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Shotgun metagenomics reveals the functional diversity of root-associated endophytic microbiomes in maize plant

Ayomide Emmanuel Fadiji, Ayansina Segun Ayangbenro, Olubukola Oluranti Babalola *

Food Security and Safety Niche, Faculty of Natural and Agricultural Sciences, North-West University, Private Mail Bag X2046, Mmabatho, South Africa

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ABSTRACT

In this study, we used shotgun metagenomics to analyze the whole DNA from maize root planted with different fertilization and without fertilization in a bid to profile the impact of fertilizer applications on the functional diversity of endophytic microbiomes. Complete DNA extraction from roots of maize plant grown on different farming sites such as organic (FK), inorganic (NK) and no fertilizer (CK) sites was carried out, and sequenced using a shotgun metagenomic approach. The raw sequenced data obtained were analyzed using an online database called MG-RAST. Through MG-RAST analysis, endophytic microbiome sequences were identified while sequences of maize origin were discarded. The prediction of the functions of the endophytic microbiomes was done using the SEED subsystem. Our results revealed that no significant difference ($P > 0.05$) exist in the relative abundance of the 28 functional groups identified within the endophytic microbiomes across the sites. Also, some functional groups and metabolic pathways associated with plant growth promotion such as carbohydrate, secondary metabolism, nitrogen metabolism, iron acquisition and metabolism alongside phosphorus metabolism were observed in the endophytes across the sites. Alpha diversity study revealed no significant difference exist among the functional groups of the endophytes across the sites, while beta diversity study indicated that there was a significant difference ($P = 0.01$) among the functional groups of the endophytes across the fertilizer sites. Going by the high abundance of functional groups observed in this study, especially in FK samples, it is evident that different farming practices influenced the functions of endophytic microbiomes. We recommend that further studies should explore the functional genes in endophytic microbiomes with the aim of assessing their usefulness in promoting sustainable agriculture.

1. Introduction

Maize is the staple food for the largest number of people in the world, particularly in South, West, East and Northern part of Africa [1]. However, in South Africa, about 8 million tons of maize grain are produced annually on almost 3.1 million ha of land. Half of this production is white maize, which is majorly consumed by humans [2]. The continuous increase in maize consumption demands increased yields and improved management practices [3]. Maize roots have been observed to passively secret natural compounds such as sugars, nucleosides, amino acid, mucilage, and organic acids, which help to entice microbes from the bulk soil to its rhizospheric environment and subsequently endosphere for plant growth promotion [4]. Notable among the organisms that are attracted to the endosphere is the endophytes.

Endophytic bacteria and fungi have been reported to be beneficial to plant growth enhancement via several mechanisms among which are fixing of nitrogen, production of ammonia, siderophore and phytohormones [5–8]. Endophytes are organisms that inhabit the tissues of plants without causing harm to the host [9,10]. Studies have shown that endophytes perform notable roles in plant growth and health improvement [11–15]. For about a decade, most researchers have concentrated on endophytic microbes from medicinal plants because these organisms are believed to possess a huge capacity to secrete many important secondary metabolites including antibiotics, antituberculosis, antimalarial, antiviral, antifungal insecticidal, anticancer, antifungal, volatile organic compounds, antiviral, immunosuppressant and plant growth improvement [8,16–19]. However, recent metagenomics studies have focused on the diversity and community structure of endophytes in maize, rice and other plants [3,20–22] while limited studies exist on the functions of these endophytes [3,22]. Considering all these functions and benefits, endophytic microbiomes are still underexplored, because they have the prospect of replacing our dependence on chemical fertilizers through

* Corresponding author.
E-mail address: olubukola.babalola@nwu.ac.za (O.O. Babalola).

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their potentials as a biofertilizer and enhancement of better agricultural practices.

In order to have an in-depth understanding of other contribution and function of these microbes, it is important to unravel their adaptations and beneficial characteristics. However, assessing the function of endophytes is faced with many challenges especially in culturing the microbes, because most of these microbes inhabit the tissue of their host and most times they are not responsive to genetic or biochemical analyses [3]. Endophytic cells inside plant tissues firmly stick to the host cells, and are often difficult to separate and isolate from plant cells alongside at the risk of being contaminated with epiphytes. However, the advent of next-generation sequencing technology has now simplified the process, in which, endophytic genomes can be extracted from the total metagenome dataset of a plant without the fear of being contaminated by plant genome [22].

