Received: 26 July 2021 | Revised: 24 November 2021 | Accepted: 18 December 2021

DOI: 10.1002/ldr.4179

RESEARCH ARTICLE

WILEY

Composition, activity and diversity of bacterial and fungal communities responses to inputs of phosphorus fertilizer enriched with beneficial microbes in degraded Brunic Arenosol

Mateusz Mącik[1] | Agata Gryta[1] | Lidia Sas-Paszt[2] | Magdalena Frąc[1]

[1]Institute of Agrophysics, Polish Academy of Sciences, Lublin, Poland

[2]Institute of Horticulture in Skierniewice, Skierniewice, Poland

Correspondence
Magdalena Frąc, Institute of Agrophysics, Polish Academy of Sciences, Doświadczalna 4, 20-290 Lublin, Poland.
Email: m.frac@ipan.lublin.pl

Abstract

Anthropogenic-induced deterioration of soil health remains a global problem, resulting in a diminished productivity of agroecosystems. In order to improve soil quality, we investigated the impact of phosphorus biofertilizer on the microbiological parameters of soil (type Brunic Arenosol) degraded as a result of inappropriate cultivation and fertilization, characterized by low pH and decrease in K and Mg content. Two-year field experiment included control treatment (FC) without microbial enrichment and FA100 (fertilizer amendment at optimal dose) and FA60 (fertilizer amendment at 40% reduced dose) treatments containing beneficial microorganisms. The results showed that the biofertilizer enhanced soil enzymatic activity (as expressed by the increased activities of urease, protease, acid phosphomonoesterase, and β-glucosidase), increased number of operational taxonomic units associated with metabolic processes (including phosphorus related pathways and degradation of xenobiotics), improved crop yield, increased bacterial diversity, and changed the quantity of phytoavailable phosphorus in the soil. Biofertilizer also stimulated the occurrence of plant growth promoting microorganisms involved in phosphorus biotransformations, decomposition of organic matter, nitrogen fixation, and protection plants against pathogens. Next generation sequencing (NGS) and Biolog analyses showed that the composition of soil microbiome was affected also by soil sampling time, suggesting seasonal variations in the preferred carbon sources and level of C-substrates utilization, and pointing the differences in the relative abundance of individual microbial groups at particular stages of the experiment. Taking into consideration the improvement of microbiological indicators of soil health, phosphorus biofertilizer seems to be effective approach to implement in sustainable agriculture linking soil microbiome quality with the general soil condition.

KEYWORDS
biofertilizer, degraded soil, phosphorus biotransformations, soil health, soil microbiome

1 | INTRODUCTION

It cannot be denied that the degradation of arable soils is an important issue influencing not only the quality and quantity of crop yields but also the functioning of whole agroecosystems (Gomiero, 2016). The intensive and unsustainable soil cultivation leads to severe alterations in its structure including a depletion of mineral components, acidification, accumulation of heavy metals or loss of organic matter (Lin

et al., 2019). These changes result in the gradual degradation of arable soils and decrease their production potential (Geng et al., 2019). Considering the application of mineral fertilizers, it should be considered that their excessive amounts remain not indifferent to a key element of soil environment architecture, namely complex communities of bacteria, archaea, and fungi (Geisseler & Scow, 2014). As described earlier, soil microorganisms play the foremost role in the basic biochemical transformations occurring below ground level and therefore their role in maintaining soil balance is invaluable (Hellequin et al., 2020; Lacerda-Júnior et al., 2019). Regarding the importance of soil microorganisms, the deterioration of soil health may be evidenced by microbiological indicators encompassing reduction of microorganisms activity (reflected by low soil enzymatic activity and poor efficiency of metabolic processes) and simplification of community structure (expressed through a small number of different taxa and low biodiversity among microorganisms performing specific roles) (Lee et al., 2020; Nunes et al., 2012; Zhang et al., 2017). It goes without saying that phosphorus is a key macronutrient which availability determines plant growth and development and, consequently, crop productivity. P is involved in enzymatic and metabolic processes (such as photosynthesis and cell division), formation of phospholipids and constitute the vital element of nucleic acids. Unfortunately, the majority of soil P (∼95%) is unavailable for plant uptake and, in addition, prolonged or inappropriate application of phosphorus mineral fertilizers may result in limitation of indigenous P (Hallama et al., 2021; Siedliska et al., 2021). With regard to the disadvantages of mineral fertilizers, the use of beneficial microorganisms capable of converting insoluble P-compounds into ortho-phosphates which can be uptaken by plants, seems to be an innovative and ecofriendly alternative or supplementation (Hallama et al., 2021; Kour et al., 2020). Unlike synthetic fertilizers, microbial based preparations do not pose a threat to the natural environment and their application remains in agreement with sustainable and organic agriculture principles which involve the limitation or complete exclusion of mineral fertilizers and artificial plant protection agents (Bhardwaj et al., 2014; Mącik, Gryta, & Frąc, 2020). It was reported that application of biofertilizers based on P-solubilizing microorganisms provides benefits including increase highly assimilable P compounds content (Alori et al., 2017), improve plant (e.g. maize) growth parameters (Zhao et al., 2014), enhance acid phosphatase activity (Heidari et al., 2019), boost nutrient (N, P, K, Mg, Fe) uptake (Chen et al., 2021), suppress phytopathogens (Mitra et al., 2020), and increase bacterial richness and diversity in the rhizosphere (Wang, Liu, et al., 2021). The phosphorus biofertilizer used in this study was enriched with the following beneficial bacterial strains: *Paenibacillus polymyxa* (CHT114AB), *Bacillus amyloliquefaciens* (AF75BB), and *Bacillus* sp. (CZP4/4). The aforementioned strains were selected with respect to their plant growth promoting properties. As described earlier, *Paenibacillus polymyxa* strains were found to suppress plant pathogens, for example, *Rhizooctonia solani*, *Fusarium oxysporum*, and *Botrytis cinerea* due to synthesis of antifungal metabolites such as fusaricidin. What is more, representatives of *P. polymyxa* carry phn genes, a gene cluster encoding proteins involved in bacterial conversion of phosphonates to phosphorus forms readily assimilated by plants (Li et al., 2020). The P solubilization properties were also reported among *Bacillus* spp. strains, which produce organic acids and phosphatases converting inorganic P-compounds into absorbable forms. Apart from increasing P uptake, *Bacillus* spp. also synthesize plant hormones (IAA, cytokinins) and siderophores chelating and reducing Fe^{3+} to Fe^{2+} ions (Radhakrishnan et al., 2017). Meanwhile, the microbial biofertilizer containing *Bacillus amyloliquefaciens* in combination with different P-based fertilizers enhanced the P uptake in maize leaves (Vinci et al., 2018).

Shifts in condition of the soil microorganisms under the influence of various substances or treatments are the subject of large-scale research since many years, including both short- and long-term studies. A result obtained from short-term study (from September 2018 to June 2019) showed that soil inoculated with microbial inoculant containing *B. subtilis* and *P. polymyxa* exhibited increased relative abundance of Acidobacteria, Actinobacteria and Chloroflexi, with a simultaneous decrease in Bacteroidetes, as compared to non-inoculated control. In the same study, the Chao1 index of bacterial diversity was significantly higher in soil amended with *B. subtilis* and *B. cereus* than in control soil (Chen et al., 2021). In long term study (2012–2016) it was reported that inoculation with *Burkholderia cepacia* ISOP5 increased the relative abundance of genes involved in P-solubilization and mineralization such as phoN (acid phosphatase), phnA (phosphonoacetate hydrolase), and phnFGHIJKLMNOP (the C-P lyase subunit). What is more, the increase in the relative abundance of genes associated with N metabolism was also observed in soil inoculated with the abovementioned strain (Wang, Peng, et al., 2021).

Owing to the fact that soil microbial communities are sensitive to environmental fluctuations, they may display seasonal responses depending on temperature, moisture, and indigenous microorganisms properties. Seasonal variations in the status of soil microbiome are also associated with the availability of carbon sources (Koranda et al., 2013). During growing season, soil microorganisms may demonstrate increased activity due to plant root exudates containing highly assimilable carbon sources such as carbohydrates and amino acids. On the other hand, low temperatures may result in reduced microbial activity leading to an accumulation of soil organic matter (SOM) (Badri & Vivanco, 2009; Canarini et al., 2019; Xu et al., 2021). Higher SOM content may then stimulate the fungal decomposition activity (Rousk & Bååth, 2007). The seasonal response is thought to be predictable as a result of the environmental signals preceding these changes, however responses may differ among ecosystems. On the other hand, the influence of various agrotechnical practices may interfere with the regularity of microbial responses arising from the succession of natural factors including temperature fluctuations and day/night length (Bleuven & Landry, 2016; Jia et al., 2020). Tracking seasonal changes in the status of soil microbiome is essential in order to estimate the stability of agroecosystems in the face of external factors such as fertilization. It is of particular importance for new farming techniques that were not formerly used (Lacerda-Júnior et al., 2019).

The contemporary concept of agriculture, ensuring high soil quality and fertility, is increasingly driven by the interrelationship of agroecosystems with soil microbial diversity. Maintaining a high

biodiversity among soil microbial communities, which is intended to stimulate crop resistance to pathogens, enhance nutrient uptake and improve microbiological indicators of soil health, is a promising strategy for sustainable soil management (Bertola et al., 2021; Hartman et al., 2018). This is particularly important for degraded soils owing to the fact that successful soil restoration is inextricably linked with the soil microorganisms welfare (Zhang et al., 2020). Even though soil fertilization with different organic amendments is widely described by researchers, the approach considering exploitation of traditional mineral fertilizers enriched with strains of beneficial microorganisms is still poorly recognized. Therefore, this study aimed determine the seasonal effects of innovative phosphorus mineral fertilizer enriched with strains of beneficial bacteria (*Paenibacillus polymyxa*—CHT114AB, *Bacillus amyloliquefaciens*—AF75BB, and *Bacillus* sp.—CZP4/4) on the biodiversity of soil microbiome and enzymatic activity of chemically degraded (very low content of K, Mg, and pH value) soil under maize cultivation. Because of an essential task of agricultural systems is to improve P recycling in the environment, for example, by decreasing the doses of phosphorus mineral fertilizers applied to the soil and incorporation of microbes that will be able to solubilize internal phosphorus present in the environment, we mainly wanted to evaluate the influence of tested treatments on soil mycobiome and microbiome as relevant soil health and quality indicators. Moreover, these types of results are missing or present as very fragmentary in literature, therefore the tested approach was innovative, especially for degraded soil. We hypothesize that the application of phosphorus biofertilizer will improve microbiological indicators of soil degradation and thus soil quality. Consequently, we assume that the obtained results will provide guidelines for sustainable soil management, based on the status of soil microbiome, and the phosphorus biofertilizer will find practical implementation in modern agriculture.

2 | MATERIALS AND METHODS

2.1 | Field experiment

The study was conducted over 2 years under field experiment conditions in 2018–2019, on agricultural land in Biszcza, Southeast Poland (50°43′N, 22°60′E). The study was located at the altitude of 211 m above sea level. The soil, classified as a Brunic Arenosol (BA), was degraded due to inadequate fertilization and cultivation, with the following physicochemical parameters: pH_{KCl} 4.8 and content of P_20_5, K_20, and Mg of 17.4 mg 100 g^{-1} (high), 2.9 mg 100 g^{-1} (very low), 1.2 mg 100 g^{-1} (very low), respectively. The additional physicochemical soil properties were investigated and described in previous studies (Boguta et al., 2021; Pertile et al., 2021; Walkiewicz et al., 2020).

The field experiment was conducted according to the method of Mącik, Gryta, Sas-Paszt, & Frąc (2020). Soil was fertilized with the phosphate mineral fertilizer SUPER FOS DAR 40 (Grupa Azoty, Puławy, Poland), nitrogen fertilizer PULREA PUŁAWSKI MOCZNIK 46N (Grupa Azoty, Puławy, Poland) and granulated potassium salt (BIALCHEM, Poland). The experiment comprised of the following treatments: FC – optimal dose of fertilizer (control treatment), FA100—optimal dose of fertilizer enriched with microorganisms and FA60–40% reduced dose of fertilizer enriched with microorganisms. The field experiment was conducted under maize cultivation (variety of P9241, FAO: K280, Z270, PIONEER).

Phosphorus mineral fertilizer was enriched with the following bacterial strains: *Paenibacillus polymyxa* (CHT114AB), *Bacillus amyloliquefaciens* (AF75BB), and *Bacillus* sp. (CZP4/4), provided by the Research Institute of Horticulture in Skierniewice, Poland. The biofertilizers were prepared according to Borowik et al. (2019), namely granules of fertilizer were coated with the mixture containing 1:1:1 each aforementioned strain. The ready-to-use biofertilizers were produced and provided by the Łukasiewicz Research Network—New Chemical Syntheses Institute (Puławy, Poland).

Each fertilization treatment included three replications plots (10 × 15 m). The soil samples were taken in autumn 2018 (A18), summer 2019 (S19), and autumn 2019 (A19) at a depth of 0–25 cm from five random sites within each plot. Afterward, samples were delivered to the laboratory and passed through a 2 mm sieve in order to get rid of impurities such as stones or plant roots. The purified soil samples were then immediately used for measurements or stored (at 4°C for Biolog and enzymatic analyses or −80°C for DNA extraction).

2.2 | Weather conditions

Weather conditions were recorded using the meteorological station in Zamość (Poland) (50°70′N, 23°25′E), located ∼50 km of the experimental site. Total rainfall in 2018 and 2019 accounted for 439.14 and 525.47 mm, respectively, while average annual air temperature were 9.3 and 9.8°C, respectively. The highest monthly rainfall during the 2-year experiment was reported in July 2018 and May 2019. Considering the growing season (April–July), the average temperature during this period was 16.8°C and 15.4°C in 2018 and 2019, respectively. Analyzing weather conditions in soil sampling months (October 2018, June 2019, and October 2019) it was reported that monthly rainfall remained at similar level and accounted for, respectively, 28.97, 23.62, and 26.4 mm. The highest temperature was observed in June 2019 (20.9°C), whereas in October 2018 and October 2019 accounted for 9.7°C and 10.8°C, respectively (Figure S1). The weather conditions were obtained through online climate database tutiempo.net (Tutiempo, 2021).

2.3 | Soil enzymatic activity, assimilated phosphorus content, and maize yield

Protease activity was assessed using the Tris–HCl (pH 8.1) sodium caseinate as a substrate and determining the release of tyrosine after incubation for 1 hr at 50°C. The concentration of tyrosine was measured calorimetrically at a wavelength of 578 nm (Ladd & Butler, 1972 with modification of Alef & Nannipieri, 1995). For urease analysis, urea was used and after 18 hr incubation at 37°C, the concentration

of ammonia was measured at 410 nm (Zantua & Bremner, 1977). Acid phosphomonoesterase activity was determined by incubating (for 1 hr at 37°C) the soil samples with p-nitrophenyl phosphate and evaluating the released p-nitrophenol (PNP) spectrophotometrically at 400 nm (Tabatabai & Bremner, 1969). β-glucosidase activity (as determined by the PNP concentration (at 400 nm) after incubation with p-nitrophenol glucoside (PNG) for 1 hr at 37°C) was assessed according to the methods of Eivazi & Tabatabai (1988) with a modification developed by Alef & Nannipieri (1995). The enzymes activities were calculated based on the dry (105°C) weight of the soil. The assimilated phosphorus content (P_2O_5) was determined by the Egner-Riehm method according to Polish Standard PN-R-04023 (1996), using a Sherwood flame photometer, Genesys 6 spectrophotometer. After maize harvest at full maturity, its yield was assessed by weighing together all plants (including grains and straw) from each plot in particular treatments.

