Rice Root-Associated Endophytic Microbiome in Correspondence To Different Levels of Salinity At Indian Sundarban Areas

Gargi Das
Jadavpur University

Paltu Kumar Dhal (paltuk.dhal@jadavpuruniversity.in)
Jadavpur University  https://orcid.org/0000-0001-5790-3268

Research Article

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Abstract

The present study attempted to analyse rice root endogenous microbial diversity and their relationship with soil salinity and physicochemical factors in the salt stressed region of Sundarbans, India using amplicon metagenomics approaches. Our investigation indicates, the unique microbiome at slightly acidic nutrient enriched non-saline zone characterized by microbial genera that reported either having plant growth promotion (Flavobacterium, Novosphingobium and Kocuria) or biocatalytic abilities (Leptotrichia) whereas high ionic alkaline saline stressed zone dominated with either salt-tolerant microbes or less characterized endophytes (Arcobacter and Vogesella). The number of genera represented by significantly abundant OTUs was higher at the non-saline zone compared to that of the saline stressed zone probably due to higher nutrient concentrations and the absence of abiotic stress factors including salinity. Physicochemical parameters like nitrogen, phosphorus and potassium were found significantly correlated with Muribaculaceae highly enriched at the non-saline zone. However, relative dissolved oxygen was found significantly negatively correlated with Rikenellaceae and Desulfovibrionaceae, enriched in the non-saline soil. This study gives a well resolved picture of microbial community composition impacted by salinity and other rhizospheric soil factors.

1. Introduction

Rice (Oryza sativa L.) being one of the major staple food for more than half of the world's population significantly contribute to the nation's food security (Kunda et al. 2021). In India, the state of West Bengal is the highest producer of rice (Biswas et al. 2020). Within West Bengal, rice is grown in about 1 million hectares of land under the coastal ecosystem comprising 'the Sundarbans' (Banerjee et al. 2018). The world's largest coastal wetland, the Sundarbans, is an ecological hotspot and recognized for its significant biodiversity (Pramanik et al. 2019). The socioeconomic status of the inhabitants of Sundarbans is mostly dependant on farming where about 70% of farmers primarily grow rice crops (Ghosh and Mistri 2020). But Sundarbans facing the Bay of Bengal is a climatically vulnerable region exposed to severe impacts from climate change. Sea-level rise, increased frequency of cyclonic storms, erratic rainfall, and inefficient centuries-old river embankments result in saline intrusion into the mainland thereby increasing the soil salinity (Nath et al. 2021). Salt stress generates reactive oxygen species (ROS), decreases osmotic potential, and reduces water availability to plant roots, followed by ion toxicity thereby hindering the growth and development of rice plants (Hussain et al. 2019). Moreover, during higher salt accumulation, Na+ ions replace other essential cations present in the soil, increasing the compactness of soil and decreasing oxygen availability in the root zone (Vaishnav et al. 2019). These changes in the soil cause nutrient deficiencies and sodium toxicity in plants thereby turning fertile paddy fields into barren lands and ultimately reducing rice yield (Vaishnav et al., 2019).

Recent promising mitigation strategies for sustainable rice production include the development of halo-tolerant plant growth-promoting endophytic bio inoculants that alleviate salt stress in plants by inducing osmotic adjustment, detoxification, modulation of phytohormones, activating stress-responsive/induced genes, and nutrient acquisition (Shabanamol et al. 2018; Singh et al. 2018; Greetatorn et al. 2019; Lata et al. 2019; Rana et al. 2020; Hernández et al. 2021; Tufail et al. 2021). However, the application of bio inoculants is not much effective because some of the microbes are unable to act efficiently in real stressed field conditions (Vaishnav et al. 2016). Therefore, there is a need for the characterization of indigenous endophytic microbial assemblages and their changes with varying rhizospheric soil conditions for better formulation of efficient bio inoculants.