Understanding the functions, host-microbe interactions, adaptations and purported beneficial traits, strongly depend on culturable endophytic microbes from maize [23,24] and other plants [25]. Cellular capacities and functions of uncultured microbial communities can be unraveled using metagenomic techniques [26]. Interestingly, cluster analysis and evaluation of plant microbial metabolism and interactions have been previously attributed to the uncultivable microorganisms [22]. However, studies in this regard concentrated on rhizospheric microbes, while studies on endophytes are restricted to tomato [27], rice [28], peony [29], grape [30], aloe [31], maize [21], *Panax* [22] among others, with limited information about the functional roles of the identified endophytic microbes. At present, to the best of our understanding, no report exists on the functional diversity of endophytic microbiomes from maize plant cultivated with different fertilizers using shotgun metagenomics. Shotgun metagenomics is preferred recently over other techniques because it enables the functional profiling of microbial communities inhabiting an environment [32]. In shotgun metagenomic sequencing, all DNA samples pulled out from a particular environment will be analyzed, instead of focusing on a distinct genomic locus. This novel globally recognized technique is dependent on 2 major steps. Firstly, the splitting of the DNA molecules into some tiny gene fragments, followed by independent sequencing. The second step involves the reassembling of gene fragments [10,33]. This study hypothesized that the functional diversity of endophytic microbiomes will increase in maize plant in cultivated with organic fertilizer sites as compared to those cultivated in inorganic site. In this study, we investigate, for the first time, the functional diversity of endophytic microbial communities in the root of maize plant using shotgun metagenomic approach.

2. Materials and methods

2.1. Seed collection

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

2.2. Experimental design and site description

The long-existing (15 years) organic and inorganic experimental fields located in North-West University School farm Molelwane, Mafikeng, North West Province (S25°47′25.24056″, E25°37′8.17464″), South Africa, was used for the study. North West Province of South African borders Botswana. This province is characterized by shrubs and trees. The mean temperatures experienced in the province ranges from 3–21°C in winter and 17–31°C in summer. The rainfall of the province is estimated at 360 mm per annum, having severe rains experienced between October and April. This major plant 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, the experimental field was divided in to three different sites. The soil samples were analyzed for pH and other soil chemical parameters. The soil samples from the experimental sites had similar chemical and physical properties (22 % Sand, 66 % Silt, 12 % clay, pH 6; 0.48 % organic C, 0.15 % total N, 101.5 ppm P, 0.962 ppm K) (Supplementary Table S1).

Two fertilization regimes were used in this study, the organic fertilizer site (FK) and inorganic fertilizer site (NK) which has been in operation for over 15 years following standard methods as described by the U S Department of Agriculture [34], while the third site is the no fertilizer site (CK). Further information about each farming site are provided in Table 1. The planting was carried out during October-December 2019. Irrigation was provided across the sites in required volumes to prevent drought stress. The weeding process was handled manually.

2.3. Root sampling

Each farming site was divided into three different regions representing three (3) replicates for root sampling purpose. Each replicate sample for sequencing came from the roots of 10 randomly selected fresh plants in each region of the sites which were pooled (Fig. 1). The plants were collected at fruiting stage of the plant growth [35]. In total, 90 plant samples 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.

2.4. Surface washing of maize roots

Surface washing was carried out on the fresh maize roots using the method described by Liu, *et al.* [36] and Correa-Galeote et al. [21]. To sure that the process of sterilization was perfectly carried out and epiphytes removed, small parts of the sterilized roots were cut and plated on yeast extract-mannitol medium using a Petri dish [37]. After 72 h incubation at 30°C the Petri dishes were checked bacterial growth. Maize roots from Petri dishes without contamination were chosen for DNA extraction [21,38].

2.5. 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 immediately extracted from the root tissue samples using the Qiagen DNeasy Plant Mini Kit. Shotgun metagenomic sequencing was done at the Molecular Research LP, Texas, USA. The preparation of library was carried out with Nextera DNA Flex kit (Illumina) following standard procedure. The actual DNA concentration in all the samples was

Table 1
General information on the selected farming sites.

