Prebiotics: A Solution for Improving Plant Growth, Soil Health, and Carbon Sequestration?

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ORIGINAL PAPER

Prebiotics: A Solution for Improving Plant Growth, Soil Health, and Carbon Sequestration?

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Abstract

Soil fertility and productivity are severely impacted by exploitation and degradation processes. These threats, coupled with population growth and climatic changes, compel us to search for innovative agroecological solutions. Prebiotics, a type of soil biostimulant, are used to enhance soil conditions and plant growth and may play a role in carbon (C) sequestration. Two commercial prebiotics, K1® and NUTRIGEO L® (referred to as SPK and SPN, respectively), were assessed for their effects on agricultural soil cultivated with *Zea mays* L., compared to untreated soil or control (SP). Analyses were performed at two harvesting dates: three weeks (D1) and ten weeks (D2) after the application of prebiotics. Plant growth parameters and soil characteristics were measured, focusing on soil organic matter, soil bacterial and fungal communities, and plant root mycorrhization. Regarding physicochemical parameters, both prebiotic treatments increased soil electrical conductivity, cation exchange capacity, and soluble phosphorus (P) while decreasing nitrates. Meanwhile, the SPN treatment was distinct in elevating specific cationic minerals, such as calcium (Ca) and boron (B), at D2. At the microbial level, each prebiotic induced a unique shift in the indigenous bacterial and fungal communities' abundance and diversity, evident at D2. Simultaneously, specific microbial taxa were recruited by each prebiotic treatment, such as *Caulobacter*, *Sphingobium*, and *Massilia* from bacteria and *Mortierella globalpina* and *Schizothecium carpinicola* from fungi in SPK as well as *Chitinophaga*, *Neobacillus*, and *Rhizomicrobium* from bacteria and *Sordariomycetes* and *Mortierella minutissima* from fungi in SPN. These biomarkers were identified as (a) saprotrophs, (b) plant growth-promoting bacteria and fungi, (c) endohyphal bacteria, and (d) endophytic and symbiotic microbiota. This result was reflected in the increase in glomalin content and mycorrhization rate in the treated soils, especially by SPN. We observed that these effects led to an increase in plant biomass (shoots by 19% and 22.8% and roots by 47.8% and 35.7% dry weights for SPK and SPN, respectively) and contributed to an increase in soil C content (organic C by 8.4% and total C by 8.9%), particularly with SPN treatment. In light of these findings, the use of prebiotics ten weeks after application not only increased plant growth by improving soil characteristics and shaping its native microbial community but also demonstrated the potential to enhance C sequestration.

Keywords Prebiotics · Biostimulants · Soil fertility · Plant growth · Microbial community · Carbon sequestration

1 Introduction

Demographic projections have estimated that the world population will exceed 9 billion people by 2050 (Igiehon and Babalola 2017). This surge will be coupled with higher nutritional demands, which must be addressed by increasing food production (Nephali et al. 2020). In response to these demands, the agricultural sector is working to increase crop yields while reducing dependency on conventional practices and their negative impacts on the environment (Rouphael and Colla 2020). This process is known as the agroecological transition, within which many alternative eco-friendly approaches are being considered, created, and applied (Therond et al. 2019). One of these new approaches is biostimulants, which, by definition, are substances that, when applied to seeds, plants, or the rhizosphere (regardless of their nutrient content, microorganism, or mix of both),

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stimulate natural processes to enhance or benefit nutrient uptake, nutrient use efficiency, abiotic and biotic stress tolerance, and/or crop quality and yield (Albrecht 2019).

Prebiotics are one type of biostimulants that typically include ingredients from natural sources, such as humic and fulvic acids, protein hydrolysates (plant- or animal-based), seaweed, plant and algal extracts, chitosan, and other biopolymers, complex organic materials (sewage sludge, compost, and manure), and inorganic compounds and minerals (silicon, iron [Fe], manganese [Mn], zinc [Zn], etc.) (Bulgari et al. 2015; Calvo et al. 2014). This type of biostimulant is widely applied through soil drenching to enhance soil fertility and plant growth (Jardin 2015; Yakhin et al. 2017). Over the last decade, the interest of researchers in studying soil prebiotics has rapidly elevated, especially with the exponential increase in the number of commercial biostimulants being released onto the market (Ricci et al. 2019; Yakhin et al. 2017). This new trend in agricultural practice reduces the dependency on synthetic fertilizers, pesticides, and herbicides and their negative impact on the soil ecosystem (Rouphael and Colla 2020). Biostimulants' application has demonstrated its efficacy in enhancing plant growth, traits, and productivity by improving soil health, structure, and properties (Berg et al. 2020; Colla et al. 2017; Nosheen et al. 2021).

Prebiotics may still hold undiscovered potentials that could aid in the agroecological transition and provide solutions to the continuing increase in carbon (C) emissions and soil exploitation/deterioration (Gupta and Staden 2021). One of these promising potentials is the enhancement of C quantity, stability, and storage in agricultural soils through sequestration (Lal 2011). Enhancing this soil process will be crucial in regulating climate change by decreasing the amount of atmospheric carbon dioxide (CO_2) and promoting soil health conservation and fertility restoration (Grace et al. 2012; Lal 2004; Tejada et al. 2011). The main sources of C in agricultural soils are plants via rhizodeposition, crop residues, and microorganisms through their biomass and activity (Meena et al. 2020). Many studies have indicated that biostimulant application led to a significant rise in plant biomass and plant residue degradation (Bulgari et al. 2015). These outcomes were explained by the effect of biostimulants on soil physicochemical characteristics and its native microbial diversity and functionality (Berg et al. 2020; Sharma et al. 2013). Biostimulants were proven to have a role in pH neutralization, cation exchange capacity (CEC), nutrient bioassimilation, mineral solubilization, and simultaneous enhancement of the organic C (OC) mineralization of crop residues and microbial biomass that were linked to changes in soil microbial communities (Castellano-Hinojosa et al. 2021; Hellequin et al. 2020). Specific members of these communities, especially bacterial and fungal, were recruited after the addition of biostimulants, which led to the support of soil C mineralization (Castiglione et al. 2021). These selected taxa were also identified as saprotrophic, endophytic, and symbiotic bacteria and fungi that have the capacity to decompose recalcitrant C sources (such as chitin and lignocellulosic material), boost fungal-bacterial symbiosis and plant mycorrhization, and fulfill a plant growth-promoting (PGP) role (Aeron et al. 2021; Igiehon and Babalola 2017; Müller et al. 2020; Simmons et al. 2014). Many studies have correlated the increase in biomass, diversity, and functionality of soil fungi with a greater C and nitrogen (N) ratio (C/N), higher C efficiency, and enhanced C accumulation in soil, resulting in higher C sequestration (Malik et al. 2016; Six et al. 2006). Indeed, the prebiotic application has been seen to support and activate the fungal microbial community, especially arbuscular mycorrhizal fungi (AMF) (Basile et al. 2020). This effect was seen to provoke a significant increase in plant photosynthesis, from 15.3% to 33.1%, in plant growth (especially the roots) by threefold, and in C storage by an average of 17.2% compared to untreated soils (Amaranthus and Jiracek 2001; Wang et al. 2016).

Based on these encouraging results concerning the role of biostimulants, research teams have started conducting more elaborate studies to assess these products in laboratory and field trials. Many of the tested products have proven their efficiency with varying intensities and modes of action, but this was not the case for others (Bello et al. 2021; Berg et al. 2020; Larkin 2008). This variability in biostimulants' application outcomes was explained by differences in their composition as well as soil characteristics, plant type, and experimental conditions (such as light, temperature, and humidity), all of which are crucial factors in determining products' functionality and efficiency (Sawaguchi et al. 2015; Shahrajabian et al. 2021). In 2015, the Paris Agreement called for action to store and increase the sequestration capacity of greenhouse gases (FAO 2016). An estimated 25% of the C present in virgin, uncultured soils has already been lost (Six et al. 2006). Nowadays, the potential of agricultural soils to regain most of the missing 25% C is attainable by reducing erosion, improving fertility, and mitigating CO_2 emissions (Six et al. 2006). Therefore, the use of soil prebiotics to increase plant growth, soil properties, and C sequestration represents an innovative approach that must be further investigated and exploited in response to the growing global population and its nutritional needs versus C emissions and associated climatic changes (Rouphael and Colla 2020; Ugena et al. 2018).

In this context, our study was designed to evaluate and elucidate the effects of two commercial prebiotics (K1® and NUTRIGEO L®) on soil mixed with organic wheat straws and cultivated with *Zea mays* L., compared to the control, at two harvesting dates after their application (three weeks and ten weeks). For this purpose, we monitored plant growth

criteria, soil physicochemical parameters, soil organic matter (OM) evolution, and soil microbial community structure and diversity, with an emphasis on indigenous microbial selection and root mycorrhization. To our knowledge, this study is the first to demonstrate prebiotics' short- and medium-term positive effects on soil fertility and microbial community and their repercussions on plant growth and C storage. Our aim is to explore new potential in prebiotics and confirm their vital role as a novel alternative approach in the agro-ecological transition toward modern, durable, and sustainable agriculture.

2 Materials and Methods

2.1 Soil Sampling and System Design

The soil was sampled from an agricultural field near Ronchois, a French commune located in the Normandy region (49°43′56.2″ N 1°36′41.6″ E). This soil is of the silty type (silt 63.36%, sand 20.49%, and clay 16.15%), with a water retention capacity of 31.68% and a pH of approximately 7.92. Prior to the experiment, the initial soil C content was determined, showing a total C (TC) content of 13.26 g kg^{-1} of dry soil, with 13.19 g representing OC and 0.07 g for inorganic C (IC). The N content in the soil was found to be 0.98 g kg^{-1} of dry soil. The sampled soil was transported to the laboratory and spread on nylon sheets to allow aeration for three days. It was then sieved at 5 mm to remove any debris or stones and subsequently stored in opaque containers at 5 °C. Two weeks before launching the experimental system (T0), all the soil was carefully sorted, hydrated to achieve a 70% humidity level, and mixed with organic wheat straws at a ratio of 2.5% by weight. Specifically, 1.8 kg of soil was mixed with 45 g of wheat residue and filled into rectangular 2 L black plastic pots (with dimensions of 10.4 cm length and width, 21 cm depth, and an empty weight of 70 g). As a result, the total C and N content introduced from the wheat straws into the soil was 10.52 g kg^{-1} of dry soil for C and 0.13 g kg^{-1} of dry soil for N. After two weeks of incubation, the pots containing the soil–straw mix were sown with organic *Zea mays* L. seeds of the variety DATABAZ (LOT: F0272 E9 09818 A) from Soufflet seeds and treated with prebiotics (T0). The prebiotic products tested in this study, namely, NUTRIGEO L® and K1®, were in liquid forms and were freely provided by the company Gaïago. NUTRIGEO L® is a mixture of plant extracts and trace elements such as boron (B) and Mn, while K1® is a mixture of specific organic acids and trace elements such as Mn and Zn. Based on this, the experimental system was built with a total of 90 pots, all containing the same soil–straw mix, and divided equally into three treatments: (i) planted soil without any treatment or the control (SP), (ii) planted soil treated with NUTRIGEO L® (SPN), and (iii) planted soil treated with K1® (SPK). Each of these treatments was composed of five blocks, with each block having three replicates. In addition, there were two harvesting dates: three weeks (D1) and ten weeks (D2) after the application of the prebiotics (T0). The two prebiotics were used in a single soil application manner through soil drenching at T0, at concentrations of 25 liters per hectare (L ha^{-1}) for NUTRIGEO L® and 5 L ha^{-1} for K1®, which are the recommended concentrations for field application. With respect to the control treatment (SP), it was simply treated with water at T0. The pots were arranged randomly in the greenhouse and were regularly watered to maintain the soil at 70% of its field retention capacity, preventing nutrient leaching. The greenhouse conditions were optimized to suit the growth requirements of the maize plant: (i) temperature ranging from 22 °C during the day to 18 °C at night, (ii) lighting with 16 hours of light and 8 hours of darkness, and (iii) humidity at 70%.