Few studies on endogenous unculturable microbiota colonizing rice-plants using Next-generation sequencing (NGS) technology have been conducted worldwide as well as in India but limited in the saline stressed zone of Sundarbans area (Long and Yao, 2020; Kunda et al. 2018, 2021; Setiawati et al. 2018; Khan et al. 2020; Kumar et al. 2021). However, these studies were undergone with a limited number of samples and even lack comparative information about non-saline soil from the same region. Additionally, an elaborate and well depth knowledge about the role of rhizospheric soil parameters for shaping endophytic microbiome diversity at saline ecosystem has not been established yet.

Nonetheless, this will be the first attempt to encounter all the above-mentioned problems with the following objectives:

1) Characterizing rice root endophytic microbial assemblages in the saline coastal zone of Sundarbans, West Bengal.

2) Identification of core and unique microbiota in rice plants grown in saline and non-saline region.

3) Predicting the contributions of relevant soil properties on rice root endophytic community composition, if any.

This study will expand the understanding of overall rice endophytes' role on the sustainability of cultivating the rice in the Sundarbans area concerning various environmental indices that may lead to prospective innovations in an agricultural field.

2. Method

2.1. Site selection and sample collection

In the present investigation, two coastal saline rice fields named Dabu and Godkhali in the Sundarbans region, West Bengal, were carefully chosen in collaboration with the expertise of ICAR-Central Soil Salinity Research Institute (CSSRI), West Bengal, India. Four different sampling sites (S1, S2, S3 and S4) from these two closely associated rice field stations representing both saline and non-saline soils were chosen so that we could avoid maximum disparity of location variation based microbial diversity (Fig S1). Total twenty-four independent rice plants (Oryza sativa; cultivar WGL20471, locally known as LalMiniket) from these four sites were collected at their vegetative stage during April 2019. The plants were randomly chosen and dug out carefully to prevent any damage to the roots. The rhizospheric soil (RS) for all samples was also carefully collected. Immediately sampled material was packaged in sterile bags (Himedia) placed on ice and brought back to the laboratory for further processing within 24 hours.
The salinity status of RS collected from these four sites were measured based on electrical conductivity (EC) of the saturated-paste extracts (ECe) using Thermo Scientific Orion Star 140 TM Series Multimeter (Thermo Fisher Scientific, U.S.). Based on the calculated result of soil salinity status, the sites were classified into two different zones: Sundarbans non-saline zone (SNSZ; S1, S2 and S3; 18 samples) and Sundarbans saline zone (SSZ; S4; 6 samples) (Table 1). Soils were considered to be saline if ECe was greater than 4 dS/m at 25°C (Shrivastava and Kumar 2015).

### Table 1

| Type                      | Site   | GPS location                      | pH   | ECe (dS/m) | RDO (mg/L) | TDS (ppm) | SO4 (mg/kg) | OC (%) | ON (mg/kg) | AP (mg/kg) | EK (mg/kg) |
|---------------------------|--------|-----------------------------------|------|------------|------------|-----------|-------------|--------|------------|------------|------------|
| Sundarbans non-saline zone (SNSZ) | S1     | 22°18’9”N, 88°4’40”E             | 7.0±0.1 | 3.07±0.07  | 137.7±0.7  | 673.2±0.7 | 158.5±0.9   | 2±0.2 | 1352.1±0.9 | 87.7±0.9   | 481.6±12.5 |
|                           | S2     | 22°18’43”N, 88°39’24”E            | 6.2±0.2 | 2.49±0.2   | 157.7±0.6  | 597.1±0.8 | 42±0.9      | 1.2±0.3| 1153.0±0.4 | 20.9±0.8   | 225.2±22.2 |
|                           | S3     | 22°18’43”N, 88°39’24”E            | 6.4±0.5 | 2.21±0.1   | 152.7±1.0  | 937.7±1.2 | 53.5±0.9    | 1.3±0.2| 1172.0±1.0 | 28.9±1.0   | 222.9±32.0  |
| Sundarbans saline zone (SSZ) | S4     | 22°13’16”N, 88°42’8”E             | 7.7±0.1 | 5±0.6      | 156.6±0.8  | 2679.1±1.9| 412.2±0.9   | 1±0.2 | 717.2±0.9  | 25.1±0.8   | 153.8±25.4  |

