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RESEARCH

Culture-Dependent Comparison of Aerobic Bacterial Morphospecies Profiles in Paddy Soils Under Different Eco-Friendly Fertilizer Technologies

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ABSTRACT

Purpose: *To compare the key structural community parameters and functional group profiles of culturable aerobic bacteria in paddy rhizosphere maintained under different fertilizer regimes at two locations with different soil types in Sri Lanka across three stages (i.e. before transplanting, at maximum tillering and grain filling stages) of the crop cycle.*

Research Method: *Three locally developed eco-friendly fertilizer technologies were tested against the recommended dosages of synthetic nitrogen and phosphorous fertilizers and no application of fertilizers using rice variety Bg 352. The field experiments were conducted at Batalagoda and Maha-Illuppallama research stations, which located at two different agro ecological zones of Sri Lanka. Based on the culturable aerobic bacterial morphospecies in rhizosheric soil, microbial community parameters and functional groups were determined and compared among the fertilizer treatments at two locations and the three soil sampling stages of crop growth.*

Findings and Values: *Abundance, richness, diversity and percentages of phosphate-solubilizing, nitrogen-fixing and Gram-positive bacteria did not differ significantly among the fertilizer regimes but the abundance varied between locations and sampling stages ($p<0.05$). The interaction effect (location x fertilizer regime x sampling stage) was significant ($p<0.05$) on species richness, diversity, percentage of phosphate-solubilizing bacteria and N-fixing bacteria. Community structure of culturable aerobic bacteria in paddy-soils is largely determined by the soil type and crop growth stage than the short-term responses to the fertilizer regimes. This study highlights the effects of short-term exposure of reduced dosages of synthetic fertilizers on culturable rice rhizospheric bacteria.*

Keywords: *Eco-friendly nutrient management technologies, Functional bacterial profiles, N-fixing bacteria, Paddy rhizosphere, Phosphate-solubilizing bacteria.*

INTRODUCTION

Soil microbiota demonstrates an inseparable relationship with the growth and development of plants and for rice (*Oryza sativa* L.) it is not an exception. Bacteria are a key component of soil microbiota and perform beneficial roles by enhancing nutrient availability to plants through fixing nitrogen and dissolving phosphate.

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Additionally, the soil dwelling bacteria act as antagonists of plant pathogenic microbes and control disease development through direct or indirect interventions (Jacoby *et al.* 2017). In addition, the contribution of soil bacteria as drivers of biogeochemical cycling of nutrients and organic matter decomposition is well documented (Pratibha *et al.* 2023). Therefore, diverse soil bacterial community can facilitate plant growth, development and vigour due to their numerous functional capabilities.

However, the structure and functional activities of soil bacterial communities are affected by many soil management factors, such as the type of fertilizer, the mode of fertilization, the level and frequency of fertilization (Chen *et al.* 2022). Huang *et al.* (2020) reported that the bacterial community structure in paddy soils was affected by the cropping system, and the effect of altering of rice soil microbial community on rice productivity was observed by Xuan *et al.* (2012). According to Hayatsu (2014), slow and controlled-release nitrogen fertilizers improve the microbial community in the rhizosphere.

Inorganic fertilizer is an indispensable input in global rice cultivation to sustain the demand of rice. In the past few decades, chemical fertilizers, particularly urea, triple super phosphate (TSP) and muriate of potash (MOP) have been widely used in paddy cultivation in Sri Lanka through the heavily subsidized fertilizer schemes. As explained by Liang *et al.* (2020), several studies have reported the reduction of diversity of soil bacterial community structure (both rhizosphere and non-rhizosphere) due to chemical fertilizer application compared to the application of organic fertilizer. A recent research was conducted to introduce effective eco-friendly fertilizer technologies (EFTs) for paddy cultivation in Sri Lanka through a package comprised of biofertilizers containing cyanobacteria (CB) and phosphate solubilizing bacteria (PSBs), a slow release fertilizer (SRF) containing urea intercalated paddy husk biochar (23% N), and a site specific determination of NPK requirement through a decision management software named as Nutrient Expert, Sri Lanka (NESL) (Amarawansa 2020, Dissanayake 2022, Kulasinghe 2022, Dandeniya *et al.* 2023). The objective of the introduction of EFTs is to reduce the present use of synthetic fertilizer in paddy cultivation without causing a negative impact on rice yield or an improvement of the present status of the rice yield.

