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Unveiling the putative functional genes present in root-associated endophytic microbiome from maize plant using the shotgun approach

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Abstract

To ensure food security for the ever-increasing world's population, it is important to explore other alternatives for enhancing plant productivity. This study is aimed at identifying the putative plant growth–promoting (PGP) and endophytic gene clusters in root-associated endophytic microbes from maize root and to also verify if their abundance is affected by different farming practices. To achieve this, we characterize endophytic microbiome genes involved in PGP and endophytic lifestyle inside maize root using the shotgun metagenomic approach. Our results revealed the presence of genes involved in PGP activities such as nitrogen fixation, HCN biosynthesis, siderophore, 4-hydroxybenzoate, ACC deaminase, phenazine, phosphate solubilization, butanediol, methanol utilization, acetoin, nitrogen metabolism, and IAA biosynthesis. We also identify genes involved in stress resistance such as glutathione, catalase, and peroxidase. Our results further revealed the presence of putative genes involved in endophytic behaviors such as aerotaxis, regulator proteins, motility mechanisms, flagellum biosynthesis, nitrogen regulation, regulation of carbon storage, formation of biofilm, reduction of nitric oxide, regulation of beta-lactamase resistance, type III secretion, type IV conjugal DNA, type I pilus assembly, phosphotransferase system (PTS), and ATP-binding cassette (ABC). Our study suggests a high possibility in the utilization of endophytic microbial community for plant growth promotion, biocontrol activities, and stress mitigation. Further studies in ascertaining this claim through culturing of the beneficial isolates as well as pot and field experiments are necessary.

Keywords Endophytic genes · Metagenome · PGP genes · Shotgun · *Zea mays*

Introduction

Maize (*Zea mays* L.) is a major staple diet of the native peasants of most African countries. Maize being a household food crop can be grown in almost all soil types (Liu et al. 2017) most times with a different degree of yield. More than fifty species are cultivated depending on the region; the species vary in taste, texture, size and shape. Plants form beneficial association with diverse microorganisms that provide them with specific benefits (Khare et al. 2018; Toju et al. 2018). Most common among these beneficial microorganisms are the endophytes. Endophytes are microorganisms that colonize the interior tissues of plants asymptomatically (Omomowo and Babalola 2019; Fadiji and Babalola 2020b), but recent studies have shown that some of them can lead to disease development in their host plant (Van Overbeek et al. 2014; Brader et al. 2017). Similar studies have also revealed that plant microbiome could have considerable side effects on the health of humans when plants are consumed raw (Blaser et al. 2013; Van Overbeek et al. 2014; Liu et al. 2017).

Some endophytes enhance plant growth, help in disease suppression, and boost stress tolerance (Syranidou et al. 2017; Sun et al. 2019; Zolti et al. 2020). Endophytes secrete a notable plant hormone, indole-3-acetic acid (IAA), which promotes plant growth and development (Mefteh et al. 2019; Fadiji and Babalola 2020a). Endophytes also aid phosphate solubilization and nitrogen uptake, which are considered the most important elements for the growth and development of

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plants (Slama et al. 2019). Similarly, they produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase which is notable for ethylene production (Alenezi et al. 2017). Furthermore, endophytes possess high ACC deaminase activity which helps in plant growth promotion (Mefteh et al. 2019). The plant also resists growth inhibition by a number of ethylene-inducing stresses (Lumactud and Fulthorpe 2018). Production of HCN by endophytes has been found to be beneficial to their host and aid an indirect increase in nutrient availability (Rijavec and Lapanje 2016). Equally, the production of siderophore by endophytes indirectly enhances plant growth promotion (PGP) and it is considered important for plants to thrive in polluted environments (Aloo et al. 2019).

A lot of current and past studies on plant endophytic microbiomes have generated a large quantity of sequenced data and numerous information on the abundance and diversity of the different taxonomic group of endophytic microbiomes present in maize plant using next-generation techniques (Liu et al. 2017; Correa-Galeote et al. 2018; Fadiji and Babalola 2020b). However, limited studies exist on the functional importance of endophytic microbiomes for plant growth, yield, and health (Sessitsch et al. 2012; Mashiane et al. 2018; Carrión et al. 2019). Furthermore, the genes responsible for most of these important functions, in most cases, are still unknown (Carrión et al. 2019).

Plant roots are often regarded as the point in which most interactivity between microorganism take place (Sessitsch et al. 2012). In this study, to unravel the plant growth–promoting and endophytic genes in the root-associated endophytic microbiomes in maize plant, we carried out shotgun metagenomic sequencing on the DNA extract from the root of maize plants cultivated with organic fertilizer, inorganic fertilizer and those without fertilizer. Shotgun metagenomics is now being embraced over similar metagenomic techniques because it enables a comprehensive functional study of entire microbial communities present in an environment (De Tender 2017). Limited studies exist on the functional genes of endophytic microbes in the plant using shotgun metagenomics (Sessitsch et al. 2012; Hong et al. 2019). However, to the best of our knowledge, no report exists on the effects of different farming practices on plant growth–promoting (PGP) and endophytic genes in endophytic microbiomes from maize plants using shotgun metagenomics. Hence, this study presents the first attempt to unveil the putative PGP and endophytic gene clusters present in root-associated endophytic microbes of maize plants alongside assessing the effect of different farming practices on their expression using shotgun metagenomics. The study hypothesized that functional genes would be more represented in endophytes from maize plants cultivated with organic fertilizer than inorganic fertilizers.

Materials and methods

Seed collection

The maize seed (WE 3127) used in this experiment was collected from North-West University School Farm Molewane, Mafikeng, North West Province, South Africa.

Experimental design and site description

The field located in North-West University School farm Molewane, Mafikeng, North West Province (S25° 47′ 25.24056″, E25° 37′ 8.17464″), South Africa, used for the study has been in existence for over 15 years. The chemical and physical properties of soil sample from this experimental field were assessed as 22% sand, 66% silt, 12% clay, pH 6; 0.48% organic C, 0.15% total N, 101.5 ppm P, and 0.962 ppm K (Supplementary Table 1). The North West province is characterized by shrubs and trees. The mean temperatures experienced in the province ranges from 3 to 21°C in winter and 17 to 31°C in summer. The rainfall in the province is estimated at 360 mm per annum, having heavy rains between October and April. The major plants cultivated in this experiment site had been the rotation of sorghum, maize, and soybean for a long time, with soybean planted in 2018. In this study, three different sites in the experimental field were used.