2.4 | Metabolic potential of soil microbial communities

The metabolic potential of soil bacterial and fungal communities was determined with the application of Biolog system using ECOplates and FFplates (Biolog Inc., Hayward, CA). The suspension containing 1 g of fresh soil and 99 ml of sterile saline peptone water was shaken for 20 min at 20°C and incubated for 30 min at 4°C (Gryta et al., 2014). Afterward, ECOplates and FFplates wells were inoculated with soil microorganisms suspension with the amount of 120 and 100 μl, respectively. After the inoculation, during 216 hr incubation period at 23°C, absorbance readings were taken every 24 hr interval at 590 nm (ECOplates) and 490 nm (FFplates).

2.5 | DNA extraction

Genomic DNA was extracted from a 0.5 g of soil sample using a FastDNA SPIN Kit for Feces (MP Biomedicals, Solon, OH) according to the manufacturer's protocol. The amount of DNA was determined spectrophotometrically at 260 nm (NanoDrop 2000/2000c Thermo Scientific, West Palm Beach, FL). The extracted DNA was then stored at −20°C and used for multiplex terminal restriction fragments length polymorphism (M-tRFLP) and next generation sequencing (NGS).

2.6 | Multiplex terminal restriction fragment length polymorphism (M-tRFLP)

The genetic diversity of soil bacterial, archaeal, and fungal communities was characterized by the multiplex terminal restriction fragment length polymorphism (M-tRFLP). M-tRFLP analysis included the following steps: multiplex PCR reaction (parameters of performed PCR are shown in Table S1), digestion of the obtained amplicons with restriction enzyme HaeIII and detection of separated terminal restriction fragments in genetic analyzer (ABI 3130). M-tRFLP was carried out as described in detail by Gryta & Frąc (2020) and Mącik, Gryta, Sas-Paszt, & Frąc (2020).

2.7 | Next generation sequencing (NGS)

The DNA sequencing was performed with the application of MiSeq platform (Illumina Inc., San Diego, CA) at Genomed S.A. (Warsaw, Poland). The PCR primer set 341F and 785R targeting the V3-V4 hypervariable region of 16S rDNA was used in bacterial metagenome analysis, whereas the primers ITS1FI2 and 5.8S were used to amplify the hypervariable fungal ITS1 rDNA region (Table S1). According to the manufacturer (NEB Inc., Ipswich, MA), the aforementioned primers and Q5 Hot Start High-Fidelity 2× Master Mix were applied to perform an amplification. The v2 Illumina kit was used for sequencing using 2 × 250 bp pair-ended technology.

2.8 | Statistical and bioinformatics analyses

Differences and similarities in carbon substrates utilization profiles between treatments were analyzed by principal component analysis (PCA) and by Jaccard's coefficient index. Jaccard's coefficient was calculated based on the number of utilized C-substrates between particular treatments; the substrate was considered when the absorbance value was greater or equal 0.25 (A ≥ 0.25). The average absorbance values from 216 hr incubation period were used. Jaccard's coefficient was calculated with the following formula: $J = N_{AB}/(N_A + N_B - N_{AB})$, where N_A-number of C-substrates utilized in profile 1, N_B-number of C-substrates utilized in profile 2, N_{AB}-number of C-substrates utilized in both profile 1 and 2 (López et al., 2019).

The relative abundance of particular terminal restriction fragments (T-RFs) was visualized as heatmaps with the matrix2png web interface (Pavlidis & Noble, 2003). TRiFLe tool was used for in silico identification of microorganisms, based on the size of the obtained T-RFs and defined nucleotide sequence sets (Junier et al., 2008). The Sorensen's similarity coefficient (QS) was calculated based on the number of shared and unique peaks between particular treatments, according to the formula: $QS = 2C/(A + B)$, where A-number of T-RFs in profile 1, B-number of T-RFs in profile 2, C-number of T-RFs common for both profile 1 and 2 (Walitang et al., 2019).

Beta diversity, based on Bray-Curtis distances was visualized using unweighted pair group method with arithmetic mean (UPGMA) clustering, principal coordinate analysis (PCoA), and non-metric multidimensional scaling (NMDS).

The bacterial functional traits, based on the 16S rDNA data, were assessed with PICRUSt (phylogenetic investigation of communities by reconstruction of unobserved states) (Langille et al., 2013) software in cooperation with KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa & Goto, 2000) online database. In addition, based on PICRUSt/KEGG results, the analysis concerning metabolic pathways related with P biotransformations in particular treatment was

TABLE 1 Changes of soil enzymatic activity, P_2O_5 content and maize yield under the influence of phosphorus biofertilizer at an optimal dose (FC), at an optimal dose enriched with microorganisms (FA100) and at a 40% reduced dose enriched with microorganisms (FA60)

	Sampling time/treatment								
	A18			S19			A19		
	FC	FA100	FA60	FC	FA100	FA60	FC	FA100	FA60
Protease activity (mg tyrosine kg^{-1} hr^{-1})	0.48 ± 0.02 b	2.50 ± 0.15 a	2.22 ± 0.79 a	3.34 ± 1.16 a	4.20 ± 1.59 a	2.22 ± 0.71 a	1.71 ± 0.29 a	2.00 ± 0.51 a	2.48 ± 0.79 a
Urease activity (μg N-NH_4 kg^{-1} hr^{-1})	46.90 ± 0.10 ab	59.86 ± 2.76 a	40.29 ± 1.94 b	41.03 ± 4.44 a	40.76 ± 5.33 a	43.81 ± 18.69 a	33.83 ± 6.30 a	50.82 ± 6.92 a	43.82 ± 3.29 a
Acid phosphomonoesterase activity (mmol PNP kg^{-1} hr^{-1})	13.79 ± 2.14 a	20.20 ± 6.13 a	19.31 ± 4.16 a	14.22 ± 1.32 a	17.85 ± 10.67 a	26.71 ± 10.35 a	16.28 ± 3.50 a	17.85 ± 0.56 a	19.39 ± 2.44 a
β-glucosidase activity (mg PNP kg^{-1} hr^{-1})	0.65 ± 0.15 a	0.74 ± 0.20 a	0.59 ± 0.22 a	0.71 ± 0.22 a	0.48 ± 0.16 a	0.76 ± 0.08a	0.60 ± 0.08b	1.21 ± 0.30 a	1.05 ± 0.05 ab
P_2O_5 content (mg 100 g^{-1})	17.77 ± 0.35 a	16.57 ± 0.68 b	16.97 ± 0.31 ab	13.10 ± 0.96 a	13.43 ± 0.49 a	13.07 ± 0.64 a	15.27 ± 0.42 a	13.90 ± 0.17 b	13.70 ± 0.10 bc
maize yield (t ha^{-1})	24.56 ± 1.70 a	26.08 ± 1.63 a	27.60 ± 1.10 a	-	-	-	11.39 ± 3.74 a	11.72 ± 3.22 a	15.20 ± 0.63 a

Note: significant differences ($p < 0.05$) were calculated for each sampling time separately. Explanation: PNP-*p*-nitrophenol, A18-autumn 2018, S19-summer 2019, A19-autumn 2019. Different letters indicate statistically significant differences between treatments.

performed. The functional characterization of soil fungal communities was conducted using the FUNGuild online database (Nguyen et al., 2016).

The differences in enzyme activities and number of functional operational taxonomic units (OTUs) between particular treatments and sampling times were determined with statistical tests. The analysis of variances (ANOVA) regarding soil sampling time and fertilization method and a post hoc Tukey test were used to calculate significant differences when ANOVA assumptions were met (β-glucosidase, PICRUSt/KEGG analyses, maize yield). The verification of ANOVA assumptions, including dataset normality and homoscedasticity of the variance, was conducted using Shapiro–Wilk and Levene tests, respectively. F-Welch test with post hoc Tukey test were used when normality of dataset was maintained but variance was not homogenous (protease, acid phosphomonoesterase, P_2O_5 content). On the other hand, when the dataset normality was violated, Kruskal-Wallis and Dunn tests were used (urease).

The automatic preliminary analysis of the NGS data, consisting on demultiplexing and the generation of fastq files, was performed on the MiSeq platform using the MiSeq Reporter (MSR) version 2.6 software (Illumina Inc., San Diego, CA). Bioinformatic analysis providing classification of reads to species taxonomic level was performed with the Quantitative Insights into Microbial Ecology (QIIME) software (Caporaso et al., 2010) based on the uCLUST algorithm (Edgar, 2010) and the GreenGenes version 13_8 database (16S V3-V4 OTUs) (DeSantis et al., 2006) and BLAST algorithm (Altschul et al., 1990) and UNITE version 8 database (ITS1 region) (Kõljalg et al., 2013; Nilsson et al., 2019).

All statistical analyses were performed with Statistica version 13.1 software (StatSoft Inc., Tulsa, OK) and R version 1.0.5.999 software (R Core Team, 2018, Vienna, Austria).

3 | RESULTS

3.1 | Soil enzymatic activity, assimilated phosphorus content, and maize yield

The changes of soil enzymatic activity in response to application of biofertilizers and sampling time are shown in Table 1. The activities of protease, urease, acid phosphomonoesterase, and β-glucosidase varied between individual fertilization treatments in a particular year.

The highest protease activity across whole experiment was observed in FA100(S19). On the other hand, the topmost increments in protease activity were reported in FA100(A18) and FA60(A18) (by 415.78% and 358.95%, respectively) as compared to control. Concerning the seasonal variations in protease activity, 2019 witnessed a decrease in aforementioned enzyme activity between controls and between FA100 treatments and an increase between FA60 treatments. It was also observed that activity of protease remained at higher levels in FA100 and FA60 in both A18 and A19.

In case of the urease, the highest activity during 2-year experiment was recorded in FA100(A18) and FA100(A19). Simultaneously, a greater variations in urease activity were observed in aforementioned sampling times in comparison with S19. In 2019 there was an upward trend in the urease activity between FA100 treatments.

FA100 and FA60 were characterized by increased acid phosphomoesterase activity as compared to controls (by 9.69%–87.85% depending on fertilization method and sampling time) throughout whole experiment. In S19 and A19, an upward trend in aforementioned enzyme activity was observed along with the decrease in the mineral fertilizer dose. No statistically significant changes occurred across the experimental period.

The highest increase in β-glucosidase activity as compared with control was recorded in FA100(A19) and FA60(A19) (by 102.28% and 75.45%, respectively). However, a reduction in the activity of β-glucosidase was observed in FA60(A18) and in FA100(S19). Statistically significant changes were noticed in A19.

When analyzing enzymatic activities throughout whole experimental period it is clearly visible that protease, β-glucosidase, urease, and acid phosphomonoesterase activities remained at higher levels in FA100 and FA60. The highest activities of protease and urease were observed in FA100. The application of biofertilizer also increased the activity of acid phosphomonoesterase in FA60. On the other hand, β-glucosidase activities were similar in both FA100 and FA60 throughout whole experiment. The statistically significant differences between treatments were reported in case of acid phosphomonoesterase activity (Figure 1).

Application of phosphorus biofertilizer increased the content of phytoavailable P_2O_5 in FA100(S19) by 2.52% as compared to FC(S19). The second year of the field experiment witnessed a decline in the quantity of P_2O_5 in the soil. Both FC(A18) and FC(A19) were characterized by the higher P_2O_5 content as compared to corresponding FA100 and FA60 treatments, whereas in FA100 and FA60 in a particular sampling time the quantity of assimilable phosphorus forms remained at a similar level (Table 1).

In both year of the field experiment, application of phosphorus biofertilizer increased average maize yield in FA100 and FA60 treatments. In 2018, in FA100 and FA60 yield increased by 6.19% and 12.38%, while in 2019 by 2.90% and 33.45%, respectively (as compared with corresponding controls). No statistically significant differences occurred between particular treatments. Noteworthy is the fact that FA60 treatments were characterized by the higher average maize yield in comparison with FA100 treatments (Table 1).

3.2 | Metabolic potential of soil microbial communities

Based on the average absorbance values of C-substrates utilization in ECO and FF Biolog plates during 216 incubation hours, the PCA was performed. In most cases, treatments from particular sampling times were clustered separately. PCA grouped the samples collected in A19 together in both plate types. Moreover, in ECOplates two groups were formed: first contained FC(S19) and FA100(S19) and second consisted of FA60(A18) and FA100(A18). Similar trend was noticed in

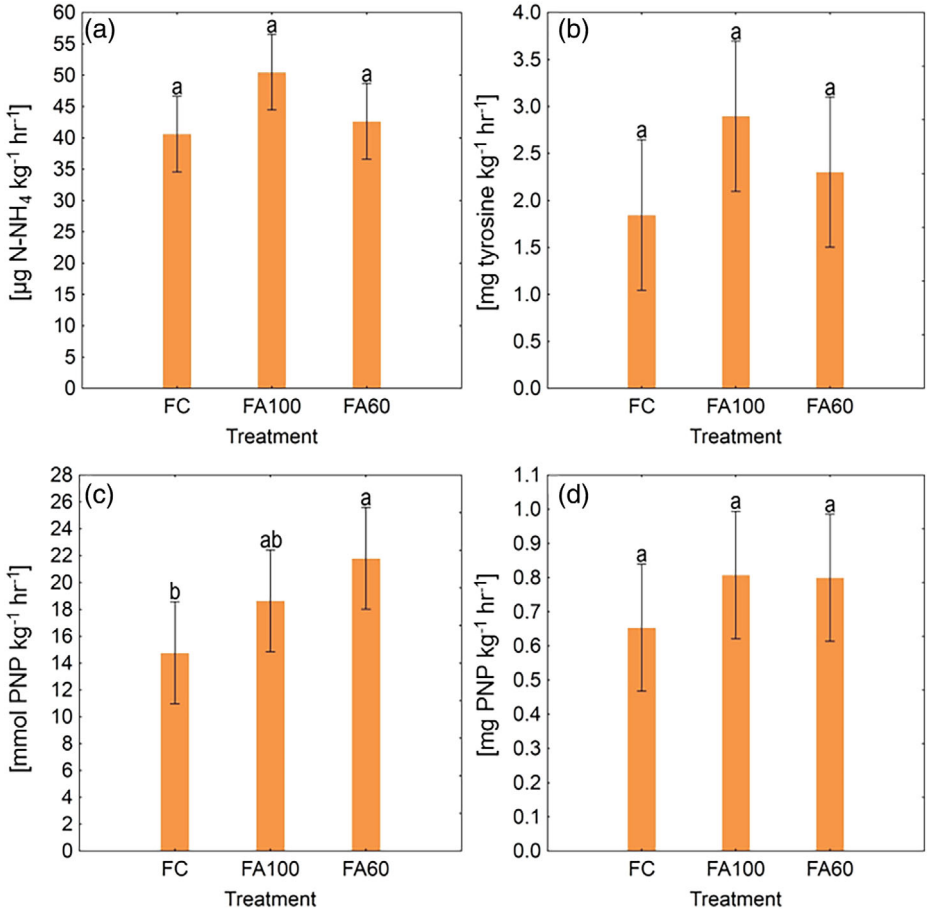

FIGURE 1 Changes in soil enzymatic activity in particular treatments throughout the experimental period. (a)-the activity of urease, (b)-the activity of protease, (c)-the activity of acid phosphomonoesterase, (d)-the activity of β-glucosidase. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), PNP-*p*-nitrophenol. Vertical bars denote 0.95 confidence intervals. Vertical bars denote 0.95 confidence intervals. Significant differences ($p < 0.05$) were calculated for all sampling times together [Colour figure can be viewed at wileyonlinelibrary.com]

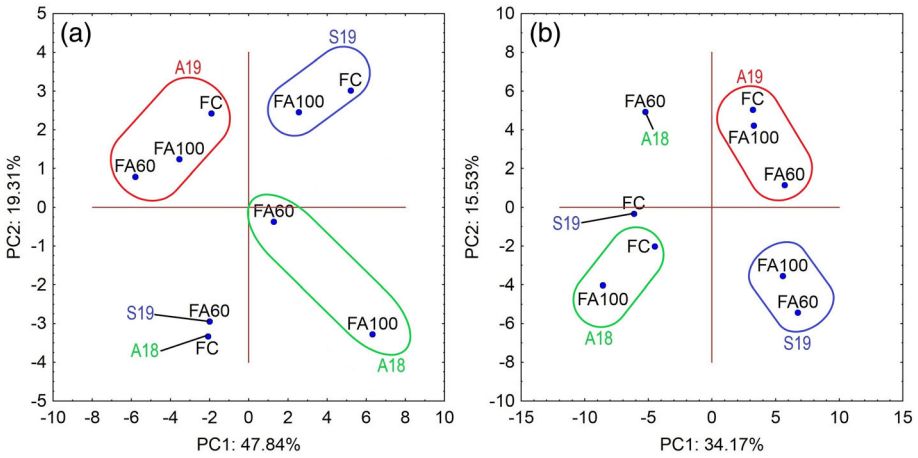

FIGURE 2 Principal component analysis (PCA) of C-substrate utilization patterns after 216 hr incubation of ECO (a) and FF (b) biolog plates. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

FF plates where FA100(S19), FA60(S19), FC(A18), and FA100(A18) also formed separated clusters (Figure 2).