2.2 Harvest and Plant Biomass Analysis

At each harvesting date, non-invasive measures were taken, such as soil electric conductivity (EC) using the HI-98331 Groline direct soil conductivity and temperature tester by Hanna instruments (France) and plant chlorophyll level using the SPAD 502 Plus Chlorophyll Meter by Konica Minolta, Inc. After taking these measurements, the system was disassembled by removing the plant from the pots and weighing and measuring the length of the plant's shoots and roots. Part of the roots was conserved for mycorrhization analysis, and the remaining parts, along with the shoot, were dried at 40 °C in an oven for three days for further analysis. Regarding the soil, the three replicates in each block were grouped and mixed thoroughly to create, in the end, five composite samples per treatment. These replicates were then sieved to 5 mm, 2 mm, and 50 µm to comply with the protocols of the different analyses to be carried out for the experiment. Depending on the specific analysis criteria, the samples were subsequently stored at room temperature for physicochemical analyses or at +4 °C, −20 °C, or −80 °C for biological analyses.

2.3 Soil Physicochemical Analysis

Soil chemical characteristics, such as pH (in water and in KCl); CEC (using the Metson method); exchangeable macro-elements (including calcium oxide [CaO], potassium oxide [K_2O], magnesium oxide [MgO], and sodium oxide [Na_2O]); trace elements (such as Zn in EDTA, Mn in EDTA, copper [Cu] in EDTA, Fe in EDTA, B in boiling water, aluminum in KCl by the Jackson method, and sulfates [$SO4^{2-}$] in water); nitrate (NO_3^-) aqueous; total limestone ($CaCO_3$); total organic C (TOC); OM; and available phosphorus (P_2O_5-Olsen) were

all measured on room temperature dried and sieved soil by Aurea Agrosciences laboratory (Ardon, France-http://www.aurea.eu). TC and IC content analysis in soil samples was performed in-house using a Shimadzu SSM-5000A/TOC-VCSH C analyzer (Shimadzu, Kyoto, Japan). Briefly, after the soil samples were dried at room temperature and sieved at 50 µm, they were introduced to the machine. To measure the TC, the solid samples of dried soil were transformed into CO_2 by complete combustion at 900 °C in an oven. For the measurement of IC, the solid samples of dried soil were previously treated with phosphoric acid to dissolve the IC and then transformed into CO_2 by combustion at 700 °C in a column using a catalyst. CO_2 was then measured using an infrared cell by comparison with a standard range.

Soil particle size fractionation and analysis were performed to study OM presence and evolution in the soil. This was achieved by starting with the dispersion of 30 g of room-temperature dried soil samples, sieved at 2 mm, and mixed with 10 glass beads in 75 mL of sodium hexametaphosphate solution (5 g L^{-1}). The soil solutions were left for 24 hours on an Eb® Universal shaker SM-30 (Edmund Bühler, GmbH, Germany) at 175 strokes per minute rotational speed. After that, the OM in the dispersed soil was carefully separated into three fractions by sieving and flotation in water (standard NF X 31-516). These three fractions are (i) F.200, which resembles the free/fast OM fraction (size between 200 and 2000 µm) that is unprotected from degradation, consisting of plant residues associated with microbial compounds, and with a rapid turnover of 2 to 3 years; (ii) F.50, the free/slow MO fraction (size between 50 and 200 µm) that is located in the soil micro-aggregates, physically protected from degradation, and with a slow turnover between 10 and 30 years; and (iii) F.Inf 50, representing the bound/slow OM fraction (size < 50 µm) that is protected from degradation due to its association with silts and clays, with a residence time exceeding 100 years. The surveillance of the quantities, C contents, and N contents of these fractions is a tool for evaluating the dynamics of OM evolution and the effects induced by prebiotics application on the soil's organic reserve, thus C sink. The samples obtained from each fraction were then weighed and passed through a Thermo Scientific FlashSmart Organic Elemental Analyzer (ThermoFisher, Waltham, USA). Briefly, combustion takes place, and the burnt-up sample generates uniform gaseous compounds of elements C, H, and N. These products are then measured by gas chromatography to determine the contents of the elements of interest in the initial samples.

2.4 Metabarcoding: Analysis of the Structure and Diversity of Soil Microbial Communities

Total genomic DNA was extracted from 0.5 g of soil samples using the FastDNA® Spin Kit for Soil (MP Biomedicals, Santa Ana, USA), following the manufacturer's protocol. The samples' extracted DNA was then quantified using an advanced spectral scanning multimode reader, Varioskan® Flash (Thermo Fisher Scientific), with the Hoechst 33258 staining dye kit from Bio-Rad, under the name of Fluorescent DNA Quantitation Kit (Bio-Rad, Hercules, USA), in accordance with the supplier's recommendations. After this step, the DNA samples were stored at −20 °C. From these DNA samples, we randomly chose four out of the five composite replicates in each treatment and sent them to Biomnigene (Besançon, France—http://www.biomnigene.com) for conducting the metabarcoding analysis using high-throughput sequencing (Illumina® MiSeq® technology), according to their protocol. Briefly, the 16S V3-V4 regions of the extracted DNA were PCR-amplified with 16S-341F (5′-CCTACGGGNGGCWGCAG-3′) and 16S-806R (5′-GGACTACHVGGGTWTCTAAT-3′) primers (Caporaso et al. 2011; Klindworth et al. 2013). Meanwhile, the ITS1 region was PCR-amplified with ITS1F-Kyo2 (5′-TAGAGGAAGTAAAAGTCGTAA-3′) and ITS1R (5′-TTTCGCTGCGTTCTTCATCG-3′) primers (Toju et al. 2012). Both 16S and ITS1 primers were merged with Illumina adapters dedicated to hybridization on the flow-cell, sequencing initiation, and indexing. The PCR products were checked, purified, quantified, and pooled. The pool was then sequenced on the Illumina MiSeq platform (2 × 251-bp paired-end reads). Sequences were demultiplexed with Cutadapt 4.0 (with the following specifications: a minimum length of 220, an overlap of 7, and a value of e equal to 0.15) and then imported into the Qiime2 package (2022.2). The primer sequences were subsequently removed from the reads. The sequences were then quality-filtered, denoised, merged, and clustered using the Qiime2 DADA2 module with default parameters. Taxonomic assignment was performed with the Qiime2 classify-sklearn module and the GTDB database (release 202) for the 16S analysis and the UNIGE database (release ver8_99_10.05.2021) for the ITS1 analysis at multiple taxonomic levels: kingdom, phylum, class, order, family, genus, and species. Tables with raw data counts of species distribution for each sample and phylogenetic trees were then generated. All obtained sequences were submitted to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information database (NCBI) under the BioProject accession numbers: PRJNA998348.

2.5 Extraction and Quantitative Determination of Soil Proteins

The quantity of proteins in the soil was determined based on the quantity of glomalin-related soil protein (GRSP) (Rillig 2004). Soil proteins were classified into three fractions: (i) Fraction 1: labile Bradford reactive soil proteins (BRSP); (ii) Fraction 2: recalcitrant BRSP; and (iii) Fraction 3: the

sum of the first two fractions, referred to as total BRSP or total glomalin (TGRSP) (Koide and Peoples 2013). The TGRSP content was calculated according to the protocol developed by (Wang et al. 2015). The process started with extracting Fraction 1, or labile BRSPs, by adding 8 mL of citrate buffer (20 mM, pH 7) to 0.5 g of fresh soil. This mixture was autoclaved for 30 minutes at 121 °C and then centrifuged for 5 minutes at 10,000 g using the Eppendorf® Centrifuge 5810R (Eppendorf, Hamburg, Germany). The supernatant contained labile BRSPs. Next, 8 mL of citrate buffer (50 mM, pH 8) was added to the remaining soil deposit. The mixture was shaken by a vortex and autoclaved for 60 minutes at 121 °C before being centrifuged for 5 minutes at 10,000 g. The supernatant obtained contained the recalcitrant BRSPs, which constituted Fraction 2. The two extracted fractions were then quantified using the classic quantification method for BRSPs. The sum of these two fractions was calculated to obtain Fraction 3 content, or the total BRSPs (Koide and Peoples 2013).

2.6 Arbuscular Mycorrhizal Fungi Analysis

For mycorrhizal root colonization measurement, a part of the fresh fine roots was collected from the root system of each seedling. The roots were gently washed under tap water and bleached with 10% (w/v) KOH at 90°C for 30 minutes. Then, they were stained in 0.05% Trypan blue at 90°C for 30 minutes, following the method of (Phillips and Hayman 1970). The percentage of root length colonized by AMF was assessed at 40x magnification, using 100 fragments of lateral roots, each approximately 1 cm in length, on microscopic slides. Mycorrhizal root colonization was evaluated using the method described by (Trouvelot et al. 1986). Finally, images of the plant roots were taken using a digital microscope VHX 5000 (KEYENCE, France).