The maize seeds were planted at different maize sites, each site with 10 m × 4 m in dimension. The planting was carried out during October–December 2019. Two fertilization sites namely organic fertilization (FK), inorganic fertilization (NK) and no fertilization (CK) were used in this study. These sites have been in existence for over 15 years. Urea (N), potassium sulfate (K), and calcium superphosphate (P) have been in use as the inorganic fertilizer and applied to the site at 150, 75, and 75 kg/ha for N, P, and K, respectively. The organic fertilizer (cattle manure) has been in a consistent application at 10,625 kg ha^{-1} approximately for the organic site for more than 15 years complying with standard procedures (USDA 2014), while no fertilizer has ever been applied to the third site. Irrigation was provided across the sites in required volumes to prevent drought stress. The weeding process was handled manually.

Root sampling

Each site was divided into three regions for sampling purposes. Each replicate sample for sequencing came from the pooled roots of 10 randomly selected from fresh plants from each region of a treatment site. The plants were collected during the fruiting stage (Xia et al. 2015). A total of 90 plant samples (representing 30 plants per site) were evaluated; the three regions represent three replicates for each sampling site. The plant samples were kept with ice and transported to the laboratory the same day where they were processed immediately.

Root surface washing

Surface washing was carried out on the maize roots using the method described by Liu et al. (2017) after soil particles have been removed. Seventy percent ethanol was used to submerge the roots for 3 min, and 2.5% solution having sodium hypochlorite was used to rinse for 5 min; again the roots were washed for 30 s with 70% ethanol and lastly washed with sterile distilled water. To ensure that the process of sterilization was correctly carried out, sterilized roots were plated on yeast extract-mannitol medium using a Petri dish (Vincent 1970). After 72 h of incubation at 30°C, the plates were checked for bacterial growth. Maize roots from Petri dishes without contamination were chosen for DNA extraction (Correa-Galeote et al. 2018).

Extraction of DNA and shotgun sequencing

The roots were cut into 1 cm using a sterile scalpel and instantly macerated using a Qiagen TissueLyser. Total metagenome DNA was extracted from the root tissue samples using the Qiagen DNeasy Plant Mini Kit (Qiagen, USA). Shotgun metagenomic sequencing was done at the Molecular Research LP, Texas, USA. The preparation of the library was carried out with Nextera DNA Flex kit (Illumina) following standard procedure. The DNA concentration in the samples was evaluated using Life Technologies' Qubit® dsDNA HS Assay Kit. The library preparation was carried out using 50 ng of the DNA. After the library has been prepared, the final concentration was measured using the Qubit® dsDNA HS Assay Kit, and the Agilent 2100 Bioanalyzer was used to ascertain the size of the library. The library size varies from 683 to 877 bp with an average of 731 bp, pooling of libraries was done using 0.6 nM ratios, and paired-end sequencing was done with 300 cycles using Illumina NovaSeq 6000 system.

Metagenome assembly and gene annotation

The obtained sequences of each metagenome were transferred to an online server called MG-RAST (Hong et al. 2019). Inside this online server, quality control analysis was carried out using the Trimmomatic v 0.33 program (Bolger et al. 2014). Other quality control processes also include the removal of chimeras, filtering of ambiguous bases, specification of minimum read size, and length filtering. After quality control analyses, annotation of the processed sequences was carried out using BLAT (Kent 2002), against M5NR database (Wilke et al. 2012), which allows nonredundant integration of several databases. Classification of the endophytic microbiomes and protein-coding genes was carried out using SEED Subsystem. The identified putative functional genes were manually selected from the SEED functional level databases with specified parameters such as a 10^{-5} e-value cut-off and a minimum 60% sequence similarity to a subsystem. No further analysis was carried on the sequences that could not be annotated. However, since the main focus of this work is on endophytic microbiomes which include bacteria, fungi, and archaea, which account for about 99% of the whole sequences, we, therefore, discard the plant and viral sequences after mapping against a reference genome database (Jayakodi et al. 2018; Hong et al. 2019). To suppress the influence of experimental error/noise, data normalization option was selected on the MG-RAST. Furthermore, the relative abundance of the functional genes was calculated in percentages, after taking the average of the independent analysis of the 3 sequences for the sampling sites FK, CK, and NK respectively using MG-RAST. These sequences can be found on NCBI SRA dataset with the accession number PRJNA607664.

Statistical analyses

Shinyheatmap via *z*-score was used to visualize the abundance and distribution of the major endophytic microbiomes at the phylum level and the plotting of the relative abundance of the identified functional genes. The abundance of each functional gene across the sites was plotted using Circos software (www.circos.ca/software). Simpson, Shannon, and Pielou indices for diversity assessment were employed for samples across the sites, and Kruskal-Wallis test was used to compare these indices. The analyses were performed with PAST version 3.20 (Chauhan et al. 2019). The Bray-Curtis-based principal coordinate analysis (PCoA) and ANOSIM were used for the β diversity study and to assess the differences in the functional genes present in the samples across the sites (Carrell and Frank 2015). The PCoA and PCA plots based on Bray-Curtis dissimilarity matrix were performed using CANOCO version 5.0.

Results

Metagenome sequencing, quality control, and protein annotation

After quality control analyses were carried out in MG-RAST, the sequenced output for CK was 334259767 bp, FK had 415505341 bp, and NK had 817699487 bp, while the mean G+C content of 44%, 44%, and 49% for CK, FK, and NK respectively (Supplementary Table S2). Among the sequences that passed the quality control check, sequences that mapped predicted proteins with known functions in the samples were 643141, 371329, and 325439 sequence reads from metagenomes originating from plants grown under inorganic fertilized (NK), organic fertilized (FK) and no fertilizer (CK) sites, respectively. The species richness was obtained by rarefaction analysis through MG-RAST (Fig. 1).