Jaccard's coefficient index, based on the absorbance values of utilized carbon substrates was calculated in order to point similarities between particular treatments. The high values of aforementioned index (0.95–1) indicate a strong resemblance of C-substrates utilization patterns. The most similar utilization profiles in ECO plates were observed between FA100(A19) and FA60(A19), and in the case of FF plates, between FC(S19) and FA100(S19) (Table 2).

3.3 | The multiplex terminal restriction fragment length polymorphism

Multiplex tRFLP fingerprinting profile showed that not only fertilization method, but also sampling time influenced the structure of soil microbiome. Soil samples taken at different times exhibited variations in the number of T-RFs, their size and relative abundance. In silico analysis with the TRiFLe software allowed to identify microbial genera based on the size of selected T-RFs. Our results showed that T-RFs

with different sizes could be represented by various microorganisms and, what is more, the same genera were identified within T-RFs which differ from each other by several base pairs.

Soil bacterial communities were characterized by the richness (number of obtained T-RFs) ranging from 4 to 15, with a simultaneous decline in samples collected in S19 (Figure 3a). However, the increase in the number of T-RFs were observed in FA60(A18) and FA100(S19) as compared to corresponding controls. In whole restriction profile there were no T-RFs common for all treatments and sampling times, nonetheless 170 bp appeared throughout the whole experiment, with the exception of FC(S19). Fragments with the highest relative abundance were 112 bp (~41–49% reported from samples collected in A19) and 128 bp (48.29%in FA60(S19)). It is worth mentioning that the relative abundance of 113 bp (assigned to *Lysobacter*, *Pseudomonas*, and *Pantoea*) increased in FA100(S19) (30.39%) as compared to FC(S19) (13.36%). The similar trend was also observed in case of 120 bp (assigned to the abovementioned microorganisms) which relative abundance in FC(A18), FA100(A18), and FA60(A18) accounted for 4.23%, 6.76%, and 6.55%, respectively. Comparing restriction profiles from particular sampling times it is clearly seen that both A18 and A19 exhibited increased diversity of obtained T-RFs and higher Sorensen coefficient values (Table 3). Analysis with TRiFLe revealed the presence of microorganisms assigned to selected DNA fragments and belonged to the following phyla: Proteobacteria (*Filomicrobium* (60 bp), *Paracoccus* (76 bp), *Rhizobium* (155 bp), *Burkholderia* (360/364 bp), *Salicola* (60 bp), *Proteus* (90/94/100 bp), *Klebsiella* (170 bp), *Shigella* (290 bp), Actinobacteria (*Micrococcus* [90/94 bp]), Bacteroidetes (*Proteiniphilum* (90/94 bp), *Flavobacterium* (170 bp), Firmicutes (*Clostridium* (170 bp), *Staphylococcus* (272 bp) Cyanobacteria [*Anabaenopsis* (256 bp]] and Gemmatimonadetes (74/80 bp).

TABLE 2 Jaccard's coefficient values

	PlateType/sampling time					
	ECOplate			FFplate		
Treatment	A18	S19	A19	A18	S19	A19
FC-FA100	0.80	0.90	0.97	0.93	0.95	0.92
FC-FA60	0.97	0.87	0.97	0.91	0.92	0.91
FA100-FA60	0.77	0.90	1.00	0.93	0.91	0.92

Note: explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019

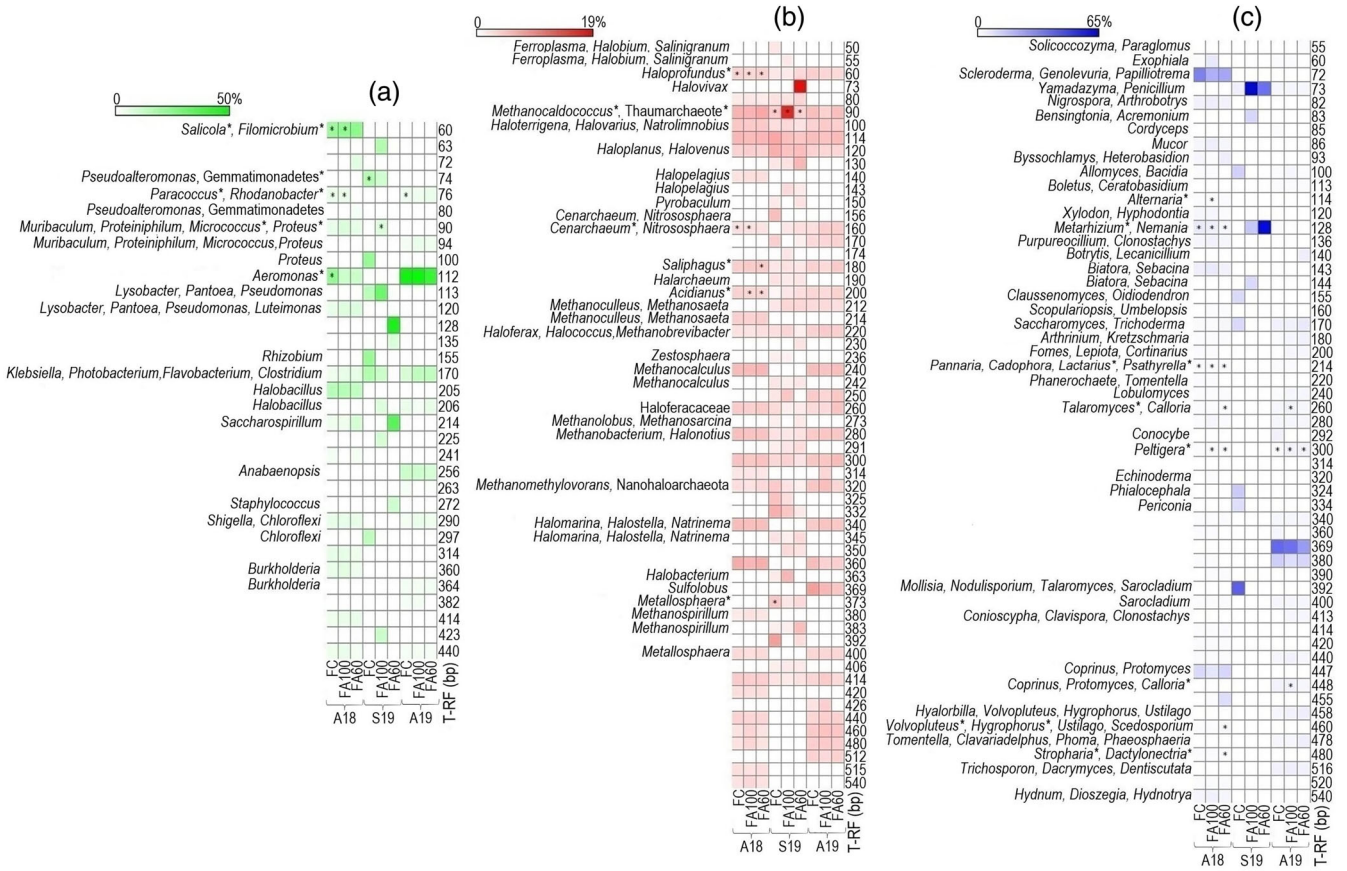

FIGURE 3 Heat maps presenting the number of terminal restriction fragments, their relative abundance and genera prediction in bacterial (a), archaeal (b) and fungal (c) communities. *The particular T-RF was present, but no identification of microorganisms occurred. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Sorensen's similarity coefficient values

	Microbial group/sampling time								
	Bacteria			Archaea			Fungi		
Treatment	A18	S19	A19	A18	S19	A19	A18	S19	A19
FC-FA100	0.963	0.429	0.957	1	0.841	0.981	0.971	0.182	0.870
FC-FA60	0.897	0	0.957	1	0.824	1	0.958	0.222	0.809
FA100-FA60	0.857	0	0.909	1	0.806	0.981	0.986	0.667	0.889

Note: explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019

In archaeal communities, richness ranged from 26 to 35, with the highest values reported in soil samples taken in S19 (33–35 T-RFs, depending on the treatment) (Figure 3b). The only treatment where the number of T-RFs was higher than the control was FA100(S19). In restriction profiles there were common T-RFs shared within all sampling times and treatments (60, 90, 100, 180, 200, 220, 320 bp) and some unique fragments which appeared only in treatments where biofertilizers were applied (55, 143, 174, 350 bp). The highest Sorensen coefficient values were noted in case of soil samples taken in A18 and A19 (Table 3). The predominant archaeal T-RFs were 73 bp (affiliated to *Halovivax*) and 90 bp (attributed to *Methanocaldococcus* and Thaumarchaeote) with the relative abundance accounted for 18.53% [in FA60(S19)] and 16.81% [in FA100(S19)], respectively. Identification with TRiFLe software showed that representatives of *Ferroplasma*, *Halobium*, *Salinigranum*, *Halopelagius*, *Cenarchaeum*, *Nitrososphaera*, *Methanoculleus*, *Methanosaeta*, and *Methanocalculus* could be assigned to different T-RFs depending on the treatment and sampling time. Our results proved that some archaea were found in all treatments during the experimental period, for example, *Haloterrigena*, *Halovarius*, *Natrolimnobius*, *Haloplanus*, and *Halovenus*. On the other hand, there were genera belonged to unique T-RFs, e.g. *Pyrobaculum* assigned to 150 bp and *Zestosphaera* connected with 236 bp.

In fungal communities, the number of T-RFs varied between 2 and 37 and similar to bacteria, A18 and A19 were characterized by increased richness and Sorensen coefficient values (Table 3) as compared to S19. The increase in number of T-RFs was observed in FA100(A18) and FA60(A18) as compared to FC(A18). Fragments that reached the highest relative abundance throughout the experimental period were 72 bp (32.52%in FC(A18)), 73 bp [64.46%in FA100(S19)], 128 bp [63.3%in FA60(S19)], 392 bp [41.54% in FC(S19)], and 369 bp (38.83%in [FC(A19)] (Figure 3c). There were no T-RFs common for all sampling times and treatments, however some of them appeared in FC, FA100 and FA60 in both A18 and A19, e.g. 180, 240, 260, 340, 380, and 400 bp. It was observed that that the relative abundance of 73 bp (assigned to *Penicillium*) increased in FA1009(S19) (64.46%), FA60(S19) (36.69%) and FA60(A19) (2.92%) as compared to FC(S19) (4.57%) and FC(A19) (2.78%). The increased relative abundance of 170 bp (affiliated to *Trichoderma*) was reported in FA60(A18) (2.08%), FA100(A19) (3.33%) and FA60(A19) (5.25%) in comparison with corresponding control treatments FC(A18) (1.76%) and FC(A19) (3.16%).

3.4 | Next generation sequencing (NGS)

3.4.1 | Bacterial and archaeal community composition and functional prediction

Among the 188 identified OTUs at the order level, 171 were shared between FA100, FA60, and FC across the all sampling times and constituted the core microbiome (Figure 4a). What is worth mentioning, FA100 and FA60 were characterized by the greater diversity than control (FC – 178, FA100–181, FA60–180 identified orders). The analysis of seasonal shifts within particular treatments showed that the core microbiome of FC (Figure 4d) consisted of higher number of identified orders as compared to FA100 and FA60. The most diverse treatments in terms of individual orders were FA100(A18), FA100(A19) (Figure 4b), and FA60(A19) (Figure 4c). The distribution of common and unique bacterial and archaeal orders in particular treatments and sampling times is presented in Table S2 and S3. Bacterial orders which were characteristic to FA100 included C20 (Chlorobi), Lactobacillales, S0208 (Chloroflexi), and VC38 (Chlorobi) while GMD14H09 (Proteobacteria) and TG3-1 (Fibrobacteres) were unique to FA60. Orders characteristic to both FA100 and FA60 included Rhodothermales, LD1-PB3 (Verrucomicrobia), Oceanospirillales, and SJA-36 (Acidobacteria) (Table S3). The bacteria which dominated treatments within all sampling times belonged to the following orders: Actinomycetales (12.69–19.08%), Rhizobiales (6.06–8.04%), Xanthomonadales (3.16–6.43%), Rhodospirillales (4.40–5.49%), Acidobacteriales (4.03–7.05%), and Solirubrobacterales (4.19–5.15%). The relative abundance of particular orders was dependent on the sampling time and fertilization method. In S19 the relative abundance of Actinomycetales, Rhizobiales, Xanthomonadales, Sphingomonadales, Burkholderiales, and Bacillales increased as compared to A18 and A19. On the other hand, the relative abundance of Rhodospirillales, Acidobacteriales, Acidimicrobiales, Thermogemmatisporales, Solibac terales, Myxococcales, and Gemmatales was higher in samples collected in autumn (Figure 4e).