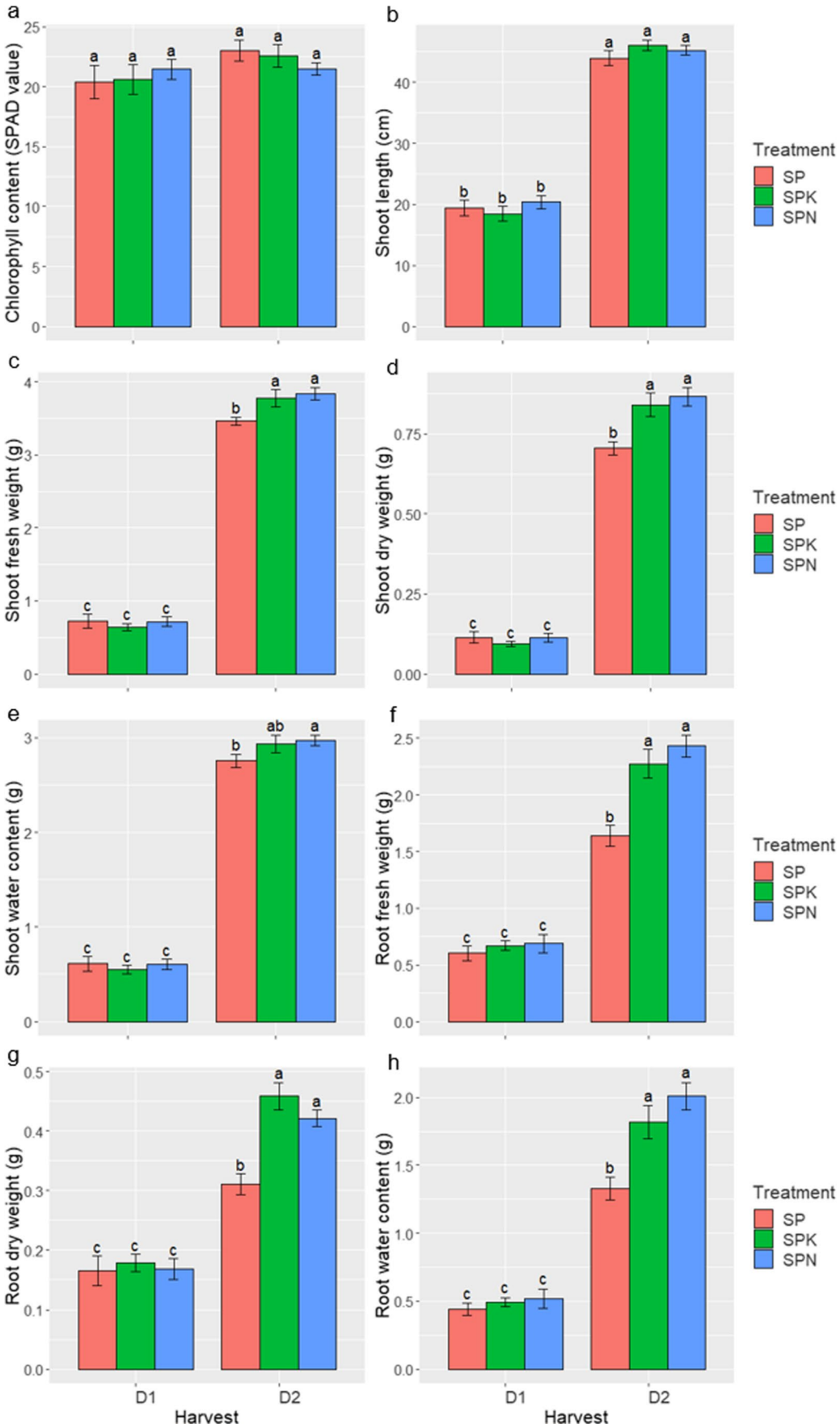
2.7 Statistical Analysis

All statistical analyses and figure creations were performed using R v.4.2.0 (http://www.r-project.org/) and MicrobiomeAnalyst (http://www.microbiomeanalyst.ca). The results presented are the mean values ± standard deviation (SD) of five determinations for each treatment across all tested plant and soil parameters. For all data except metabarcoding ones, the analysis of variance (ANOVA) was carried out using the "AOV" function of the package "stats" (R Core Team 2020). Multiple comparisons of treatments were then conducted using the least significant difference (LSD) test, with the grouping of treatments performed using the "LSD.test" function of the agricolae package (Mendiburu and Yaseen 2020). For this, the default value for the alpha argument, representing the 5% significance level ($p \leq 0.05$), and the Bonferroni method for adjusting the probability value were used. With respect to metabarcoding data, after receiving the OTUs/sample raw reads matrix and phylogenetic tree from Biomnigene, we created the metadata matrix and uploaded all data files to the Microbiome Analyst server (Chong et al. 2020; Dhariwal et al. 2017). Next, a data integrity check was performed using the "SanityCheckData" function, where OTUs with identical values (i.e., zero) in all samples and OTUs that appeared in only a single sample were excluded. Data filtering was then applied using the "ApplyAbundanceFilter" function based on the mean abundance value and the "ApplyVarianceFilter" function based on the SD (Ho et al. 2019). Data normalization was implemented using the "PerformNormalization" function, and data were rarefied to the minimum library size (Weiss et al. 2017). We calculated alpha diversity by taxonomic richness (observed OTUs) and the Chao1 index at a p-value < 0.05. The Shannon and Simpson indices were used to estimate evenness between samples at a p-value < 0.001. All diversity indices were compared among Treatments–Harvests using the t-test/ANOVA statistical method. For beta diversity, a Bray–Curtis dissimilarity was used to measure the distance between each pair of samples. This explicit comparison of microbial communities (pairwise) based on their composition was tested using permutational multivariate analysis of variance (PERMANOVA; 999 permutations) and plotted by principal coordinate analysis (PCoA). For microbial biomarker discovery and interpretation, a linear discriminant analysis effect size (LEfSe) analysis was conducted at the featured taxonomic level with a 0.1 p-value cutoff and a 0.5 log LDA score (Segata et al. 2011).

3 Results

3.1 Effect of Prebiotics on Maize Growth and Development

We studied the effect of two prebiotics on maize growth in relation to their date of application by monitoring several plant physiological parameters. Focusing on the maize's aerial part, the two prebiotic applications (SPK and SPN) did not demonstrate any significant difference ($p < 0.05$) in all plant shoot parameters when compared to untreated soil (SP) in the first harvesting date (D1) (Fig. 1a–e). Conversely, in the second harvesting date (D2), the SPK and SPN treatments significantly increased ($p < 0.05$) shoot fresh weight (SFW) by 9.1% and 10.9%, shoot dry weight (SDW) by 19% and 22.8%, and shoot water content (SWC) by 6.6% and 7.9%, respectively, in comparison to the control (SP) (Fig. 1c–e). Meanwhile, chlorophyll content (SPAD) and shoot length (SL) were not affected by the two prebiotic applications at D2. Moving to the subterranean part of the plant, we detected the same pattern as in the aerial


a
Chlorophyll content (SPAD value)
b
Shoot length (cm)
c
Shoot fresh weight (g)
d
Shoot dry weight (g)
e
Shoot water content (g)
f
Root fresh weight (g)
g
Root dry weight (g)
h
Root water content (g)
Treatment
SP
SPK
SPN
D1
D2
Harvest

◂**Fig. 1** Histograms representing the effects of the application of two soil prebiotics on **a** SPAD (leaf's chlorophyll content), **b** shoot length (SL), **c** shoot fresh weight (SFW), **d** shoot dry weight (SDW), **e** shoot water content (SWC), **f** root fresh weight (RFW), **g** root dry weight (RDW), and **h** root water content (RWC). D1, first harvesting date, three weeks after application of prebiotics; D2, second harvesting date, ten weeks after application of prebiotics; SP, plants without any treatment (control); SPK, plants treated with K1®; SPN, plants treated with NUTRIGEO L®. Each treatment is denoted by a color where red is for SP, green for SPK, and blue for SPN. Data presented are means ± SD. Bars sharing the same letters in each graphic are not significantly different ($p < 0.05$) according to the ANOVA LSD test

part, where no significant differences ($p < 0.05$) were present between the treatments at D1. A significantly greater improvement ($p < 0.05$) in plant root biomass was seen in the prebiotic treatments (SPK and SPN) at D2 in comparison to the untreated plants (SP) (Fig. 1f–h). This improvement was reflected in the increase of root fresh weight (RFW) by 38.7% and 48.3%, root dry weight (RDW) by 47.8% and 35.7%, and root water content (RWC) by 36.6% and 51.2% by plants treated with SPK and SPN, respectively (Fig. 1f–h).

3.2 Effect of Prebiotics on Soil Physicochemical Characteristics

Several soil parameters were measured in soil fractions sampled from all treatments at both harvesting dates to monitor the short- and medium-term effects of prebiotics on soil physicochemical characteristics and, thus, fertility. pH measurements showed slight variation, with pH-water ranging between 7.87 and 8.08. No significant differences ($p < 0.05$) were found between treatments in both harvests (D1 and D2), but a small increase ($p < 0.05$) in pH was noted in untreated soil (SP) from D1 to D2 (Table 1). Considering EC, it was generally higher at D1, and at D2, both prebiotic treatments exhibited higher EC values than SP (0.11 mS cm^{-1}), with a significant difference observed ($p < 0.05$) for SPK (0.15 mS cm^{-1}) (Table 1). Moving to CEC, there was no significant difference between the treatments at D1. However, at D2, both prebiotic treatments increased CEC compared to SP (9.65 meq $100g^{-1}$), with SPN showing a significant difference at 10.45 meq $100g^{-1}$ ($p < 0.05$) (Table 1).

Regarding soil C content, there were no significant differences ($p < 0.05$) between all treatments (SP, SPK, and SPN) at D1 (Table 1). In contrast, at D2, OC, OM, and TC increased significantly ($p < 0.05$) in the SPN treatment within a respective range of 8.4%, 8.3%, and 8.9% compared to SP. With respect to IC, no significant changes ($p < 0.05$) were observed between all treatments at both D1 and D2 (Table 1).

For soil N content, no significant difference in total N (TN) was observed between all treatments in either D1 or D2. Instead, there was a general significant ($p < 0.05$) decrease with an average of 37.9% between the two harvesting dates (Table 1). In contrast, NO_3^- in soil showed a significant general increase from D1 to D2 by an average of 58.9%. Within each harvesting date, NO_3^- did not vary significantly ($p < 0.05$) between treatments at D1, but at D2, it significantly decreased in SPK and SPN (18% and 20.4%, respectively, $p < 0.05$) compared to SP (Table 1).

The soil's major elements ($CaCO_3$, CaO, K_2O, MgO, Na_2O, and P_2O_5) did not show significant differences ($p < 0.05$) between treatments at both harvests (Table 1). However, CaO increased significantly in SPN (7.35 g kg^{-1}, $p < 0.05$) at D2 compared to SP (5.76 g kg^{-1}) (Table 1). Considering Na_2O, SPK showed a significantly greater quantity ($p < 0.05$) compared to SP by 6.3% (Table 1). As for P_2O_5, it significantly increased ($p < 0.05$) in SPK (8.3%) and SPN (2.1%) between D1 and D2 (Table 1).

With respect to trace elements, no significant differences were found in the concentrations of Mn, Fe, Al, SO_4^{2-}, Zn, Cu, and B between all treatments at both harvests (Table 1). However, Zn content was significantly greater in SP at D2 (3.72 mg kg^{-1}, $p < 0.05$) compared to D1 (3.35 mg kg^{-1}). In contrast, Cu significantly decreased ($p < 0.05$) in SPK by 12.7% from D1 to D2. SPN showed significant increases ($p < 0.05$) in B content compared to SP at both harvesting dates (D1: +58.3%; D2: +46.2%) (Table 1).

3.3 Effect of Prebiotic on the Evolution of Soil Organic Matter

Soil particle size fractionation was carried out on all the samples at D2. This analysis enabled us to divide soil OM into three distinct fractions based on their size and characteristics. The results obtained showed that SPN induced a significant ($p < 0.05$) increase (24.4%) in the weight of F.200 compared to SP. With respect to the masses of the following OM fractions: F.50 and F.Inf 50, no significant ($p < 0.05$) changes were observed between all the treatments (Table 2). The same trend was reported for the C content measured in the three separated OM fractions. SPN increased the C content of F.200 significantly ($p < 0.05$) by 27.1% in comparison to SP, with no repercussions on the other fractions (Table 2). Regarding the N content measured in the different OM fractions, no differences were noticed between any of the treatments across all the fractions (F.200, F.50, and F.Inf 50) (Table 2).

