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Microcystin influence on soil-plant microbiota: Unraveling microbiota modulations and assembly processes in the rhizosphere of *Vicia faba*

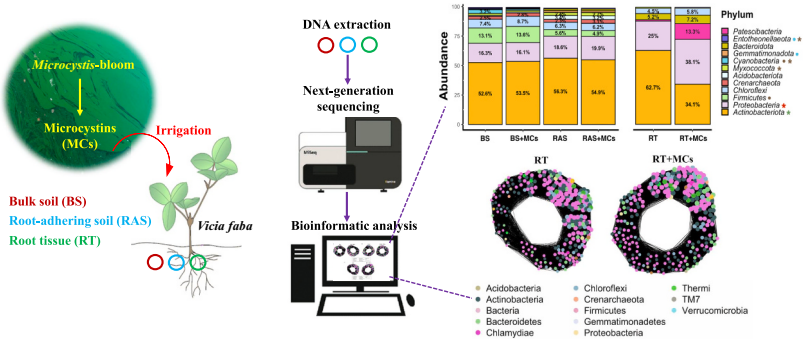
El Mahdi Redouane [a,b], Andrés Núñez [c,d], Wafa Achouak [e,*], Mohamed Barakat [e], Anoop Alex [b,f], José Carlos Martins [b], Zakaria Tazart [a,g], Richard Mugani [a], Soukaina El Amrani Zerrifi [a,h], Mohammed Haida [a], Ana M. García [c], Alexandre Campos [b], Majida Lahrouni [a], Khalid Oufdou [i], Vitor Vasconcelos [b,f], Brahim Oudra [a]

[a] *Water, Biodiversity and Climate Change Laboratory, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech 40000, Morocco*
[b] *CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, Terminal de Cruzeiros do Porto de Leixões, Matosinhos 4450-208, Portugal*
[c] *Escuela Técnica Superior de Ingenieros Industriales, Universidad Politécnica de Madrid (ETSII-UPM), Madrid 28006, Spain*
[d] *Departamento de Genética y Microbiología, Facultad de Biología, Universidad de Murcia, Campus de Espinardo, Murcia 30100, Spain*
[e] *Aix Marseille University, CEA, CNRS, BIAM, Lab of Microbial Ecology of the Rhizosphere, (LEMiRE), Saint Paul Lez Durance 13115, France*
[f] *Department of Biology, Faculty of Sciences, University of Porto, Porto 4169-007, Portugal*
[g] *AgroBioSciences, Plant Stress Physiology Laboratory, Mohammed VI Polytechnic University, Benguerir 43150, Morocco*
[h] *Higher Institute of Nurses Professions and Health Techniques of Guelmim, Guelmim 81000, Morocco*
[i] *Laboratory of Microbial Biotechnologies, Agrosciences, and Environment (BioMAgE), Labeled Research Unit-CNRST N°4, Faculty of Sciences Semlalia, Cadi Ayyad University, Marrakech 40000, Morocco*

HIGHLIGHTS

- MCs reshaped the microbiota structure in soil and root systems.
- MCs reduced the abundance of *Actinobacteria*-related taxa in the root system.
- MCs increased the abundance of *Proteobacteria*-affiliated taxa.
- MCs increased the abundance of potentially-degrading methylotrophic bacteria
- MCs influenced the microbiota network and assembly in soil and root systems.

GRAPHICAL ABSTRACT



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ABSTRACT

Microcystins (MCs) are frequently detected in cyanobacterial bloom-impacted waterbodies and introduced into agroecosystems via irrigation water. They are widely known as phytotoxic cyanotoxins, which impair the growth and physiological functions of crop plants. However, their impact on the plant-associated microbiota is scarcely tackled and poorly understood. Therefore, we aimed to investigate the effect of MCs on microbiota-inhabiting bulk soil (BS), root adhering soil (RAS), and root tissue (RT) of *Vicia faba* when exposed to 100 μg L^{-1} MCs in a greenhouse pot experiment. Under MC exposure, the structure, co-occurrence network, and assembly processes

* Corresponding author.
E-mail address: wafa.achouak@cea.fr (W. Achouak).

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of the bacterial microbiota were modulated with the greatest impact on RT-inhabiting bacteria, followed by BS and, to a lesser extent, RAS. The analyses revealed a significant decrease in the abundances of several Actinobacteriota-related taxa within the RT microbiota, including the most abundant and known genus of *Streptomyces*. Furthermore, MCs significantly increased the abundance of methylotrophic bacteria (*Methylobacillus*, *Methylotenera*) and other Proteobacteria-affiliated genera (e.g., *Paucibacter*), which are supposed to degrade MCs. The co-occurrence network of the bacterial community in the presence of MCs was less complex than the control network. In MC-exposed RT, the turnover in community composition was more strongly driven by deterministic processes, as proven by the beta-nearest taxon index. Whereas in MC-treated BS and RAS, both deterministic and stochastic processes can influence community assembly to some extent, with a relative dominance of deterministic processes. Altogether, these results suggest that MCs may reshape the structure of the microbiota in the soil-plant system by reducing bacterial taxa with potential phytobeneficial traits and increasing other taxa with the potential capacity to degrade MCs.

1. Introduction

Toxic cyanobacterial blooms (cyanoblooms) have been increasing in prevalence in freshwater ecosystems worldwide. These profuse blooms have been fast-occurring in recent decades due to the synergistic effect of global warming and eutrophication (Huo et al., 2019; Ibelings et al., 2021). Cyanobloom-forming genera (mainly *Microcystis*) produce and release cyanobacterial toxins (cyanotoxins) into water bodies meant for human consumption and irrigation purposes (Harke et al., 2016; Massey et al., 2018; Campos et al., 2021). Microcystins (MCs) are the most widespread and noxious cyanotoxins that are widely known as detrimental biotoxins to aquatic and terrestrial living organisms (Mehinto et al., 2021; Nowruzi et al., 2021).

Senescence and lysis of MC-producing cells cause a spike in MC release into water bodies during bloom formation and decay (Song et al., 2007; Zhang et al., 2009). MCs are chemically stable in water and persist for up to 251 days (Zastepa et al., 2014; Redouane et al., 2019). Nevertheless, they are susceptible to biodegradation and hence drive shifts in indigenous MC-degrading microbiota in the aquatic ecosystem (Giaramida et al., 2013; Ding et al., 2020). Overall, the structure and function of freshwater microbiota are found to be affected by the seasonal production of MCs and other cyanotoxins during the outbreak of cyanoblooms (Lezcano et al., 2017; Parulekar et al., 2017; Su et al., 2017).