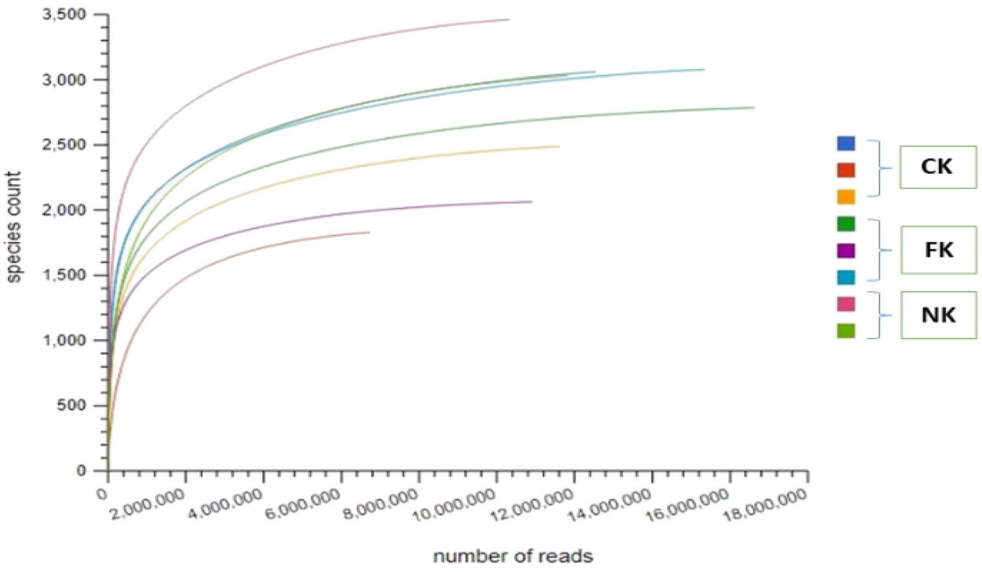

Fig. 1 Rarefaction curves used to ascertain the species richness sequences across the sites. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

Distribution of endophytic microbiomes in the maize plant

Although twenty-nine (28) bacterial phyla were identified from the samples, 23 phyla were dominant in the sites, some of which include Firmicutes, Proteobacteria, and Bacteroidetes. Three (3) archaea phyla were also identified, namely Crenarchaeota, Euryarchaeota, and Thaumarchaeota. In contrast, two (2) fungal phyla, namely Ascomycota and Basidiomycota, were identified. The most dominant phyla of endophytic microbiomes identified are presented in Fig. 2. No significant difference (p = 0.292, Kruskal-Wallis) was observed among the identified dominant endophytic microbiomes across the sampling sites.

Functional genes in maize endophytic microbiomes

A total of 58 functional genes were detected in maize endophytic microbiomes across the fertilizer sites. The identified functional genes were classified into plant growth–promoting and endophytic genes.

Plant growth–promoting (PGP) genes in maize endophytic microbiomes

Twenty-two (22) out of the putative genes were identified as PGP genes, notable among them include genes involved in snitrogen fixation (*nifH*), siderophore (*pchB* and *entF*), HCN biosynthesis (*hcnB*), ACC deaminase (*acdS*), phenazine (*phzF*), phosphate solubilization (appA), butanediol (*butB*), methanol utilization (*xoxF*), acetoin (*budC*), nitrogen metabolism (*glnA*, *glnB*, *gltB*, *gltD*, and *nirB*), and IAA biosynthesis (*ipdC* and *iaaM*). We also identify genes involved in stress tolerance such as glutathione, catalase, and peroxidase (*btuE*, *gst*, *katE*, and *sod1*) (Fig. 3; Supplementary Table S3). No significant differences ($p > 0.05$) were observed in the abundance of these functional genes across the sampling sites. Principal component analysis (PCA) was used to show the distribution of PGP genes present in endophytic microbes across the farming sites with FK sites having the highest distribution (Fig. 4).

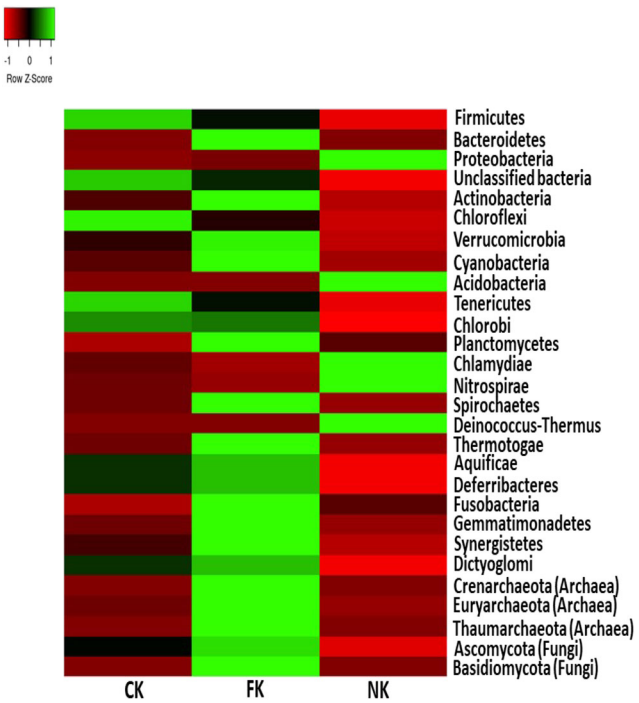

Fig. 2 Heatmap showing the distribution of endophytic microbiomes in maize samples across the sites. The scale bar represents color saturation gradient based on the relative abundances with z-score transformed relative abundance of the endophytic bacteria taxa. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