3.4 Effect of Prebiotics on the Abundance and Diversity of Maize Rhizosphere Microbiota

After analyzing the metabarcoding data from the high-throughput sequencing results, the bacterial and fungal composition of the maize rhizosphere was examined at different taxonomic levels (Fig. 2a, b). A notable change

Table 1 Results of prebiotics treatments (SPK and SPN) effect in comparison to the control (SP) on soil physico-chemical characteristics in the two harvesting dates (D1 and D2)

	Unite	D1			D2		
		SP	SPK	SPN	SP	SPK	SPN
pH water		7.93±0.06b	7.99±0.04ab	8.01±0.06ab	8.01±0.06a	7.99±0.06ab	7.99±0.05ab
pH KCl		7.59±0.04b	7.62±0.04ab	7.62±0.03ab	7.65±0.05a	7.63±0.03ab	7.64±0.02ab
EC	mS cm^{-1}	0.17±0.01ab	0.17±0.04a	0.16±0.01abc	0.11±0.01d	0.15±0.01bc	0.14±0.02cd
CEC	méq $100g^{-1}$	10.6±0.34a	10.39±0.59a	10.25±0.23a	9.65±0.36b	10.12±0.34ab	10.45±0.44a
OC	g kg^{-1}	18.78±1.07ab	18.3±1.47b	19.36±0.86ab	18.42±0.47b	19.02±1.72ab	19.96±0.52a
OM	%	3.23±0.18ab	3.15±0.26b	3.33±0.15ab	3.17±0.08b	3.27±0.3ab	3.43±0.09a
IC	g kg^{-1}	0.31±0.1a	0.43±0.24a	0.42±0.19a	0.36±0.08a	0.37±0.09a	0.5±0.13a
TC	g kg^{-1}	19.09±1.04ab	18.73±1.4b	19.78±0.76ab	18.78±0.43ab	19.39±1.65ab	20.46±0.57a
TN	g kg^{-1}	0.99±0.15a	0.95±0.06a	0.96±0.13a	0.58±0.15b	0.6±0.08b	0.58±0.11b
NO_3^-	mg $100g^{-1}$	0.09±0.03c	0.09±0.01c	0.08±0.01c	0.24±0.03a	0.2±0.03b	0.19±0.03b
$CaCO_3$	%	0.82±0.26a	0.86±0.69a	0.98±0.39a	0.6±0.28a	1.08±0.33a	1.14±0.43a
CaO	g kg^{-1}	6.25±1.15a	6.36±1.13ab	6.91±0.66ab	5.76±0.69b	6.99±0.88ab	7.35±1.14a
P_2O_5	mg kg^{-1}	95.65±2.89ab	92.95±6.18b	97.22±1.53ab	95.8±4.13ab	100.69±6.84a	99.24±2.92a
K_2O	mg kg^{-1}	567.2±56.41a	578.8±48.84a	600.4±125.72a	568.2±68.23a	587±18.76a	583.6±60.97a
MgO	mg kg^{-1}	110.6±4.39a	110.6±4.22a	112.2±10.06a	108.8±6.42a	113±2.74a	111.8±4.09a
Na_2O	mg kg^{-1}	21.24±1.32c	24.38±2.44ab	22.58±1.11bc	26.4±2.64a	26.8±1.92a	26.96±2.65a
Zn	mg kg^{-1}	3.35±0.28b	3.5±0.17ab	3.5±0.17ab	3.72±0.21a	3.59±0.21ab	3.66±0.21a
Mn	mg kg^{-1}	35.66±5.18a	37.31±8.78a	32.77±2.85a	33.65±4.34a	34.25±3.78a	37.47±6.84a
Cu	mg kg^{-1}	2.13±0.17ab	2.15±0.23a	2.11±0.21ab	1.87±0.14b	1.88±0.09b	2.04±0.29ab
Fe	mg kg^{-1}	34.74±19.75a	36.49±24.91a	24.79±6.52a	19.17±6.13a	21.65±6.26a	31.88±22.55a
B	mg kg^{-1}	0.17±0.03b	0.17±0.01b	0.27±0.08a	0.16±0.02b	0.17±0.02b	0.23±0.02a
Al	mg kg^{-1}	2±0a	2.01±0.03a	2.12±0.27a	2±0a	2±0.01a	2±0.01a
SO_4^{2-}	mg kg^{-1}	15.06±0.46a	15.51±1.81a	16.35±0.63a	15.47±1.52a	14.61±1.4a	15.77±0.84a

Means within columns followed by different lowercase letters are significantly different at $p < 0.05$ according to two-way ANOVA LSD test

D1 first harvesting date after three weeks of prebiotics application, *D2* second harvesting date after ten weeks of prebiotics application, *SP* untreated soil (control), *SPK* soil treated with K1®, *SPN* soil treated with NUTRIGEO L®, *EC* electrical conductivity, *CEC* cation exchange capacity, *OC* organic carbon, *OM* organic matter, *IC* inorganic carbon, *TC* total carbon, *TN* total nitrogen, NO_3^- Nitrates, $CaCO_3$ calcium carbonate or total limestone, *CaO* calcium oxide or exchangeable calcium, P_2O_5 phosphorus pentoxide or Olsen available phosphorus, K_2O potassium oxide or exchangeable potassium, *MgO* magnesium oxide or exchangeable magnesium, Na_2O sodium oxide or exchangeable sodium, *Zn* zinc, *Mn* manganese, *Cu* copper, *Fe* iron, *B* boron, *Al* aluminum, SO_4^{2-} sulfates

in the bacterial community composition was observed in the prebiotic-treated soil, particularly between SPN and the control at D2. Although the difference in the relative abundances of bacterial communities between treatments was not clearly evident at the phylum level, a deeper analysis at the class level revealed significant distinctions (Tables A1–A2). Specifically, the SP-D2 samples were dominated by *Alphaproteobacteria* (30.6% vs. 31.5% in SPK and 35.9% in SPN), *Actinomycetia* (14.3% vs. 14.5% in SPK and 17.2% in SPN), *Bacilli* (14.3% vs. 14% in SPK and 18.6% in SPN), *Vicinamibacteria* (10.8% vs. 10.5% in SPK and 2.1% in SPN), *Gammaproteobacteria* (9.8% vs. 8.5% in SPK and 7% in SPN), *Bacteroidia* (6.8% vs. 5.8% in SPK and 7% in SPN), *Verrucomicrobiota* (2.7% vs. 2.1% in SPK and 1% in SPN), and *Acidimicrobiia* (1.9% vs. 2.3% in SPK and 2.4% in SPN) (Fig. 2a, Table A2). In addition, differences between treatments at D2 were noticed in low abundant classes (≤ 2% of relative abundance), such as *Gemmatimonadetes*, *Thermoleophilia*, and *Acidobacteriae* (Fig. 2a, Table A2). At this level of taxonomic resolution, there was less difference in the bacterial composition of the major phyla among the three treatments detected at D1 (Fig. 2a). The difference at the first harvest lay in the less abundant taxa, which were more prevalent in the soils treated with both prebiotics than in the control (Tables A1–A2). In parallel, fungal diversity exhibited a similar tendency in the variation of community composition between the prebiotic-treated soil and the control, mainly at D2. To discern a clear and significant difference in the relative abundance of the fungal community, we delved into the sequencing depth until reaching the order level (Tables A3–A4). Consequently, we observed that the last harvest was dominated by *Sordariales* (58.9% in SP vs. 37.7% in SPK and 26.1% in SPN), Not_Assigned which is an unidentified order from the phylum *Ascomycota* (19.4% in

Table 2 Results of prebiotics treatments (SPK and SPN) effect in comparison to the control (SP) on the evolution of organic matter (OM) in the different soil partitions separated by particle size fractionation at the second harvesting date (D2)

Harvest	D2		
Treatment	SP	SPK	SPN
Weight F.200	6.61±0.69b	6.81±1.00b	8.22±0.51a
Weight F.50	8.83±2.27a	7.99±1.09a	7.37±1.05a
Weight F.Inf 50	848.45±20.53a	853.76±7.31a	855.36±11.08a
Carbon F.200	2.55±0.23b	2.61±0.45b	3.24±0.27a
Carbon F.50	1.96±0.35a	1.85±0.13a	1.84±0.29a
Carbon F.Inf 50	14.74±0.94a	14.93±0.50a	15.32±1.19a
Nitrogen F.200	0.10±0.01a	0.10±0.03a	0.11±0.02a
Nitrogen F.50	0.14±0.02a	0.13±0.01a	0.12±0.01a
Nitrogen F.Inf 50	1.06±0.11a	1.05±0.16a	1.09±0.09a

The unite for the weight of each fraction and carbon and nitrogen content in each is g kg^{-1} of dry soil. Means within columns followed by different lowercase letters are significantly different at $p \leq 0.05$ according to the two-way ANOVA LSD test

F.200 soil granulometric fraction ranging between 2 mm and 0.2 mm in size, *F.50* soil granulometric fraction ranging between 0.2 mm to 0.05 mm in size, *F.Inf 50* soil granulometric fraction its size is inferior to 0.05 mm, *D2* the second harvesting date after ten weeks of prebiotics application, *SP* untreated soil (control), *SPK* soil treated with K1®, *SPN* soil treated with NUTRIGEO L®

SP vs. 32.6% in SPK and 37% in SPN), *Hypocreales* (13.5% in SP vs. 16.3% in SPK and 20.8% in SPN), *Mortierellales* (3.55% in SP vs. 5.1% in SPK and 6.7% in SPN), and *Agaricales* (0.8% in SP vs. 5.6% in SPK and 5.1% in SPN) (Fig. 2b, Table A4). The variation in fungal relative abundance among the treatments (SP, SPK, and SPN) was less pronounced at D1 (Fig. 2b, Table A4). Considering the less abundant fungal taxa, their presence and percentages fluctuated between non-treated and prebiotic-treated soil in D1 and D2, depending on the taxa. They were more common in SP and SPN than in SPK (Tables A3–A4).

Considering the alpha diversity, at D1, the two prebiotic-treated soils (SPK and SPN) were associated with higher bacterial taxonomic richness compared to untreated soil (SP), regardless of the method used (Chao1 and OTUs or observed). However, at D2, SPK showed a significant decrease in its richness in comparison to SP ($p < 0.003$) (Fig. A1a–b). On the other hand, the bacterial taxonomic evenness at both harvests (D1 and D2) did not vary significantly between the three treatments (SP, SPK, and SPN) in the two calculated indices (Shannon and Simpson, with p-values < 0.002) (Fig. A1c–d). A different trend was observed for fungal alpha diversity measures. Starting with the richness, it did not change in both indices ($p < 0.005$) between the three treatments (SP, SPK, and SPN) in the two harvests (D1 and D2) (Fig. A1e–f). Moving to the fungal community distribution (evenness), it also did not change between treatments (SP, SPK, and SPN) at D1. At D2, the fungal evenness increased non-significantly for SPK and significantly for SPN in comparison to SP (Shannon with $p < 0.004$ and Simpson with $p < 0.005$) (Fig. A1g–h). In summary, the alpha diversity of the SPN-treated soil appears to be significantly different from that of SP and SPK, especially at D2.