MCs find their way into agricultural soil via contaminated irrigation water, where they can adversely impact soil-plant homeostasis (Corbel et al., 2014; Redouane et al., 2019). On the one hand, they interfere with microbially-mediated carbon and nitrogen metabolisms in soil, disrupt plant-rhizobia symbiosis, and hence decrease root nodulation and nitrogen uptake (Lahrouni et al., 2012; Cao et al., 2017). On the other hand, they stimulate the microbial degradation of MCs, which could shield the soil-plant system from their adverse effects (Cao et al., 2018; Redouane et al., 2021a, 2021b). Moreover, MC-tolerant rhizobia are useful to mitigate MC-toxicity in legume-rhizobia systems (Lahrouni et al., 2016; Redouane et al., 2021a). Yet, very little is known about the impact of MCs on soil microbiota. It was found that microbiota structure shifts in the *Medicago sativa*-rhizosphere under MC exposure, with an increase in the abundance of betaproteobacteria, to which several MC-degrading species are affiliated (El Khalloufi et al., 2016; Li et al., 2017).

Soil microbiota plays a prominent role in plant nutrition and protection against biotic and abiotic stressors. To the best of our knowledge, the impact of MCs on microbiota structure and functions has not been fully investigated to date. Therefore, this study aims to assess their effects on the bacteria of bulk soil alongside the rhizosphere soil and roots of *Vicia faba*. A 16S rRNA amplicon-sequencing technique was used for studying the structure, richness, and microbial network and assembly processes of soil and root-inhabiting microbiota when exposed to MCs and compared to unexposed specimens.

2. Materials and methods

2.1. Extraction, purification and analysis of microcystin congeners

Cyanobloom material, containing 75.3 % of *Microcystis aeruginosa* cells, was collected in October 2018 from the Lalla Takerkoust lake-reservoir, Marrakesh, Morocco (31° 36′ N, 8° 2′ W, 664 m). The crude extract of *Microcystis* bloom was prepared as described by Lahrouni et al. (2012), and the MCs contained within were pre-purified on LiChrolut octadecyl-silica cartridges (LiChrolut® RP-18, 1 g/6 mL, Sigma-Aldrich, Munich, Germany), as described by Triantis et al. (2016). Afterwards, MCs profiling was determined by ultra-high-pressure liquid chromatography coupled to a mass spectrometer (UPLC-MS/MS), according to Beltrán et al. (2012).

2.2. Experimental setup and samples collection

Farmland soils (0–20 cm depth) were collected from Lalla Takerkoust town, Marrakesh, Morocco (31° 22′ N, 8° 7′ W, 612 m) for a 30-day trial of exposure to MCs. Following that, soil samples were air-dried for 48 h, sieved (2-mm mesh size), and transferred to black plastic grow bags (2.5 kg soil per bag). A soil portion was analyzed for biological and physicochemical properties, and the results were previously reported in Redouane et al. (2021b). It was a silty clay loam soil (28.92 % clay, 64.5 % silt, and 7.03 % sand), with 3.8 g organic matter kg^{-1}, non-saline (EC = 0.42 $dS.m^{-1}$), and slightly alkaline (pH 8.17). The total bacterial count was 3.28×10^7 cfu g $soil^{-1}$ (dry weight). The soil was microcystin-free, as proven by UPLC-MS/MS, and it had no prior history of phytosanitary treatments that may impede microbiota homeostasis in the soil and root systems.

Certified seeds of *Vicia faba* L., var. *Alfia 321*, from INRA (Marrakesh, Morocco), were surface-disinfected in sodium hypochlorite (6 %, 10 min), and they were thoroughly washed in sterile deionized water. Following this step, water-imbibed seeds were dark-germinated at 25 °C for 48 h in glass petri dishes. Then, uniformly sprouted seeds with 1-cm-long radicles were transplanted into grow bags containing farmland soil. A total of 10 bags were planted, each containing a single seedling (Redouane et al., 2021b). The remaining 10 bags were kept unplanted to assess the impact of MCs on the bulk soil microbiota (Fig. S1).

MCs were pre-purified as described in Section 2.1 and spiked in irrigation water to a final dose of 100 μg L^{-1} as an average concentration that was proven to be toxic to a range of soil bacterial taxa (Lahrouni et al., 2012; El Khalloufi et al., 2016). At the 2-leaf stage, seedlings (planted soil) as well as unplanted soil were irrigated with MC-containing water at 2-day intervals (100 mL per plant), whereas control groups were irrigated with MC-free water (Fig. S1). The greenhouse trial was carried out in a randomized complete block design over the period March–April 2019, under ambient conditions of temperature, photoperiod, luminosity, and humidity.

After the 30-day exposure to MCs, plants were delicately uprooted using sterile gloves. Root-adhering soil (RAS), representing the rhizosphere compartment, was then collected. Subsequently, root tissue (RT)

underwent rinsing and shaking in sterile deionized water to eliminate any residual root-adhering soil. Afterwards, composite samples of bulk soil (BS) were collected with a sterile spatula. Both root (RT) and, soil (BS and RAS) samples were further freeze-dried and sieved (2-mm mesh size) for DNA extraction.

2.3. DNA extraction, 16S rRNA gene amplification and sequencing

Bacterial DNA was extracted in triplicates from 50 mg of freeze-dried roots and soils using the DNeasy® PowerSoil® isolation kit (QIAGEN, Madrid, Spain) and following the manufacturer's instructions. Extracted DNA was tested for purity ($A_{260/280\ nm} \sim 1.8$) using a NanoDrop Spectrophotometer (DeNovix, Wilmington, USA) and for integrity by electrophoresis on agarose gel. Next-generation sequencing of DNA samples was performed at LGC Genomics GmbH (Berlin, Germany). For the soil samples, the universal primers U341F/U806R 5′-CCTAYGGGRBGCASCAG-3′ and 5′-GGACTACNNGGGTATCTAAT-3′ (Sundberg et al., 2013) were used for amplification of the 16S rRNA gene by targeting the bacterial/archaeal V3-V4 region. As for the root samples, DNA amplification targeting V5-V6 region was performed with chloroplast-excluding primer sets 799F/1115R 5′-AACMGGATTAGATACCCKG-3′ (Chelius and Triplett, 2001) and 5′-AGGGTTGCGCTCGTTG-3′ (Reysenbach and Pace, 1995) in order to reduce contamination by host (plant) sequences. Amplicons were sequenced with the Illumina MiSeq platform using the paired-end read library (2 × 300 bp) protocol.

2.4. Bioinformatics and statistical analysis

Adaptors and primer sequences were trimmed, read-paired sequences were merged, and chimera sequences were filtered out using the default parameters of DADA2 pipeline (v1.17.5) (Callahan et al., 2016) in the R environment (R Development Core Team, 2020). SILVA database (release 138) (Quast et al., 2013) was used for the taxonomic assignment of the Amplicon Sequence Variants (ASVs). Normalization of the data was performed with the R package "metagenomeSeq" (Paulson et al., 2013) and global data analysis with "phyloseq" (McMurdie and Holmes, 2013). To get around the problem of using different sets of primers, we used Qiime2's q2-fragment-insertion plugin, developed precisely to deal with this type of situation and produce a coherent phylogenetic tree.