### 2.2. Measurement of the environmental parameters and nutrient content of the rhizospheric soils

Soil pH, relative dissolved oxygen (RDO) and total dissolved solids (TDS) were determined in 1:5 soil water suspensions using a Thermo Scientific Orion Star 140 TM Series Multimeter (Thermo Fisher Scientific, U.S.). Sulphate (SO4) was measured using Photometer MD 600 (Lovibond, Britain). Organic carbon (OC) was determined by the dichromate oxidation method of Walkley (1934). Organic nitrogen (ON) of soil was determined in 2.0M KCl extract by Kjeldahl distillation (Bremner 1960). Soil organic matter (SOM) was analysed according to Tabatabai (2015). Available phosphorus (AP), exchangeable potassium (EK) were determined by the Olsen method and NH4OAc extract-flame photometric method (Wichers et al. 1944, Watanabe and Olsen 1965) respectively.

### 2.3. Surface sterilization, metagenome extraction and 16S rRNA gene based amplicon metagenomic sequencing

The roots of the rice plants were surface sterilized according to methods by (Sessitsch et al. 2012) with minor modifications. After sterility check, the surface sterilized roots were frozen with liquid nitrogen and grounded to a fine powder using sterile mortar and pestle. Metagenomic DNA was extracted using Power Plant Pro DNA isolation kit (Mo Bio) following the manufacturer's instructions. The hypervariable V3-V4 regions of bacterial 16S rRNA gene were amplified using universal primers 341F (GCCTACGGGNGGCWGCAG) and 806R (ACTACHVGGGTATCTAATCC), amplicon libraries were prepared, purified and amplicon sequencing data were generated on the Illumina MiSeq platform in a 2 × 300 bp paired-end run (Nayak et al. 2021). Raw paired-end primer trimmed sequences were provided by Eurofins, Germany. The raw FASTAQ sequences were submitted to NCBI under BioProject PRJNA681119.

As we are only focused on the sequences of bacterial 16S rRNA gene, but there could be good chances of contamination with mitochondrial and chloroplast DNA sequences, other prominent strategies were adapted to exclude these contaminations.

### 2.4. Sequence data analysis

All the raw FastQ datasets were processed following the protocol of (Hassenrück et al. 2016). The sequence reads were quality trimmed based on minimum quality score 15, the window size of 4 bases and the minimum length of 100bp by using trimmomatic v0.32 (Bolger et al. 2014). Then trimmed sequences were merged using PEAR v0.9.5 (Zhang et al. 2014). Finally, clustered into OTU (operational taxonomic unit) using swarm v2.0 (Mahé et al. 2014). Sequence OTUs were taxonomically assigned using SINA (SILVA Incremental Aligner) v1.2.11 of the SILVA rRNA project reference database (release 128) with a minimum similarity alignment of 0.8 (Elmar et al. 2011). The obtained OTUs were further curated to exclude the absolute singleton, mitochondria/ chloroplast/ eukaryotic DNA using well standardised R scripts (Kunda et al. 2018).

### 2.5. Statistical analysis

Principal component analysis (PCA) was conducted to ordinate the sampling sites based on their measured RS physicochemical parameters. The measured environmental parameters and nutrient contents that significantly differ among the two zones were examined using the Wilcoxon rank sum test. A diversity indices were calculated based on species richness estimators (average number of OTUs, Chao1 and ace) and the diversity index (average inverse Simpson Index, Shannon diversity) to assess overall microbial diversity within the zones after randomly rarefying the data set repeatedly to the minimum library size (28588 sequences). Significant differences in α diversity indices were assessed using nonparametric, unpaired p-value adjusted Wilcoxon tests. The relationship between α diversity and soil parameters (if any) was also assessed by the Spearman's Rank correlation test.