In examining beta-diversity, we found a significant difference at D2 in both bacterial and fungal community compositions ([PERMANOVA] F-value: 2.3112; R-squared: 0.39099; p-value < 0.001 and F-value: 4.7974; R-squared: 0.57129; p-value < 0.001) between the different treatments, especially between SPN and SP. The Principal Coordinate Analysis (PCoA) plots illustrate this divergence between treatments, where it was greater in fungal community compositions (Axis2 = 30.8%) than in bacterial ones (Axis2 = 10.5%) (Fig. 3a, b). This divergence between treatments is not present in D1 for both bacterial and fungal beta-diversities. The PCoA also confirmed a clear separation between treatments in the two soil microbial community compositions with respect to their harvesting dates (D1 and D2). This separation was primarily obvious in the fungal communities (Axis1 = 58.7%) and, to a lesser extent, in the bacterial communities (Axis1 = 24.8%) (Fig. 3a, b).

LEfSe showed differentially abundant taxa as biomarkers, using the Kruskal–Wallis rank sum test ($p < 0.1$), with an LDA score > 0.5. The SPN treatment had the largest number of biomarker species (nine bacteria and six fungi) compared to SPK (six bacteria and five fungi) and SP (six bacteria and two fungi) (Fig. 4a, b). Soil treated with SPN was specifically colonized by certain bacterial species of *Alphaproteobacteria* (*Sphingopyxis*, *Microvirga*, and *Rhizomicrobium*), *Bacteroidetes* (*Flavobacterium* and *Chitinophaga*), *Bacillota* (*Neobacillus* and *Niallia*), and *Actinobacteriota* (*Nocardiodes* and *Nonomurea*), as well as fungal species of *Ascomycota* (*Sordariomycetes*, *Microdochium*, *Cladosporium tenuissimum*, and *Pseudogymnoascus roseus*), *Basidiomycota* (*Conocybe anthracophila*), and *Mortierellomycota* (*Mortierella minutissima*). In turn, the SPK-treated soil was colonized by particular species of *Alphaproteobacteria* (*Caulobacter*, *Sphingobium*, and *Dongia*), *Bacteroidota* (*Pedobacter*), *Betaproteobacteria* (*Massilia*), and *Acidobacteria* (*Solibacter*) from the bacterial community, and species of *Ascomycota* (*Schizothecium carpinicola*, *Zymoseptoria ardabiliae*, *Fusarium sporotrichioides*, and *Cercophora mirabilis*) and *Mortierellomycota* (*Mortierella globalpina*) from the fungal community. Meanwhile, the biomarker species present in the control (SP) were mainly from *Alpha* (*Sphingomicrobium*, *Devosia*, and *Hyphomicrobium*) and *Gammaproteobacteria* (*Pseudomonas* and *Pseudoxanthomonas*) bacterial classes and *Ascomycota* fungal phyla (*Acremonium furcatum* and *Fusarium domesticum*) (Fig. 4a, b).

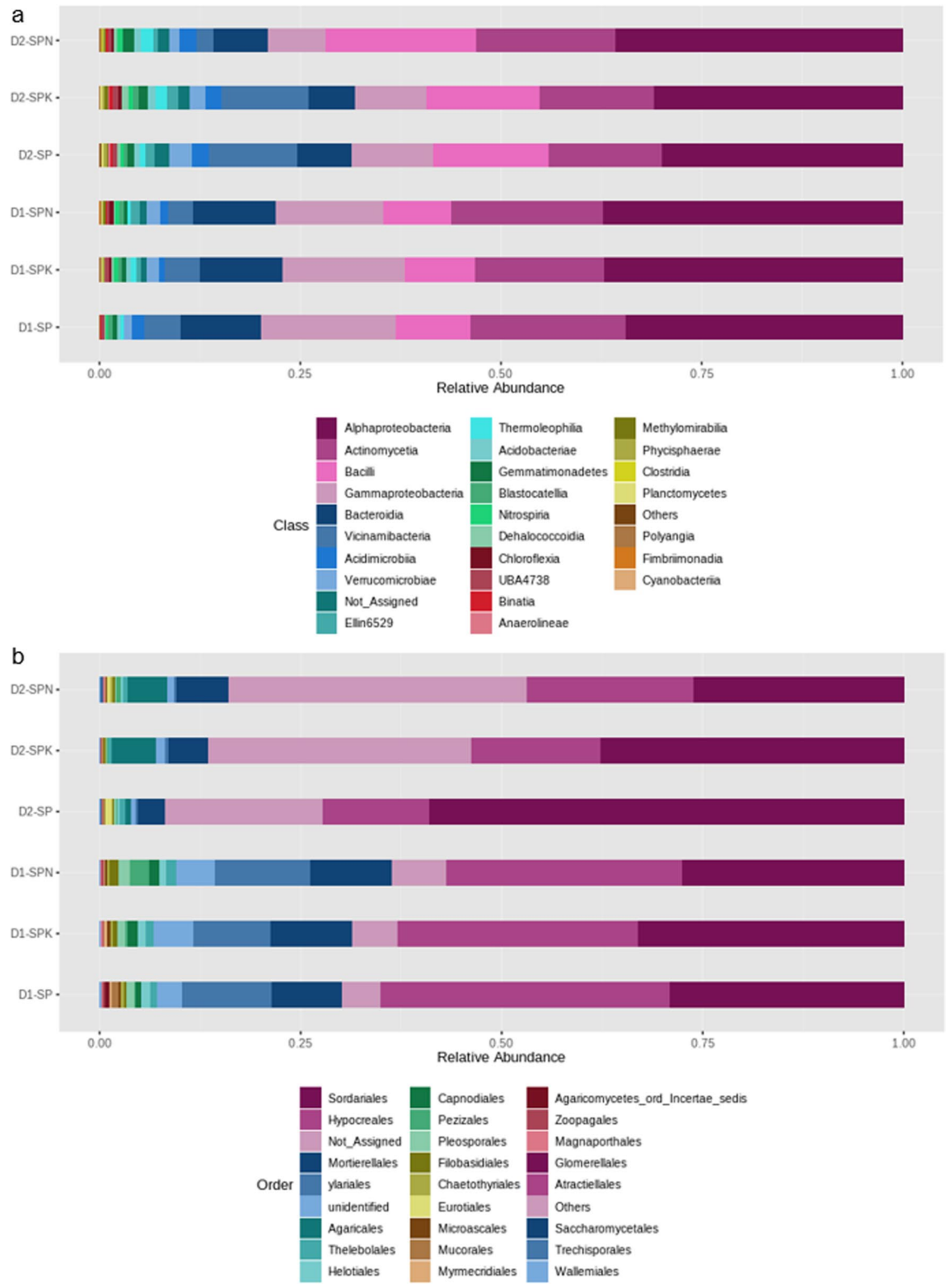


Fig. 2 Bar graphs representing the effects of the application of two soil prebiotics on soil microbial communities' relative abundance **a** bacteria at class level and **b** fungi at order level. D1, first harvesting date, three weeks after application of prebiotics; D2, second harvesting date, ten weeks after application of prebiotics; SP, plants without any treatment (control); SPN, plants treated with NUTRIGEO L®; SPK, plants treated with K1®. Each color represents a bacterial class or a fungal order. Data presented are in percentages (%)

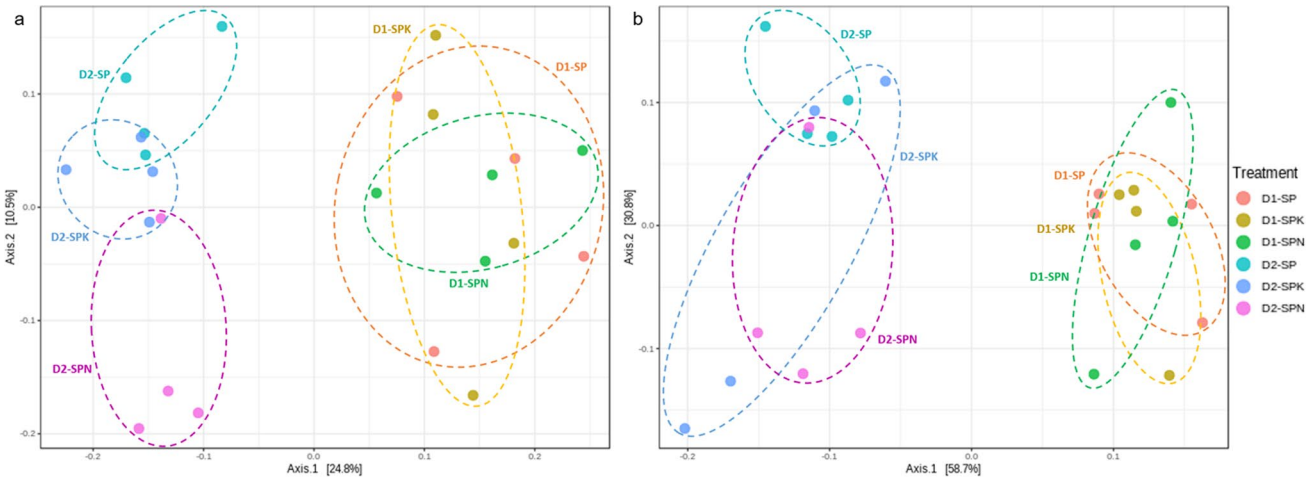


Fig. 3 PCoA plots of beta-diversity of **a** bacterial and **b** fungal soil communities in the different treatments (SP for control, SPK for NUTRIGEO L®, and SPN for K1®) at the two harvests (D1: after three weeks of prebiotics application; D2: after ten weeks of prebiotics application). The dots represent the samples from each condition (harvesting date plus treatment) where the red ones are from D1-SP, orange from D1-SPK, green from D1-SPN, cyan from D2-SP, blue from D2-SPK, and violet from D2-SPN. Samples from each condition are grouped by a 95% confidence ellipse that have the same color as the samples. PERMANOVA with a p-value < 0.001

3.5 Effect of Prebiotics on Soil Arbuscular Mycorrhizal Fungi and Maize Root Colonization

We used two methodologies to study the abundance of AMF in soil and the colonization rate of maize plant roots in response to prebiotic application. The first method involved measuring the quantity of glomalin or TGRSP at D1 and D2. Glomalin is the most cited protein among all soil proteins and is specific to AMF. Hence, the quantity of this protein in the soil is a good indicator of the biomass of the AMF present. Our results showed no significant ($p < 0.05$) change in the total glomalin content measured in SP, SPK, and SPN soils at D1, whereas at D2, both prebiotic applications led to an increase in TGRSP, with the increase being significantly ($p < 0.05$) greater in the SPN treatment (by 37.9%) than in SP (Fig. 5a).

The second method involved measuring the AMF mycorrhization rate of the plant roots at D2 through microscopic observation and then calculating the mycorrhization frequency (MF), intensity (MI), and arbuscular abundance (AA). Starting with MF, we noticed a significant ($p < 0.05$) increase in the number of mycorrhizal fragments in the two prebiotic treatments, with a greater effect in SPN (94.8%) than in SPK (89.5%), compared to SP (72.2%) (Figs. 5b and 6a–f). The same trend was observed in MI, with prebiotic application having a greater impact on mycorrhizal colonization. MI was 9.2% in SP and increased significantly ($p < 0.05$) to 24.2% in SPK and 36.1% in SPN (Figs. 5c and 6a–f). The strongest effect of prebiotics was detected on AA, where exclusively the SPN treatment significantly ($p < 0.05$) elevated the number of arbuscules by 21.7%, thus increasing symbiotic activity by more than four times compared to the control (4.9%) (Figs. 5d and 6b, c, e, f).