Information	Organic fertilizer site (FK)	Inorganic fertilizer site (NK)	No fertilizer site (CK)
Years of existence	over 15 years	over 15 years	over 15 years
Type of fertilizer continually used	Cattle manure	NPK Urea is used as fertilizer N, Potassium sulfate taken as fertilizer K, and calcium superphosphate as P fertilizer	No fertilizer application
Constant fertilizer dosage over the years	10,625 kg ha^{-1}	150 N, 75 P_2O_5 and 75 K_2O all in kg ha^{-1}	Nil
Maize cultivar planted	WE 3127	WE 3127	WE 3127
Dimension adopted for the study on each site	10m × 4m	10m × 4m	10m × 4m

Fig. 1. Representative samples of maize roots used in the study.

evaluated making use of the Life Technologies Qubit® dsDNA HS Assay Kit. The library preparation was carried out using 50 ng of the DNA. The samples passed through fragmentation and adapter sequences were added. These adapters were then used for limited-cycle PCR with specific indices being added to the samples. After the library has been prepared, the final concentration was measured using the Qubit® dsDNA HS Assay Kit, and the Agilent 2100 Bioanalyzer was 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 were done using 0.6 nM ratios, and the paired-end sequencing was done with 300 cycles via the Illumina NovaSeq 6000 system.

2.6. Data analysis

The obtained sequences of each metagenome were transferred to an online server called MG-RAST [22]. Inside this online server, quality control of the raw data was carried out. This include, removal of the adapter and low reads sequences from the sequenced data using the Trimmomatic v 0.33 program [39] for the quality trimming of the sequenced data. The quality control process also includes the removal of artificial sequences, filtering of ambiguous bases, specification of minimum read size, and length filtering. After quality control analysis, annotation of the processed sequences was carried out using BLAT [40], against M5NR database [41], which allows nonredundant integration of several databases. Classification of the microbiomes was carried out using SEED Subsystem (Supplementary Figure S1). Also, profiling of the functional categories of endophytic microbiomes was performed using the SEED Subsystem level 1, 2 and 3 databases with specified parameters such as a 10^{-5} e-value cut-off and minimum 60% sequence similarity to a subsystem. No further analysis was carried on the sequences that could not be annotated. However, since our concentration is on endophytic microbiomes, which accounts for a large percentage of the whole sequences, we, therefore, discard maize plant sequences. To suppress the influence of experimental error/noise, data normalization option was selected on the MG- RAST. The functional table obtained was aggregated to functional level and uncategorized sequence reads were kept for statistical analysis. Furthermore, the relative abundance of the functional categories was calculated in percentages, after the independent analysis of the 9 sequences using MG-RAST. For the statistical analysis, the average figure of the obtained relative abundance of the 3 replicates for each sampling sites (CK, FK and NK) were used. These standard sequences can be found on NCBI SRA dataset with the accession number PRJNA607664.

2.7. Statistical analyses

The abundance and distribution of the major endophytic microbiomes at the phyla level were visualized using a column bar graph via Microsoft Excel software. Shinyheatmap via z-score was used for the plotting of heatmaps using the relative abundance of the functional groups. Shannon and Pielou indices for diversity assessment were employed for samples across the fertilizer sites and Kruskal–Wallis test was used to compare these indices. The analyses were performed via PAST version 3.20 [42]. The Euclidean based principal coordinate analysis (PCoA) and ANOSIM through 999 permutations were used for the β diversity study and for the assessment of functional differences in the samples across the fertilizer sites respectively [43]. A Euclidean based PCA was used to assess the distribution of the different functional categories from samples across the fertilizer sites. CANOCO version 5.0 was used for the plotting of both PCoA and PCA graphs.

3. Results

3.1. Metagenome sequencing, quality control and protein annotation

A total of 56,087,796,311 sequenced reads were recorded for the three (3) sampling sites, with individual sequence reads of samples as CK (4,839,895,527), FK (2,977,205,570) and NK (48,270,695,214) respectively. After quality control analyses were carried out in MG-RAST, the sequenced reads for CK was 334,259,767 having a mean G + C content of 44 %, FK had 415,505,341 having a mean G + C content of 44 % and while NK had 817,699,487 with a mean G + C content of 49 % (Supplementary Table S2). Among the sequences that passed the quality control check, sequences that mapped for identified proteins in the samples were 325,439 (CK), 371,329 (FK) and 643,141 (NK), respectively (Supplementary Table S2).