Predictive functional profiling of the bacterial communities, based on the 16S rDNA data, was conducted with PICRUSt (Langille et al., 2013) and KEGG (http://www.kegg.jp/) and revealed that the majority of identified OTUs was assigned to "Metabolism" (∼54%) followed by "Environmental Information Processing" (∼15%), "Genetic Information Processing" (∼13%), "Genes and Proteins" (∼10%) and "Cellular Processes" (∼5%) (Figure S2). In A18 number of

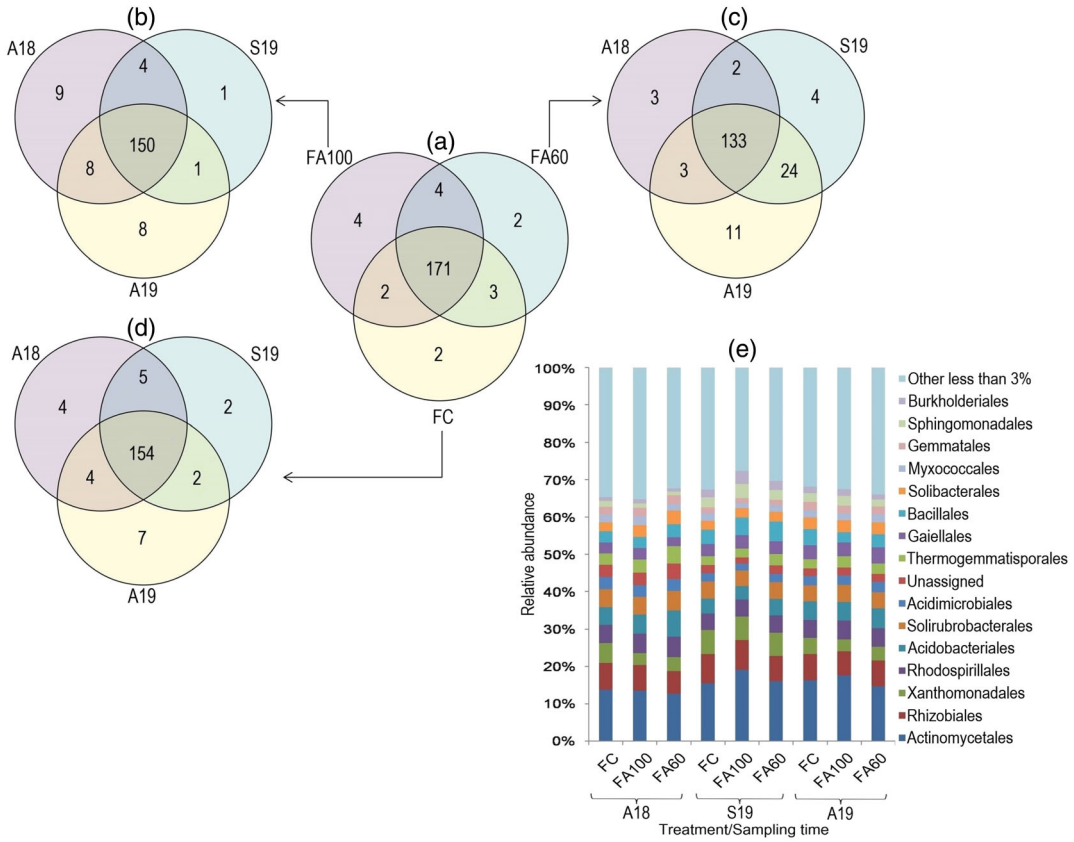

FIGURE 4 Venn diagrams showing the distribution of shared and unique bacterial and archaeal operational taxonomic units (OTUs) identified at the order level. (a)-treatments from all sampling times, (b)-seasonal variations in FA100 treatment, (c)-seasonal variations in FA60 treatment, (d)-seasonal variations in FC treatment, (e)-the relative abundance of dominant bacterial orders throughout the experimental period. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

functional sequences in particular main KEGG classes remained at similar level in all treatments. The application of biofertilizers in S19 contributed to the increase in the number of OTUs assigned to each main KEGG class in FA100 and FA60, however, FC showed a relatively similar number of functional OTUs to samples taken in A18. An upward trend in the number of OTUs was also observed in A19 not only in FA100 and FA60, but also in controls as compared to treatments from S19. Throughout the experimental period, FA60(A19) was characterized by the greatest number of OTUs associated with each main KEGG class (Figure S3). The metabolism-related pathways encompassed 12 subclasses, of which amino acids and carbohydrates biotransformations displayed the highest number of OTUs. On the other hand, the lowest number of OTUs identified with metabolic processes was assigned to pathways associated with biosynthesis of other secondary metabolites and glycans. It was also observed that FA100 and FA60 in S19 and A19 were characterized by increased number of sequences connected with xenobiotics degradation and metabolism of terpenoids and polyketides (Figure S4). In-depth analysis of PICRUSt metabolic pathways revealed that some OTUs were assigned to P-related processes including glycerophospholipid metabolism, inositol phosphate metabolism, oxidative phosphorylation, pentose phosphate pathway, phosphatidylinositol signaling system, phosphonate and phosphinate metabolism, and phosphotransferase system (PTS). The application of phosphorus biofertilizer in S19 increased the number of functional OTUs associated with phosphorus processes in FA100 and FA60 treatments as compared to control soil and A18 samples. It is worth mentioning that the identical trend was maintained in soil samples collected in A19. Among P-related pathways, the highest number of OTUs was assigned to oxidative phosphorylation, followed by pentose phosphate pathway and glycerophospholipid metabolism. Analyzing variations throughout the experimental period, FA100(A19) and FA60(A19) were characterized by the highest number of functional OTUs associated with phosphorus biotransformations (Figure S5). Some seasonal variations in bacterial functional profiles were visualized with PCA plots, namely treatments from particular sampling times formed separated clusters (Figure S6).

3.4.2 | Fungal community composition and functional guilds prediction

The composition of soil fungal communities was analyzed, similarly as bacterial and archaeal communities, at the order level. Our data

showed that the core fungal microbiome, common for all treatments across the experimental period, consisted of 106 detected OTUs out of the total 131 defined orders (Figure 5a). Other identified OTUs were distributed among particular fertilization methods. The soil environment was dominated by representatives from the following orders: Eurotiales, Hypocreales and Mortierellales, which accounted for 13.19–23.56%, 7.99–14.01%, and 4.22–7.88%, respectively, according as sampling time and treatment (Figure 5e). In FC and FA100 the presence of 120 various fungal orders were reported, whereas for FA60 this number was 115. Regarding seasonal variations, a similar trend to the bacterial community was observed, namely core microbiome of FC encompassed higher number of orders than FA100 and FA60. What is more, A18 and A19 within each treatment were characterized by higher number of specific OTUs (Figure 5b–d). The distribution of common and unique fungal orders in particular treatments and sampling times is presented in Tables S4 and S5. Fungal orders specific for FA100 included Coryneliales, GS02 (Rozellomycota), Microstromatales, Rozellomycotina_ord_Incertae_sedis, and Teloschistales whereas Gomphales and Hyaloraphidiales were unique to FA60. Orders characteristic for both FA100 and FA60 encompassed Endogonales, Falcocladiales, Myriangiales, and Tilletiales (Table S5). The soil environment was dominated by representatives from the following orders: Eurotiales, Hypocreales, and Mortierellales, which accounted for 13.19–23.56%, 7.99–14.01%, and 4.22–7.88%, respectively, according as sampling time and treatment. Samples collected in S19 were characterized by the increased relative abundance of Mortierellaes, Sordariales, Filobasidiales, and Agaricales, whereas the relative abundance of Eurotiales, Chaetothyriales, Umbelopsidales, and Pleosporales remained higher in A18 and A19 samples. In case of Hypocreales, their contribution to the creation of soil fungal communities was higher in both S19 and A19 as compared to A18 (Figure 5e).

A functional prediction of the fungal community showed that the soil environment was dominated by the representatives of saprotrophs (29.12–39.57%), followed by saprotrophs-symbiotrophs (5.93–9.23%), pathotrophs-saprotrophs-symbiotrophs (5.78–8.91%), and pathotrophs (3.20–6.87%). It is worth mentioning that FA100 and FA60 in both S19 and A19 were characterized by the lower relative abundance of pathotrophs as compared to corresponding controls. At the same time, the increments in relative abundance of saprotrophs-symbiotrophs were observed in FA100(A18) and FA100-FA60(A19) (Figure S7). A deeper look in fungal ecological guilds revealed that the saprotrophs population was mainly dominated by undefined

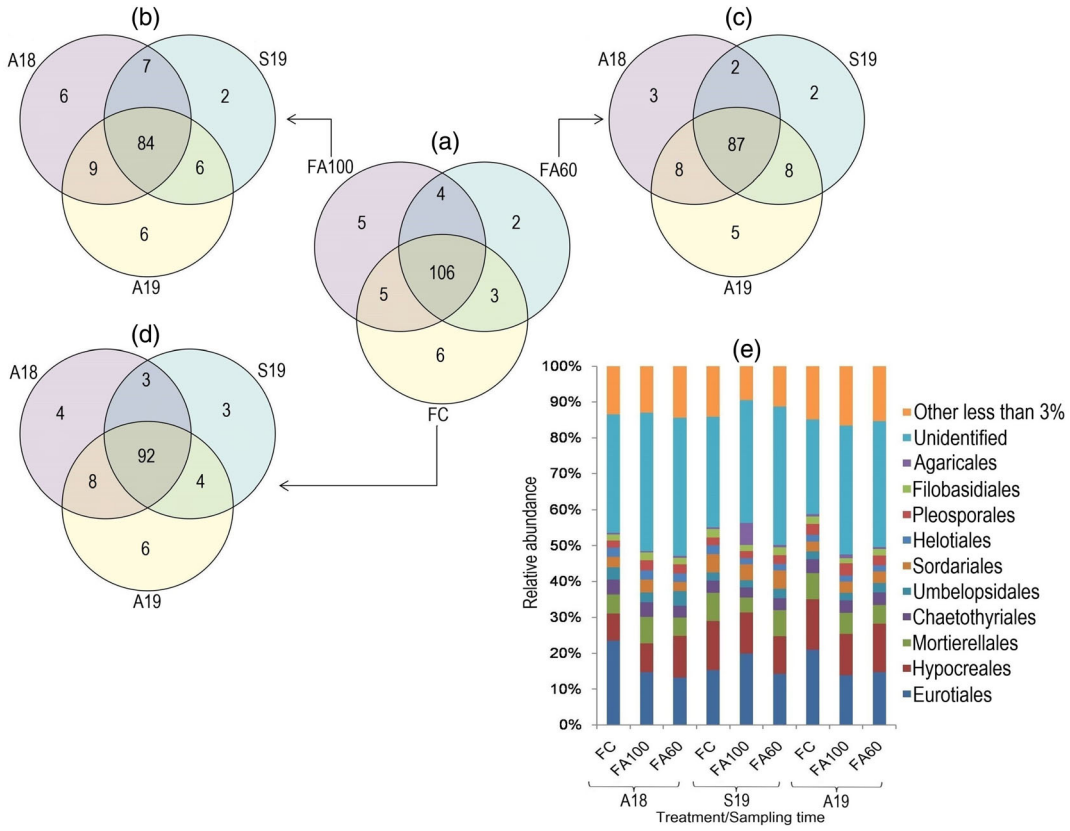

FIGURE 5 Venn diagrams showing the distribution of shared and unique fungal operational taxonomic units (OTUs) identified at the order level. (a)-treatments from all sampling times, (b)-seasonal variations in FA100 treatment, (c)-seasonal variations in FA60 treatment, (d)-seasonal variations in FC treatment, (e)-the relative abundance of dominant fungal orders throughout the experimental period. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

saprotrophs which accounted for ~50–60% of the total aforementioned guild, whereas endophyte-litter saprotroph-soil saprotroph-undefined saprotroph was the dominant group among saprotrophs-symbiotrophs (7.13–13.72%). Pathotrophs were mainly represented by animal and plant pathogens, however, the relative abundance of animal pathogens decreased in FA100 and FA60 in both S19 and A19 in comparison with corresponding controls. Lower contribution of plant pathogens in fungal community structure was also observed in FA60(A18) and FA100(S19). The relative abundance of selected fungal ecological guilds, important in terms of soil health, increased in FA100 and FA60 treatments. Such changes included the following guilds: wood saprotroph (FA60(A18), FA100-FA60(A19)), dung saprotroph-ectomycorrhizal-litter saprotroph-undefined saprotroph (FA60(A19)), ectomycorrhizal-wood saprotroph (FA100(A19)), endophyte-litter saprotroph-wood saprotroph (FA100-FA60 across whole experimental period), and arbuscular mycorrhizal (FA100-FA60 (A18), FA60(S19), and FA60(A19) (Figure S8).

3.4.3 | Beta diversity of soil microbial communities

Visualization of the beta diversity distribution between treatments and sampling times was demonstrated by the PCoA plots and UPGMA dendrograms on the basis of Bray–Curtis dissimilarity index. In PCoA plots the clear separation of treatments, depending on the soil sampling time, for both bacteria (Figure 6a) and fungi (Figure 6b) was reported. Interestingly, in most cases it was possible to distinguish inner clusters in which controls would be isolated from FA100 and FA60.

UPGMA dendrogram for bacteria showed two well-defined clusters: one included S19 treatments and second one encompassed differentiated A19 and A18. In both S19 and A19, the FC was separated from FA100 and FA60 (Figure 6c). Similar trend, namely two main groups were distinguished in case of fungal communities, however, some differences in comparison with bacteria were noticed. First main cluster was composed by A18 treatments and second, including three sub-clusters, by S19 and A19 treatments. FA100 and FA60 treatments from particular sampling time were clustered together and were clearly isolated from controls. What is worth mentioning, there is a possibility to distinguish another inner cluster which would encompass FA100(A19), FA60(A19), FC(S19), and FC(A19) (Figure 6d).

The relationships between soil microbial communities in particular treatments and sampling times were also investigated with the application of NMDS (Figure 7). In bacterial metagenome, a clear separation of A18 treatments from S19 and A19 along the axis 2 was noticed. Regarding the clustering along axis 1, all three controls remained at the higher part of axis 1, while FA60(A18) and FA60(S19)

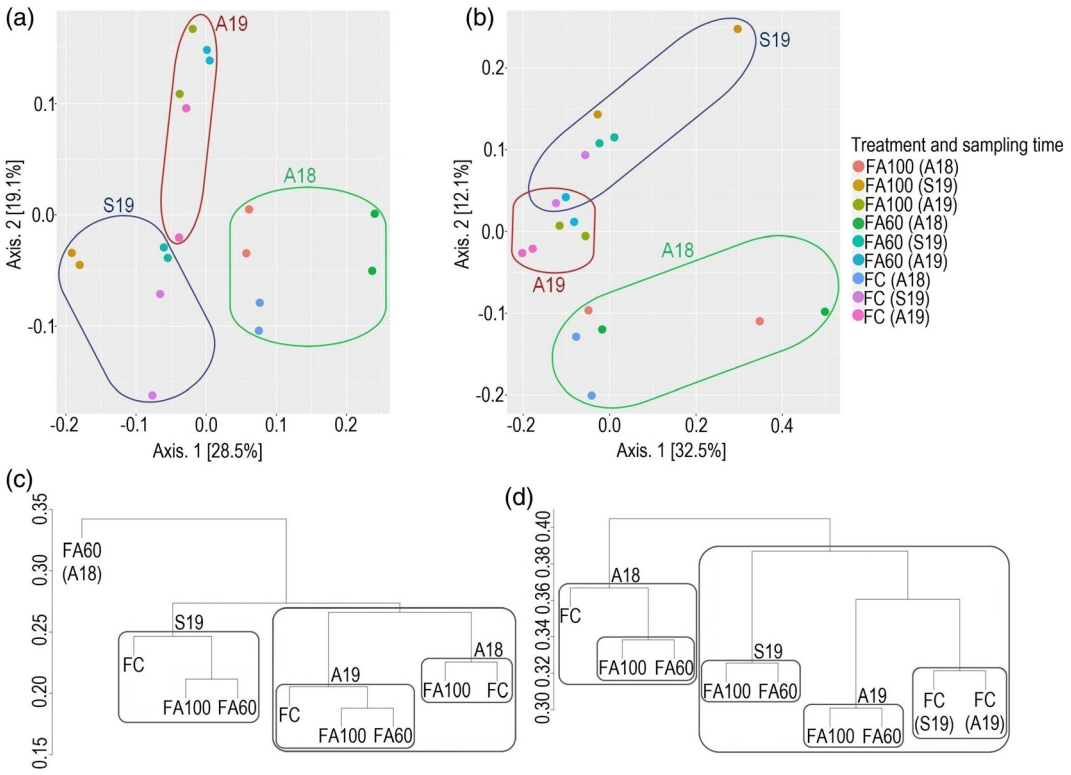

FIGURE 6 Principal coordinates analysis plots (a and b) and UPGMA dendrograms (c and d) based on the bray-Curtis distances for bacterial (a and c) and fungal (b and d) communities. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