Prior to β-diversity calculation, the dataset was pruned to exclude the rare biosphere by retaining only those OTUs that were present in at least two sequences within more than 10% of samples. The β-diversity patterns were not changed by this reduction in the data sets (Mantel test, r = 0.99, P = 0.001). The change in the composition of the microbial communities (β-diversity) between two zones was explored by analysis of similarity (ANOSIM). This was confirmed by
calculating Bray-Curtis dissimilarity coefficients, thereby generating cluster dendrogram as well as non-metric multidimensional scaling (NMDS). The envfit function of the vegan package was further used to get the correlation of the most significant variable with overall microbial communities (Clarke and Ainsworth 1993; Oksanen et al. 2016).

The sequence counts were further clr-transformed using aldex.clr function of the R package ALDEx2, using median of 128 Monte Carlo Dirichlet instances (Fernandes et al. 2014). Differently abundant OTUs among SNSZ and SSZ were identified in the reduced data set of each size fraction at a parametric FDR-adjusted and a non-parametric unadjusted significance threshold of 0.05 were plotted as Dot plot.

Correlation analyses of soil physicochemical factors and the microbial community at phylum level were carried out based on Spearman correlation coefficient using R package Hmisc (Miscellaneous and Yes 2021).

The core and unique microbial communities of the two different studied zones: SNSZ and SSZ were also identified in genus level using venny2.1 on the pruned OTUs https://bioinfogp.cnb.csic.es/tools/venny/

All statistical analyses were performed in R (R version 3.6.2 in R studio version 1.2.5033) using additional R packages like ‘vegan’ R package, ALDEx2 and gplots (Oksanen et al. 2016; Warne et al. 2016).

3. Results
3.1. Measurement of environmental and nutrient parameters of rhizospheric soil samples

SSZ was characterized by higher EC (5.6 dS/m) to that of SNSZ (2.6 dS/m). Other measured environmental parameters (pH, RDO, SO\textsubscript{4} and TDS) were higher at SSZ while nutrient contents (OC, ON, AP, EK and SOM) were elevated at SNSZ (Table 1).

All these soil parameters except RDO and AP were significantly different among SNSZ and SSZ (Wilcoxon rank sum test, p<0.05) probably indicating the influence of measured parameters on SNSZ and SSZ soil types.

PCA analysis indicates, first two principal components (PC1 and PC2) captured 93.99% of the variation among sampling sites based on environmental and nutrient parameters (Fig. 1). PC1 (62.95%) separated samples into two clusters except for two samples (S2A and S2E). Interestingly, cluster1 comprised samples from SSZ while cluster2 denoted SNSZ. PC1 is mainly contributed to TDS, OC, ON, AP and SOM while the second principal component PC2 which accounted for 31.04% of the variation had a better correlation with pH, EC\textsubscript{e} and SO\textsubscript{4}.

3.2. Endophytic microbial diversity and community composition inhabiting in rice root samples:

In total, we obtained 50, 04,200 raw reads from the amplicon sequencing which were quality trimmed to procure 47, 33,273 (average 197220) number of high quality pair-end reads corresponding to 33, 09,331 (average 137889) merged sequences that led to 521843 swarm OTUs. Further elimination of singleton, mitochondria, chloroplast and unclassified OTUs at phylum level we obtained a total 40408 OTUs (Table S1).

Each of the bacterial species richness estimators (nOTUs, Chao1 and ace) were significantly higher in SNSZ compared to SSZ (Wilcoxon Test, p-value < 0.5). Bacterial species evenness indicated by abundance based coverage estimator invS and Shannon's diversity index measuring combined species richness and evenness were higher for SNSZ (Fig S2).