3.2. Functional analysis of endophytic microbiomes associated with maize plant

The results obtained at SEED subsystem level 1 showed 28 key functional categories attributed to the endophytic microbiomes from all the sites. The functional categories such as carbohydrates (C), cell division and cell cycle (CDCC), cell wall and capsule (CWC), clustering-based subsystems (CBS), cofactors, vitamins, prosthetic groups and pigments (CVPGP), DNA metabolism (DNAM), dormancy and sporulation (DS), fatty acids, lipids, and isoprenoids (FLI), iron acquisition and metabolism (IAM), membrane transport (MT), metabolism of aromatic compounds (MAC), and miscellaneous (Mis), motility and chemotaxis (MC), nitrogen metabolism (NM), phages, prophages, transposable elements, and plasmids (PPTP), photosynthesis (P), potassium metabolism (PoM), regulation and cell signaling (RCS), secondary metabolism (SecM), stress response (SR), and virulence, disease and defense (VDD) dominated samples from the organic fertilizer site (FK) (Fig. 2). However, functions associated with nucleosides and nucleotides (NN), protein metabolism (ProM), RNA metabolism (RNAM), and respiration (R) predominated samples from the no fertilizer site (CK). While, amino acids and derivatives (AAD), iron acquisition and metabolism (IAM), motility and chemotaxis (MC), phosphorus metabolism (PM), and sulfur metabolism (SulM) were abundant in samples from inorganic fertilizer site (NK). The differences observed in all the functions identified did not vary significantly ($P > 0.05$) across the sites (Supplementary Table S3). PCA was used to assess how the distribution of the identified functional categories between the CK, FK and NK sites (Fig. 3), and this revealed that eighteen (18) major functional categories dominated samples from the inorganic fertilizer sites (FK), six (6) functional categories dominated the NK site while four (4) dominated samples from the CK site.

The functions unknown predominated the SEED Subsystem level 2 hierarchy for annotation of the gene across all the samples. The relative abundances for unknown protein in the samples were the most dominant with 17.149% (CK), 21.65% (FK) and 25.55% (NK) samples (Fig. 4).

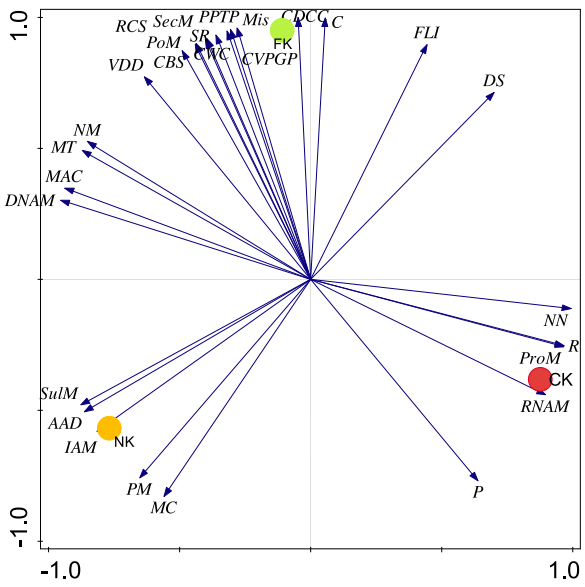

Fig. 3. PCA graph showing the functional analysis of endophytic microbiomes. The vector represents the impact of each metabolic process. Axis 1 (60.3%) and Axis 2 (39.7%) explained the variations based on Euclidean dissimilarities. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site.