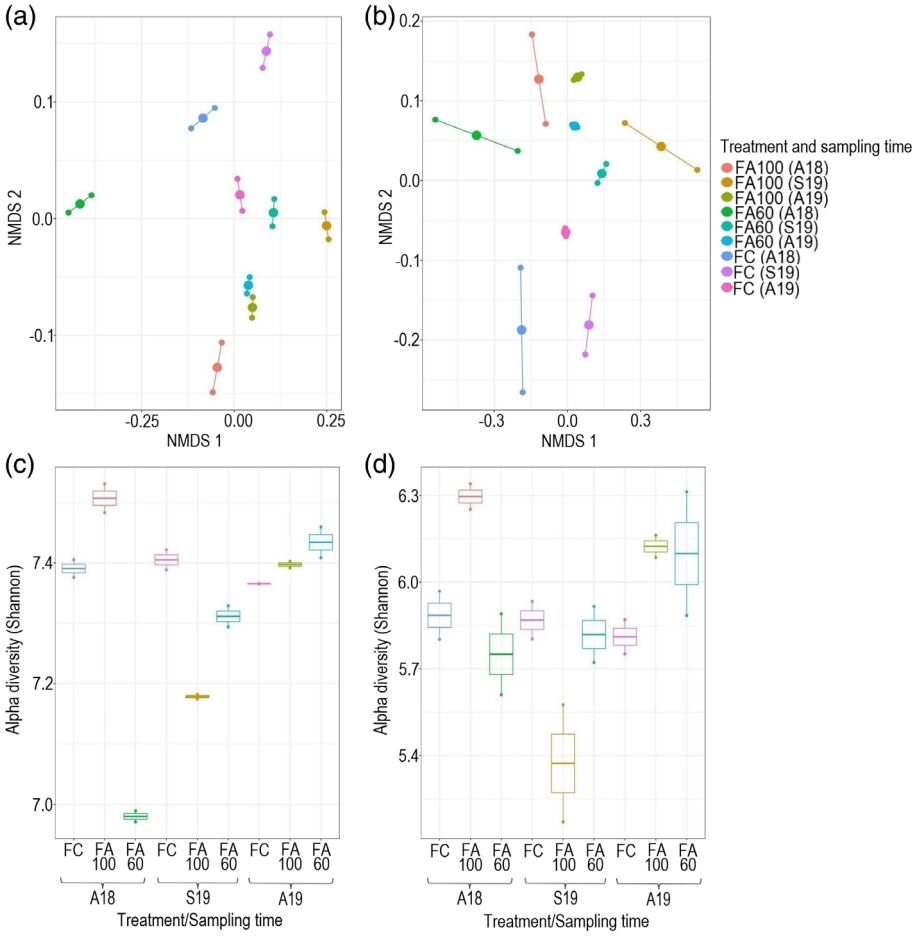

FIGURE 7 Nonmetric multidimensional scaling (NMDS) plots (a and b) and Shannon diversity (c and d) for soil bacterial (a and c) and fungal (b and d) communities. In (a) and (b) large dots represent centroids and small dots represent individual samples connect to the centroids. Explanation: FC-optimal dose of fertilizer, FA100-optimal dose of fertilizer enriched with microorganisms, FA60-fertilizer enriched with microorganisms (dose reduced by 40%), A18-autumn 2018, S19-summer 2019, A19-autumn 2019 [Colour figure can be viewed at wileyonlinelibrary.com]

were isolated from FA100(A18) and FA100(S19) and occupied the higher and the lower part of aforementioned axis, respectively. It was also noticed that individual replications of FA100(A18) treatments were the furthest apart in the ordination space; on the other hand points corresponding to FA60(A19) and FA100(A19) were relatively closely related (Figure 7a). In fungal communities the NMDS ordination plot showed a clear clustering of treatments along the two axes. Microbiologically enriched treatments were located at the higher part of axis 2, whereas the controls occupied the lower part of this axis. Similar trend as in bacterial communities was observed, namely A18 treatments were placed at the left side of axis 1, while the opposite extreme of this axis was positioned by S19 and A19 treatments (Figure 7b). The highest dispersion between individual replications was observed in case of A18 samples. Relatively far apart were placed points corresponding to FA100(S19) and FC(S19), however these distances were smaller as compared to A18 treatments. The closest points to each other in the ordination space were A19 samples.

The alpha diversity was expressed by the Shannon diversity index (H). The highest H values in bacterial communities were reached in FA100(A18) and FA60(A19) (Figure 7c), while in fungal metagenome in FA100(A18) and FA100-FA60(A19) (Figure 7d). Fungal communities were characterized by the higher variation in H as compared to bacterial communities, whereas H reached higher values in case of 16*S* rDNA data.

4 | DISCUSSION

Enzymatic activity is unquestionably one of the bioindicator of soil quality and reflects shifts in soil environment under the influence of various treatments, both organic and inorganic (Kwiatkowski et al., 2020). This study revealed variations in the activity of selected soil enzymes: protease, urease, acid phosphomonoesterase, and β-glucosidase. Analyzing the results in individual treatments throughout experimental period it was found that FA100 and FA60 were characterized by enhanced activity of protease, urease, acid phosphomonoesterase, and β-glucosidase. According to Mengual et al. (2014) degraded soil inoculated with beneficial bacterial strains (*Bacillus megaterium*, *Enterobacter* sp., *Bacillus thuringiensis*, and *Bacillus* sp.) and sugar beet was characterized by the increased dehydrogenase, protease, urease, and β-glucosidase activity. The fluctuations in the activity of investigated enzymes in this study may arise from the fact that this parameter is sensitive to changes in the soil environment within 2 years after the exposure to a specific factor (Dick & Kandeler, 2005). It is particularly noteworthy that in the FA100(A19) and FA60(A19) treatments, the activity of all analyzed enzymes remained at higher levels as compared to control treatment, which may indicate that some stability was achieved among the microbial communities and the pathways of N, P, and C compounds mineralization were adjusted to changes in underground environment caused by introduction to the soil microorganisms

combined with fertilizer granules. Adaptation of microorganisms to the new fertilization technique may also be supported by recording the highest protease, acid phosphomonoesterase, and β-glucosidase activities in second year of field experiment. The increased acid phosphomonoesterase activity maintained throughout the experimental period in FA100 and FA60 treatments may be explained as the introduction of a specific fertilizer combined with beneficial bacterial strains directed and stimulated soil microorganisms towards phosphorus metabolism. Enhanced acid phospomonoesterase, β-glucosidase, and protease activity may be also associated with the acceleration of metabolic processes as reflected by the increased number of functional OTUs (in FA100 and FA60) assigned to phosphorus, carbohydrates, and amino acids biotransformations.

It is commonly known that weather conditions are one of the factors affecting the status of soil microbiome (Furtak et al., 2020). In our study rainfall remained at similar level on each of the three sampling times which may suggest that it had no effect on soil microbiome properties. It is worth mentioning that S19 recorded a relatively high temperature, possibly stimulating the activity of soil microorganisms. According to Heidari et al. (2019) acid phosphatase activity increased in soil along with the temperature which may be supported by the enhanced activity of abovementioned enzyme in FA60(S19). Higher temperature may also stimulate metabolic processes and occurrence of particular microbial orders in S19. Nevertheless, it should be considered that the application of phosphorus biofertilizer affected soil microorganisms to a greater extent than weather conditions.

Application of phosphorus biofertilizer resulted in changes in the bioavailability of P in the soil. The increase in the quantity of available P in FA100(S19) remains in agreement with Wang, Liu, et al. (2021) who observed that soil samples inoculated with *Bacillus subtilis*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* were characterized by the higher content of assimilable phosphorus forms as compared to non-inoculated control. However, the decline in P_2O_5 content in particular FA100 and FA60 treatments may be related to enhanced acid phosphomonoesterase activity and more efficient exploitation of P compounds.

In meta-analysis conducted by two researchers (Schmidt & Gaudin 2018) it was found that the biofertilizers increased maize yield by an average 15.3% in field experiments and 18.4% in pot studies. Increased maize yield in FA100 and FA60 may be related with the enhanced soil enzymatic activity and hence with the higher nutrients availability. It is possible that the microorganisms provided in the biofertilizers accelerated the solubilization of phosphorus compounds, resulting in improved plant productivity. Furthermore, it was described that amino acids are plant growth stimulants and phosphorus biofertilizers, by increasing the number of OTUs associated with amino acids metabolism, may improve crop yield (Moe, 2013; Wang, Liu, et al., 2021). Increased maize yield in 2018 as compared to 2019 may be also related to higher P_2O_5 content in the soil. Simultaneously, a reduction in the quantity of available P may also be linked to the depletion of nutrients necessary for plant growth. Our study showed that the phosphorus biofertilizer application was more efficient in FA60 treatments, creating an opportunity to reduce mineral fertilizer doses along with achieving higher yields.

The metabolic potential of soil microorganisms toward C-substrates utilization showed a seasonal responses to an application of biofertilizers. In most cases, a clear clustering of treatments according to sampling time was observed. However, the apparent discrimination of FC and FA60 among A18 and S19 in PCA ordination space may indicate that both bacteria and fungi modify their metabolic pathways differently in response to biofertilizers application and this response depends on the stage of growing season. As Zhang, Tang, et al. (2010) described, soil bacteria associated with non-inoculated *P. tabulaeformis* seedlings were significantly separated from samples inoculated with ectomycorrhizal fungi in terms of functional profiles. The clear separation of S19 from A18 and A19 may be related to the intensification of photosynthesis in summer and the releasing of the products of this process to the soil. Simultaneously, the separation of FA60 may be connected with the competition for nutrients between indigenous microbiota and microorganisms provided with biofertilizers (Kandasamy et al., 2019). The fact that seasonality affected the metabolic properties of particular soil microbial communities is evidenced by the higher differences in Jaccard's coefficient reported in both ECO and FF plates in A18 and S19, respectively. As Koranda et al. (2013) mentioned, microbial communities differ in utilization of C-compounds depending on the season and the previously mentioned separation may be related to the fact that various chemical compounds were the preferred carbon sources in the particular treatments at different stages of the experiment. In winter, the preferred carbon source was cellulose, whereas utilization of glucose increased in summer. Nevertheless, the highest number of functional OTUs associated with amino acids and carbohydrates metabolism throughout the experimental period point that certain compounds are exploited primarily by microorganisms, regardless of sampling time and fertilization techniques. According to Jacoby et al. (2017) plants exudates are rich in sugars and amino acids and the high bioavailability of aforementioned components may be related to their enhanced metabolism in comparison with other compounds. On the other hand, the accomplishment of a metabolic balance and adjustment to a new fertilization practice among microbiome inhabiting studied soil may be confirmed by clustering of A19 treatments together in both ECO and FF plates and relatively high values and small differences of Jaccard's coefficient between FC, FA100, and FA60. Grouping all A19 treatments together may be also associated with the fact that FC(A19), FA100(S19), and FA60(S19) were characterized with the highest number of functional OTUs associated with metabolic processes. It is thought that soil microorganisms remain more responsive in warm season (Xu et al., 2021), however, our results showed that application of biofertilizer may prolong the activity of soil microbiome and moreover, enhance it despite autumn temperature decline. Seasonal variations in functional and metabolic profiles of soil microorganisms were also supported by the PCA grouping based on the number of functional OTUs assigned to a particular biochemical pathways.

Investigation of soil microorganisms biodiversity involves not only soil enzymatic activity and functional profiles, but also genetic variability. Our research showed that the application of biofertilizers affected the composition of soil microbiome, however, the response

depended on particular treatment, sampling time, and microbial group. The observed variations in genetic restriction profile referred to both the number of T-RFs in the restriction profiles and their relative abundance. The increase in the number of T-RFs in FA100 and FA60 may suggest the positive impact of applied biofertilizers on the genetic diversity of soil microorganisms. Similar results were observed by Trabelsi et al. (2011, 2012) and Kandasamy et al. (2019) where bacterial richness increased in soil inoculated with beneficial microorganisms. Soil inoculated with *Ensifer meliloti* 4H41, *Rhizobium gallicum* 8a3 and consortium of these strains exhibited the increased number of T-RFs, accounted for 51, 46, and 36, respectively, as compared to non-inoculated control (13 T-RFs) (Trabelsi et al., 2012). On the other hand, the decrease in the number of T-RFs in bacterial and fungal communities in S19 may be a result of sensitivity of microorganisms to a new fertilization regime. According to Zhang, Sun, et al. (2010) *Rhizobium* spp. inoculation lowered the bacterial richness in the rhizosphere of faba bean. In archaea community, the number of T-RFs remained at a relatively similar level during the whole experimental period, which may indicate greater stability of aforementioned group in the face of modified environmental conditions. The high Sorensen's coefficient values between treatments in A18 and A19 point to the close similarity in the distribution of terminal restriction fragments among soil bacteria, fungi, and archaea communities (Walitang et al., 2019). On the contrary, in S19 treatments lower values of above-mentioned index result from the presence of many unique T-RFs that were specific for a particular fertilization method.

The assignment of selected T-RFs to a various microbial genera with TRiFLe software revealed the presence of some ecologically important microorganisms. Among archaea, members of *Nitrososphaera* genus are ammonia oxidizing archaea (AOA) which are thought to be a key element in the nitrogen biogeochemical cycle (Mukhtar et al., 2019), *Ferroplasma* spp. species are involved in iron cycling (Golyshina, 2011) and representatives of *Methanocaldococcus* spp., *Methanosaeta* spp., *Methanosarcina* spp., and *Methanomethylovorans* spp. are well-described methanogens (Cha et al., 2013; Ferry, 2020; Mori et al., 2012; Susanti et al., 2019). Beneficial microorganisms among bacteria may be represented by strains of *Rhizobium* spp. and *Lysobacter* spp. Members of *Rhizobium* spp. genus are commonly known legume plants symbionts and nitrogen fixers (Lindström & Mousavi, 2020), whereas *Lysobacter* spp. was found to exert plant growth promoting properties and was considered as a biocontrol agent against *Fusarium graminearum* (Chen et al., 2020). It is worth emphasizing that the aforementioned microorganisms were also characterized in terms of phosphate dissolving abilities. *Lysobacter enzymogenes* LE16 increased water-soluble phosphorus content in the soil and boosted P uptake in lettuce seedling (Chen et al., 2019), *Burkholderia cepacia* SCAUK0330 solubilized phosphate from insoluble $Ca_3(PO_4)_2$ in a liquid medium (Zhao et al., 2014), *Pseudomonas aeruginosa* KR270346 and KR27034 increased the available P content in the soil and enhanced phosphatase activity (Linu et al., 2019), whereas *Pantoea agglomerans* IALR1325 synthesized extracellular enzymes (acid phosphatase and phytase) and dissolved $Ca_3(PO_4)_2$ and $Ca_5(PO_4)_3OH$ (Mei et al., 2021). The application of phosphorus biofertilizer in S19 increased the relative abundance of 113 bp and 120 bp T-RFs within which *Lysobacter* spp., *Pantoea* spp. and *Pseudomonas* spp. representatives were identified, what may suggest that the phosphorus biofertilizer promotes the occurrence of microorganisms involved in P solubilization. The identified bacterial genera were assigned to different phyla, confirming the high diversity among studied community. The analysis of fungal community showed that selected T-RFs may be attributed to strains that exhibit plant growth promoting properties (*Penicillium* spp.) (Naziya et al., 2020), saprotrophs involved in SOM decomposition (*Cortinarius* spp., *Conocybe* spp.) (Barnes et al., 2016; Müller et al., 2020) biocontrol agents (*Metarhizium* spp., *Trichoderma* spp., *Clonostachys* spp.) (Faria et al., 2017; Sun et al., 2020), plants and algae/cyanobacteria symbionts (*Boletus* spp., *Bacidia* spp.) (Malíček et al., 2018; Treindl & Leuchtmann, 2019) and microorganisms used in bioremediation (*Solicoccozyma* spp.) (Stosiek et al., 2019). Interestingly, representatives of *Penicillium* spp. and *Trichoderma* spp. were also found to exhibit P-solubilizing abilities. *Penicillium guanacastense* was able to solubilize phosphorus in microbiological media containing $AlPO_4$, $Ca_3(PO_4)_2$, and $FePO_4{\cdot}4H_20$, while *Trichoderma koningiopsis* synthesized organic acids to solubilize $Ca_3(PO_4)_2$ at high pH stress (Qiao et al., 2019; Tandon et al., 2020). The afore-described properties are significant for land rehabilitation based on microbial-derived products as they contribute to the improvement of nutrient availability, reduce the number of potentially harmful organisms and detoxify pollutants.