Paddy soil is a rich microcosm in terms of microbial community structure, which includes aerobic and anaerobic microorganisms with diverse physiological characteristics. The present study focuses on aerobic bacterial population in paddy soils treated under five different fertilizer management approaches (regimes) by culture-dependent methods. Culture-dependent methods have the limitation of recovering only a few groups of microorganisms and hence, do not reflect the real existence of microorganisms in environmental samples. Therefore, culture-independent methods based on molecular biology (i.e. PCR and gel electrophoresis, nucleic acid hybridization) have emerged to reveal numerous unculturable microorganisms in a community (Hill *et al.* 2000). Nevertheless, data obtained purely by molecular methods are not completely error-free. Biased PCR amplification of certain sequence types in samples and difficulties in assigning phenotypes to the detected taxons are some of the deficiencies of molecular-based culture-independent methods (Chin *et al.* 1999). Therefore, neither method is superior to the other and to obtain a more complete picture of the structural and functional profile of the soil microbial communities, a combination of both methods is necessary (Hill *et al.* 2000). Despite the inherent limitations, culture-based methods with modified/improved culturing techniques have been widely, traditionally and routinely used to capture the diversity of targeted groups with specific physiological requirements in soil samples, when focusing especially on soil health under resource-limited setups (Hill *et al.* 2000).

Soil microbial populations can be positively influenced by eco-friendly fertilizer technologies. Being a sensitive biomarker of soil and plant health, scientific evidence of soil bacterial population by EFTs would provide a strong background when deciding the most effective EFTs. Based on available literature, majority of the studies have been focused on the effect

of soil microbiota by a single type of EFT. Limited research findings are available on the types of EFTs used in the present study which were designed by aiming reduced usage of synthetic fertilizer without a negative impact on rice yield. Further, temporal changes of the microorganisms in paddy soil, over a growth season, due to the above types of EFTs have not been determined. Therefore, the present study was conducted to compare the community parameters of culturable aerobic bacteria populations in paddy soils collected from fields of two agroecological zones of Sri Lanka which were maintained under different fertilizer regimes. It was also aimed to compare the soil bacterial populations possessing beneficial traits towards plant growth promotion under the different fertilizer regimes.

MATERIALS AND METHODS

Description of the soil sampling sites

Soil samples were collected from experimental rice fields in Batalagoda (BG) (7.5324°N, 80.4340°E) and Maha-Illuppallama (MI) (8.1134°N, 80.4702°E) during the 2020/21 rice cultivation season (Maha season). Batalagoda (BG) and Maha-Illuppallama (MI) are located respectively in the low country intermediate zone (50 m above mean sea level) and low country dry zone (138 m above mean sea level) and represent two major rice-producing regions of Sri Lanka. The soil type in the paddy fields at both locations is classified as Low-Humic Gley (LHG), belonging to the great group Tropaqualfs, under the order Alfisols and sub-order Aqualfs (Mapa *et al.* 2005, 2009). Field experiments were conducted in these fields during the 2019/20 Maha season, and the soil analyses for studying aerobic bacterial populations were performed in the 2020/21 Maha season. During the 2020/21 Maha season, Batalagoda experienced an average rainfall of 146.5 mm, with maximum and minimum daily temperatures of 29.9°C and 21.6°C, respectively. In the same season, Maha-Illuppallama reported an average rainfall of 94.08 mm, along with maximum and minimum daily temperatures of 30.4°C and 21.8°C, respectively.

Crop establishment and fertilizer regimes

Rice seedlings (var. Bg 352) were transplanted in replicate plots (each having dimensions of 6 m × 3 m) which received five different nutrient management treatments (Table 1). Out of the five treatments, three were eco-friendly fertilizer technologies (EFTs), developed for potential reduction of the current use of inorganic nitrogen (N) and phosphorus (P) fertilizers recommended by the Department of Agriculture, Sri Lanka (DoA). These EFTs were designed using a slow-release fertilizer (SRF) as a pelleted urea–rice husk biochar composite (Dissanayake 2022), biofertilizers containing cyanobacteria (CB) (Amarawansa 2020) and phosphate solubilizing bacteria (PSB) (Dandeniya *et al.* 2023), and a decision-making software tool, Nutrient Expert Sri Lanka (NESL), to determine the site-specific nutrient requirement (Kulasinghe 2022). In addition, the current recommended rates of N and P fertilizers as urea (U) and triple super phosphate (TSP) as per DoA recommendations served as the positive control. A negative control treatment was maintained without addition of any form of N and P fertilizers. Each treatment (Table 1) was replicated three times at both locations. Potassium (K) requirement was supplied using muriate of potash (MOP) to all treatments as the objective was to reduce only synthetic N and P fertilizers. T1 and T2 received the DoA-recommended K rate for both locations, whereas T3, T4 and T5 received the site-specific K requirement. Urea, TSP, MOP, SRF and cyanobacteria were applied to the paddy field after transplanting, while PSB were applied to nursery trays at the seedling stage. To avoid cross-contamination between treatments, each field plot was partitioned using polythene sheets (450 gauge) secured with a mud bund. The treatments were repeated in the same fields with necessary measures taken to prevent soil cross-contamination during all agronomic operations, including land preparation and irrigation.