There was a positive correlation of a diversity for SO\textsubscript{4} (p = 0.6, p-value= 0.009), ON (p = 0.52, p-value= 0.02) and EK (p = 0.49, p-value= 0.04) among SNSZ soil type. A significant negative association of RDO (p = -0.52, p-value= 0.02) for a diversity among SNSZ soils was also observed. But no such correlations were found for SSZ.

The rice root endophytic bacterial communities from studied zones of the Sundarbans region were dominated by Alphaproteobacteria (36.1% among all samples; higher abundance at SSZ), Bacteroidia (22%; higher abundance at SNSZ), Clostridia (13.8%; SNSZ) and Gammaproteobacteria (12%; SNSZ) representing 84% of the total number of sequences (Fig. 2).

Although bacterial genus Rhizobium (57.9% of all the sequences) was the most abundant in both the zones, SSZ showed higher relative abundance followed by Bacteroides. On the other hand, higher proportions of Muribaculaceae Incertae Sedis, Lachnospiraceae NK4A136 group and Pseudomonas were found in SNSZ (Fig 2). Among 77 total common bacteria termed as core bacteria, the aforementioned bacterial genera were the most dominant common bacterial groups prevalent in both zones. Apart from them, 7 bacterial genera found exclusively at SSZ were Vogesella, Arcobacter, Saccharimonadaceae Incertae Sedis, Gmella, Woesearchaeia Incertae Sedis, Burgeyella and GCA-900066575 (Lachnospiraceae family) while 8 bacterial genera were found only in SNSZ: Undibacterium, Peptococcaceae Incertae Sedis, Prolixibacteraceae Incertae Sedis, Flavobacterium, Kocuria, Novosphingobium, Leptotrichia and Shewanella (Fig S3).

To further identify the OTUs responsible for the patterns in endophytic bacterial community composition, the differences in the proportion of individual OTUs between the SNSZ and SSZ were tested using ALDEx2. Total 17 differently abundant OTUs were detected among the two zones which represented 3.2% of SSZ and 2.9% of SNSZ. The differently abundant OTUs were mostly dominated by Bacteroidia (6 OTUs), Gammaproteobacteria (4 OTUs), Alphaproteobacteria (2 OTUs), Bacilli (2 OTUs), Camphylobacteria (2 OTUs) and a single OTU of Verrucomicrobiae. The cases which showed more than one differently abundant OTUs per class often exhibited divergent patterns between the zones. Among bacterial class Bacteroidia, Odoribacter (OTU9) and Bacteroides (OTU8), Muribaculaceae Incertae Sedis (OTU 15) were the significantly abundant OTUs dominant at SNSZ while Bacteroides (OTU49) was higher at SSZ. Under
3.3. Changes composition of endophytic rice root microbial community

At OTU level, variation in microbial communities between SNSZ and SSZ was explored using ANOSIM which didn't show any significant difference (ANOSIM, R = 0.08, p-value>0.05). This finding was supported by cluster dendrogram based on Bray-Curtis dissimilarities which also unable to show any distinctive pattern in bacterial community composition between SNSZ and SSZ (Fig. 2). The NMDS ordination didn't show any distinct separation between SNSZ and SSZ (Fig: 3). EnviT function showed soil parameters ECr, EK, RDO, AP, ON and TDS appeared to be strongly correlated with the patterns in bacterial community composition (Table S2).

3.4. Connecting environmental parameters and microbial community composition

The correlation between the physicochemical parameters and relative microbial abundance at the family level was evaluated based on Spearman correlation coefficient analysis (Fig. 4). The abundance of the family Muribaculaceae at SNSZ was positively correlated with nutrient parameters ON, AP and EK (r > 0.5; p < 0.05). In addition, AP was positively correlated with the abundance of Rikenellaceae and EK with Ruminococcaceae (r > 0.5; p < 0.05). However, RDO concentration was significantly positively correlated with Rhizobiaceae (r >0.5; p < 0.5) and significantly negatively correlated with Rikenellaceae and Desulfovibrionaceae (r <0.5; p > 0.05).