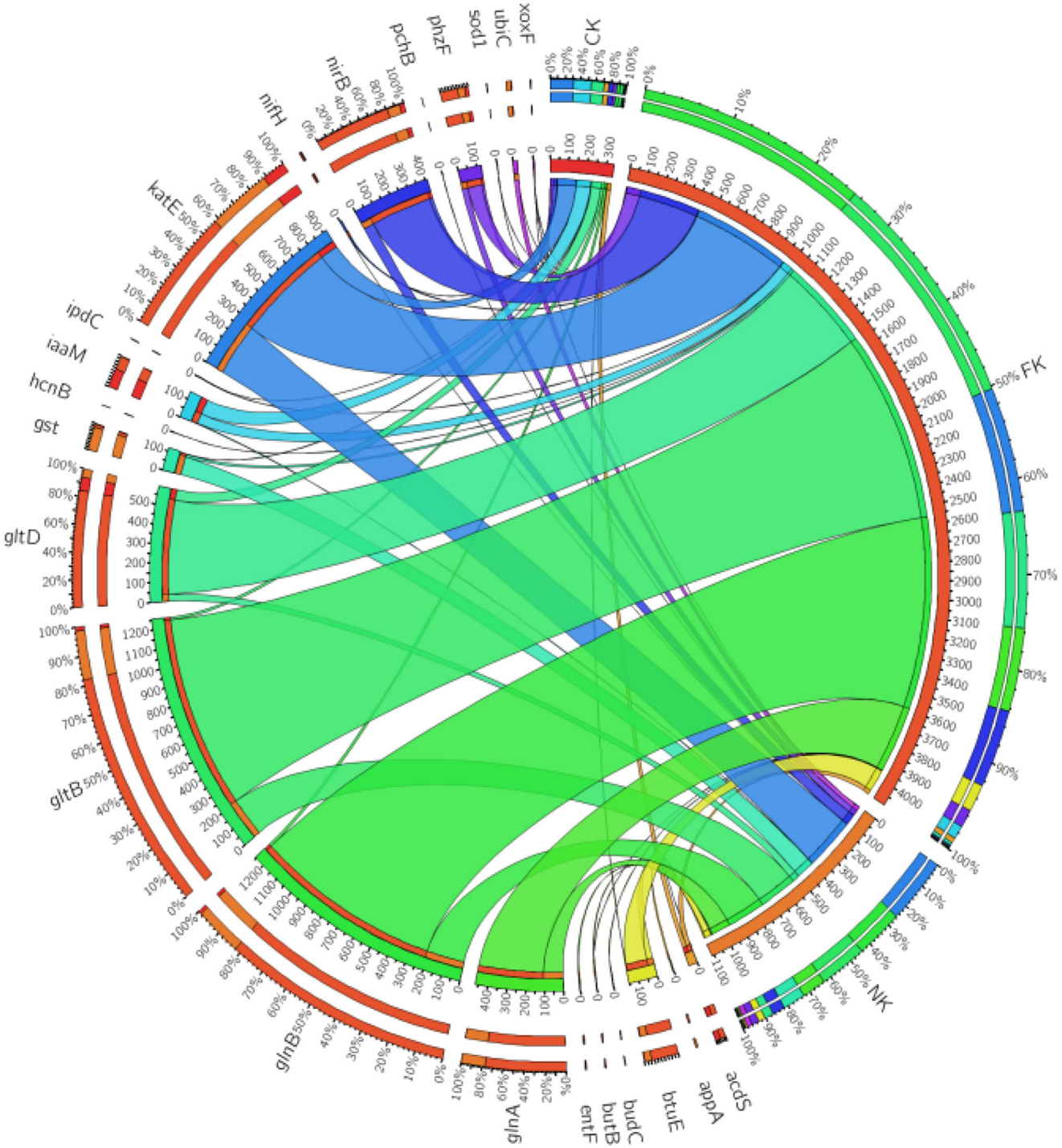

Fig. 3 Relative abundance of Genes involved in plant growth promotion observed across the sampling sites plotted using Circos software. NK= samples from inorganic fertilizer site, FK= samples from organic fertilizer site, CK= samples from no fertilizer site

Endophytic genes in maize endophytic microbiomes

Furthermore, 36 genes out of the functional genes were associated with endophytic behaviors. Notable among these genes are those involved in chemotaxis and motility such as aerotaxis (*aer*), regulator of proteins (*cheC*, *cheD*, *cheV*, and *cheZ*) and motility (*flhA*, *flhB*, *flhF*, and *fliL*). Others include, transcriptional regulators such as nitrogen regulation (*nifA* and *nadR*), regulation of carbon storage (*sdiA*), formation of biofilm (*crp*), reduction of nitric oxide (*norR*) and regulation of beta-lactamase resistance (*ampR*). Also, we identified genes involved in secretion systems such as type III secretion (*yscJ*),

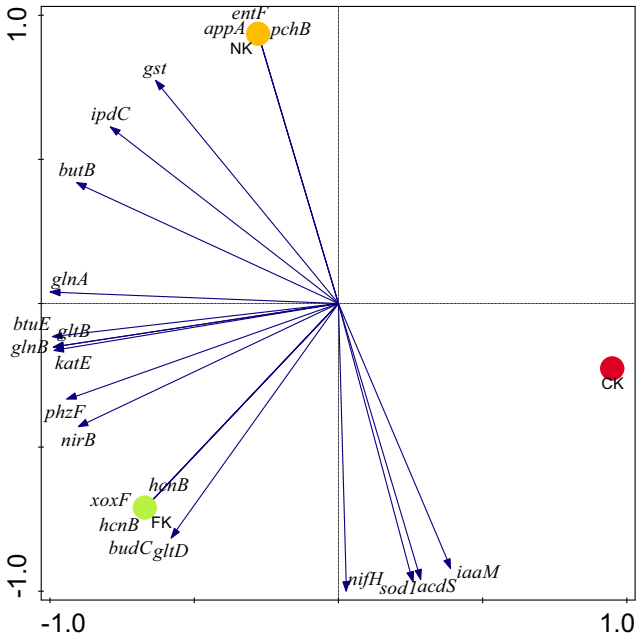

Fig. 4 PCA graph of plant growth–promoting genes. The vector arrow represents the influence of plant growth–promoting genes. Axis 1 (71%) and axis 2 (29%) explained the variations based on Bray-Curtis dissimilarity matrix. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

type IV conjugal DNA (*virB2*), and type I pilus assembly (*fimA*). Furthermore, genes involved in transport system were identified to include twitching movement, phosphotransferase system (PTS), an ATP-binding cassette (ABC), multidrug transporter, tricarboxylic transporter (*tctA*), and methyldicarboxylate (*dctA*) (Fig. 5; Supplementary Table S4). No significant differences ($p > 0.05$) were observed in the abundance of these endophytic genes across the sampling sites. Principal component analysis (PCA) was used to show the distribution of endophytic genes present in the endophytic microbes across the farming sites with FK sites having the highest distribution (Fig. 6).

Alpha (α) and beta (β) diversity estimation of functional genes in endophytic microbiomes across the sampling sites

Shannon, Simpson, and evenness indices were used to estimate the alpha diversity of the functional genes across the sampling sites. The alpha diversity results showed that there were no significant differences (p value = 0.162 and 0.09, Kruskal-Wallis) among the identified PGP and endophytic genes across the sampling sites (Table 1). However, beta diversity using analysis of similarity (ANOSIM) revealed a significant difference (p value = 0.01; $R = 0.67$) among the functional genes across the sites. PCoA further revealed a clear separation across the sites (Fig. 7A and B).