Collection of soil samples

Soil samples were collected from each replicate field plot under the five different nutrient management regimes. At the time of sample collection, all the replicate plots have been maintained under their respective treatment for

Table 1. Details of the nutrient treatments used in the study at Batalagoda(BG) and Maha-Illuppallama (MI).

Treatment	Description of the treatment	Batalagoda(BG)			Maha-Illuppallama(MI)		
		Urea	TSP	MOP	Urea	TSP	MOP
T1	No application of N or P sources (negative control)	0.0	0.0	61.1	0.0	0.0	61.1
T2	N as Urea and P as TSP were applied according to DoA recommendation (positive control)	227.8	55.6	61.1	227.8	55.6	61.1
T3	N as Urea and P as TSP were applied as per the site-specific requirement determined by NESL software	238.9	50.0	77.8	238.9	72.2	77.8
T4*	65% of N and P decided by the NESL software as site-specific requirement were applied using SRF and TSP respectively	0.0	38.9	77.8	0.0	55.6	77.8
T5**	65% of the site-specific requirement of N and P decided by the NESL software was applied using SRF and TSP. The remaining P and N requirement was supplemented through biofertilizers containing CB and PSB	0.0	38.9	77.8	0.0	55.6	77.8

Note: *At both locations for T4 treatment SRF was added instead of Urea at a rate of 413.8 kg/ha.
**T5 treatment at both locations was applied with SRF (413.8 kg/ha) + CB (40 kg/ha) instead of Urea and PSB (30 kg/ha) as the remaining P requirement.

two consecutive growing seasons (one-year duration). Soil sampling was done in the second season at three time points; before transplanting, at maximum tillering stage (36 days after transplanting) and grain filling stage (63 days after transplanting). Before transplanting stage, the field was not submerged and soil samples were collected to represent a 10 cm depth. At the maximum tillering and grain filling stages there was standing water in the fields and soil samples were collected from the rhizosphere of rice plants at an approximate depth of 10 cm and 10 cm from the base of the plant. Four samples were collected from each replicate plot

at each collection time. The collected samples were transported on ice and used for culturing the bacteria within 48 h.

Isolation of culturable aerobic bacteria

Culturable aerobic soil bacteria in the five nutrient management regimes and the two locations (BG and MI) were assessed using the serial dilution method. A composite soil sample (i.e 10g) was prepared from the four samples collected from each replicate plot at each sampling time and suspended in 100 mL of sterile distilled water. The soil suspension was serially diluted, and aliquots (20 µL) from the 10^{-4} dilution level were plated on Nutrient Agar plates. Nutrient Agar, being a non-selective medium, provides ample nutrients to support the growth of a wide variety of microorganisms and is widely used for the isolation of heterogeneous groups of soil bacteria (Thapa *et al.* 2017). Following three days of incubation at room temperature (28±2°C), the number of morphologically different colonies (morphospecies) and the total colonies of each morphospecies were counted. Gram status of the isolated morphospecies was determined using the 3% KOH test.

Screening for phosphate-solubilizing bacteria

A representative colony of each bacterial morphospecies isolated from different nutrient regimes and both locations was inoculated on PVK medium (Surange *et al.* 1997) and incubated for one week at room temperature (25–30°C). Phosphate-solubilizing bacteria formed a clear halo around their colonies on PVK medium. Seven days after incubation, the percentage abundance of phosphorus-solubilizing bacterial morphospecies in each treatment at each location was recorded. The phosphate solubilization index (SI) was calculated for each morphospecies following the method described by Edi-Premono *et al.* (1996).

Screening for nitrogen-fixing bacteria

Nitrogen-fixing ability of representative colonies of each bacterial morphospecies was screened on nitrogen-free medium supplemented with bromothymol blue (NFb medium) (Baldani *et al.* 2014). Isolates that produced blue zones on the medium after seven days of incubation were identified as nitrogen fixers, and their percentage abundance was recorded.