We also observed abundance of bacterial family Muribaculaceae at slightly acidic nutrient enriched non-saline soils positively correlated with nutrient parameters ON, AP and EK. SNSZ comprising Rikenellaceae was positively correlated with AP which was in line with the previous finding where the abundance of this family increased with higher phosphorus concentrations (Latif et al. 2018). Moreover, RDO was found to be significantly positively correlated with one of the most abundant family Rhizobiaceae which was well compared with the previous investigation where their abundance was usually observed near the oxic surface of the polygon, primarily in the studied rim but was significantly negatively correlated with Rikenellaceae and Desulfovibrionaceae (Liebner et al. 2008).

4. Discussion

4.1. Comparison between rhizospheric soil properties

As indicated from measured environmental and nutrient parameters, SNSZ can be termed as slightly acidic nutrient enriched soil as they are characterized by lower pH and elevated amounts of OC, ON, AP and EK, SOM which is in line with previous investigations (Asadu et al. 2010; Pan et al. 2013; Zörb et al. 2014; Lamizadeh et al. 2016) while SSZ recorded higher ECe and pH as that of previous findings (Haldar and Deb Nath; Kushwaha et al. 2020; Yang et al. 2020). As reported by (Chinnusamy et al. 2005), Na, Cl and SO₄ are the major salt ions responsible for ion toxicity probably support our data of higher SO₄ concentrations and dissolved solids, TDS at saline rice paddy field, SSZ. Therefore, soils of SSZ can be referred to as high ionic alkaline saline soils. Thus, SSZ is characterized by ion toxicity and osmotic potential imbalance due to high salinity while non-saline SNSZ is marked by a balance of soil nutrients and organic matter.

4.2. Variation in microbial richness and evenness across the studied zones

In this study, we found a significant decrease in species richness and evenness estimators along SSZ compared to SNSZ indicating bacterial community richness decreases with increasing salinities which support the previous findings (Zhao et al., 2020). However, the differences in these estimators within SSZ and SNSZ are probably not only because of salinity as well as various other environmental and nutrient parameters. Since other physicochemical factors also changed along with the salinity of the Sundarbans, the observed bacterial community composition may be a result of parameters that correlate with salinity.

4.3. Relative abundance of microbial taxa found in two zones

In this study, among seven bacterial genera which are unique to SSZ, Arcobacter was previously reported as rice endophytes from the same region (Kunda et al. 2018). Gemella and Saccharimonadaceae were reported to be present in heavy metal contaminated saline soil (Marzan et al., 2017). The abundance of Vogesella is frequently observed in alkaline and rhizospheric soil of saline tolerant pokkali rice plant, therefore its presence as an endophyte in rice plants at SSZ is quite familiar (Rameshkumar et al., 2020). Archaeal family Woesarearchaeia Incertae Sedis and Bergeyella were previously observed in the saline marine environment but recognized as rice root endophytes from the first time in this study (Cho and Hwang 2011; Fernandes et al. 2020).

In contrast, among eight unique bacterial genera found at SNSZ, Flavobacterium strain, Kocuria and Novosphingobium well studied for plant growth promoting (PGP) capabilities were reported to promote rice growth by encoding several plant microbe interacting genes, regulating phytohormone levels, nutrient acquisition, redox potential, ion homeostasis, Shewanella and Undibacterium identified as rice endophytes responsible for biogeochemical Fe and S cycles in the tested rice paddy soils (Rodrigo 2011; Krishnan et al. 2016; Kunda et al. 2018; Li et al. 2020; Lian et al. 2020; Wang et al. 2021). To our knowledge, few genera (Peptococcaceae, Prolificibacteraceae and Leptotrichia) identified in our study have not been reported previously as rice endophytes and most probably are endophytes at non-saline zone. However, Cas13a from Leptotrichia was successful in conferring resistance to one of the RNA viruses, Turnip Mosaic Virus (TuMV) in Nicotiana benthamiana (Farhat et al. 2019). Our investigation indicates, the unique microbiome at non-saline zone characterized by microbial genera that reported either having PGP abilities or acting as potential biocontrol agents whereas saline stressed SSZ dominated with either salt-tolerant microbes or less characterized endophytes.