A broader analysis of the genetic diversity of microorganisms in soil fertilized with phosphorus biofertilizer was conducted using NGS and metataxonomic approach. Venn diagrams were drawn in order to expose the number and the seasonal distribution of OTUs identified at order level within microbial communities. The high number of OTUs between individual treatments indicates the presence of many commonly occurring microorganisms, regardless of fertilization regime. Particularly noteworthy is the fact the studied soil was dominated by Rhizobiales and Actinomycetales, some representatives of which were previously characterized for their beneficial features toward plant growth and development and improvement of soil quality. Rhizobiales, apart from nitrogen-fixing properties, synthesize plant hormones and improve nutrient uptake. Actinomycetales are involved in P solubilization in the soil, suppress pathogens such as *Pythium ultimum* and *Erwinia carotovora*, excrete siderophores, increase nutrient uptake and boost plant growth (AbdElgawad et al., 2020; Erlacher et al., 2015). Beneficial properties including synthesis of plant hormones (abscisic acid, gibberellins, and zeatin) were also found among *Sphingomonas* spp. which belong to the Sphingomonadales (Sun et al., 2021). Furthermore, the highest relative abundance of aforementioned orders was reported in FA100 in S19, which may suggest the beneficial impact of microbiologically enriched phosphorus biofertilizer. Taking into consideration fact that the relative abundance of these bacterial orders increased also in FC(S19) as compared to FC(A18), application of phosphorus biofertilizer in optimal dose may stimulate seasonal changes in bacterial communities. Simultaneously, the fungal community comprised mainly of Eurotiales, Hypocreales and Mortierellales. It is worth mentioning that most representatives of Eurotiales are

saprotrophs and exert high diversity in metabolic abilities (Geiser et al., 2006), Hypocreales encompasses wide range of entomopathogenic fungi (Barnett & Johnson, 2013) and some species within Mortierellales (e.g. *Mortierella globalpina*, *Mortierella elongata*) are involved in phosphorus cycling in soil (Mącik, Gryta, Sas-Paszt, & Frąc, 2020; Ozimek & Hanaka, 2021). The domination of saprotrophic Eurotiales remains in agreement with FUNGuild results which showed that saprotrophs was main ecological fungal guild throughout the experiment.

Considering the increase in the number of bacterial order level OTUs in FA100 and FA60, it can be assumed that the application of biofertilizers contribute to a favorable shift in the composition of microbiome. The obtained results remained in agreement with Shen et al. (2015) who observed increase in bacterial diversity in soil amended with biofertilizer in 2-year experiment. However, the positive impact of biofertilizers on genetic diversity of soil microorganisms have been described by the researchers. In study conducted by Dong et al. (2019) it was reported that application of biofertilizer increased bacterial diversity, increased the relative abundance of *Bacillus*, *Burkholderia*, *Rhizobium*, and *Streptomyces* which are considered as a potentially beneficial microorganism and lowered the relative abundance of *Fusarium*. A result obtained from Wang, Liu, et al. (2021) showed that soil amended with *Bacillus subtilis* was characterized by higher number of OTUs and increased Chao1 and Shannon indices as compared to non-inoculated control. Nevertheless, in case of fungi, total number of OTUs remained at the same amount in FC and FA100, with slight decrease in FA60, which may be associated with the less content of nutrients provided with the 40% reduced dose of mineral fertilizer. It is worth emphasizing that one the fungal orders specific for FA100 was Teloschistales, which comprise more than a 1000 lichenized species (Gaya et al., 2015). The analysis of the seasonal shifts in soil microbiome within particular treatments showed that the A18 and A19 treatments were characterized by the higher number of specifically occurring OTUs, which is inextricably linked with the greater genetic diversity. Furthermore, the greater seasonal variation in FA100 and FA60 may also be indicated by the lower number of OTUs constituting the core microbiome in the aforementioned treatments as compared to control. Overall, the relative abundance of identified orders was affected to greater extent than their number. A similar trend at the phylum and class level was observed in fungal communities in study conducted by Wen et al. (2020). The increase in the genetic diversity in soil microbiome may be also supported by the higher Shannon index values, especially in FA100-FA60(A19) as compared to control. Analyzing H values in soil microbiome it was reported that bacteria were characterized by the greater diversity than fungi, which is also confirmed by the number of identified OTUs at order level.

Predictive functional profiling of soil bacterial communities, based on the 16S rDNA data, revealed that the application of phosphorus biofertilizer affected all metabolic pathways identified with KEGG database, also biotransformations and processes associated with P-compounds. Improved energy metabolism was evidenced by, i.e., enhanced oxidative phosphorylation, which may indicate the increased ATP synthesis, main energy carrier in intracellular metabolic processes (Chen & Nielsen, 2019). The higher number of OTUs associated with lipid metabolism was linked with the enhanced glycerophospholipid metabolism. Gram negative bacteria cell wall membranes, which determines survival under stress conditions and the proper cell functioning, are mainly composed of phospholipids (Chi et al., 2020). Hence, the higher metabolism of abovementioned P-containing compounds may result in better adaptation of soil bacteria to not only environmental factors but also new fertilization regime (Srour et al., 2020). Another P-related pathways identified among 'Metabolism' main KEGG class included phosphonate and phosphinate metabolism, pentose phosphate pathway and inositol phosphate metabolism. Certain microorganisms (Planctomycetes, Cyanobacteria, and Firmicutes) were found to utilize phosphonates, thus increasing the amount of available P in the soil (Tapia-Torres et al., 2016) and, interestingly, representatives of Firmicutes (*Clostridium* spp., *Staphylococcus* spp.) and Cyanobacteria (*Anabaenopsis* spp.) were identified with TRiFLe software in this study. According to Bore et al. (2017) intensification of pentose phosphate pathway is inextricably linked with increased NADPH synthesis, a compound involved in protein and lipid biosynthesis. On the other hand, phosphorylated inositol forms may constitute the P source in the soil (Herrou & Crosson, 2013). P-associated processes were also found in 'Environmental Information Processing' and encompassed "Phosphotransferase system" (PTS) and "Phosphatidylinositol signaling system". PTS is a translocation system which combines carbohydrates (e g. glucose, fructose, lactose, mannitol) uptake with their phosphorylation. In general, PTS system provide bacteria with a favourable pathway to obtain sugars from the environment (Erni, 2013). On the other hand, phosphatidylinositol may be a signal mediator in salt stress response in mycobacteria (Morita et al., 2010).

Apart from phosphorus compounds transformations, an important aspect, in terms of improving soil health, is the removal of contaminants such as xenobiotics (Mishra et al., 2021) The higher number of sequences assigned to xenobiotics biodegradation and metabolism may indicate that phosphorus biofertilizer stimulate bacterial bioremediation potential toward specific substances. What is more, the high genetic diversity of soil microbiome inhabiting studied soil may be confirmed by the occurrence of some metabolic pathways. According to Xun et al. (2019) genes involved in 'Xenobiotics biodegradation and metabolism' and 'Metabolism of terpenoids and polyketides' are carried by a small group of soil microorganisms (Actinobacteria), hence biofertilizer may stimulate the activity of microbial groups demonstrating highly specialized functions.

Fungal functional profile, constructed based on the ITS data, revealed the presence of ecological guilds useful for soil health improvement. Saprotrophic fungi (involved in decomposition of substances including wood, dung, and plant litter) are known to increase nutrient content, improve water retention and participate in carbon cycling (Clocchiatti et al., 2020), whereas ectomycorrhizal and arbuscular mycorrhizal fungi stimulate rooting and provide plants with essential mineral components, including phosphorus (Frąc et al., 2018).

β-diversity expressed by PcoA, UPGMA, and NMDS analyses showed that the sampling time was main factor influencing the composition of soil microbiome. The distinct clustering of treatments according to sampling time was associated with the relative abundance of particular microbial groups. Based on the UPGMA clustering, bacterial communities were more similar to each other in A19 and A18, so application of biofertilizer may resulted in only temporary changes that did not persist over time. This may also be related to the rapid adaptation of the soil bacteria to the new fertilization regime and stimulation of the occurrence of selected microbial groups during warm season. On the other hand, changes in the relative abundance did not reflect variations in functional profiles, as the A19 treatments were associated with the highest number of functional OTUs. Analyzing shifts in the fungal communities, treatments were separated according to the year, so it may indicate that the introduction of biofertilizer resulted in variations in the relative abundance that persisted from summer to autumn, which may be also supported by PCoA analysis. This may also point to slower adjustment of fungal communities to agrotechnical procedures such as fertilization. Moreover, the distinction of FA100 and FA60 from FC showed that biofertilizer shaped the soil environment and microbial communities tried to adopt to the new conditions. Grouping treatments according to soil sampling time was also observed in study conducted by Yang et al. (2020), where soil samples (under wheat cultivation), inoculated with mixture of beneficial bacteria (including *Azospirillam brasilense*, *Bacillus subtilis*, *Bacillus licheniformis,* and *Bacillus mucilaginosus*) formed clusters (in PCoA and UPGMA) encompassing samples collected in tillering period, turning green period, grain filling period, and maturity period, respectively.

In NMDS ordination space the close promixity of smaller points indicates that treatments are more similar to each other in the composition of microorganisms; simultaneously greater distances between points reflects greater variations in the biodiversity of bacterial and fungal communities (Galitskaya et al., 2021). In our study, the distances between smaller points corresponding to individual samples were relatively short within A19 treatments as compared to other sampling times, which may infer that some balance in the composition of the soil microbiome has been achieved. According to Wang, Liu, et al. (2021) microbial communities, faced with different fertilization techniques, may remain more stable in long-term studies as compared to short experimental period. Taking into consideration growing number of OTUs along with the sampling time, we hypothesize that the application of biofertilizers in long-term studies may induce changes in microbial communities at the gene level. The distinction of mineral fertilization from organically amended soil in NMDS plots was also reported in study conducted by Francioli et al. (2016).

5 | CONCLUSIONS

Unsustainable land management in agroecosystems may result in soil degradation and reduction in the quality of soil microbiome. In view of the importance of soil microorganisms for the proper functioning of arable lands, we proposed the application of phosphorus mineral fertilizer enriched with strains of beneficial bacteria and highlighted the improvement of microbiological indicators of soil health including soil enzymatic activity, occurrence of microorganisms exerting plant growth promoting properties and increased genetic diversity among microbial communities. We have evaluated the effect of phosphorus biofertilizer expressed by the enhanced maize yield and increased number of OTUs associated with phosphorus processes. We have also emphasized seasonal changes in the status of soil microbiome. Throughout the experimental period we have observed variations in soil environment leading to certain stability in the autumn 2019 which may be a result of adaptation of indigenous microbiota to new fertilization regime. Our results indicate that phosphorus biofertilizer is an ecofriendly and effective alternative to traditional mineral fertilizers and has potential applications in sustainable agriculture and soil management practices based on microbial-derived preparations.

ACKNOWLEDGMENTS

We are grateful to Agnieszka Rutkowska from the Institute of Soil Science and Plant Cultivation in Puławy, Poland for calculating doses of mineral fertilizers and biofertilizers used in this study.

The work was supported by The National Centre for Research and Development within the framework of the project BIOSTRATEG, contract number BIOSTRATEG3/347464/5/NCBR/2017 "BIO-FERTIL."

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available within manuscript and supplementarry files.

ORCID

Magdalena Frąc https://orcid.org/0000-0001-9437-3139

REFERENCES

AbdElgawad, H., Abuelsoud, W., Madany, M. M. Y., Selim, S., Zinta, G., Mousa, A. S. M., & Hozzein, W. N. (2020). Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. *Biomolecules*, *10*, 1675. https://doi.org/10.3390/biom10121675

Alef, K., & Nannipieri, P. (1995). Enzyme activities. In K. Alef & P. Nannipieri (Eds.), *Methods in applied soil microbiology and biochemistry* (pp. 311–373). London: Academic Press. https://doi.org/10.1016/b978-012513840-6/50022-7

Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology*, *8*, 971. https://doi.org/10.3389/fmicb.2017.00971

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Badri, D. V., & Vivanco, J. M. (2009). Regulation and function of root exudates. *Plant, Cell and Environment*, *32*(6), 666–681. https://doi.org/10.1111/j.1365-3040.2009.01926.x

Barnes, C. J., Maldonado, C., Frøslev, T. G., Antonelli, A., & Rønsted, N. (2016). Unexpectedly high beta-diversity of root-associated fungal communities in the Bolivian Andes. *Frontiers in Microbiology*, 7, 1377. https://doi.org/10.3389/fmicb.2016.01377

Barnett, K., & Johnson, S. N. (2013). Living in the soil matrix: Abiotic factors affecting root herbivores. In S. N. Johnson, I. Hiltpold, & T. Turlings (Eds.), *Advances in insect physiology: Behaviour and physiology of root herbivores*, *vol. 45* (pp. 1–52). London: Academic Press. https://doi.org/10.1016/B978-0-12-417165-7.00001-5

Bertola, M., Ferrarini, A., & Visioli, G. (2021). Improvement of soil microbial diversity through sustainable agricultural practices and its evaluation by-omics approaches: A perspective for the environment, food quality and human safety. *Microorganisms*, *9*, 1400. https://doi.org/10.3390/microorganisms9071400

Bhardwaj, D., Ansari, M. W., Sahoo, R. K., & Tuteja, N. (2014). Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial Cell Factories*, *13*, 66. https://doi.org/10.1186/1475-2859-13-66

Bleuven, C., & Landry, C. R. (2016). Molecular and cellular bases of adaptation to a changing environment in microorganisms. *Proceedings of the Royal Society B: Biological Sciences*, *283*, 20161458. https://doi.org/10.1098/rspb.2016.1458

Boguta, P., Skic, K., Sokołowska, Z., Frąc, M., & Sas-Paszt, L. (2021). Chemical transformation of humic acid molecules under the influence of mineral, fungal and bacterial fertilization in the context of the agricultural use of degraded soils. *Molecules*, *26*, 4921. https://doi.org/10.3390/molecules26164921

Bore, E. K., Apostel, C., Halicki, S., Kuzyakov, Y., & Dippold, M. A. (2017). Microbial metabolism in soil at subzero temperatures: Adaptation mechanisms revealed by position-specific 13C labeling. *Frontiers in Microbiology*, *8*, 946. https://doi.org/10.3389/fmicb.2017.00946

Borowik, K., Rusek, P., Schab, S. & Rusek, Ł. (2019). Biofertilizer and method of its production. Patent Application No. 431350. Puławy, Poland.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., & Wanek, W. (2019). Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*, *10*, 157. https://doi.org/10.3389/fpls.2019.00157

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Cha, I. T., Min, U. G., Kim, S. J., Yim, K. J., Roh, S. W., & Rhee, S. K. (2013). *Methanomethylovorans uponensis* sp. nov., a methylotrophic methanogen isolated from wetland sediment. *Antonie Van Leeuwenhoek*, *104*(6), 1005–1012. https://doi.org/10.1007/s10482-013-0020-4

Chen, D., Huang, J., & Yuan, L. (2019). A new function of the biocontrol bacterium *Lysobacter enzymogenes* LE16 in the mineralization of soil organic phosphorus. *Plant and Soil*, *442*, 299–309. https://doi.org/10.1007/s11104-019-04175-x

Chen, D. M., Yang, H. J., Huang, J. G., & Yuan, L. (2020). *Lysobacter enzymogenes* LE16 autolysates have potential as biocontrol agents—*Lysobacter* sp. autolysates as biofungicide. *Journal of Applied Microbiology*, *129*(6), 1684–1692. https://doi.org/10.1111/jam.14752