3.3. Alpha and Beta diversity assessment of the functional groups across the sampling sites

The diversity of the functional groups at level 1 of the SEED subsystem was evaluated using the evenness index and Shannon index, and they were observed not to differ significantly ($P > 0.05$) (Table 2). Using the Kruskal–Wallis test, the extent of the differences in diversity between all samples from each site were assessed and no significant difference was observed ($P = 0.77$). The PCoA plot revealed a clear difference in the abundance of the 28 functional categories identified at SEED Subsystems level 1 in FK as compared to CK and NK (Fig. 5). Similarity test

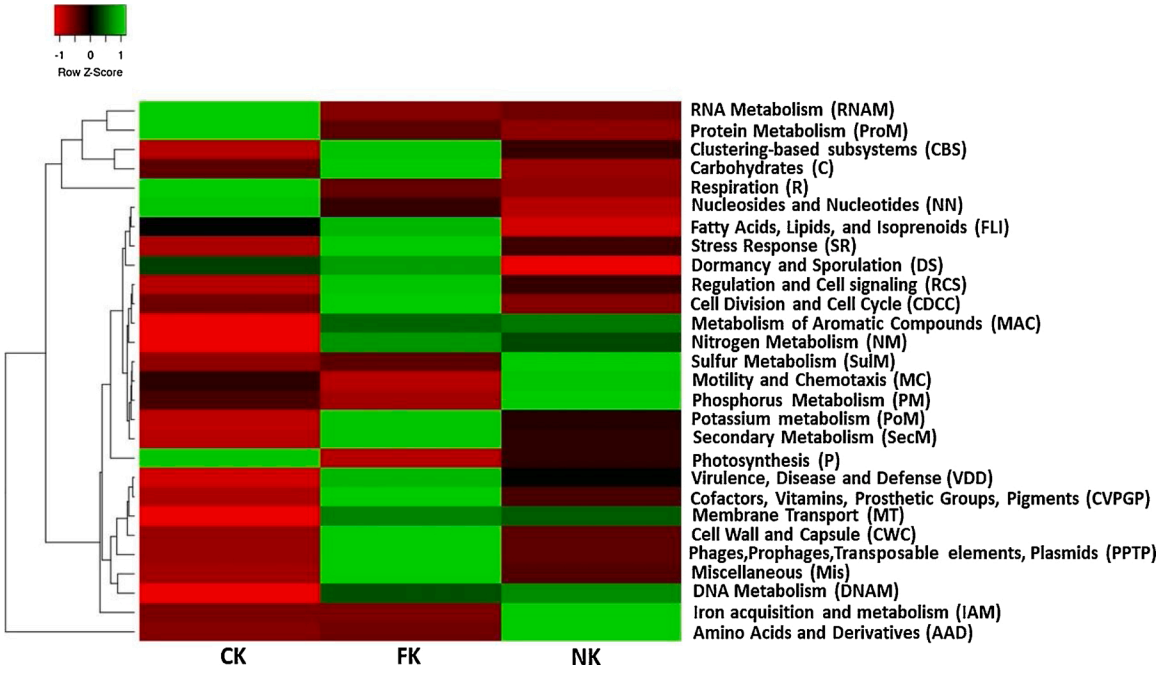

Fig. 2. Sequences similar to key metabolisms in samples from the maize plant in different sites. Relative abundance is indicated with the different colors as represented with the scale bar with z-score. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site.