The core microbiota, Rhizobium and Pseudomonas are well reported as N₂-fixing plant-growth-promoting root-nodule endophytic bacteria commonly found in rice and saline soil (Mirza et al. 2006; Kunda et al. 2018). While Muribaculaceae Incertae Sedis as rice root endophytes have not been reported yet. Among
other common genera found abundantly in both the zones, endophytes Bacteroides, Lachnospiraceae NK4A136 group dominating in lower pH SNSZ is according to finding by the author (Huang et al. 2018) who identified this genus as the key contributor to decrease in pH for reductive soil disinfection.

Among the 17 most differently abundant OTUs, SNSZ associated bacterial communities mainly belonged to genera Pantoea and Pseudomonas. Endophytic diazotrophic Pantoea promote growth of rice plants even in salinity stress by making biologically fixed nitrogen and present in almost all the samples of rice collected from all around the world (Prakamhang et al., 2009; Kunda et al., 2018). They vigorously colonize rice and exhibit useful properties like phytohormone production viz. abscisic acid, gibberellic acid, cytokinin and indole-3-acetic acid and phosphate solubilization in rice plants (Prakamhang et al., 2009). Pseudomonas sp. found in the rhizospheric region of rice plants have Ca-P solubilizing and IAA production ability, act as biocontrol agents which produce HCN, pyoluteorin, pyrrolnitrin, 2, 4-diacetylphloroglucinol and phenazines chiefly phenazine-1-carboxylic acid and phenazine-1-carboxamide (Prakamhang et al. 2009). Ammonia oxidizing Odoribacter and Bacteroides endophytes were also enriched in SNSZ. The number of genera represented by significantly abundant OTUs was higher at the non-saline zone compared to that of the saline stressed zone probably due to higher nutrient concentrations and the absence of abiotic stress factors including salinity may responsible for hampering rice growth. This data corroborates the result of differences in α-diversity indices where the species diversity was higher at the non-saline than the saline prone zone.

Some of the aforementioned endophytes have been reported as prominent candidates for biofertilizers in salinity affected rice paddy fields which include Rhizobium sp., Pantoea sp. and Pseudomonas sp (Gupta et al. 2012). These endophytic diazotrophs are reported to have better N2 fixing capability than their counterparts i.e., rhizospheric diazotrophs because they escape competition with soil microbes for nutrients and achieve close contact with the plant tissues (Prakamhang et al., 2009). Also, the diversity and the spatio-temporal distribution of diazotrophs is essential to improve our understanding of biogeochemical cycles in the mangrove ecosystem. Thus, rice endophytes could provide an immense agricultural benefit which is of definite ecological and economic significance.

### 4.4. Influence of environmental parameters and nutrient concentrations on microbial community composition

Bray-Curtis dissimilarity analysis revealed no significant differences for endophytic microbiome profiles between rice plants grown in SSZ and SNSZ in a particular geographical location which is also supported by ANOSIM indicator. This finding may support the idea that the endophytic community composition is dependent not only on the surrounding environment but also on the host genotype (Hilholm et al. 2002). The change in microbial community composition was not significant could be due to the same host genotype selected from the same region. In this study, a single variety of cultivar (Oryza sativa; cultivar WGL20471, locally known as LalMiniket) has been selected for both the zones thereby conferring the same genotype of rice plant harbouring the endogenous microbial communities. However, variation in endophytic microbial diversity was observed between the saline stressed and non-saline zones because of differences among their physicochemical parameters.