4 Discussion

In this study, we investigated the short- and medium-term effects of two commercial soil prebiotics: K1® (referred to as SPK), which consists of specific organic acids and trace elements, and NUTRIGEO L® (referred to as SPN), comprising a mixture of plant extracts and trace elements. These prebiotics were compared with the control group labeled SP. Our assessments focused on several aspects, including *Zea mays* L. plant growth, soil physicochemical characteristics, and microbial structure and diversity. Simultaneously, we examined the impact of applying these prebiotics on soil OM evolution, soil bacterial and fungal selection, mycorrhization rate, and their combinatorial role in enhancing C content. By examining these parameters, we aimed to gain a comprehensive understanding of the effects of these prebiotics on plant growth, soil health, and microbial communities as well as their potential contributions to C enrichment in the soil.

4.1 Prebiotics' Application Led to an Increase in Plant Growth

The definition, concept, and classification of plant biostimulants are still developing. Generally, they are described as bioproducts, distinct from fertilizers, that promote plant growth when applied in low quantities (Kauffman et al. 2007). The effects that biostimulants, particularly prebiotics, can have on plants encompass

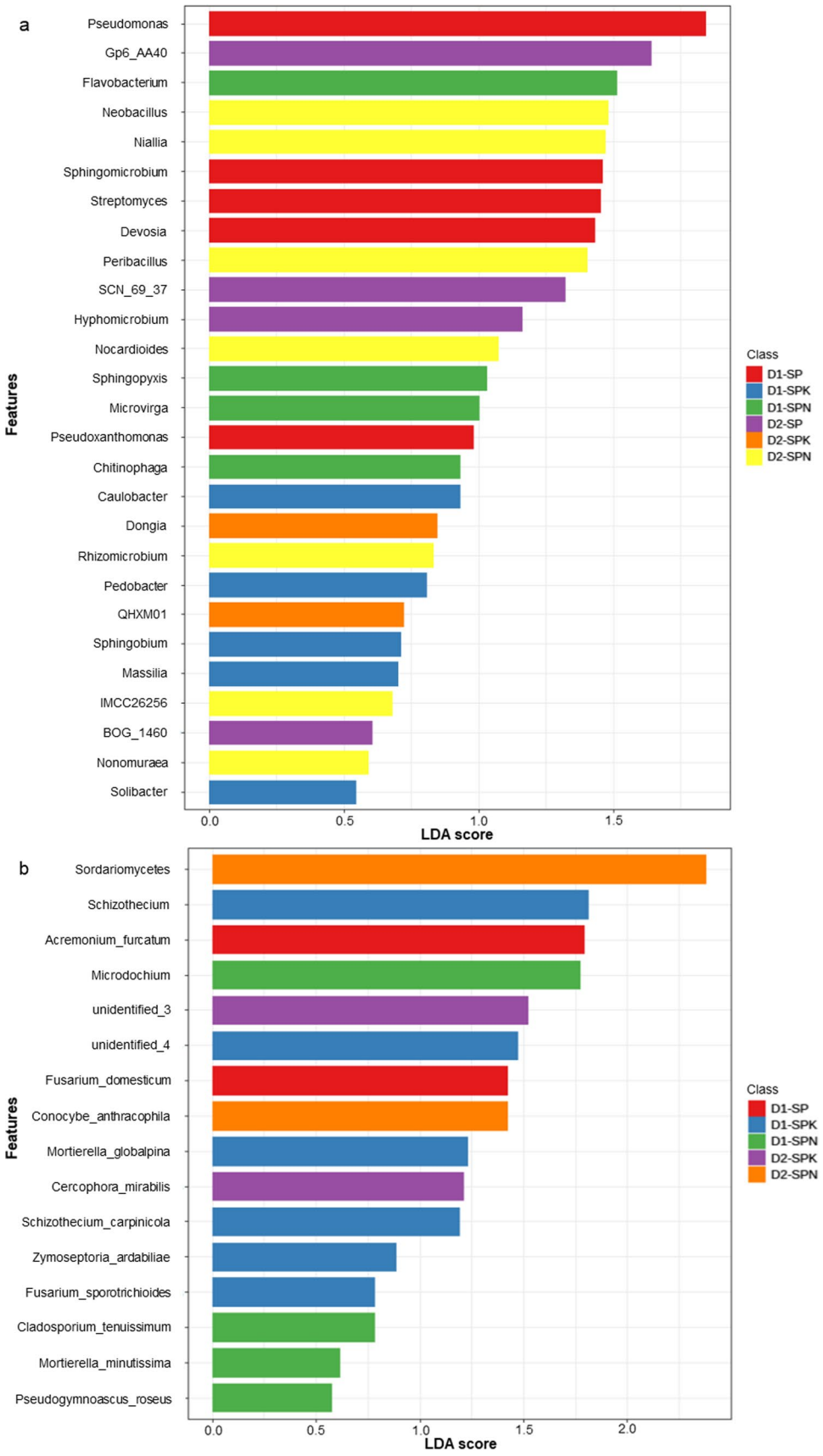


Fig. 4 Bar graph of LEfSe analysis representing the differentially abundant **a** bacterial taxa and **b** fungal taxa as biomarkers to different treatments (SP for control, SPK for K1®, and SPN for NUTRIGEO L®) at both harvesting dates (D1: after three weeks of prebiotics application; D2: after ten weeks of prebiotics application). Each feature is the name of a microbial taxa or biomarker. In class each color refers to a condition (harvest date plus treatment) where the red stands for D1-SP, blue for D1-SPK, green for D1-SPN, violet for D2-SP, orange for D2-SPK, and yellow for D2-SPN. Kruskal–Wallis rank sum test ($p < 0.1$) with LDA score > 0.5

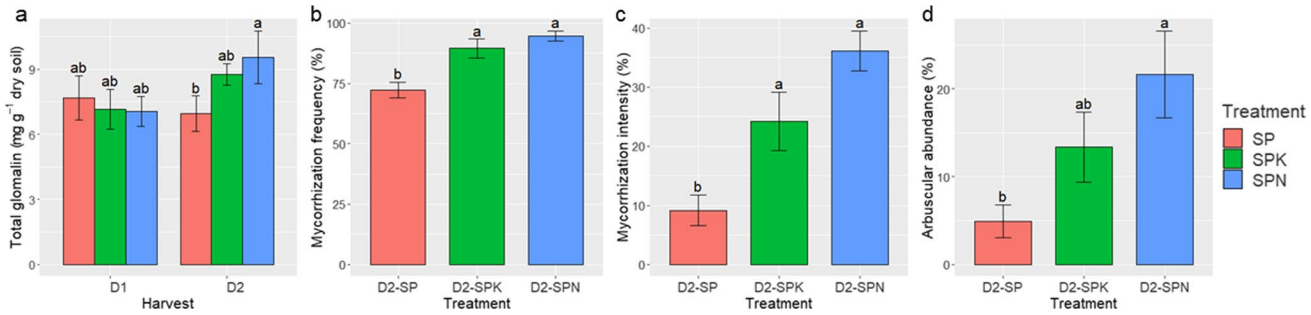


Fig. 5 Histograms representing the effects of the application of two soil prebiotics on **a** Glomalin total fractions, **b** mycorrhization intensity (MI), **e** mycorrhization frequency (MF), and **f** arbuscular abundance (AA). D1, first harvesting date, three weeks after application of prebiotics; D2, second harvesting date, ten weeks after application of prebiotics; SP, plants without any treatment (control); SPK, plants treated with K1®; SPN, plants treated with NUTRIGEO L®. Each treatment is denoted by a color where red is for SP, green for SPK, and blue for SPN. Data presented are means ± SD. Bars sharing the same letters in each graphic are not significantly different ($p < 0.05$) according to the ANOVA LSD test

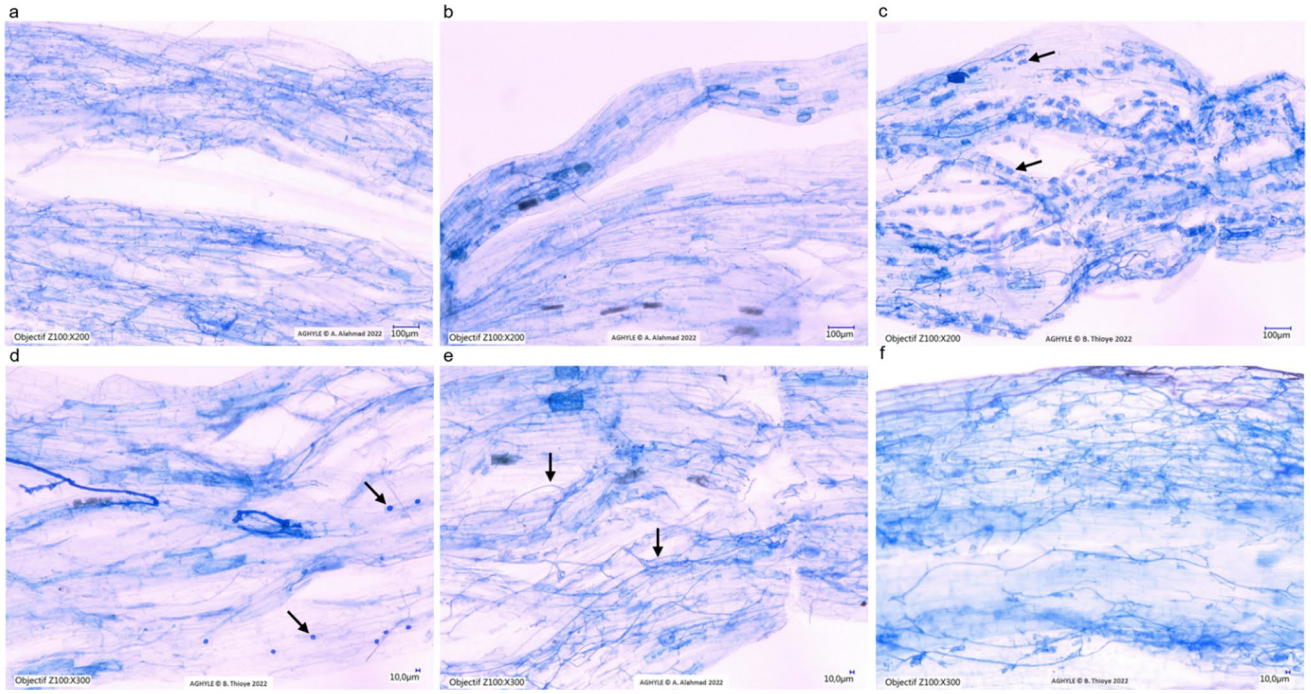


Fig. 6 Microscopic images representing the effects of two soil prebiotics after ten weeks of their application on *Zea mays* root's mycorrhizal colonization. Stained roots are shown with a Z100:X200 objective (**a**–**c**) and a greater magnification with a Z100:X300 objective (**d**–**f**). **a** and **d** display SP, plants without any treatment (control); **b** and **e** display SPK treatment, plants treated with K1®; **c** and **f** display SPN treatment, plants treated with NUTRIGEO L®. Black arrows on the images point to the different mycorrhization structures; arrows on **c**, are pointing at the exchange surface between the fungi and the root cell, the arbuscules; arrows on **d** are pointing at storage structures of the fungi, hyphal swellings known as vesicles mostly containing lipids; arrows in **e** are pointing at hyphae, fungal filaments penetrating the root cell, and forming the exchange network. Digital microscope VHX 5000 (KEYENCE, France)