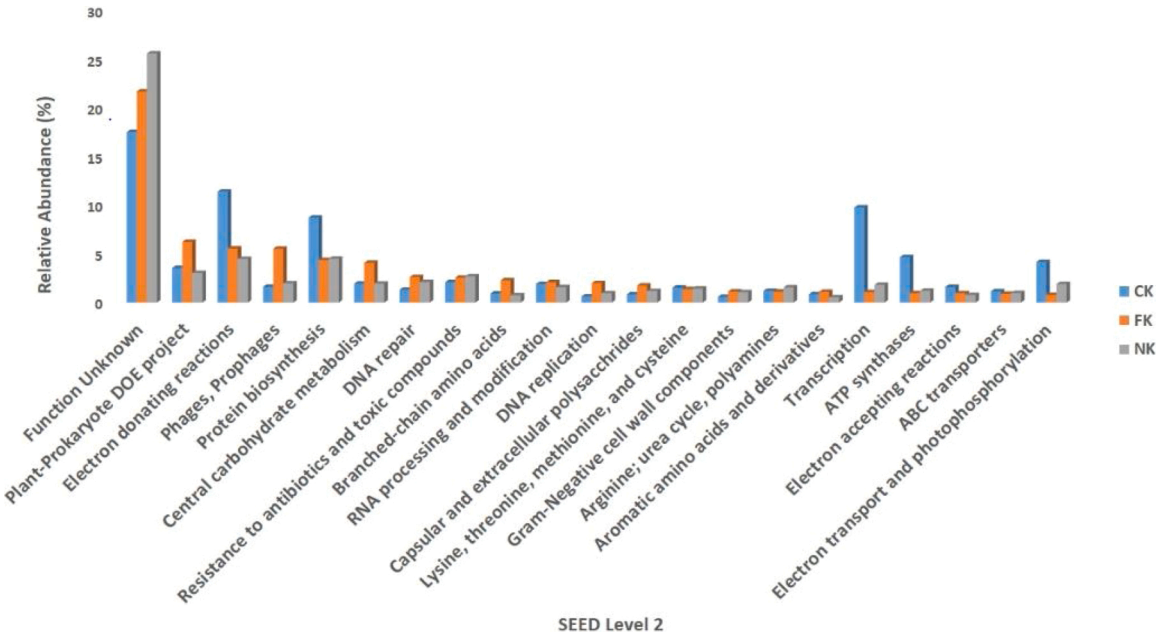

Fig. 4. Functional groups obtained at level 2 of the SEED subsystems. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site.

Table 2
Evenness and Diversity examination of the functional categories endophytic microbiomes at level 1 of the SEED subsystem from each site.

Indices	CK	FK	NK	P-value
Shannon_H	2.73 ± 0.17	2.94 ± 0.16	2.82 ± 0.16	0.77
Evenness_e^H/S	0.55 ± 0.08	0.68 ± 0.09	0.60 ± 0.09	

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.

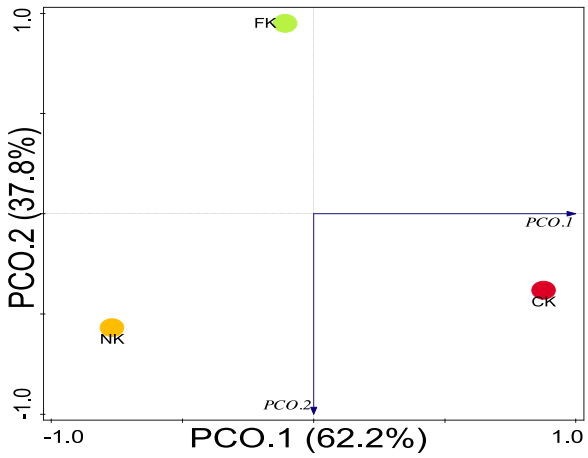

Fig. 5. PCoA graph for the functional groups identified at the SEED subsystem level for all the endophytic microbiomes from each site based on Euclidean dissimilarities. NK = samples from inorganic fertilizer site, FK = samples from organic fertilizer site, CK = samples from no fertilizer site.

using one-way ANOSIM revealed a significant difference in the 28 functional categories from samples across the sites (P = 0.01 and R = 0.67).

4. Discussion

In this study, we assessed the effect different farming practice on the functional diversity of endophytic microbiomes in root of maize plant. To actualize this the whole DNA from maize root planted with different fertilization and without fertilization were extracted and sequenced using shotgun metagenomics. The raw sequenced data obtained were analyzed using an online database called MG-RAST. Using MG-RAST analysis, endophytic microbiome sequences were identified while sequences attributed to maize plant were discarded. The major endophytic microbiome phyla identified in this study are basically of bacterial, fungi and archaea origin as shown in Figure S1. The SEED subsystem analysis was then used to predict the functions of identified endophytic microbiomes using different fertilizer applications.

The SEED is a categorization system that assembles functional gene groups into a hierarchy with 5 levels of subsystems. Level 1 which is the highest level of the subsystems includes different metabolisms such as catabolism and anabolism, while the lower levels show specific pathways or genes involved in the metabolisms [44]. In this study, though the abundance of microbiomes varies across the different fertilizer sites, the functional difference identified at subsystems level 1 across the sites do not differ significantly (P > 0.05) (Supplementary Table S3). Eighteen (18) major functional categories dominated samples from the organic fertilizer sites (FK), six (6) functional categories dominated the inorganic (NK) site while four (4) dominated samples from the on fertilizer (CK) site. This agrees with the claim that bacteria are grouped based on functional relatedness rather than taxonomic relatedness [45], the study suggests that the key level at which to address the assembly and structure of bacterial communities may not be "species" but rather the more functional level of genes. Other studies also revealed the functional roles of endophytes do not rely on their taxonomic classification but depend strongly on the environmental factors and host types [22,46].