Amplicon sequence variants (ASVs) from soil and root samples were rarefied for the analyses of diversity indexes. The Chao1 and Observed indexes were used to assess species richness, and Shannon's and Evenness indexes were used to assess alpha-diversity. The statistical differences between the abundances of particular taxonomic groups were analyzed by selecting the accumulative abundances of the corresponding ASVs of the taxa and using pairwise comparisons by Welch's test. The differences for the diversity indexes were similarly analyzed, using the diversity values obtained for each sample. Principal Coordinate Analysis (PCoA) and permutational multivariate analysis of variance (PERMANOVA) were conducted using UniFrac distances ($p < 0.001$) to explore dissimilarities between soil and root compartments.

Co-occurrence networks were constructed based on pairwise Pearson correlations calculated between bacterial OTUs by using the base R function cor. The p-values were then adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995), and only edges with a p-value below 0.01 were retained. To describe the topology properties of the networks, a set of network indexes including number of nodes, number of edges, graph density, network diameter and average path length were calculated with the R package "igraph" (version 1.2.11).

To explore the structure of bacterial community assembly processes by deterministic or stochastic processes, the β-nearest taxon index (βNTI; Stegen et al., 2012) was calculated using the R package "picante" (version 1.8.2). The βNTI was calculated for pairwise phylogenetic turnover among communities to estimate the proportion of determinism. $|\beta NTI| > 2$ indicates that observed turnover between a pair of communities is governed primarily by determinism, which could be divided into homogeneous selection ($\beta NTI < -2$) and heterogeneous selection ($\beta NTI > +2$). On the contrary, $|\beta NTI| < 2$ indicates that observed differences in phylogenetic composition between a pair of communities are governed primarily by stochasticity.

3. Results and discussion

3.1. Microcystis bloom analysis

The analysis of the cyanobloom collected in October 2018 using UPLC-MS/MS showed a total MC content of about 14.67 μg mL^{-1}. MC profiling revealed three main variants: MC-YR (53.47 %), MC-LR (44.18 %), and MC-RR (2.35 %). Hence, the primary influence of MCs on bacterial microbiota in soil and root systems may be attributed to MC-YR and -LR, given their prevalence as the most dominant congeners in the extract (approximately 97.65 %). Certain bacterial genera appear to be particularly sensitive to MC-LR and MC-YR, exhibiting significant growth inhibition, as evidenced in the study conducted by Valdor and Aboal (2007).

3.2. Microcystin impact on bacterial composition and diversity in soil and root systems

An Illumina-based sequencing of the 16S rRNA gene amplicons was conducted to fully analyze the bacterial microbiota in the bulk soil (BS) and the soil-root system of *Vicia faba* (RAS and RT) under MC exposure. An average of 5289 and 2239 ASVs were generated and identified for analysis in soil (BS and RAS) and root (RT) compartments, respectively. We have primarily evaluated the effect of MCs on the microbiota core of the three compartments, as shown by the Venn diagram (Fig. S2). In MC-free BS and RAS, a total of 840 ASVs (416 defined genera) were found, of which 754 (373 genera) were shared. In these two compartments, the bacterial core was well-conserved, accounting for >99.2 % of the total abundance in each compartment. Interestingly, MCs did not change significantly the number of ASVs or genera (839 and 434, respectively), nor the shared ones (769 and 383), suggesting a low effect of these cyanotoxins in the soil compartments. Focusing on the RT, the number of ASVs and bacterial genera slightly increased when treated with MCs (700 and 337, respectively). Similarly, MC-treated and untreated RT shared many ASVs (665), which contributed to >99.5 % of each one. Overall, it seems that the bacterial core in soil and root compartments was mostly represented by species adapted to MCs. Furthermore, MCs may not change the bacterial composition upon short-term exposure in our study.

The richness (Chao1, observed) and diversity (Shannon, evenness) indices of bacterial communities were markedly higher in the BS and RAS compared with those of the RT (Fig. 1A). Also, the number of different ASVs (Chao1, observed) was similar between BS and RAS, while the bacterial diversity was significantly higher in the latter (Welch's test, $P < 0.05$). Likewise, El Khalloufi et al. (2016) observed a decrease in the bacterial richness and diversity from the bulk soil to the root system of *Medicago sativa* using the Shannon, observed species, and Chao1 indices. The exposure to MCs had no significant effect on the richness and diversity of the bacterial microbiota in the soil compartments (BS and RAS). Although it increased the diversity found in the RT (Welch's test, $P < 0.05$) without affecting the number of species (Chao1, observed; $P > 0.05$). These findings suggest a redistribution in the abundances of the species present in this compartment in response to MCs instead of changing the species composition. However, El Khalloufi et al. (2016) have noticed the opposite trend: an increase and decrease in bacterial richness in the root system and bulk soil, respectively, under MC exposure. Furthermore, in a study conducted by Petrou et al. (2020), MCs had no significant effect on the alpha-diversity of the bacterial microbiota in the rhizosphere of *Raphanus sativus*.