Data collection and calculations

Abundance of aerobic bacterial colonies was quantified as colony-forming units per gram of soil (CFU/g) on a dry-weight basis for all isolation combinations (treatment × collection stage × location). Species richness was determined based on the number of distinct morphospecies. Diversity was quantified using the Shannon–Wiener index (H'):

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i)$$

where: S = total number of species (morphospecies), p_i = proportion of individuals belonging to the i^{th} species, $\ln$ = natural logarithm.

The solubilization index (SI) was calculated using the formula from Edi-Premono *et al.* (1996):

$$\text{SI} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}} \quad (1)$$

Data analysis

Bacterial abundance data were log-transformed prior to analysis. All data related to richness, abundance, diversity, and SI were subjected to Levene's test to verify homogeneity across samples before performing ANOVA. The Kruskal–Wallis test was used to analyze PSI data.

RESULTS AND DISCUSSION

Significance of the effect of fertilizer treatments, location, and sampling stage on bacterial community parameters

The community parameters were differently affected by the main effects of sampling

location, sampling stage, and fertilizer treatments (regimes), including their three-way interaction. The main effects of sampling location and sampling stage were significant ($p<0.05$) on all estimated community parameters, namely richness, abundance, and diversity. None of the community parameters assessed differed significantly among the five treatments (fertilizer management regimes). Interestingly, only the richness and diversity of bacterial morphospecies varied significantly ($p<0.05$) due to the interaction effect of treatment, location, and stage of sampling. The mean gravimetric soil moisture contents (% w/w) before transplanting, at maximum tillering, and at grain-filling stages were 64.21 ± 7.2, 71.47 ± 0.51, and 77.44 ± 10.14, respectively, for BG; and 65.16 ± 0.77, 51.75 ± 0.26, and 59.33 ± 6.78, respectively, for MI.

Bacterial abundance

The abundance of bacterial morphospecies differed significantly ($p<0.05$) between locations and among the sampling stages. The effects of fertilizer treatment and the three-way interaction among the main factors were not significant ($p<0.05$). Considering the significant difference between the two locations, the abundance of culturable bacteria at the three sampling stages for each location is presented separately in Fig. 1a and Fig. 1b. At both locations, a significantly higher abundance of aerobic bacterial colonies was observed in soil samples collected prior to transplanting compared to the other two sampling stages, which showed no significant differences from each other.

Richness of bacterial morphospecies

Interestingly, bacterial richness differed significantly ($p<0.05$) due to the interaction effect of fertilizer regime, location, and sampling stage. Only at MI did the sampling stage, with reference to crop growth, show a highly significant influence ($p<0.0001$) on bacterial richness (Fig. 2). Bacterial richness prior to the transplanting stage was higher in MI fields than in BG fields. Within the MI fields, a significantly higher richness (Fig. 2b) was observed prior to transplanting compared to the other two stages. The mean richness values between the maximum tillering and grain-filling stages at MI were not significantly different from each other.

Diversity of bacteria morphospecies

The diversity of the bacterial morphospecies was significantly affected ($p<0.05$) by the interaction effect of fertilizer regime and location and sampling stage. Diversity of the bacterial morphospecies in BG, determined by Shannon-Weiner Index, did not differ among the treatments or among the stages of sampling (Fig. 3a). However, the diversity of the bacterial morphospecies demonstrated a different image at MI (Fig. 3b) because at MI, the sampling stage had a significant influence ($p<0.0001$) on the diversity of bacterial morphospecies (Fig. 3b). In there, diversity index was significantly higher in all five treatments in the soils collected before transplanting (Fig. 3b). Except for T1 and T4 treatments, the diversity index did not differ significantly in the latter two stages of sampling. The negative control fertilizer treatment (T1) and T3 treatment reported a significantly higher diversity index at the last stage of soil sampling compared to the samples collected at the maximum tillering stage. Contrary, sample under T4 treatment showed a significant decrease of the diversity index at the grain filling stage compared to the sample at maximum tillering stage (Fig. 3b).