Our results from the alpha diversity analysis showed the functions exhibited by the metagenomes in all the sites approached the theoretical limit of 2.81, indicating that virtually all the subsystems are present in the samples from all the sites [26]. In addition, low evenness value was observed (approximately 0.61, Table 2), indicating that there are few dominant metabolic processes (Such as protein metabolism,

clustering-based subsystems and respiration) in each site. Through shotgun metagenomics, we were able to show the different predominant metabolisms and distinct characteristics of the identified functional categories in the endophytic metagenomes. Our results further revealed that all the functional groups attributed to the endophytes from all the sampling sites did not differ significantly ($P > 0.05$) (Table 1). PCoA plot showed distinct separations ($R = 0.67$) between all the fertilizer sites (Fig. 5). This was further checked with ANOSIM which revealed that there was a significant difference between the functional groups of the endophytes across the fertilizer sites (P-values = 0.01).

Furthermore, PCA was used to test the hypothesis that different farming practices have a major impact on the metabolic pathways of endophytes (Fig. 3). The variance between the different sites obtained in this study is an indication that major functions are predicted by endophytic metagenomes. The position occupied by each metagenome in the PCA graph revealed the makeup of sequences linked to each subsystem, with the vectors showing that metabolism has more considerable influence on the distribution. Going by this results, it is easier to predict which metabolism is important to the endophytes identified in each fertilizer site. For instance, amino acids and derivatives (AAD), iron acquisition and metabolism (IAM), motility and chemotaxis (MC), phosphorus metabolism (PM), and sulfur metabolism (SulM) were abundant and specific in endophytes from inorganic fertilizers sites (NK) as compared with endophytes found within FK and NK sites.

Our results also revealed that each fertilizer site has some predominant categories of functional gene attributed to them. Dominant sequences associated with fatty acid, lipids and isoprenoids metabolism, carbohydrate metabolism, stress response, phage, prophage, nitrogen metabolism and secondary metabolism were observed in samples from the organic fertilizers sites (FK) (Supplementary Table S3, Fig. 2). This is much expected because organic fertilizers sites (FK) are known to have higher organic matter and the plant cultivated in such site is expected to have a higher level of carbon [47]. Endophytes will successfully thrive well in such an environment because they depend solely on the plant as their energy source. Therefore, higher fatty acid, lipids and isoprenoids metabolism, and carbohydrate from organic fertilizer samples as obtained in this study are in agreement with the earlier reports by Sharma and Chetani [47] and Lin, *et al.* [48] where high level of carbon are reported as part of the major characteristics of organic farming sites.

This was further buttressed at lower levels with the abundance of sequences linked with major metabolic pathways involved in the carbon cycle, such as glycolysis, gluconeogenesis and TCA cycle, being dominant in FK samples (Supplementary Figure S2A). Higher stress response, phage, and prophage observed in the organic fertilizer sites were much as expected, these attributes can help in plant protection against many environmental factors. This agrees with earlier studies in which agricultural practices with organic fertilizer increases soil microbial activities and enhances plant resistance to disease and pest attack [49,50]. Similarly, sequences related to nitrogen metabolism were abundant in endophytes from FK site. This is expected because studies have shown that organic fertilizers produce nitrogen in usable form, which helps in plant growth promotion without causing root burn in plants or destroying beneficial microorganisms inhabiting the soil [47]. At a lower level, sequences associated with metabolic processes involved in the nitrogen cycle like allantoin utilization, ammonia assimilation, nitrogen fixation, denitrification, nitrate and nitrite ammonification, alongside nitrilase (Supplementary Figure S2B) were dominant in FK samples. This agrees with an earlier study in which high nitrate and ammonium were observed in tomato cultivated with organic fertilizer [51]. In addition, sequences associated with secondary metabolism were dominant in FK samples. At a lower level, auxin associated trait such as auxin biosynthesis (Supplementary Figure S2E) was identified which have been reported in plant growth promotion [22].