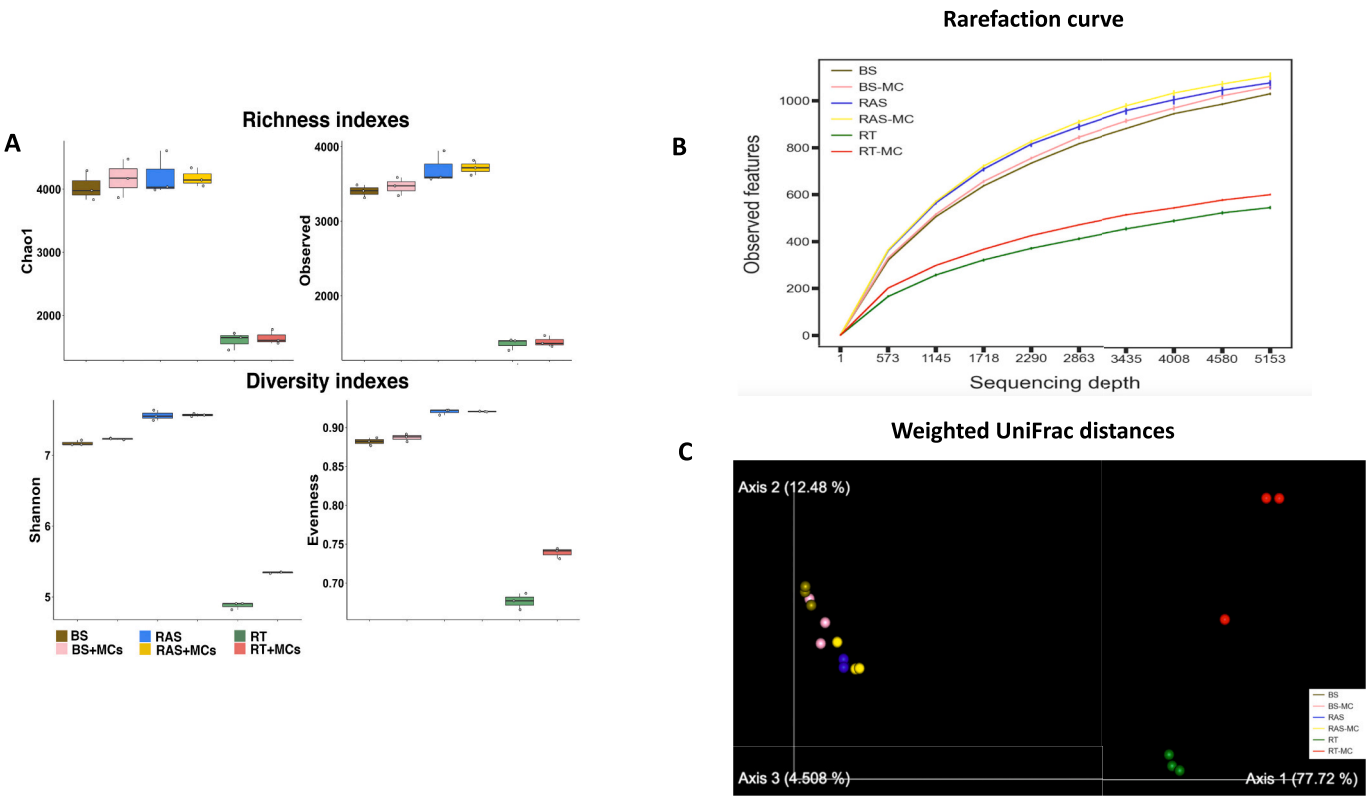

Fig. 1. Microbial community alpha and beta-diversity in *Vicia faba* rhizosphere and bulk soil in the presence and absence of microcystins (MCs). A) Microbial alpha-diversity Box Plots: Box plots depict the microbial alpha-diversity within various compartments, showcasing the richness and evenness of microbial communities. B) Rarefaction curves, constructed using the Shannon index, provide insights into the alpha-diversity trends. The Wilcoxon rank-sum test is employed for comparing alpha-diversity indices among different conditions, and statistical significance is demonstrated in the rarefaction curves; C) Non-Metric Multidimensional Scaling (NMDS) plots visually represent the beta-diversity of bacterial communities in different compartments, root (RT) and root-adhering soil (RAS) of *Vicia faba*, and bulk soil (BS) with and without MCs. Each dot corresponds to an individual sample (cf legend). The statistical significance of differences in beta-diversity is determined by PERMANOVA, with a p-value < 0.001, emphasizing the robustness of observed variations.

The results of diversity analysis also showed that under the same sequencing depth, the abundance and diversity of the root-associated microbiota were slightly higher in the presence of MCs (Fig. 1B). Visual comparisons of individual plots from principal coordinate analysis (PCoA) based on the weighted UniFrac distances (Fig. 1C) revealed subtle dissimilarities between BS and RAS, indicating some level of variation in microbial community composition. The first axis of the PCoA explained 77.72 % of the variance in bacterial composition. However, the root compartment (RT) exhibited a distinct separation from both BS and RAS, suggesting a pronounced dissimilarity in microbial communities. This observation indicates that the plant has recruited a specific microbial community in the RT compartment that differs significantly from that of BS and RAS. Furthermore, when subjected to MCs, notable changes were observed in both the abundance and community membership of the root-associated microbial community. This was confirmed by PERMANOVA analysis using weighted UniFrac distances ($p < 0.001$), highlighting the substantial influence of MCs on the composition of the root-associated microbial community. Further in-depth investigations are required to fully understand the impact of MCs on the intra-group and inter-group diversities of soil and root-associated microbiota.

3.3. Structure of bacterial microbiota in the soil and root systems

Bacterial taxa abundance and profiling differed between soil and root compartments across this study. Actinobacteriota, Proteobacteria, Firmicutes, and Chloroflexi were the most dominant phyla in BS and RAS, with an accumulative abundance of between 85 and 92 % across MC-treated and untreated soils (Fig. 2A). These phyla are found to be dominant in different terrestrial ecosystems and under various conditions across bulk and rhizospheric soils (Huaidong et al., 2017; Fonseca et al., 2018; Essel et al., 2019; Ren et al., 2021). Among these, Firmicutes was the only phylum that was statistically different between the two soils, as shown by Welch's test at $P < 0.05$ (Fig. 2A). However, other less abundant phyla showed a similar difference, among which Cyanobacteria and Deinococcota were higher in BS, whereas Desulfobacterota, Entotheonellaeota, Gemmatimonadota, Methylomirabilota, and Nitrospira were higher in RAS (Welch's test, $P < 0.05$; Fig. 2A).

Moreover, other taxa were higher in BS, such as the Bacillaceae family (Firmicutes) and three of its abundant species (*Bacillus foraminis*, *B. niacin*, and *B. nealsonii*), the Rubrobacteriaceae, and the Geodermatophilaceae (including the *Geodermatophilus obscurus*) within the Actinobacteriota phylum (Fig. 2 B). On the contrary, Pseudomonadota-related Xanthomonadaceae and Actinobacteriota-related Gaiellaceae and Nocardioidaceae were higher in the RAS of *Vicia faba* (Fig. S5). BS and RAS show different physicochemical and biological properties that are partly driven by plant-mediated interaction through rhizodeposition (Hinsinger et al., 2005; Medina-Sauza et al., 2022). Therefore, the RAS microbiota may differ from that of the adjacent BS (Fan et al., 2018). In regard to the root-inhabited microbiota (RT), they were also dominated by members of the phyla Actinobacteriota and Proteobacteria, with >72 % of accumulative abundance. However, other taxa such as the Bacteroidota and Patescibacteria phyla and the Actinobacteriota-affiliated Streptomycetaceae family and *Streptomyces* genus were predominant in this compartment (Figs. 2A, S3 and S4). These taxa are found to be abundant and dominant within the root endophytobiome, as previously reported in several studies (Rana et al., 2020).