various aspects of plant growth and development throughout the crop's life cycle (Calvo et al. 2014). Some of the demonstrated effects include improving the plant's metabolic efficiency, leading to increased yields and enhanced crop quality (Jardin 2015). Biostimulants also contribute to facilitating nutrient assimilation, translocation, and utilization, thus enhancing the production of quality attributes such as biomass, sugar content, color, and fruit seeding (Yakhin et al. 2017). In addition, they can optimize water use efficiency, improve the specific physicochemical properties of the soil, and promote the development of beneficial soil microorganisms (Rouphael and Colla 2020). Our findings revealed that soil prebiotic treatments improved maize plant growth and development,

specifically in the areas of shoot and root fresh weights (SFW and RFW) and dry weights (SDW and RDW), compared to the control (SP). This effect was significant ten weeks after the application of prebiotics (D2) and was more pronounced in the plant roots than in the shoots. Other researchers have made similar observations on *Zea Mays* L. growth after using humic acids or leonardite-humate and lignosulfonate-based biostimulants (Ertani et al. 2019; Monda et al. 2018). In the most recent study, the application of prebiotics increased root growth by 51–140% and, to a lesser extent, leaf growth by 5%–35%. The researchers also mentioned the enhanced photosynthesis detected by the elevated SPAD values, resembling the chlorophyll content in response to biostimulants. However, this was not seen in the effects of the prebiotics that we tested (Ertani et al. 2019). The enhancement of root growth through the application of prebiotics has been explained by their ability to mimic some hormonal functions, thereby enhancing the adventitious rooting process and, consequently, the root biomass (Kim et al. 2019). Equivalent results were obtained in a greenhouse experiment in which a vegetal-based biopolymer was applied to the melon by drenching. The use of biostimulants increased both leaves and, to a greater extent, root growth. This increase was explained by a change in the root-metabolic profile, in which brassinosteroids and their interactions with other hormones appeared to be significant (Lucini et al. 2018). Other studies failed to demonstrate the efficacy of prebiotics in open-field and controlled conditions trials. Niewiadomska and her colleagues tested the effect of two commercial biostimulants applied to white lupine for three years. During the entire growing season, the indexes of the lupine crops, biological soil fertility, and enzymatic activity were not significantly stimulated by biostimulant application as compared with the control treatments (Niewiadomska et al. 2020). Furthermore, a study conducted on butterhead lettuce treated with three different amino acids indicated that only L-methionine treatment had positively increased plant growth by 23%, whereas the other two imposed a negative impact (Khan et al. 2019). The effects of biostimulants on plant growth reported in the literature vary widely. These variations can be explained by differences in the products' extraction and production processes, the variation in active and inactive constituents within their composition, and the differences in application doses, methods, and conditions (Baltazar et al. 2021; Monda et al. 2018). Thus, further research must be conducted, delving deeper into the molecular and functional levels, specifically in terms of gene expression and the metabolomic pathways triggered or altered by biostimulants in a range of experimental systems. All affecting factors must be taken into consideration (Calvo et al. 2014).

4.2 Prebiotics' Application Enhanced Soil Physicochemical Characteristics

The effects of prebiotics' application on plant growth are attributed to their biostimulant functionality, which enhances soil properties and nutrient uptake, thus improving its ecological services and fertility (Verma et al. 2021). Our results also demonstrated this, where prebiotic application induced a distinctive and favorable impact on soil physicochemical characteristics. The soil pH did not change in response to prebiotics' application; it remained neutral, tending to be basic (≈ 8) throughout the experiment's duration. Similar results have been reported in other studies about the role of biostimulants in neutralizing and/or stabilizing soil pH, while others have indicated that biostimulants cause a decrease in pH (Hellequin et al. 2020; Ioppolo et al. 2020; Obieze et al. 2020). We also noticed that both prebiotics increased soil EC and CEC in comparison to untreated soil. Specifically, SPK had a more pronounced effect on EC, while SPN had a greater impact on CEC. This observation aligns with a study performed on *Physalis ixocarpa* treated with a nopal-based biofertilizer in conjunction with bamboo biochar and a study conducted on turfgrass treated with a rhizogenic biostimulant (Cruz-Méndez et al. 2021; Yousfi et al. 2021). Soil EC is a measure of the amount of salts in soil and is an excellent indicator of nutrient availability, soil texture, and available water capacity (Friedman 2005). On the other hand, CEC is a measure of negatively charged sites on the surface that help in holding positively charged ions and nutrients (Solly et al. 2020). This is an important indicator of the potential for fixing and storing cations in soil, thus affecting soil fertility and buffer capacity (Sharma et al. 2015). The higher the EC and CEC, the more clay and organic particles are present in the soil, and the more cations can be absorbed, released, and made available to the roots (Domingues et al. 2020; Romaneckas et al. 2023). Crus-Méndez and colleagues indicated that their combination of treatments improved soil OM and overall soil conditions (Cruz-Méndez et al. 2021). Concordant with these findings, the prebiotics and, primarily, SPN increased OC by 6.77 g C per kg of dry soil as well as OM and TC contents significantly in treated soil ten weeks after application. Taking into consideration that the C input from prebiotics was negligible (i.e., 5.56 and 9.98 µg C per kg of dry soil in SPK and SPN, respectively), this was corroborated by the indifference observed in the measured C forms between all treatments (SP, SPK, and SPN) at D1. In harmony with these observations, Ioppolo et al. (2020) also noted that drenching citrus fruit processing wastewaters on the soil during incubation for 56 days increased the total and labile C contents. Marks and his team have further elucidated the role of algal biofertilizers in improving soil quality and increasing soil C, positively affecting C sequestration (Marks et al. 2019).

Regarding N, we did not observe any difference in TN content between all treatments (SP, SPK, and SPN) at both harvesting dates; instead, we observed an overall decrease from D1 to D2 due to plant uptake. This result contradicts those stated by other researchers on the effect of biostimulants in increasing N fixation and N content in the soil. However, these studies were conducted on bare soil, where no consumption of N is taking place, and soil cultivated with leguminous plants, which play a significant role in the N cycle (Gou et al. 2023; Marks et al. 2019; Niewiadomska et al. 2020). Alternatively, a decrease in nitrates, a major nutrient for plant growth, has been noticed in treated soil at D2 compared to the control. In a detailed study on bare and planted soils to investigate the biostimulant action of dissolved humic substances (DHS), researchers discovered that DHS enhanced NO_3^- uptake rates in maize roots and modulated several genes involved in N acquisition, including the upregulation of NO_3^- reductase (Vujinović et al. 2019). These changes were accelerated in planted soil, indicating that the effects of biostimulants on soil N are highly dependent on plant–soil interactions and biostimulant types and properties (Hellequin et al. 2020; Sawaguchi et al. 2015; Vujinović et al. 2019). In our case, soil prebiotics are not a source of N in soil but rather play a role in the N cycle and uptake efficiency. Indeed, soil prebiotics have increased maize plant growth, elevating N uptake (Matos et al. 2020; Fiorentino et al. 2018). In conjunction with the above-mentioned effects, the utilization of biostimulants was seen to enhance nutrient solubility and availability in soil and facilitate their uptake by the plant, thus increasing their use efficiency (Castellano-Hinojosa et al. 2021; Leoni et al. 2019; Rouphael and Colla 2020). This was reflected in our results with an increase in the soil's nutrient content, such as available P (at D2 by SPK and SPN), Ca (at D2 by SPN followed by SPK), Na (at D1 by SPK followed by SPN), and SO_4^{2-} (in SPN) in addition to trace elements such as Zn (at D1 by SPK and SPN), B (at D1 and D2 by SPN), and Fe and Cu (at D2 by SPN). B was imported into the soil because it is a component of the SPN solution. The rise of the other minerals, such as Ca, especially at D2, suggests that they are not part of the prebiotic's composition. This rise may be due either to the direct effect of prebiotics on soil physicochemical characteristics and/or to soil microbial activity, both of which impact mineral solubility and bioavailability in soil (Chen et al. 2020; Cruz-Méndez et al. 2021; Wu et al. 2005; Yousfi et al. 2021). In fact, metal oxides are considered primary OM-stabilizing soil constituents, and they play a role with Ca in the C cycle by ternary complex formation (Sowers et al. 2018). In addition, Ca promotes humic acid sorption to Fe minerals. Therefore, a positive correlation is observed between exchangeable Ca in soil, SOC, and CEC due to their simple co-occurrence (Trivedi et al. 2018). This is complementary to our findings considering the effects of biostimulants application, especially SPN, in elevating these soil characteristics. This physicochemical hypothesis must be explored further to better understand its mechanisms and strengthen its argument, particularly in terms of soil microbial community diversity and functionality.

4.3 Prebiotics' Application Shaped the Soil's Bacterial and Fungal Communities and Recruited Specific Endogenous Microorganisms with Different Ecological Services

Biostimulants could play a role in soil revitalization and the simultaneous enhancement of root exudation and OC mineralization of crop residues and microbial biomass. These processes were linked to changes in soil microbial communities (Backer et al. 2018; Hellequin et al. 2020). Specific members of these communities, especially those from bacteria and fungi (but not from archaea), were recruited after the addition of biostimulants. These selected microorganisms had a positive role in plant residue degradation, soil C stability and storage, nutrient availability, plant-microbial interactions, symbiotic associations, and plant viability (Castiglione et al. 2021; Diacono and Montemurro 2010; Palla et al. 2022; Powlson et al. 2016). We witnessed modifications imposed by prebiotic application on soil microbial community structure and diversity. As a short-term effect (D1), SPN and SPK treatments increased the bacterial alpha diversity, specifically the richness, with no tangible variations detected in soil microbial communities' structure (relative abundance and beta-diversity) for both bacteria and fungi. This is consistent with other studies' results (Hellequin et al. 2020; Wang et al. 2022). At D2, the difference in the microbial community beta-diversities between the treated and untreated soil became clear. This deviation was more pronounced between SPN and SP than between SPK and SP. Nevertheless, prebiotics began to recruit specific members of these communities in a short-term application effect and continued this process in the medium term, as shown by another study (Castiglione et al. 2021). These indigenous bacterial and fungal taxa or biomarkers, selected differently according to the prebiotic characteristics (compositional and functional), play a key role in defining these biostimulants' identity, mode of action, and efficacy (Sible et al. 2021). They provide a logical explanation of these prebiotics' effects on soil ecology, services, and fertility, especially C storage, and their repercussions on plant growth and development (Rouphael et al. 2018). Starting with the bacterial community, SPN treatment induced the proliferation of *Alphaproteobacteria*, *Actinobacteriota*, *Firmicutes*, and *Bacteriodota*, to the advantage of *Acidobacteriota* and other low-abundant phyla. From these induced phyla, SPN selected specific taxa, such as *Flavobacterium*, *Neobacillus*, *Niallia*, *Nocardiodes*, and *Nonomurea*, which

