022). Identifying the key metabolic functions associated with the disease-suppressive activity of composts may be crucial to enable disease suppression prediction. Metagenomic approaches like shotgun sequencing will aid in identifying functional genes associated with disease suppression, as shown in studies of suppressive soils (Carrión et al., 2019; Tracanna et al., 2021). However, challenges such as high costs and the under-representation of compost microbial diversity in genome catalogs (Quince et al., 2017) limit their current application to composts. Therefore, we need to increase our knowledge not only of the compost microbial communities, but also of the individual compost microorganisms, of which we know only a small fraction. Understanding their biology and their ecological functions will help to unlock the potential of compost as a means to suppress soilborne pathogens.

In this study, we identified bacterial and fungal indicators that provide a promising

starting point to develop diagnostic tools for disease-suppressive composts, but also for a targeted isolation of interesting candidate microorganisms, which might mediate disease suppression. Testing these microorganisms for their disease-suppressive activity, both individually and in consortia, along with genomic and metabolic studies, could help to uncover key functions associated with disease suppression (Lutz et al., 2020). These efforts would enhance our understanding of the microorganisms and the mechanisms driving disease suppression and contribute to the overarching goal of developing reliable predictors for disease-suppressive composts. Moreover, disease-suppressive compost microorganisms could be added or fostered during the composting process to produce composts with higher disease-suppressive activity. Taken together, these approaches could contribute to a more successful and widespread use of compost to sustainably improve and maintain soil and plant health.

CRediT authorship contribution statement

Anja Logo: Writing — original draft, Writing — review & editing, Methodology, Visualization, Formal analysis, Conceptualization, Investigation. **Benedikt Boppré**: Investigation, Methodology, Writing — review & editing. **Jacques Fuchs**: Conceptualization, Resources, Funding acquisition. **Monika Maurhofer**: Writing — review & editing, Supervision. **Thomas Oberhänsli**: Project administration, Methodology, Conceptualization, Funding acquisition, Writing — review & editing. **Barbara Thürig**: Writing — review & editing, Methodology, Conceptualization, Funding acquisition. **Franco Widmer**: Project administration, Funding acquisition, Conceptualization. **Pascale Flury**: Writing — original draft, Writing — review & editing, Methodology, Conceptualization, Supervision, Funding acquisition. **Johanna Mayerhofer**: Writing — original draft, Writing — review & editing, Formal analysis, Conceptualization, Supervision, Methodology, Funding acquisition.

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Conflicts of Interest

No conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used *Writing Assistant* by Mohammed Ziara (ChatGPT 4o, OpenAI) and DeepL in order to fix spelling, grammar and enhancing clarity of the text. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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