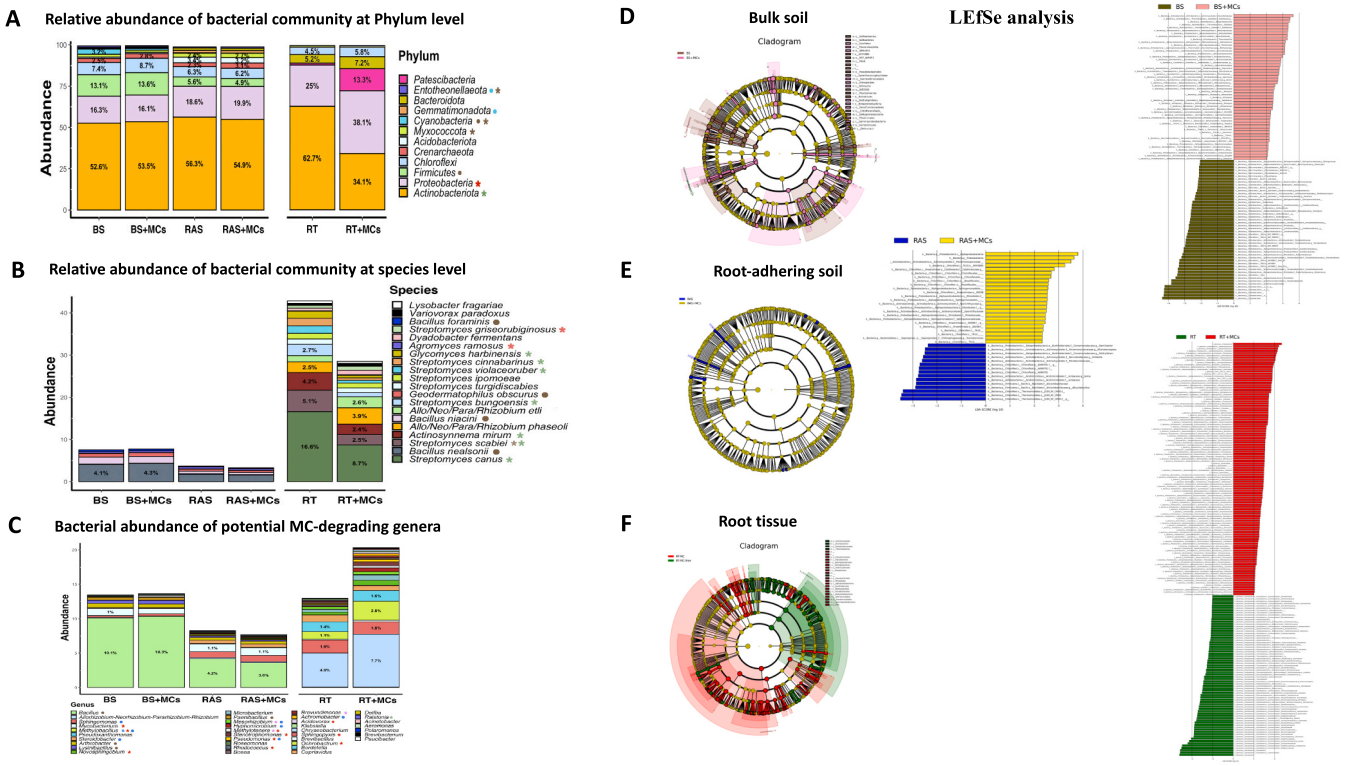

Fig. 2. Exploring the impact of microcystins (MCs) on microbial community beta-diversity and differential taxonomic biomarkers in the rhizosphere of *Vicia faba* and bulk soil. A–C) Relative abundance of OTUs of bacterial communities, A) at the phylum level, B) at the species level, C) highlights potential MC-degrading genera under MC exposure. The analysis is conducted in various compartments, including the root tissue (RT) and root-adhering soil (RAS) in the rhizosphere of *Vicia faba* and bulk soil (BS). Statistical significance is determined using Welch's test ($P < 0.05$). D, E, F) LEfSe Analysis; the bar chart displays the outcomes of Linear Discriminant Analysis Effect Size (LEfSe) conducted on bacterial communities in the RT, RAS, and BS under the presence and absence of MCs. The chart represents log-transformed Linear Discriminant Analysis (LDA) scores of bacterial taxa identified by LEfSe analysis, with a threshold of 2.0 for the log-transformed LDA score, providing insights into significant taxonomic biomarkers.

3.4. Microcystin impact on the abundance of soil-inhabiting microbiota

Overall, the exposure to MCs did not significantly affect the relative abundance of the predominant phyla represented by Actinobacteriota, Proteobacteria, Firmicutes, and Chloroflexi in the BS and RAS (Fig. 2A). Previous studies showed a similar pattern in bulk and rhizosphere soils exposed to abiotic stressors and dominated by the phyla Actinobacteriota, Proteobacteria, Firmicutes, and Chloroflexi (El Khalloufi et al., 2016; Huaidong et al., 2017; Benidire et al., 2020). However, less abundant phyla represented by Entotheonellaeota, Cyanobacteria, and Desulfobacterota significantly decreased in abundance in MC-treated BS (Figs. 2A and S3A). The Linear Discriminant Analysis Effect Size (LEfSe) method confirmed these findings (Fig. 2D). Various taxa affiliated with these phyla are known for their prominent roles in (a) nutrient cycling and mobilization (Liu et al., 2016; Begmatov et al., 2021); (b) carbon sequestration (Muñoz-Rojas et al., 2018); (c) soil stability and fertility (Chamizo et al., 2018; Gonçalves, 2021); (d) suppression of abiotic and biotic stressors (Poveda, 2021; Zhang et al., 2021a); (e) and release of plant growth-promoting substances (Karthikeyan et al., 2007; Gonçalves, 2021). Therefore, MCs may disrupt and alter the plant-microbiota homeostasis in agroecosystems in a way that affects soil quality and crop performance. A similar decrease was evident for Myxococcota-inhabiting bulk soil after being exposed to MCs, as shown in Fig. 2A–D. In a study conducted by Petrou et al. (2020), *Polyangium* and *Aetherobacter* genera affiliated to Myxococcota were reduced in abundance in MC-treated rhizosphere soil of radish at 12 μg L^{-1} MC-LR. MCs may thus impede the development of bacterial taxa belonging to Myxococcota (formerly Deltaproteobacteria), known as biocontrol agents against soilborne plant pathogens, owing to their predatory trait (Bhat et al., 2021). Moreover, this phylum constitutes an important source of new bioactive metabolites with multiple pharmaceutical applications (Weissman and Müller, 2009; Bhat et al., 2021). Furthermore, Actinobacteriota-related Nocardioidaceae was increased in abundance, while that of Gaiellaceae was decreased in BS and RAS, respectively, when they were treated with MCs (Figs. 2D, S5). The former plays key roles in the cleanup of hydrocarbon pollutants from soil and was found to thrive in polluted habitats (Azadi and Shojaei, 2020; Zhang et al., 2021b), whereas the latter takes part in phosphorus cycling and mobilization (Wang et al., 2022a). Significant alterations in bacterial composition were observed in the RAS following exposure to MCs. Specifically, certain Actinobacteria (*Lamia*, *Kribella*), Betaproteobacteria (*Ramlibacter* and *Methylibium*) and Chloroflexi at order level (Chloroflexi and Thermomicrobia), exhibited depletion. In contrast, other Chloroflexi lineages (Chloroflexales, Roseiflexales and Anaerolineae), and Alphaproteobacteria (Sphingomonadacae, Rhizobiaceae) showed enrichment (Fig. 2E). Conclusively, MCs did not affect the abundance of the dominant phyla and most of their related taxa in BS and RAS. However, these cyanotoxins seemed to reduce some other taxa, playing pivotal roles in nutrient cycling and maintaining soil-plant homeostasis against contaminants and pathogens.