It is generally accepted that the growth of rice plants are strongly influenced by their endophytic bacteria and soil health status. Thus, an elaborate study on rice endophytic communities associated with soil characteristics on saline stressed and non-saline paddy fields is helpful to predict any relationship between them. From NMDS envfit, it was found that ECv, EK, RDO, AP, ON and TDS coincided most strongly with microbial community composition in the studied zones. Thereby proving the role of environmental and nutrient parameters in shaping microbial communities at the studied zones.

As we are the very first one to report about these observations, the present study could be a pillar to new approaches in the field of interdisciplinary methods to know about endophytic communities in rice roots along with coastal saline soil and their trait based relationships.

### 5. Conclusion

This study provides the first insight into the diversity of rice root endophytic microbes in the saline stressed zone of Sundarbans, West Bengal along with the non-saline zone from the same region. From our findings, the saline zone could be represented as high ionic alkaline saline soils while non-saline zone is slightly acidic nutrient enriched soils. Although the variation in endophytic microbial community composition between saline and non-saline zones was not significant possibly due to the same variety of cultivar chosen for this study conferring similar plant based genotypic makeup from the same regions.

However, this study reports, the unique microbiome at non-saline zone characterized by microbial genera that reported either having PGP abilities or acting as potential biocontrol agents whereas saline stressed SSZ dominated with either salt-tolerant microbes or less characterized endophytes. An attempt was also made to correlate the association of endophytic microbial diversity and rhizospheric soil environmental and nutrient parameters in the studied zones. Physicochemical parameters like ECv, EK, RDO, AP, ON and TDS coincided most strongly with microbial community composition in the studied paddy zones which could drive their microbial communities.

### Declarations

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#### Author Contributions

PKD allocate funds and critically analyse manuscripts. PKD and GD designed experiments. GD performed the experiments. GD and PKD curated the metagenomic data, analysed data and assisted in data interpretation. PKD and GD wrote the manuscript. Both the authors critically revised the article and
gave their approval of the submitted version

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**Data Availability:**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate:** Not applicable.

**Consent for publication:** Not applicable.

**Competing Interests:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

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Figures
Figure 1

Principal component analysis (PCA) of measured environmental parameters at Site1 (S1), Site2 (S2), Site3 (S3) and Site4 (S4). Based on ECe, electrical conductivity of the saturated-paste extracts, samples were grouped into two distinct zones, shown as shaded hulls: Sundarbans non-saline zone (violet) and Sundarbans saline zone (cyan). TDS, total dissolved solids; SO4, sulphate; OC, organic carbon; SOM, soil organic matter; AP, available phosphorus; EK, exchangeable potassium; RDO, relative dissolved oxygen; ON, organic nitrogen.

Figure 2
Bacterial community composition on class and genus level. Order of plots from top to bottom: hierarchical cluster dendrogram based on Bray-Curtis dissimilarity, bar plot of relative sequence proportions of dominant bacterial class, heatmap of dominant genera map (white: no sequences) and total sequence proportion of genera displayed in heatmap.

Figure 3

Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis dissimilarity of the bacterial community of each sampled site. Arrows indicate envt correlations of environmental parameters with bacterial community composition. The shaded hulls denote Sundarbans non-saline zone (SNSZ; violet) and Sundarbans saline zone (SSZ; cyan).

Figure 4

Heatmap of dominant genera map (white: no sequences) and total sequence proportion of genera displayed in heatmap.
Correlation Heatmap indicating spearman rank correlation between the relative abundance of bacterial phyla and soil physicochemical parameters. Horizontal ordinate represents soil physicochemical properties, vertical ordinate represent bacterial community abundance information. Negative correlation and positive correlation were represented by blue color and red color respectively, * indicates p<0.05, r<=-0.5 and r>=0.5

**Supplementary Files**

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- FigS1Map.tif
- FigS2Alphadiversity.tif
- FigS3Venndiagram.tif
- FigS4Dotplot.tif
- TableS1Sequencecount.docx
- TableS2NMDSenvfit.docx