Discussion

In this study, the shotgun metagenomic analysis was carried out on maize root cultivated with different fertilization levels and without fertilization. Using MG-RAST, the sequenced data were analyzed by recognizing sequences that are for the endophytic microbiome while discarding the whole genome sequences for maize plants. In the bacteria sequence, we identified dominant bacteria phyla such as *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Tenericutes*, *Planctomycetes*, *Cyanobacteria*, and *Chlorobi*. The identified fungi are *Ascomycota* and *Basidiomycota*, while we found *Crenarchaeota*, *Euryarchaeota*, and *Thaumarchaeota* as the dominant endophytic archaea. Most of the identified endophytic bacteria, fungi, and archaea have been reported as notable plant growth–promoting microbes and they possess important genes linked with plant growth and health promotion (Müller et al. 2015; Correa-Galeote et al. 2018; Hong et al. 2019; Xia et al. 2019). These PGP genes are involved in nitrogen metabolism, mitigation of environmental stress, phosphate solubilization, methanol utilization, and nutrient accessibility (Fig. 3). Previous studies have revealed that endophytes promote the growth of plant via direct and indirect mechanisms. Some direct mechanisms employed include the production of phytohormones, phosphate solubilization, siderophore production, and 1-Aminocyclopropane-1-carboxylate (ACC) utilization (Babalola 2010; Singh and Dubey 2018; Fadiji and Babalola 2020a). The indirect mechanisms include the induction of plant resistance, secretion of secondary metabolites, hyper-parasitism, and biocontrol activities (Olanrewaju et al. 2017; Latz et al. 2018; Fadiji and Babalola 2020a).

In this study, from the metagenomes obtained for endophytic microbes, we observed gene *ipdC* and *iaaM* which encodes indole-3-pyruvate decarboxylase and tryptophan 2-monooxygenase, respectively, which participates in the biosynthesis of an important hormone called IAA from tryptophan (Sugawara et al. 2015) represented only in FK and CK sites. Our result is consistent with Da Costa et al. (2013), in which IAA traits significantly improved the growth of rice cultivated in a moderate nutrient environment. Indole acetic acid (IAA) is a plant hormone that triggers plant cell division, differentiation, and extension. It stimulates seed and tuber germination, increases the rate at which root and xylem develop, enhances lateral initiation, and controls the rate of vegetative growth and formation of adventitious root (Singh and Dubey 2018; Carrión et al. 2019). It also helps in the formation of pigments and biosynthesis of metabolites, controlling responses to gravity, light and fluorescence, photosynthesis, and resistance to extreme conditions (Hassan 2017). Similarly, we identified *acdS* gene, which encodes ACC deaminase which

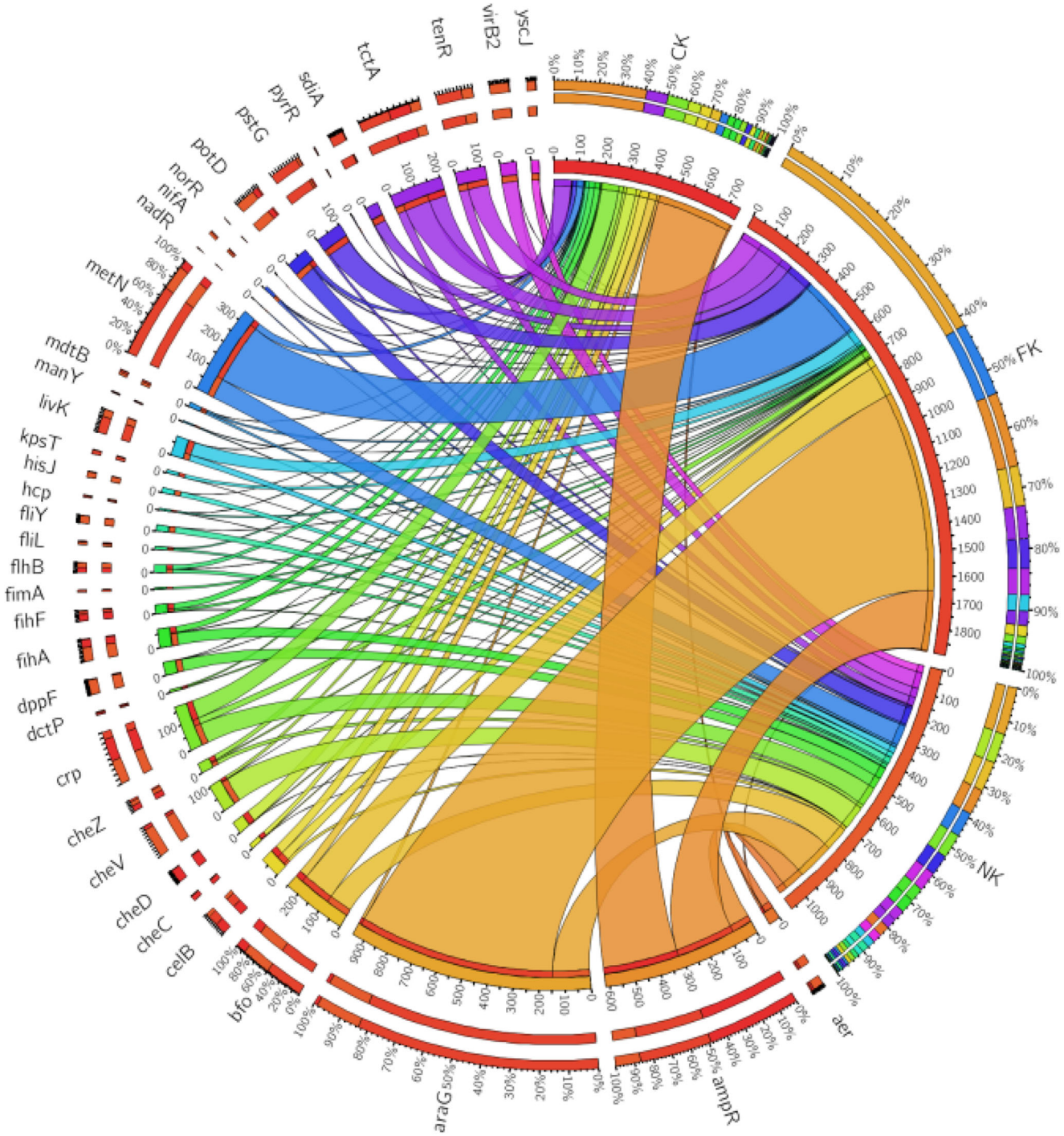

Fig. 5 Relative abundance of genes involved in endophytic behaviors observed across the sampling sites plotted using Circos software. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