3.5. Microcystin impact on the abundance of root-inhabiting microbiota

Root-inhabiting microbiota are divided into root-adhering bacteria (rhizoplane bacteria) and endophyte bacteria (endosphere bacteria), which colonize the root surface and the internal tissues, respectively. MCs have dramatically disturbed root-inhabiting microbiota, altering chiefly actinobacteria-related taxa at the phylum, family, genus, and species levels. Our results showed a significant decrease in the abundance of the Actinobacteriota phylum (Fig. 2F), besides the five related

dominant families (Streptomycetaceae, Pseudonocardiaceae, Nocardioidaceae, Micromonosporaceae, and Solirubrobacteraceae), and genera (*Streptomyces*, *Actinosynnema*, *Lechevalieria*, *Actinorectispora*, and *Glycomyces*) (Figs. 2F and S4C). In line with our findings, the root actinobacteria of *Medicago sativa* seemed to be sensitive to MCs, as previously reported in El Khalloufi et al. (2016), raising concerns about root actinobacteriomes given their pivotal role in sustainable agriculture. They have been extensively explored for their anti-phytopathogen and growth-promoting potentials (de Oliveira et al., 2010; Passari et al., 2016; Cui et al., 2022), besides their ability to mitigate abiotic stress and decontaminate polluted soils (Yandigeri et al., 2012; Baoune et al., 2018). *Streptomyces* is known as the most abundant and important actinobacterial genus, showing promising potential as key actors in sustainable agriculture and a major source of bioactive metabolites (Olanrewaju and Babalola, 2019). In this study, this genus was significantly decreased in MC-treated roots, including some of its dominant and beneficial species (*Streptomyces cinnabarinus*, *S. violaceoruber*, *S. bungoensis* and *S. canus*) as depicted in Figs. S4C and 2B. Acute or chronic exposure to contaminants might trigger the dissociation of bacterial endophytes from host plants, as shown in the study carried out by Kandalepas et al. (2015). However, the impact of environmental stressors on endophyte-plant symbioses is widely unexplored and necessitates further investigations. Furthermore, the decrease in actinobacteria-affiliated taxa may have been due to the acute toxicity of high-dose MCs in the root tissues. It was proven in several previous studies that roots accumulate more MCs than aerial parts, since they constitute the first point of contact with the toxin in contaminated soils (Mohamed and Al Shehri, 2009; Saqrane et al., 2009; Corbel et al., 2016; Lee et al., 2017; Redouane et al., 2021a). Interestingly, MCs increased the abundance of the Proteobacteria phylum and the related *Methylobacillus* genus (Figs. 2D, F and S4C). This stimulatory effect may have resulted from the decline of their antagonists and competitors or from their capability to catabolize MCs (El Khalloufi et al., 2016). Moreover, *Streptomyces griseorubiginosus* and *Agromyces ramosus* were stimulated in *Vicia faba* roots under MC exposure (Fig. 2B). The former was observed to thrive in polluted habitats and degrade organic contaminants (Sauvêtre et al., 2020). *A. ramosus*, has been described as a strong competitor that can exclude most of the Gram-positive bacteria (Casida, 1983), which may explain the decline observed in several actinobacterial taxa (Gram-positive taxa) in MC-treated roots. These results speculate that MCs remodel and shift microbiota profiling in the RT in a way that favors the development of specific taxa above others. That is to say, MC-tolerant taxa can take advantage of the sensitivity of their competitors to thrive and dominate in the RT.

3.6. Potential coexistence of MC-degraders within soil and root microbiota

Microorganisms are capable of degrading MCs in agroecosystems (Cao et al., 2018), so they may remain unaffected or even increase in abundance. In our study, Actinobacteriota, Proteobacteria, and Firmicutes were the most abundant phyla in BS and RAS, and their abundance remained intact under MC exposure. In contrast, the phylum Proteobacteria was increased in abundance in MC-treated roots (Fig. 2A). To the best of our knowledge, several studies have been reported on MC-degrading bacteria affiliated with the abovementioned phyla (Li et al., 2017; Massey and Yang, 2020; Dexter et al., 2021). We have noticed an increase in the relative abundance of *Mesorhizobium*, *Methylotenera*, and *Brevundimonas* genera in the BS and *Mycobacterium*, *Stenotrophomonas*, *Pseudomonas*, *Rhodococcus*, *Acidovorax*, *Sphingopyxis*, *Ochrobactrum*, *Novosphingobium* and notably methylotrophic bacteria such as *Methylobacillus*, *Methylotenera*, *Methylovorus*, *Methylibium*, *Methylopila*, *Methylobacterium*, genera in the RT when treated with MCs (Fig. 2C, F). All these genera were affiliated with the Actinobacteriota and Proteobacteria phyla (Table S1) and have been reported previously to degrade MCs, chiefly in aquatic ecosystems (Massey and Yang, 2020).