act as plant growth-promoting rhizobacteria (PGPR) (Gupta et al. 2020; Kolton et al. 2016; Pérez-Jaramillo et al. 2017; Sungthong and Nakaew 2015; Tang et al. 2023). They are able to secrete carbohydrates, active enzymes, phytohormones, and secondary metabolites, and they can degrade complex organic compounds (such as chitin, lignocellulosic material, and proteins) as well as fungal cell walls. These phyla enhance N and P cycles, induce biofilm formation, and promote maize root colonization (Kraut-Cohen et al. 2021; Lazcano et al. 2021; Liu and Poobathy 2021; Saxena et al. 2020; Wolińska et al. 2019). Consequently, these biomarker OTUs improve nutrient cycling in soil and their acquisition by plants, along with C stabilization and storage and plant growth. Therefore, they are considered biofertilizers and indicators of healthy soil (Liu et al. 2019; Saxena et al. 2020; Wolińska et al. 2020). Other SPN-selected bacteria, such as *Microvirga* and *Rhizomicrobium*, act as microsymbionts and improve Fe reduction, root colonization, microbial interaction, and rhizomicrobial activity, which have been proven to increase maize growth (Ma et al. 2017; Msaddak et al. 2017; Nie et al. 2018; Xue et al. 2017). Interestingly, the biomarker genus *Chitinophaga* was noteworthy because, in addition to its similar role as *Neobacillus* in chitin degradation and the deconstruction of dead fungal material, they are considered endosymbionts or endohyphal bacteria (EHB) that increase fungal growth and plant–fungal interactions (McKee et al. 2019; Shaffer et al. 2017). SPK treatment, on its part, increased the relative abundance of *Acidobacteriota*, *Actinobacteriota*, and *Chloroflexota*, but its biomarker taxa were not chosen from them except for one belonging to *Acidobacteriota*. Almost all SPK-selected bacterial taxa (*Caulobacter*, *Pedobacter*, *Shingobium*, *Massilia*, *Solibacter*, and *Dongia*) are PGPR and degrade complex organic compounds (such as cellulose, chitin, and lignin) and play a role in N, P, and C cycles (Boss et al. 2022; Palla et al. 2022; Wilhelm 2018; Wu et al. 2016; Yang et al. 2021; Zheng et al. 2017). Meanwhile, some of them possess unique, interesting functions that are correlated with our experimental system and outcomes. Such as *Caulobacter*, considered a hub species that produce exopolysaccharides (EPS), and *Pedobacter*, known to produce indole-3-acetic acid, both are known to increase maize growth (Luo et al. 2019; Tan et al. 2020). In addition, *Solibacter* was observed to proliferate in response to straw addition, degrading vast types of C sources (Yu et al. 2019). Proceeding to the fungal community, both prebiotic treatments (SPK and SPN) increased the abundance of *Mortierellomycota*, *Basidiomycota*, the unidentified group, and the rest of the phyla in place of predominant *Ascomycota*, from which most of the selected fungal taxa descend. We noticed that several biomarker taxa were pathogenic fungi and present in all conditions, such as *Acremonium furcatum* and *Fusarium domesticum* in SP, *Zymoseptoria ardabiliae* and *Fusarium sporotrichioides* in SPK, and *Microdochium* and *Cladosporium tenuissimum* in SPN (Bashir et al. 2014; Köhl et al. 2007; Perincherry et al. 2019; Stukenbrock et al. 2012; Summerbell et al. 2011; Zhou et al. 2022). The rest of the prebiotics' selected fungal species were beneficial microorganisms for soil and plants. The SPK treatment biomarkers were *Cercophora mirabilis*, *Schizothecium carpinicola*, and *Mortierella globalpina*, and they are all known as saprotrophic, symbiotic, endophytic, and plant growth-promoting fungi (PGPF) (DiLegge et al. 2019; Lv et al. 2022; Tayyab et al. 2019; Zhang et al. 2021). *Mortierella* species stand out with their status as valuable decomposers and their potential to improve the bioavailability of P and Fe in soil, increase nutrient uptake efficiency, influence soil microbiota (fungal–endobacterial symbiosis) by supporting the performance of beneficial microorganisms, significantly enhancing crop yield, and synthesizing phytohormones with a biocontrol effect against plant pathogens, hence associated with healthy soils (Büttner et al. 2021; DiLegge et al. 2019; Ozimek and Hanaka 2021). In parallel, *Mortierella minutissima*, a species from the same healthy soil bioindicator genus, was also detected in the SPN biomarker taxa, implementing all of the aforementioned vital effects on the soil-plant quantum (Ozimek and Hanaka 2021; Trytek and Fiedurek 2005). SPN treatment has also selected other fungal taxa, such as *Conocybe anthracophila*, *Pseudogymnoascus roseus*, and *Sordariomycets*, which are considered saprotrophic decomposers (Lee et al. 2019; Rosa et al. 2020; Sengul Demirak et al. 2021). In addition, the last two SPN biomarkers were proved to be symbionts (endophytes and epiphytes), fungicolous, able to form ericoid mycorrhizal associations, actively consume root exudates, and their surge was correlated with the increase in AMF (Clocchiatti et al. 2021; Lee et al. 2019; Minnis and Lindner 2013).

4.4 Prebiotics' Application Increased the Mycorrhization Rate of Maize Roots by Arbuscular Mycorrhizal Fungi

Results on microbial community dynamics, favoring plant-fungal symbiosis after prebiotics' application, were consistent and complementary with those we found regarding these products' effects on plant mycorrhization. Other studies have also indicated these findings (Basile et al. 2020; Caser et al. 2019; Jardin 2015).The prebiotic application with a greater impact of SPN over SPK showed an increase in the quantity of proteins in the soil TGRSP, or total glomalin (Koide and Peoples 2013). TGRSP has been shown to be produced by AMF and is implemented in OM pools and C storage in soil. It plays an important role in maintaining soil structure and fertility, thus considered a biological indicator of soil quality (Fokom et al. 2012; Gałązka and Grządziel 2018; Vasconcellos et al. 2016). Concomitantly, the SPN treatment followed by SPK notably elevated the mycorrhization rate

of maize roots by AMF, reflected by the increase in MI and MF and the multiplication by four times AA in comparison to SP. A large body of scientific literature shows that favoring the presence and activity of soil fungi, especially AMF, improves plant nutrient acquisition or bioassimilation (N, P, and K), leading to an increase in root biomass, plant growth, and yield (Amaranthus and Jiracek 2001; Amatussi et al. 2020; Buragohain et al. 2017; Wang et al. 2015). At the same time, soil fungi have a fundamental role in soil stability and C sequestration, explained by three mechanisms: (i) the improvement of C-use efficiency (CUE), (ii) molecules composed by fungi or produced by fungi that are more resistant to degradation (such as melanin, chitin, and glomalin), and (iii) the aggregating function of hyphae and glomalin (Fokom et al. 2012; Lehmann et al. 2020; Rillig 2004; Six et al. 2006). Thus, biostimulants play a role in microbial regulation in agroecosystems and soil restoration by targeting root microbial communities, particularly fungi and their mycorrhizal association (Calvo et al. 2014; Tejada et al. 2011; Tekaya et al. 2021). This modulation has been considered one of the strategies applied to elevate the TOC stored in the soil, which is used to measure C sequestration (Wilson et al. 2009; Zhu et al. 2022).

4.5 The Overall Positive Effect of Prebiotics' Application, Specifically NUTRIGEO L®, on Soil Carbon Storage

From the overall effects of prebiotics' application, we deduce that they not only increased plant growth through the enhancement of soil physicochemical characteristics and its microbial community structure and diversity but also led to the improvement of soil fertility, stability, and C storage (Debska et al. 2022). We observed that the intensity of the effects of SPK and SPN on plant growth, C dynamics, and the incorporation of fresh OM in the soil differ compared to the control. SPN shows larger magnitudes of changes compared to the other treatments. Pointedly, NUTRIGEO L®'s application first increased EC and CEC, and thus the prevalence of divalent cations such as Ca and Fe. This increase in positively charged elements may affect soil with a pH $\geq$ 7, altering the environmental cycling of C via ternary complex formation, which increases OC sorption concentration (Sowers et al. 2018). Second, this prebiotic treatment modulated the soil microbial community toward the degradation of recalcitrant OM sources, in our case, organic wheat straws added to the soil, recruiting specific bacterial and fungal taxa that can decompose lignin, chitin, and cellulose instead of labile C (Hellequin et al. 2018; Rezgui et al. 2021). Simultaneously, these microorganisms play a vital role in microbial interactions and rhizomicrobial activity amplification, enhancing fungal-bacterial symbiosis on one hand and root mycorrhization on the other. This effect, which specifically addressed the soil fungal community, particularly the saprophytes, and AMF, has positive repercussions on C stability and storage (Nottingham et al. 2013). These hypotheses were justified by the significant elevation of OC, OM, and TC contents in the SPN-treated soil ten weeks after the prebiotics' application. Moreover, the soil particle size fractionation results confirm the SPN treatment's effect in increasing the amount of the F.200 fraction and its C content, consisting of plant residues associated with microbial compounds with a rapid turnover of two to three years (Ioppolo et al. 2020). The increase in this OM fraction is considered the first and essential step in the pathway of increasing C stability and storage in soil, a time-dependent process that is gravely impacted by agricultural practices (Dou et al. 2016; Piccolo et al. 2019; Tivet et al. 2013). Several studies have reported no discernible effect on soil properties, particularly OM content and C storage, following the utilization of biostimulants (Mueller and Kussow 2005; Wadduwage et al. 2023). This lack of impact can be primarily attributed to the substantial variations in biostimulant types, compositions, and applications, alongside the inherent diversity of soil characteristics (Ricci et al. 2019). Moreover, disparities in experimental criteria across studies, including the presence or absence of plants, plant types, growth conditions, harvesting dates, and other relevant factors, may have further contributed to the divergent outcomes observed (Grandy et al. 2009; Sawaguchi et al. 2015; Vujinović et al. 2019).

5 Conclusion

In view of global environmental and health concerns, the imperative to reduce chemical inputs in agriculture is opening up new prospects for the utilization of biostimulants. The effect of biostimulants on plant growth has been demonstrated in several studies. In our research, we elucidated the modes of action of two tested prebiotics, especially their impact on microbial communities and soil properties. These prebiotics, K1® and NUTRIGEO L® (SPK and SPN), when combined with organic wheat straws, demonstrated a positive influence on maize growth and soil fertility by modulating native microbial communities. Each prebiotic recruited a distinct consortium of microorganisms, defining its unique modes of action. For instance, SPK attracted bacteria and fungi that promoted plant growth and nutrient cycling, while SPN selected other microbial taxa that enhanced nutrient uptake and mycorrhizal associations. Furthermore, SPN increased the availability of essential elements, cation exchange capacity, and carbon content, contributing to carbon sequestration. This pioneering study underscores the short- and medium-term effects of prebiotics on soil fertility, microbial populations, and their subsequent influence on plant development and carbon storage. Future research should delve deeper into comprehending the mechanisms of action of these prebiotics, exploring their

specific components and interactions. These findings offer promising solutions and unlock new perspectives in sustainable agriculture, addressing pressing challenges related to soil degradation, population growth, and climate change.

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Declarations

Competing Interests The author, Aude Bernardon-Mery, is an employee of the company Gaïago SAS. There are no financial interests to consider except that this paper could lead to an increase in the sales of the products. If the company's requirements do not align with the ethics and rigor of research and scientific fundamentals, the laboratory has reserved the right to terminate the contract immediately. Apart from what has been stated above, the authors have no relevant financial or non-financial interests to disclose.

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