was moderately represented in CK and FK sites and poorly represented in NK site. Our result suggests that at CK and FK sites, the interactions between endophytes and maize plants significantly enhanced growth promotion. This enzyme helps hydrolyze 1-aminocyclopropane-1-carboxylic acid (ACC) and reduces ethylene production in plants (Singh and Dubey 2018; Hong et al. 2019). Ethylene is an important plant hormone that enhances seed germination, and it is the major regulator of bacterial colonization in the tissues of the plant (Iniguez et al. 2005; Babalola 2010). However, its excessive accumulation can be detrimental to plant health and growth (Eid et al. 2019; Yurgel et al. 2019). Some studies also reported that ACC deaminase could likewise hydrolyze ACC into ammonia and α-

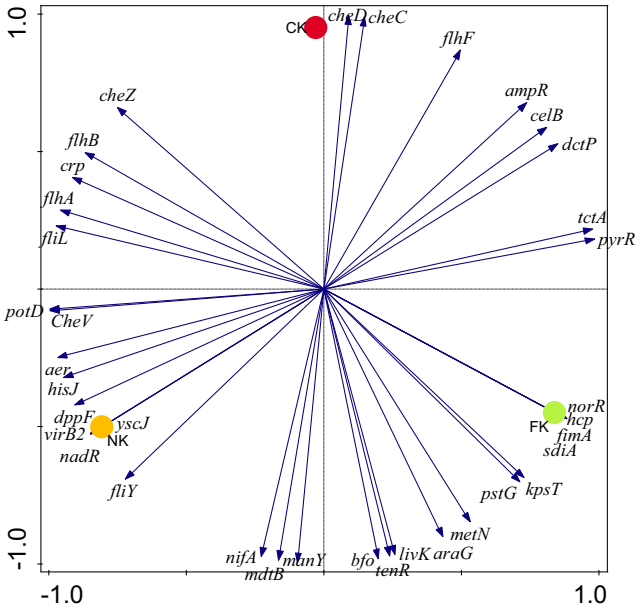

Fig. 6 PCA graph of endophytic genes. The vector arrow represents the influence of plant endophytic genes. Axis 1 (51.4%) and axis 2 (48.6%) explained the variations based on Bray-Curtis dissimilarity matrix. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

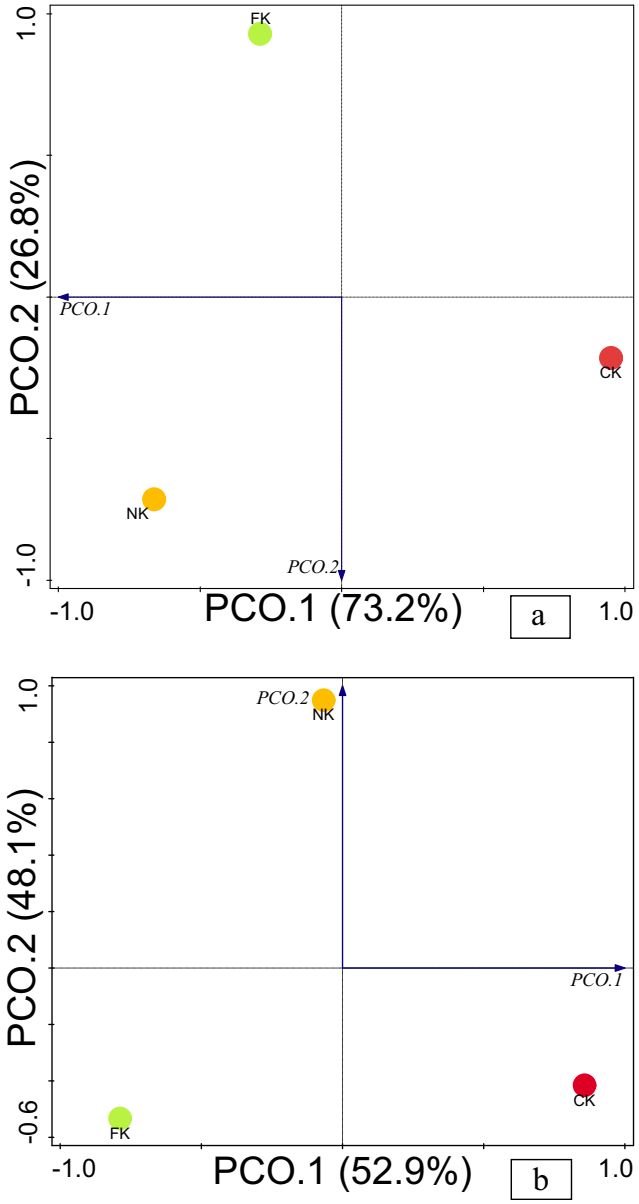

Fig. 7 Principal coordinate analysis (PCoA) plot of (A) plant growth–promoting genes (B) endophytic genes compositions across the maize sites based on Bray-Curtis dissimilarity matrix. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

ketobutyrate, which supply nitrogen for microbial growth (Xing et al. 2012). Endophytic bacteria genera such as *Bacillus*, *Arthrobacter*, and *Microbacterium* (belonging to *Firmicutes* and *Actinobacteria*) isolated from *Capsicum annum* L. exhibited ACC deaminase activity by supporting growth in plants exposed to drought stress (Sziderics et al. 2007).