Chen, Y., Li, S., Liu, N., He, H., Cao, X., Lv, C., Zhang, K., & Dai, J. (2021). Effects of different types of microbial inoculants on available nitrogen and phosphorus, soil microbial community, and wheat growth in high-P soil. *Environmental Science and Pollution Research*, *28*(18), 23036–23047. https://doi.org/10.1007/s11356-020-12203-y

Chen, Y., & Nielsen, J. (2019). Energy metabolism controls phenotypes by protein efficiency and allocation. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(35), 17592–17597. https://doi.org/10.1073/pnas.1906569116

Chi, X., Fan, Q., Zhang, Y., Liang, K., Wan, L., Zhou, Q., & Li, Y. (2020). Structural mechanism of phospholipids translocation by MlaFEDB complex. *Cell Research*, *30*(12), 1127–1135. https://doi.org/10.1038/s41422-020-00404-6

Clocchiatti, A., Hannula, S. E., van den Berg, M., Korthals, G., & de Boer, W. (2020). The hidden potential of saprotrophic fungi in arable soil: Patterns of short-term stimulation by organic amendments. *Applied Soil Ecology*, *147*, 103434. https://doi.org/10.1016/j.apsoil.2019.103434

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., & Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, *72*(7), 5069–5072. https://doi.org/10.1128/AEM.03006-05

Dick, R. P., & Kandeler, E. (2005). Enzymes in soils. In D. Hillel (Ed.), *Encyclopedia of soils in the environment* (pp. 448–456). Amsterdam: Elsevier. https://doi.org/10.1016/B0-12-348530-4/00146-6

Dong, L., Li, Y., Xu, J., Yang, J., Wei, G., Shen, L., Ding, W., & Chen, S. (2019). Biofertilizers regulate the soil microbial community and enhance *Panax ginseng* yields. *Chinese Medicine*, *14*, 20. https://doi.org/10.1186/s13020-019-0241-1

Edgar, R. C. (2010). Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, *26*(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Eivazi, F., & Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, *20*(5), 601–606. https://doi.org/10.1016/0038-0717(88)90141-1

Erlacher, A., Cernava, T., Cardinale, M., Soh, J., Sensen, C. W., Grube, M., & Berg, G. (2015). Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. *Frontiers in Microbiology*, *6*, 53. https://doi.org/10.3389/fmicb.2015.00053

Erni, B. (2013). The bacterial phosphoenolpyruvate: Sugar phosphotransferase system (PTS): An interface between energy and signal transduction. *Journal of the Iranian Chemical Society*, *10*(3), 593–630. https://doi.org/10.1007/s13738-012-0185-1

Faria, M., Martins, I., Souza, D. A., Mascarin, G. M., & Lopes, R. B. (2017). Susceptibility of the biocontrol fungi *Metarhizium anisopliae* and *Trichoderma asperellum* (Ascomycota: Hypocreales) to imbibitional damage is driven by conidial vigor. *Biological Control*, *107*, 87–94. https://doi.org/10.1016/j.biocontrol.2017.01.015

Ferry, J. G. (2020). *Methanosarcina acetivorans*: A model for mechanistic understanding of aceticlastic and reverse methanogenesis. *Frontiers in Microbiology*, *11*, 1806. https://doi.org/10.3389/fmicb.2020.01806

Frąc, M., Hannula, S. E., Belka, M., & J dryczka, M. (2018). Fungal biodiversity and their role in soil health. *Frontiers in Microbiology*, *9*, 707. https://doi.org/10.3389/fmicb.2018.00707

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F., & Reitz, T. (2016). Mineral vs. organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Frontiers in Microbiology*, *7*, 1446. https://doi.org/10.3389/fmicb.2016.01446

Furtak, K., Grządziel, J., Gałązka, A., & Niedźwiecki, J. (2020). Prevalence of unclassified bacteria in the soil bacterial community from floodplain meadows (Fluvisols) under simulated flood conditions revealed by a metataxonomic approachss. *Catena*, *188*, 104448. https://doi.org/10.1016/j.catena.2019.104448

Galitskaya, P., Biktasheva, L., Blagodatsky, S., & Selivanovskaya, S. (2021). Response of bacterial and fungal communities to high petroleum pollution in different soils. *Scientific Reports*, *11*, 164. https://doi.org/10.1038/s41598-020-80631-4

Gardes, M., & Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. *Molecular Ecology*, *2*(2), 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x

Gaya, E., Fernández-Brime, S., Vargas, R., Lachlan, R. F., Gueidang, C., Ramírez-Mejía, M., & Lutzoni, F. (2015). The adaptive radiation of lichen-forming Teloschistaceae is associated with sunscreening pigments and a bark-to-rock substrate shift. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(37), 11600–11605. https://doi.org/10.1073/pnas.1507072112

Geiser, D. M., Gueidan, C., Miadlikowska, J., Lutzoni, F., Kauff, F., Hofstetter, V., Fraker, E., Schoch, C. L., Tibell, L., Untereiner, W. A., & Aptroot, A. (2006). Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae. *Mycologia*, *98*(6), 1053–1064. https://doi.org/10.3852/mycologia.98.6.1053

Geisseler, D., & Scow, K. M. (2014). Long-term effects of mineral fertilizers on soil microorganisms—A review. *Soil Biology and Biochemistry*, *75*, 54–63. https://doi.org/10.1016/j.soilbio.2014.03.023

Geng, Y., Cao, G., Wang, L., & Wang, S. (2019). Effects of equal chemical fertilizer substitutions with organic manure on yield, dry matter, and nitrogen uptake of spring maize and soil nitrogen distribution. *PLoS One*, *14*(7), e0219512. https://doi.org/10.1371/journal.pone.0219512

Golyshina, O. V. (2011). Environmental, biogeographic, and biochemical patterns of archaea of the family Ferroplasmaceae. *Applied and Environmental Microbiology*, *77*(15), 5071–5078. https://doi.org/10.1128/AEM.00726-11

Gomiero, T. (2016). Soil degradation, land scarcity and food security: Reviewing a complex challenge. *Sustainability*, *8*, 261. https://doi.org/10.3390/su8030281

Gryta, A., & Frąc, M. (2020). Methodological aspects of multiplex terminal restriction fragment length polymorphism-technique to describe the genetic diversity of soil bacteria, archaea and fungi. *Sensors*, *20*, 3292. https://doi.org/10.3390/s20113292

Gryta, A., Frąc, M., & Oszust, K. (2014). The application of the biolog EcoPlate approach in Ecotoxicological evaluation of dairy sewage sludge. *Applied Biochemistry and Biotechnology*, *174*, 1434–1443. https://doi.org/10.1007/s12010-014-1131-8

Hallama, M., Pekrun, C., Pilz, S., Jarosch, K. A., Frąc, M., Uksa, M., Marhan, S., & Kandeler, E. (2021). Interactions between cover crops and soil microorganisms increase phosphorus availability in conservation agriculture. *Plant and Soil*, *463*, 307–328. https://doi.org/10.1007/s11104-021-04897-x

Hartman, K., van der Heijden, M. G. A., Wittwer, R. A., Banerjee, S., Walser, J. C., & Schlaeppi, K. (2018). Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome*, *6*, 114. https://doi.org/10.1186/s40168-017-0389-9

Hauben, L., Vauterin, L., Swings, J., & Moore, E. R. B. (1997). Comparison of 16S ribosomal DNA sequences of all *Xanthomonas* species. *International Journal of Systematic Bacteriology*, *47*(2), 328–335. https://doi.org/10.1099/00207713-47-2-328

Heidari, E., Mohammadi, K., Pasari, B., Rokhzadi, A., & Sohrabi, Y. (2019). Combining the phosphate solubilizing microorganisms with biochar types in order to improve safflower yield and soil enzyme activity. *Soil Science and Plant Nutrition*, *66*(2), 255–267. https://doi.org/10.1080/00380768.2019.1704180

Hellequin, E., Monard, C., Chorin, M., Le bris, N., Daburon, V., Klarzynski, O., & Binet, F. (2020). Responses of active soil microorganisms facing to a soil biostimulant input compared to plant legacy effects. *Scientific Reports*, *10*, 13727. doi: https://doi.org/10.1038/s41598-020-70695-7

Herrou, J., & Crosson, S. (2013). Myo-inositol and D-ribose ligand discrimination in an ABC periplasmic binding protein. *Journal of Bacteriology*, *195*(10), 2379–2388. https://doi.org/10.1128/JB.00116-13

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—Current knowledge and future directions. *Frontiers in Plant Science*, *8*, 1617. https://doi.org/10.3389/fpls.2017.01617

Jia, T., Guo, T., Yao, Y., Wang, R., & Chai, B. (2020). Seasonal microbial community characteristic and its driving factors in a copper tailings dam in the Chinese Loess Plateau. *Frontiers in Microbiology*, *11*, 1574. https://doi.org/10.3389/fmicb.2020.01574

Junier, P., Junier, T., & Witzel, K. P. (2008). TRiFLe, a program for in silico terminal restriction fragment length polymorphism analysis with user-defined sequence sets. *Applied and Environmental Microbiology*, *74*(20), 6452–6456. https://doi.org/10.1128/AEM.01394-08

Jurgens, G., Lindström, K., & Saano, A. (1997). Novel group within the kingdom Crenarchaeota from Boreal forest soil. *Applied and Environmental Microbiology*, *63*(2), 803–805. https://doi.org/10.1128/aem.63.2.803-805.1997

Jurgens, G., & Saano, A. (1999). Diversity of soil archaea in boreal forest before, and after clear-cutting and prescribed burning. *FEMS Microbiology Ecology*, *29*(2), 205–213. https://doi.org/10.1016/S0168-6496(99)00013-6

Kandasamy, S., Liu, E. Y. R., Patterson, G., Saldias, S., Ali, S., & Lazarovits, G. (2019). Introducing key microbes from high productive soil transforms native soil microbial community of low productive soil. *MicrobiologyOpen*, *8*(10), e895. https://doi.org/10.1002/mbo3.895

Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto encyclopedia of genes and genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

KEGG (2021). Kyoto Encyclopedia of Genes and Genomes. Retrieved from http://www.kegg.jp/. no doi

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research*, *41*(1), e1. https://doi.org/10.1093/nar/gks808

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K. H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., & Richter, A. (2013). Seasonal variation in functional properties of microbial communities in beech forest soil. *Soil Biology & Biochemistry*, *60*, 95–104. https://doi.org/10.1016/j.soilbio.2013.01.025

Kour, D., Rana, K. L., Yadav, A. N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H. S., & Saxena, A. K. (2020). Microbial biofertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. *Biocatalysis and Agricultural Biotechnology*, *23*, 101487. https://doi.org/10.1016/j.bcab.2019.101487

Kwiatkowski, C. A., Harasim, E., Feledyn-Szewczyk, B., & Antonkiewicz, J. (2020). Enzymatic activity of loess soil in organic and conventional farming systems. *Agriculture*, *10*, 135. https://doi.org/10.3390/agriculture10040135

Lacerda-Júnior, G. V., Noronha, M. F., Cabral, L., Delforno, T. P., De Sousa, S. T. P., Fernandes-Júnior, P. I., Melo, I. S., & Oliveira, V. M. (2019). Land use and seasonal effects on the soil microbiome of a Brazilian dry forest. *Frontiers in Microbiology*, *10*, 648. https://doi.org/10.3389/fmicb.2019.00648

Ladd, J. N., & Butler, J. H. A. (1972). Short-term assays of soil proteolytic enzyme activities using proteins and dipeptide derivatives as substrates. *Soil Biology and Biochemistry*, *4*(1), 19–30. https://doi.org/10.1016/0038-0717(72)90038-7

Langille, M. G. I., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., Clemente, J. C., Burkepile, D. E., Vega Thurber, R. L., Knight, R., Beiko, R. G., & Huttenhower, C. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, *31*(9), 814–821. https://doi.org/10.1038/nbt.2676

Lee, S. H., Kim, M. S., Kim, J. G., & Kim, S. O. (2020). Use of soil enzymes as indicators for contaminated soil monitoring and sustainable management. *Sustainability*, *12*, 8209. https://doi.org/10.3390/su12198209

Li, J. Y., Gao, T. T., & Wang, Q. (2020). Comparative and functional analyses of two sequenced *Paenibacillus polymyxa* genomes provides insights into their potential genes related to plant growth-promoting features and biocontrol mechanisms. *Frontiers in Genetics*, *11*, 564939. https://doi.org/10.3389/fgene.2020.564939

Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z., & Lin, W. (2019). The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards. *PLoS One*, *14*(5), e0217018. https://doi.org/10.1371/journal.pone.0217018

Lindström, K., & Mousavi, S. A. (2020). Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology*, *13*(5), 1314–1335. https://doi.org/10.1111/1751-7915.13517

Linu, M. S., Asok, A. K., Thampi, M., Sreekumar, J., & Jisha, M. S. (2019). Plant growth promoting traits of indigenous phosphate solubilizing *Pseudomonas aeruginosa* isolates from Chilli (*Capsicum*annuum L.) rhizosphere. *Communications in Soil Science and Plant Analysis*, *50*(4), 444–457. https://doi.org/10.1080/00103624.2019.1566469

López, D. N., Camus, P. A., Valdivia, N., & Estay, S. A. (2019). Integrating species and interactions into similarity metrics: A graph theory-based approach to understanding community similarity. *PeerJ*, *7*, e7013. https://doi.org/10.7717/peerj.7013

Mącik, M., Gryta, A., & Frąc, M. (2020). Biofertilizers in agriculture: An overview on concepts, strategies and effects on soil microorganisms. In D. L. Sparks (Ed.), *Advances in agronomy*, *vol. 162* (pp. 31–87). Academic Press. https://doi.org/10.1016/bs.agron.2020.02.001

Mącik, M., Gryta, A., Sas-Paszt, L., & Frąc, M. (2020). The status of soil microbiome as affected by the application of phosphorus biofertilizer: Fertilizer enriched with beneficial bacterial strains. *International Journal of Molecular Sciences*, *21*, 8003. https://doi.org/10.3390/ijms21218003

Malíček, J., Palice, Z., Vondrák, J., Łubek, A., & Kukwa, M. (2018). *Bacidia albogranulosa* (Ramalinaceae, lichenized Ascomycota), a new sorediate lichen from European old-growth forests. *MycoKeys*, *44*, 51–62. https://doi.org/10.3897/mycokeys.44.30199

Marchesi, J. R., Sato, T., Weightman, A. J., Martin, T. A., Fry, J. C., Hiom, S. J., & Wade, W. G. (1998). Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology*, *64*(2), 795–799. https://doi.org/10.1128/AEM.64.2.795-799.1998

Mei, C., Chretien, R. L., Amaradasa, B. S., He, Y., Turner, A., & Lowman, S. (2021). Characterization of phosphate solubilizing bacterial endophytes and plant growth promotion in vitro and in greenhouse. *Microorganisms*, *9*, 1935. https://doi.org/10.3390/microorganisms9091935

Mengual, C., Schoebitz, M., Azcón, R., & Roldán, A. (2014). Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. *Journal of Environmental Management*, *134*, 1–7. https://doi.org/10.1016/j.jenvman.2014.01.008

Mishra, S., Lin, Z., Pang, S., Zhang, W., Bhatt, P., & Chen, S. (2021). Recent advanced technologies for the characterization of xenobiotic-degrading microorganisms and microbial communities. *Frontiers in Bioengineering and Biotechnology*, *9*, 632059. https://doi.org/10.3389/fbioe.2021.632059

Mitra, D., Anđelković, S., Panneerselvam, P., Senapati, A., Vasić, T., Ganeshamurthy, A. N., Chauhan, M., Uniyal, N., Mahakur, B., & Radha, T. K. (2020). Phosphate-solubilizing microbes and biocontrol agent for plant nutrition and protection: Current perspective. *Communications in Soil Science and Plant Analysis*, *51*(5), 645–657. https://doi.org/10.1080/00103624.2020.1729379