Although sequences linked with phosphorus metabolism, iron acquisition and metabolism, sulfur metabolism and motility and chemotaxis were dominant in inorganic fertilizer sites (NK), they do not differ significantly ($P < 0.05$) across the sites. High application of inorganic fertilizers can be linked to high phosphorus and sulfur metabolism observed in the NK site, though inorganic fertilizers have reported to have some side effects on microorganisms in the environment [47,52]. This was further confirmed at lower levels with notable metabolic processes such as thioredoxin disulfide reductase, alkanesulfonate assimilation, sulfatide metabolism, inorganic sulfur assimilation, and galactosylceramide (Supplementary Figure S2C). Equally, some key metabolic pathways involved in phosphate metabolism observed at the lower level are alkylphosphonate utilization, phosphorus uptake and phosphate binding DING proteins (Supplementary Figure S2D).

Moreover, sequences associated with iron acquisition and metabolism were dominant in NK samples. At the lower level, this was further confirmed with notable metabolic pathways (such as bacillibactin siderophore, iron siderophore sensor and receptor system, siderophore pyochelin, siderophore achromobactin, siderophore enterobactin, siderophore assembly kit, siderophore pyoverdine, siderophore yersiniabactin biosynthesis, siderophore [Alcaligin-like], and siderophore staphylobactin (Supplementary Figure S2E). Iron is important in the synthesis of siderophore which are believed to abundant in inorganic fertilizer sites [53], as observed in this study. This agrees with a study by Rajkumar, *et al.* [54] where high iron for the roots of plants increased the production of siderophores by phytosiderophore-iron complex. Siderophore linked traits have been reported in plant growth promotion, this suggests that the association between endophytes and maize plants can enhance the growth of the root [22,38]. Also, siderophore biosynthesis has been reported in inducing systemic resistance of the plant to pathogens [55].

Sequences associated with motility and chemotaxis were also observed in NK samples. Motility and chemotaxis can aid the performance of endophytes; in that, it will enhance movement, networking and regulation of nutrient acquisition within the host [46,56]. Equally, clustering-based subsystems were observed to be predominant in all the samples, especially samples from FK sites. Clustering-based subsystems have been reported to harbor several functional genes whose functions are unknown [57]. In this study, they were the second most abundant functional category in all the samples (Fig. 2, Supplementary Table S3). This high distribution of clustering-based subsystems coupled with unknown function at level 2 of the subsystem (Fig. 4) showed that are many notable endophytic genes are present in endophytes whose functions are not yet explored.

5. Conclusions

We carried out the first functional diversity study of endophytic microbiomes in maize plant using shotgun metagenomics. Our study has shown that the functional diversity of endophytic microbiome in maize plant is influenced by different farming practices. To a greater extent that major functional categories were most abundant in endophytic microbiome from organic fertilizer sites (FK). Alpha diversity study revealed no significant differences exist among functional groups of the endophytes across the sites, while beta diversity study indicated that there was a significant difference among the functional groups of the endophytes across the fertilizer sites. Our study presents a high abundance of functional groups whose functions are unknown, indicating the prospect of identifying peculiar genes from the endophytic microbiomes. Therefore, we advocate for further studies that will explore the functional genes in endophytic microbiomes. Also, this study indicates that traits associated with plant growth promotion were highly represented in endophytes from plants cultivated with organic fertilizer. Our findings suggest a basis for the improvement of maize cultivation by exploring the beneficial properties of endophytes, this study advocate for the use of organic fertilizers in maize cultivation and in promoting sustainable agriculture.

Credit authorship contribution statement

Ayomide Emmanuel Fadiji handled the literature findings, carried out the planting and laboratory work, performed the all necessary analyses, interpreted the results, and wrote the manuscript. Ayansina Segun Ayangbenro provided technical input and proofread the manuscript. Olubukola Oluranti Babalola initiated the next-generation sequence research, supervised Ayomide Emmanuel Fadiji, helped shape the research, verified the analytical methods, secured funds for the study and commented on the manuscript at all stages.

Data accessibility

Sequence data obtained in this work have been deposited in the NCBI Sequence Read Archive under accession number PRJNA607664.

Contribution

Authors have contributed equally to this work, and there is no conflict of interest among them

Funding

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Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cpb.2021.100195.

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