In accordance with these findings, El Khalloufi et al. (2016) have registered an increase in the abundance of species affiliated with the *Methylobacillus*, *Pseudomonas*, and *Acidovorax* genera in MC-treated bulk soil and roots of *Medicago sativa*. Furthermore, we have summarized in Table S1 all the bacterial genera found in our investigation and known for their potential ability to degrade MCs. For instance, *Ochrobactrum*, *Acidovorax*, *Acinetobacter*, *Bordetella*, *Cupriavidus*, *Delftia*, *Klebsiella*, *Polaromonas*, *Ralstonia*, and *Paucibacter* were only detected in the RT of *Vicia faba*. Contrariwise, *Brevibacterium*, *Brevibacillus*, and *Lysinibacillus* were only present in BS and RAS. Interestingly, *Brevibacterium* and *Paucibacter* were only detected under MC exposure in the rhizosphere and roots, respectively (Fig. S3). *Paucibacter*-related species were largely studied for their bloom-lysing and MC-degrading potentials (Rapala et al., 2005; Morón-López et al., 2017; Van Le et al., 2022). Thus, the abovementioned genera may exhibit such potential and could be further isolated and used in consortia for alleviating MC-impairing effects in agroecosystems. To date, most of the MC-degraders have been found in aquatic ecosystems, and reports about such bacteria in agricultural soils are absent. The farmland soil used in this trial has been exposed to MCs via contaminated irrigation water over the last two decades. Thus, the microbiota within has eventually evolved over time to degrade MCs. The acquisition of such a catabolic trait may occur through a horizontal gene transfer mechanism between MC-degrading microbiota from MC-containing waters and native soil microbiota. Furthermore, we have previously carried out a greenhouse trial in which we grew faba bean plants in the same farmland soil used in our investigation. We noticed that the plants accumulated less MCs in microorganism-rich soil than those in microorganism-free soil, which suggests a potential bio-removal of the toxins by native MC-degrading microbiota (Redouane et al., 2021b). Roots accumulate MCs at concentrations higher than those in the soil compartment, as evidenced by several studies (Corbel et al., 2016; Lee et al., 2017; Redouane et al., 2021b). Thus, MC-degrading taxa, chiefly Proteobacteria, may thrive in such a toxin-rich environment. Moreover, Proteobacteria may outcompete other root taxa that are MC-sensitive under toxin-induced stress, resulting in an increase in their relative abundance.

3.7. Microcystin impact on microbial community co–occurrence network

We employed a comprehensive co-occurrence network analysis to gain deeper insights into the ecological dynamics of microorganisms in the BS, RAS, and RT under MC exposure. This approach allowed us to explore the intricate patterns of microbial interactions and their responses to the presence of MCs. In the microbial network of the BS exposed to MCs, we observed an increase in the number of nodes (276 vs 267) and edges (15,878 vs 14,918), indicating a higher number of operational taxonomic units and their interactions. However, the network exhibited a lower density (0.418 vs 0.420), suggesting that the connections between species became sparser. This suggests a more complex and diverse microbial community with weaker species interactions. Furthermore, the higher average path length (1.763 vs 1.761) suggests that information or signals take longer to propagate through the network, indicating a potential decrease in communication or coordination efficiency between species (Table 1, Fig. 3A). Conversely, in the RAS, the presence of MCs resulted in a decrease in both the number of nodes and edges, while the density and average path length remained unchanged. This suggests a reduction in overall microbiota diversity in response to MC exposure (Table 1, Fig. 3B).

In the RT compartment, the presence of MCs led to an increase in the number of nodes (271 vs 264) but a decrease in the number of edges (15,135 vs 15,273) in the microbial network. This suggests that the interactions between microbial taxa are weaker or less frequent, resulting in a lower network density (0.414 vs 0.440). The lower network density indicates a reduction in the number of connections or interactions among the microbial taxa, which may indicate a less structured or less tightly connected microbial community. This reduced connectivity

Table 1
Topological properties of co-occurrence networks of bacterial communities at the ASVs level (n = 3). BS: bulk soil, RAS: root-adhering soil, RT: root tissue, MCs: microcystins.

	Number of nodes	Number of edges	Density	Transitivity	Diameter	Average path length
BS	267	14,918	0.420	0.759	3	1.761
BS + MCs	276	15,878	0.418	0.759	3	1.763
RAS	268	14,630	0.409	0.768	3	1.784
RAS + MCs	254	13,126	0.409	0.749	3	1.785
RT	264	15,273	0.440	0.769	3	1.728
RT + MCs	271	15,135	0.414	0.762	3	1.772