Moe, L. A. (2013). Amino acids in the rhizosphere: From plants to microbes. *American Journal of Botany*, *100*(9), 1692–1705. https://doi.org/10.3732/ajb.1300033

Mori, K., Iino, T., Suzuki, K. I., Yamaguchi, K., & Kamagata, Y. (2012). Aceticlastic and NaCl-requiring methanogen "*Methanosaeta pelagica*" sp. nov., isolated from marine tidal flat sediment Koji. *Applied and Environmental Microbiology*, *78*(9), 3416–3423. https://doi.org/10.1128/AEM.07484-11

Morita, Y. S., Yamaryo-Botte, Y., Miyanagi, K., Callaghan, J. M., Patterson, J. H., Crellin, P. K., Coppel, R. L., Billman-Jacobe, H., Kinoshita, T., & McConville, M. J. (2010). Stress-induced synthesis of phosphatidylinositol 3-phosphate in mycobacteria. *Journal of Biological Chemistry*, *285*(22), 16643–16650. https://doi.org/10.1074/jbc.M110.119263

Mukhtar, H., Lin, Y. P., Lin, C. M., & Lin, Y. R. (2019). Relative abundance of ammonia oxidizing archaea and bacteria influences soil nitrification responses to temperature. *Microorganisms*, *7*, 526. https://doi.org/10.3390/microorganisms7110526

Müller, K., Kubsch, N., Marhan, S., Mayer-Gruner, P., Nassal, P., Schneider, D., Daniel, R., Piepho, H., Polle, A., & Kandeler, E. (2020). Saprotrophic and ectomycorrhizal fungi contribute differentially to organic P mobilization in beech-dominated forest ecosystems. *Frontiers in Forests and Global Change*, *3*, 47. https://doi.org/10.3389/ffgc.2020.00047

Naziya, B., Murali, M., & Amruthesh, K. N. (2020). Plant growth-promoting fungi (PGPF) instigate plant growth and induce disease resistance in *Capsicum annuum* L. upon infection with *Colletotrichum capsici* (Syd.) Butler & Bisby. *Biomolecules*, *10*, 41. https://doi.org/10.3390/biom10010041

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Nilsson, R. H., Larsson, K. H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F. O., Tedersoo, L., Saar, I., Kõljalg, U., & Abarenkov, K. (2019). The UNITE database for molecular identification of fungi: Handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, *47*(D1), D259–D264. https://doi.org/10.1093/nar/gky1022

Nunes, J. S., Araujo, A. S. F., Nunes, L. A. P. L., Lima, L. M., Carneiro, R. F. V., Salviano, A. A. C., & Tsai, S. M. (2012). Impact of land degradation on soil microbial biomass and activity in Northeast Brazil. *Pedosphere*, *22*(1), 88–95. https://doi.org/10.1016/S1002-0160(11)60194-X

Ozimek, E., & Hanaka, A. (2021). *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. *Agriculture*, *11*, 7. https://doi.org/10.3390/agriculture11010007

Pavlidis, P., & Noble, W. S. (2003). Matrix2png: A utility for visualizing matrix data. *Bioinformatics*, *19*(2), 295–296. https://doi.org/10.1093/bioinformatics/19.2.295

Pertile, G., Lamorski, K., Bieganowski, A., Boguta, P., Brzezińska, M., Polakowski, C., Skic, K., Sokołowska, Z., Baranowski, P., Gackiewicz, B., Rutkowska, A., Trzciński, P., Sas-Paszt, L., & Frąc, M. (2021). Immediate effects of the application of various fungal strains with urea fertiliser on microbiome structure and functions and their relationships with the physicochemical parameters of two different soil types. *Applied Soil Ecology*, *163*, 103972. https://doi.org/10.1016/j.apsoil.2021.103972

Polish Standard PN-R-04023 (1996). Agrochemical soil analisis—Determination of assimilated phosphorus content. Warsaw, Polish Committee for Standardization.

Qiao, H., Sun, X. R., Wu, X. Q., Li, G. E., Wang, Z., & Li, D. W. (2019). The phosphate-solubilizing ability of *Penicillium guanacastense* and its effects on the growth of *Pinus massoniana* in phosphate-limiting conditions. *Biology Open*, *8*, bio046797. https://doi.org/10.1242/bio.046797

R Core Team. (2018). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing Retrieved from https://www.r-project.org/. no doi

Radhakrishnan, R., Hashem, A., & Abd Allah, E. F. (2017). *Bacillus*: A biological tool for crop improvement through bio-molecular changes in adverse environments. *Frontiers in Physiology*, *8*, 667. https://doi.org/10.3389/fphys.2017.00667

Rousk, J., & Bååth, E. (2007). Fungal and bacterial growth in soil with plant materials of different C/N ratios. *FEMS Microbiology Ecology*, *62*(3), 258–267. https://doi.org/10.1111/j.1574-6941.2007.00398.x

Schmidt, J. E., & Gaudin, A. C. M. (2018). What is the agronomic potential of biofertilizers for maize? A meta-analysis. *FEMS Microbiology Ecology*, *94*(7), fiy094. https://doi.org/10.1093/femsec/fiy094

Schmidt, P. A., Bálint, M., Greshake, B., Bandow, C., Römbke, J., & Schmitt, I. (2013). Illumina metabarcoding of a soil fungal community. *Soil Biology and Biochemistry*, *65*, 128–132. https://doi.org/10.1016/j.soilbio.2013.05.014

Shen, Z., Ruan, Y., Chao, X., Zhang, J., Li, R., & Shen, Q. (2015). Rhizosphere microbial community manipulated by 2 years of consecutive biofertilizer application associated with banana *Fusarium* wilt disease suppression. *Biology and Fertility of Soils*, *51*(5), 553–562. https://doi.org/10.1007/s00374-015-1002-7

Siedliska, A., Baranowski, P., Pastuszka-Woźniak, J., Zubik, M., & Krzyszczak, J. (2021). Identification of plant leaf phosphorus content at different growth stages based on hyperspectral reflectance. *BMC Plant Biology*, *21*, 28. https://doi.org/10.1186/s12870-020-02807-4

Srour, A. Y., Ammar, H. A., Subedi, A., Pimentel, M., Cook, R. L., Bond, J., & Fakhoury, A. M. (2020). Microbial communities associated with long-term tillage and fertility treatments in a corn-soybean cropping system. *Frontiers in Microbiology*, *11*, 1363. https://doi.org/10.3389/fmicb.2020.01363

Stosiek, N., Terebieniec, A., Ząbek, A., Młynarz, P., Cieśliński, H., & Klimek-Ochab, M. (2019). N-phosphonomethylglycine utilization by the psychrotolerant yeast *Solicoccozyma terricola* M 3.1.4. *Bioorganic Chemistry*, *93*, 102866. https://doi.org/10.1016/j.bioorg.2019.03.040

Sun, M., Chen, B., Wang, H., Wang, N., Ma, T., Cui, Y., Luan, T., Chun, S., Liu, C., & Wang, L. (2021). Microbial interactions and roles in soil fertility in seasonal freeze-thaw periods under different straw returning strategies. *Agriculture*, *11*, 779. https://doi.org/10.3390/agriculture11080779

Sun, Z. B., Li, S. D., Ren, Q., Xu, J. L., Lu, X., & Sun, M. H. (2020). Biology and applications of *Clonostachys rosea*. *Journal of Applied Microbiology*, *129*(3), 486–495. https://doi.org/10.1111/jam.14625

Susanti, D., Frazier, M. C., & Mukhopadhyay, B. (2019). A genetic system for *Methanocaldococcus jannaschii*: An evolutionary deeply rooted Hyperthermophilic Methanarchaeon. *Frontiers in Microbiology*, *10*, 1256. https://doi.org/10.3389/fmicb.2019.01256

Tabatabai, M. A., & Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology and Biochemistry*, *1*(4), 301–307. https://doi.org/10.1016/0038-0717(69)90012-1

Tandon, A., Fatima, T., Deepali, A. S., Tripathi, P., Srivastava, S., & Singh, P. C. (2020). Phosphate solubilization by *Trichoderma koningiopsis* (NBRI-PR5) under abiotic stress conditions. *Journal of King Saud University - Science*, *32*(1), 791–798. https://doi.org/10.1016/j.jksus.2019.02.001

Tapia-Torres, Y., Rodríguez-Torres, M. D., Elser, J. J., Islas, A., Souza, V., García-Oliva, F., & Olmedo-Álvarez, G. (2016). How to live with phosphorus scarcity in soil and sediment: Lessons from bacteria. *Applied and Environmental Microbiology*, *82*(15), 4652–4662. https://doi.org/10.1128/AEM.00160-16

Trabelsi, D., Anmar, H. B., Mengoni, A., & Mhamdi, R. (2012). Appraisal of the crop-rotation effect of rhizobial inoculation on potato cropping systems in relation to soil bacterial communities. *Soil Biology and Biochemistry*, *54*, 1–6. https://doi.org/10.1016/j.soilbio.2012.05.013

Trabelsi, D., Mengoni, A., Anmar, H. B., & Mhamdi, R. (2011). Effect of on-field inoculation of *Phaseolus vulgaris* with rhizobia on soil bacterial communities. *FEMS Microbiology Ecology*, *77*(1), 211–222. https://doi.org/10.1111/j.1574-6941.2011.01102.x

Treindl, A. D., & Leuchtmann, A. (2019). A king amongst dwarfs: *Boletus edulis* forms ectomycorrhiza with dwarf willow in the Swiss Alps. *Alpine Botany*, *129*(2), 185–189. https://doi.org/10.1007/s00035-019-00218-2

Tutiempo (2021) Climate Zamość/Poland; Retrieved from https://en.tutiempo.net/climate/ws-125950.html.

Vilgalys Mycology Lab. (1992). Conserved primer sequences for PCR amplification and sequencing from nuclear ribosomal RNA. Retrieved from https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/. no doi

Vinci, G., Cozzolino, V., Mazzei, P., Monda, H., Savy, D., Drosos, M., & Piccolo, A. (2018). Effects of *Bacillus amyloliquefaciens* and different phosphorus sources on maize plants as revealed by NMR and GC-MS based metabolomics. *Plant and Soil*, *429*, 437–450. https://doi.org/10.1007/s11104-018-3701-y

Walitang, D. I., Kim, C. G., Jeon, S., Kang, Y., & Sa, T. (2019). Conservation and transmission of seed bacterial endophytes across generations following crossbreeding and repeated inbreeding of rice at different geographic locations. *MicrobiologyOpen*, *8*(3), e00662. https://doi.org/10.1002/mbo3.662

Walkiewicz, A., Brzezińska, M., Bieganowski, A., Sas-Paszt, L., & Frąc, M. (2020). Early response of soil microbial biomass and activity to biofertilizer application in degraded Brunic Arenosol and Abruptic Luvisol of contrasting textures. *Agronomy*, *10*, 1347. https://doi.org/10.3390/agronomy10091347

Wang, J., Liu, L., Gao, X., Hao, J., & Wang, M. (2021). Elucidating the effect of biofertilizers on bacterial diversity in maize rhizosphere soil. *PLoS One*, *16*(4), e0249834. https://doi.org/10.1371/journal.pone.0249834

Wang, Y., Peng, S., Hua, Q., Qiu, C., Wu, P., Liu, X., & Lin, X. (2021). The long-term effects of using phosphate-solubilizing bacteria and photosynthetic bacteria as biofertilizers on peanut yield and soil bacteria community. *Frontiers in Microbiology*, *12*, 693535. https://doi.org/10.3389/fmicb.2021.693535

Wen, Y.-C., Li, H.-Y., Lin, Z.-A., Bing-Qiang, Z., Sun, Z.-B., Yuan, L., Xu, J.-K., & Li, Y.-Q. (2020). Long-term fertilization alters soil properties and fungal community composition in Fluvo-Aquic soil of the North China Plain. *Scientific Reports*, *10*, 7198. https://doi.org/10.1038/s41598-020-64227-6

White, T. J., Bruns, T. D., Lee, S., & Taylor, J. (1990). Analysis of phylogenetic relationships by amplification and direct sequencing of ribosomal genes. In M. A. Innis, D. H. Gelfond, J. J. Sainsky, & T. J. White (Eds.), *PCR protocols: A guide to methods and applications* (pp. 315–322). London: Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Xu, C., Du, C., Jian, J., Hou, L., Wang, Z., Wang, Q., & Geng, Z. (2021). The interplay of labile organic carbon, enzyme activities and microbial communities of two forest soils across seasons. *Scientific Reports*, *11*, 5002. https://doi.org/10.1038/s41598-021-84217-6

Xun, W., Li, W., Xiong, W., Ren, Y., Liu, Y., Miao, Y., Xu, Z., Zhang, N., Shen, Q., & Zhang, R. (2019). Diversity-triggered deterministic bacterial assembly constrains community functions. *Nature Communications*, *10*, 3833. https://doi.org/10.1038/s41467-019-11787-5

Yang, W., Gong, T., Wang, J., Li, G., Liu, Y., Zhen, J., Ning, M., Yue, D., Du, Z., & Yue, D. (2020). Effects of compound microbial fertilizer on soil characteristics and yield of wheat (*Triticum aestivum* L.). *Journal of Soil Science and Plant Nutrition*, *20*, 2740–2748. https://doi.org/10.1007/s42729-020-00340-9

Zantua, M. I., & Bremner, J. M. (1977). Stability of urease in soils. *Soil Biology and Biochemistry*, *9*(2), 135–140. https://doi.org/10.1016/0038-0717(77)90050-5

Zhang, H., Wang, R., Chen, S., Qi, G., He, Z., & Zhao, X. (2017). Microbial taxa and functional genes shift in degraded soil with bacterial wilt. *Scientific Reports*, *7*, 39911. https://doi.org/10.1038/srep39911

Zhang, H. H., Tang, M., Chen, H., & Zheng, C. L. (2010). Effects of inoculation with ectomycorrhizal fungi on microbial biomass and bacterial functional diversity in the rhizosphere of *Pinus tabulaeformis* seedlings. *European Journal of Soil Biology*, *46*(1), 55–61. https://doi.org/10.1016/j.ejsobi.2009.10.005

Zhang, N. N., Sun, Y. M., Li, L., Wang, E. T., Chen, W. X., & Yuan, H. L. (2010). Effects of intercropping and *Rhizobium* inoculation on yield and rhizosphere bacterial community of faba bean (*Vicia faba* L.). *Biology and Fertility of Soils*, *46*(6), 625–639. https://doi.org/10.1007/s00374-010-0469-5

Zhang, Z., Han, X., Yan, J., Zou, W., Wang, E., Lu, X., & Chen, X. (2020). Keystone microbiomes revealed by 14 years of field restoration of the degraded agricultural soil under distinct vegetation scenarios. *Frontiers in Microbiology*, *11*, 1915. https://doi.org/10.3389/fmicb.2020.01915

Zhao, K., Penttinen, P., Zhang, X., Ao, X., Liu, M., Yu, X., & Chen, Q. (2014). Maize rhizosphere in Sichuan, China, hosts plant growth promoting *Burkholderia cepacia* with phosphate solubilizing and antifungal abilities. *Microbiological Research*, *169*(1), 76–82. https://doi.org/10.1016/j.micres.2013.07.003

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Mącik, M., Gryta, A., Sas-Paszt, L., & Frąc, M. (2022). Composition, activity and diversity of bacterial and fungal communities responses to inputs of phosphorus fertilizer enriched with beneficial microbes in degraded Brunic Arenosol. *Land Degradation & Development*, *33*(6), 844–865. https://doi.org/10.1002/ldr.4179