Furthermore, we identified *appA* gene, which encodes for 4-phytase, an important enzyme involved in P mineralization in endophytes from FK and NK sites but with high abundance in the samples from NK site. This may occur due to the high phosphorus content present in the inorganic fertilizer. Our results, therefore, agree with an earlier report where inorganic fertilizer application improved phosphate-solubilizing traits in microbes (Da Costa et al. 2013). Phosphorus is one of the major elements necessary for plant growth and other biological processes such as enhancing organ development in plants, glucose transport, and stimulation of cell growth, but plants cannot directly utilize phosphorus found in the soil without being solubilized to phosphate (Ahemad and Kibret 2014; Singh and Dubey 2018). Endophytes play a major role in

Table 1 Diversity and evenness assessment of putative functional genes in endophytic microbiome with different treatments in the sampling sites

Diversity indices	CK	FK	NK	*p* value
Plant growth–promoting genes				
Simpson_1-D	0.82 ± 0.01	0.77 ± 0.01	0.94 ± 0.01	0.162
Shannon_H	2.36 ± 0.09	2.01 ± 0.05	2.96 ± 0.06	
Evenness_e^H/S	0.37 ± 0.40	0.23 ± 0.03	0.61 ± 0.04	
Endophytic genes				
Simpson_1-D	0.81 ± 0.02	0.83 ± 0.01	0.84 ± 0.01	0.09
Shannon_H	1.94 ± 0.09	1.95 ± 0.03	2.04 ± 0.05	
Evenness_e^H/S	0.50 ± 0.10	0.37 ± 0.04	0.43 ± 0.04	

Mean ± standard error ($n = 3$). *p* values based on Kruskal-Wallis test. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site

phosphate solubilization and also enhance its availability for plant use through redox changes, chelation, mineralization of organic phosphorus, and acidification (Van Der Heijden et al. 2008). Many endophytic microbes solubilize phosphate complexes and convert them into forms like ortho-phosphate, which is readily available for use (Otieno et al. 2015). Organic phosphorous mineralization coupled with the secretion of phytase was reported in *Streptomyces* sp. an endophyte (belonging to the phylum *Actinobacteria*) isolated from *Triticum aestivum* which significantly improved plant growth (Jog et al. 2014). We also found genes *budC*, *butB*, and *xoxF*, which codes for acetoin reductase, butanediol dehydrogenase, and methanol dehydrogenase, respectively. These genes were poorly represented in samples from NK and CK sites, but are more frequent in samples from the FK site. A recent study by Hong et al. (2019) revealed that 2,3-butanediol and acetoin are novel volatile molecules that aid increased root length in *Panax ginseng*. It was also reported that 2,3-butanediol and acetoin could enhance plant growth (Hardoim et al. 2015). Similarly, we found a poor representation of genes *pchB* and *entF*, which encodes isochorismate pyruvate lyase and enterobactin synthetase component F, respectively, in samples from NK sites only. These genes are associated with iron and siderophore production and have been reported to be beneficial in plant growth promotion (Tsurumaru et al. 2015; Singh and Dubey 2018). For iron to be utilized by plants, it must undergo solubilization. A study by Rajkumar et al. (2009) revealed that the provision of iron for the roots of plants was increased by the production of siderophores by phytosiderophore-iron complex or bacterial siderophore-iron complex. *Streptomyces tendae* F4 (belonging to *Actinobacteria*) has been reported to be a great producer of siderophore which enhances the growth of cowpea (*Vigna unguiculata*) exposed to nickel stress (Dimkpa et al. 2009).

Nitrogen fixation is often performed by endophytes in a bid to improve the fitness of their host, especially in an environment deficient of nitrogen. This is one of the mechanisms endophyte use to reduce the effect of environmental stress on the host plant (Ruppel et al. 2013). In this study, we found gene *nifH*, which encodes nitrogenase involved in nitrogen fixation alongside *glnA*, *glnB*, *gltB*, *gltD*, and *nirB* that are associated with nitrogen metabolism. These genes were well represented in all samples, though more expressed in FK samples. Our result is similar to an earlier study in which organic fertilizer significantly improved nitrogen metabolism in peanuts (Purbajanti et al. 2019). Numerous root-associated endophytes are involved in nitrogen fixation, such as *Acetobacter* spp., *Herbaspirillum* spp., and *Azoarcus* spp., all belonging to the phylum *Proteobacteria* (Eid et al. 2019). An endophytic bacterium, *Paenibacillus* P22 (belonging to the *Firmicutes*), was also reported to fix nitrogen as well as effect changes in host plant metabolism (Hardoim et al. 2015). Similarly, we found gene *hcnB*, which encodes hydrogen cyanide synthase in only FK samples. This gene enhances HCN biosynthesis and HCN has been reported to be beneficial in the growth promotion of the host plant (Rijavec and Lapanje 2016).

Furthermore, we identified genes *ubiC* and *phzF*, which encodes chorismate lyase and phenazine biosynthesis respectively in endophytes across the sites. These genes encode phenazine and 4-hydroxybenzoate which help in antibiosis and biocontrol activities in the host (Enagbonma and Babalola 2020). Similarly, plant growth and health are affected by environmental stresses, and endophytes can protect its host from both abiotic and biotic stresses (Hardoim et al. 2015; Azad and Kaminskyj 2016; Omomowo and Babalola 2019). We identified genes involved in the mitigation of stress such as *btuE*, *gst*, *katE*, and *sod1* which codes for glutathione peroxidase, glutathione-S-transferase, catalase, and superoxide dismutase, respectively. The genes *btuE*, *gst*, and *katE* were represented in all samples and more frequent in the FK samples while *sod1* was poorly expressed only in CK and FK samples. In a bid to overcome stresses, plants produce antioxidant defense mechanisms which include non-enzymatic and enzymatic components, which help to avert the buildup of reactive oxygen species (ROS) (Miller et al. 2010). The enzymatic components are catalase, superoxide dismutase, glutathione reductase, and ascorbate peroxidase, while non-enzymatic components include ascorbic acid, glutathione, and cysteine (Vardharajula et al. 2017). However, the frequent abundance of PGP genes in samples from organic fertilizer site (FK) confirmed our hypothesis. PCA plot showed that each site has different plant growth–promoting (PGP) genes and is responsible for a combined 71% variance witnessed across the fertilization sites (Fig. 4). The position occupied by each plant growth–promoting (PGP) gene reflects their functional makeup, while the vector arrows showed the PGP gene most influenced by the distribution. Employing this indicator, it is easier to detect which of the identified PGP genes is more dominant in the endophytes from each sampling site as compared to others.

Additionally, we identified genes that are linked to the endophytic behavior of microorganisms inside the plant hosts (endophytic genes). Some of these genes include those involved in motility, regulation of transcription, transport system, and secretion systems (Fig. 5). We identified genes *aer*, *cheZ*, *cheC*, *cheD*, and *cheV*, which encodes aerotaxis and regulator proteins that were moderately expressed in all samples across the sites. Similarly, we found genes *flhA*, *flhB*, *flhF* and *fliL*, which encodes motility mechanisms and flagellum biosynthesis that could help endophytes survive in their host. These genes are connected with chemotaxis, motility, and adhesion, and were represented in all samples. One major factor that drives the colonization of microbes is the capacity to detect and respond to environmental cues (Hartmann et al. 2009; Porter et al. 2011). The response regulator proteins and flagellum biosynthesis were moderately present in all samples across the sites. The presence of these genes might contribute to a successful endophytic lifestyle in plants.