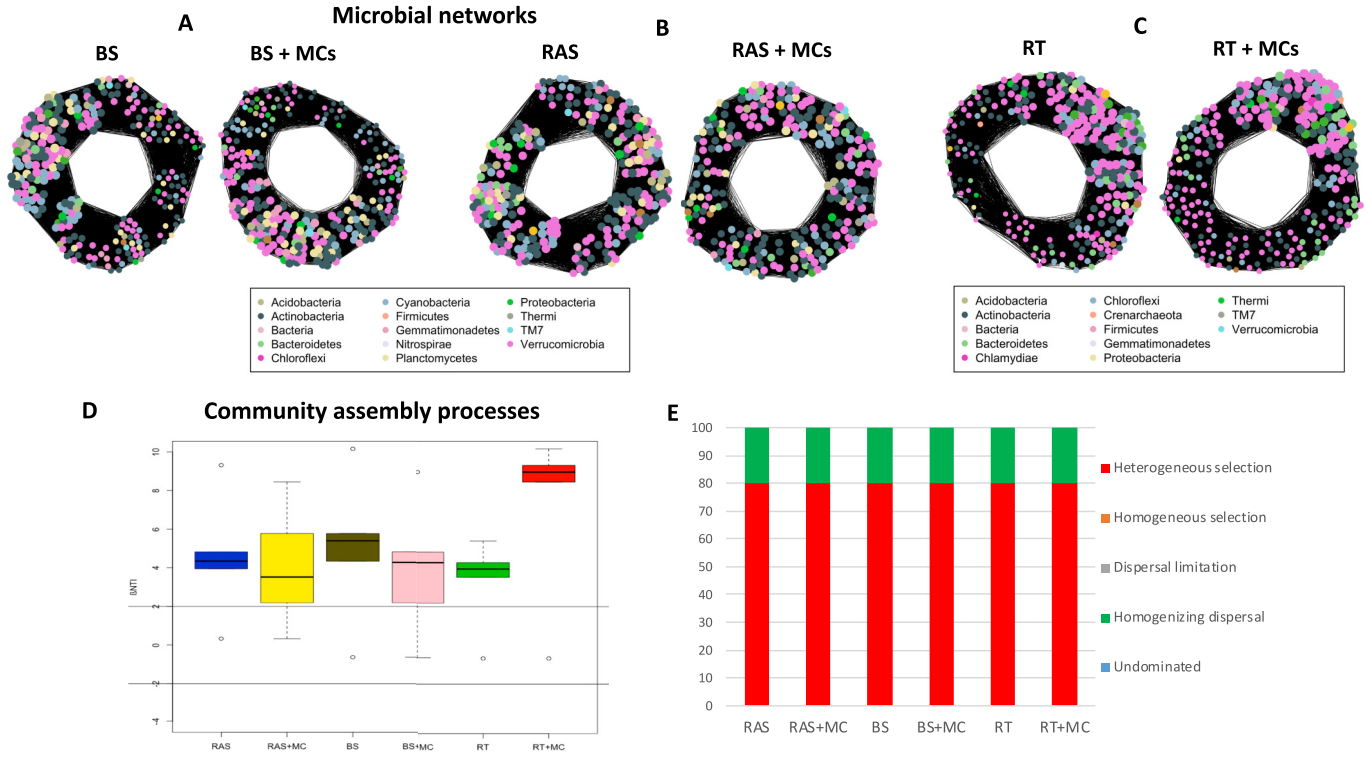

Fig. 3. Impact of microcystins (MCs) on microbial interaction network and assembly processes in the rhizosphere of *V. faba*. Co-occurrence network analysis illustrating the correlation of OTUs abundance within the bacterial communities A) in the bulk soil (BS), B) in the root adhering soil (RAS), C), and in the root tissue (RT) of *V. faba* in the presence and absence of MCs. Each dot in the network represents a node, corresponding to a distinct OTU representing a microbial population. Strong Pearson correlations, filtered at a 0.05 p-value threshold, are depicted by black lines. Nodes are color-coded according to their major taxonomic classes. The size of each node reflects its significance, determined by the number of connections (degree), betweenness, and closeness within the network. D) Assessment of assembly processes: the relative contributions of deterministic (βNTI $\geq$ 2) and stochastic (βNTI $\leq$ 2) processes on the bacterial assembly across the soil-plant root continuum of *V. faba* are evaluated using a null model. Horizontal lines indicate upper and lower significance thresholds at βNTI $<$ 2 and $>$2. E) Relative importance of 5 ecological processes for bacterial communities' assembly, along the BS, RAS and RT of *V. faba* in the presence and absence of MCs. These processes include heterogeneous selection (βNTI $<$ −2), homogeneous selection (βNTI $>$ 2), dispersal limitation (|βNTI| $<$ 2 and RCBray $>$ 0.95), homogenizing dispersal (|βNTI| $<$ 2 and RCBray $<$ −0.95), and undominated (|βNTI| $<$ 2 and |RCBray| $<$ 0.95).

could potentially affect the stability and resilience of the community to environmental changes. Furthermore, the higher average path length (1.772 vs 1.728) suggests that the distance between nodes in the network is longer, making it more challenging for signals to propagate efficiently through the network (Table 1, Fig. 3C). To cope with contaminant-induced stress, microbiota adjust their structure, function, and interactions (Berendsen et al., 2012; Vandenkoornhuyse et al., 2015; Benidire et al., 2020). Also, contaminants may destabilize the microbial community, showing lower diversity, a simpler network, and fewer ecological niches, as evidenced by Wang et al. (2022b).

3.8. Microcystin impact on microbial assembly processes

Ecological processes governing microbial assembly play a crucial role in shaping the microbial communities within specific habitats, including the plant root microbiota. One commonly used metric in microbial ecology, the beta-nearest taxon index (β-NTI), allows us to assess the degree to which differences in community composition between two samples deviate from neutral expectations. This index provides insights into the relative contributions of deterministic processes (e.g., environmental filtering, niche differentiation) versus stochastic processes (e.g., dispersal limitation, ecological drift) in driving community turnover.

Null model analysis revealed that the interplay between deterministic (|βNTI| $\geq$ 2) and stochastic (|βNTI| $<$ 2) processes in the assembly of microbiota in both bulk soil (BS) and *V. faba*-associated compartments was impacted by exposure to MCs (Fig. 3D). Our examination of β-NTI values without constraints disclosed that values $>$2 in BS, root-adhering soil (RAS), and root tissue (RT) signaled a clustered pattern of community composition, suggesting a higher likelihood of co-occurrence among closely related taxa than expected by chance. This implies a

relative prevalence of heterogeneous selection (Fig. 3E).

However, in the presence of MCs, we observed more diverse β-NTI values ranging between 0 and 8 in BS and RAS. This indicates the influence of both deterministic and stochastic processes in community assembly, with deterministic processes predominately characterized by a prevalence of heterogeneous selection (Fig. 3D–E). Interestingly, within the root compartment, the presence of MCs led to elevated β-NTI values, indicating a heightened influence of deterministic processes on community composition turnover (Fig. 3D). This discovery underscores the potential impact of MCs on the assembly of the root microbiota, emphasizing the significant role played by environmental factors in shaping the structure of microbial communities.

Based on the results of this study, MCs may cause a shift in microbial structure by selectively inhibiting the growth of sensitive species while promoting the proliferation of tolerant and degrading ones. Also, MCs may alter the microbial network by disrupting trophic interactions and interspecies communication. MC-sensitive species may encounter decreased competitive ability in a way that impacts their interspecies interactions within the community. Meanwhile, MC-tolerant species may take advantage of newly available resources, altering the stability of the microbial network. Furthermore, MCs can act as a selection pressure, influencing microbial assemblages by altering colonization, dispersal, and succession patterns. Long-term exposure may impact soil and root ecosystem processes, such as nutrient cycling. Overall, the influence of MCs on diversity, microbial networks, and assembly emphasizes the intricate interplay between environmental factors within soil-root systems. Therefore, understanding these dynamics is essential for predicting and mitigating the eco-risk of cyanoblooms and their associated cyanotoxins.

4. Conclusions

This study illuminates the substantial influence of MCs on the structure of the soil- and root-inhabiting microbiota, revealing their potential role in shaping microbial dynamics and interactions. On the one hand, MCs were found to reduce the abundances of several bacterial taxa known as plant-beneficial and disease-suppressive agents. On the other hand, they increased the abundances of specific bacterial phyla and genera, playing potential roles in MC-degradation and toxin-mediated resistance. This may be useful for deploying synthetic microbial communities for sustainable agriculture in MC-affected agro-ecosystems. Furthermore, our results provide valuable insights into the structural changes occurring within bacterial co-occurrence networks in the rhizosphere, shedding light on the potential impacts of MCs on microbial community dynamics and interactions, as well as the assembly process of the root microbiota. Further functional research, leveraging metatranscriptomic analyses, will undoubtedly contribute to our understanding of the mechanisms driving plant-associated microbiota-microcystin interactions. Such knowledge will be instrumental in the development of sustainable strategies aimed at managing microcystin-associated issues in both agricultural and natural ecosystems.

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CRediT authorship contribution statement

El Mahdi Redouane: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrés Núñez:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology. **Wafa Achouak:** Writing – review & editing, Methodology, Formal analysis. **Mohamed Barakat:** Validation, Software, Formal analysis, Data curation. **Anoop Alex:** Methodology, Investigation. **José Carlos Martins:** Methodology, Investigation. **Zakaria Tazart:** Validation, Investigation. **Richard Mugani:** Investigation. **Soukaina El Amrani Zerrifi:** Investigation. **Mohammed Haida:** Investigation. **Ana M. García:** Writing – review & editing, Funding acquisition. **Alexandre Campos:** Funding acquisition, Project administration, Resources. **Majida Lahrouni:** Writing – review & editing, Supervision. **Khalid Oufdou:** Writing – review & editing, Supervision, Resources. **Vitor Vasconcelos:** Resources, Writing – review & editing. **Brahim Oudra:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170634.

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