Similarly, we identified putative genes involved in transcriptional regulation in all endophytic microbiomes across all sites. The genes are *nifA* involved in nitrogen regulation, *sdiA* involved in the regulation of carbon storage, *crp* involved in the formation of biofilm, *norR* involved in the reduction of nitric oxide, and *ampR* involved in the regulation of beta-lactamase resistance. Others include *tenR* involved in thiamine metabolism, *pyrR* involved in pyrimidine regulator, and *nadR* involved in NAD regulation, which were more frequently detected in samples from sites FK and NK. Transcriptional regulators are of great importance to prokaryotes because they aid cellular homeostasis, response to environmental stress, new niche colonization, and adaptation flexibility (Berg et al. 2005). Regulatory genes involved in stress response, carbon metabolism, vitamins, and nucleotides alongside carbon and nitrogen metabolism might be of great support to endophytic lifestyles in plants (Hardoim et al. 2015; Rodríguez-Blanco et al. 2015). However, it is important to state that plant pathogens and nodule-forming symbionts that survive inside the inner tissues of plants use an entirely different mechanism from that of endophytes, indicating that all groups of microbes inhabiting the tissues of plants have set of regulatory genes peculiar to their behavioral feedback (Hardoim et al. 2015; Olanrewaju et al. 2019).

Furthermore, nutrient transport is an important role for organisms to survive and thrive inside plants (Mitter et al. 2013). In this study, we observed genes for phosphotransferase system (PTS) such as *cleB*, *pstG*, and *manY*, which encodes cellobiose, glucose, and mannose respectively from endophytes across the sites. We also found genes for ATP-binding cassette (ABC) such as capsular polysaccharide (*kspT*), spermidine/putrescine (*potD*), dipeptide (*dppF*), branched-chain amino acid (*livK*), cystine (*fliY*), methionine (*metN*), histidine (*hisJ*), and L-arabinose (*araG*), which were represented in all samples, but more abundant in endophytes from site FK. Others include multidrug transporter (*mdtB*), tricarboxylic transporter (*tctA*), and methyldicarboxylate (*dctp*). Genes involved in the uptake of peptides, organic ions, amino acids, carbohydrate, and capsular polysaccharides were detected among endophytes across the sites. The results showed a complex nature of the nutrient transport systems among the identified endophytes, reflecting different strategies of nutrient acquisition, which might support life inside the plant. The identified genes are similar to the ones detected in *Burkholderia* spp. (belonging to proteobacteria) (Santoyo et al. 2016).

Interestingly, protein secretion plays a major role in plant-microbe interactions (Downie 2010; Reinhold-Hurek and Hurek 2011). In this study, we identify a putative gene involved in type III secretion systems (*yscJ*) in all our samples across the sites but more abundant in samples from the NK site. Though identified in endophyte, the gene has an earlier record of being involved in phytopathogens and nodule-forming symbionts than in endophytes (Hardoim et al. 2015). This secretion system is often used by phytopathogens for manipulating their host metabolism (Abramovitch et al. 2006). In like manner, we found a gene associated with type IV conjugal DNA-protein transfer secretion systems (*vrB2*) which were poorly represented in all our samples but more abundant in samples from the NK site. The type IV secretion system has earlier been reported to be involved in DNA conjugation and host colonization (Salomon et al. 2014). Our results also revealed the presence of genes *hcp* and *fimA* in endophyte across the sampling sites and only at FK site, respectively. This suggests that endophytes in samples from FK sites are more abundant than other samples. They encode secreted proteins and pilus assembly protein, respectively. The genes are used most times for adhesion through type I pilus assembly, and twitching movement. These systems are suspected to play an important role in most successes recorded in host colonization by endophytes (Meng et al. 2005; Böhm et al. 2007). However, the frequent expression of these endophytic genes in samples from organic fertilizer sites (FK) confirmed our hypothesis. PCA plot showed that each site has different putative endophytic genes and is responsible for a combined 51% variance witnessed across the fertilization sites (Fig. 6). The position occupied by each putative endophytic gene reflects the makeup of their sequences and the vector arrows showed the putative endophytic gene most dominant and influenced by the distribution. Employing this clue, it is not difficult to detect which of the identified putative endophytic genes is more dominant in the endophytes from each sampling site as compared to others.

Shannon, Simpson, and Evenness indices calculated for the differences observed among the functional genes across the sites were not significant ($p > 0.05$) (Table 1). The alpha diversity analysis carried out on the functional genes from endophytes across the sampling sites revealed that the genes do not differ significantly (Kruskal-Wallis, p value = 0.162, 0.09). The PCoA graph showed a clear separation of the sampling sites (CK, FK, and NK). The ANOSIM test further establishes the difference in the samples across the sites with the separation strength (R) of 0.67 and that the identified putative functional genes differ significantly ($p = 0.01$) across the sites.

Conclusion

In summary, to the best of our understanding, this is the first attempt to identify putative functional genes in endophytic microbiomes in root of maize plants using shotgun metagenomics. The study identified genes that are putatively involved in plant growth–promoting and endophytic behavior (endophytic genes) in endophytes across the sites. These genes were more abundant in endophytes from maize plants cultivated in the organic fertilizer site (FK), which indicates that organic farming practices could be instrumental in the

achievement of sustainable agriculture. However, there is a need for an extensive study of the mechanism of actions and colonization of endophytes in the endosphere. This will enhance their application in agricultural management practices such as biocontrol, plant nutrition, and bioremediation. The sequenced data also revealed the abundance of more PGP genes in endophytic bacteria than endophytic fungi and archaea, thus presenting them as a potential source of biofertilizers for sustainable agriculture.

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Authors' contributions AEF handled the literature findings, carried out the planting and laboratory work, performed the analyses, interpreted the results, and wrote the manuscript. ASA provided technical input, result interpretation, and proofread the manuscript. OOB initiated the metagenomic research, provided academic and technical inputs to the co-authors, critiqued and helped shape the research, verified the analytical methods, and secured funding for the research.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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