Significance of the effect of treatments, location, and sampling stage on functional groups of bacteria

The present study focused on bacteria capable of solubilizing phosphate and fixing nitrogen among the isolated morphospecies. Although morphospecies positive for nitrogen fixation and phosphate solubilization were observed in all soil samples, the percentage of N-fixing bacteria and PSB did not differ among the treatments at both locations. The interaction effect (location $\times$ fertilizer management regime $\times$ sampling stage) had a significant influence on the percentage of PSB ($p<0.0374$) and N-fixing bacteria ($p<0.0001$). Between the two locations and among the sampling stages, the percentage of PSB differed significantly ($p<0.0008$ and $p<0.0004$, respectively). The percentage of

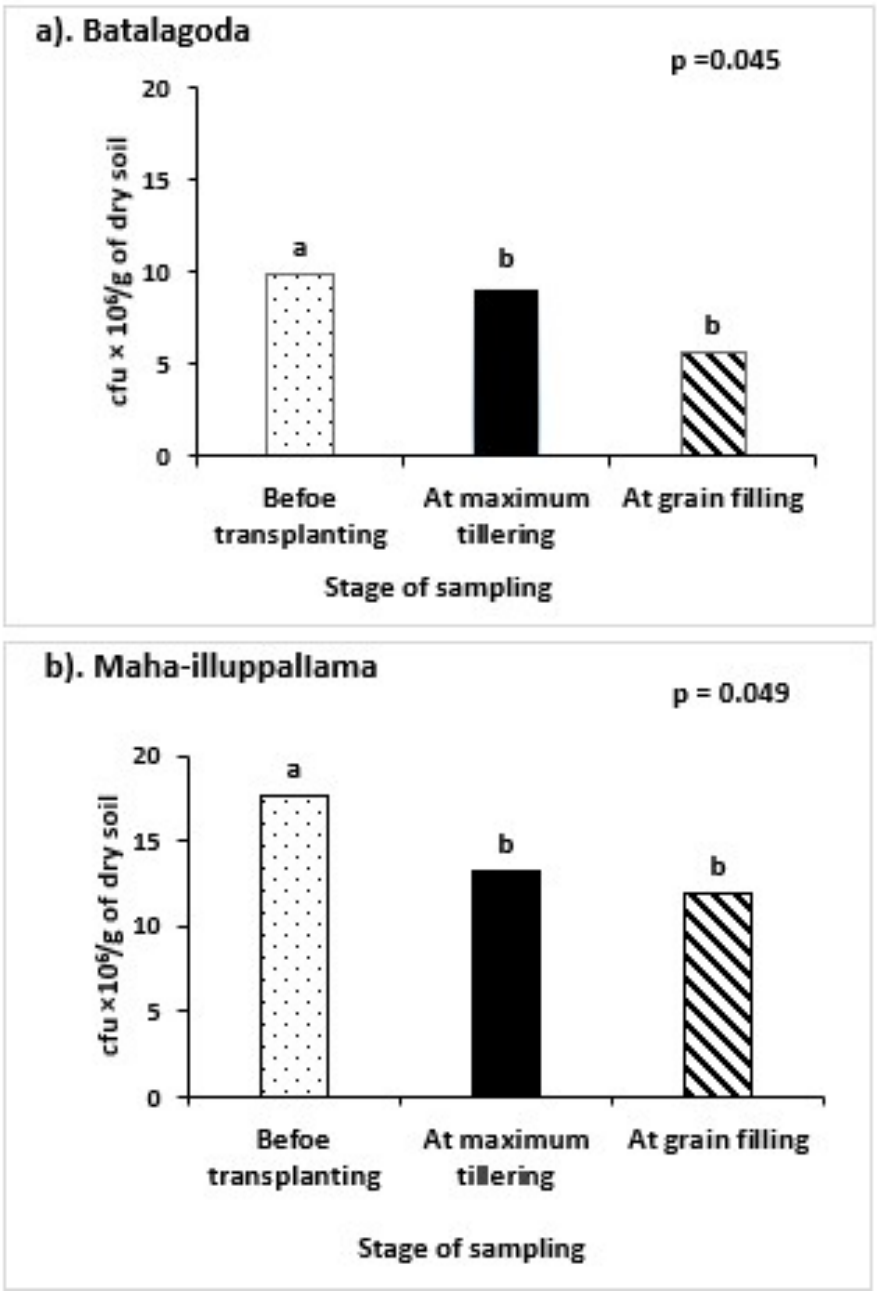

Figure 1. Abundance of colony forming units (CFU) of aerobic bacteria in soil from Batalagoda (a) and Maha-Illuppallama (b) fields at the three sampling stages as assessed by spread plating technique using nutrient agar medium. 'p' value indicates the significance of sampling time on the bacteria abundance in ANOVA.

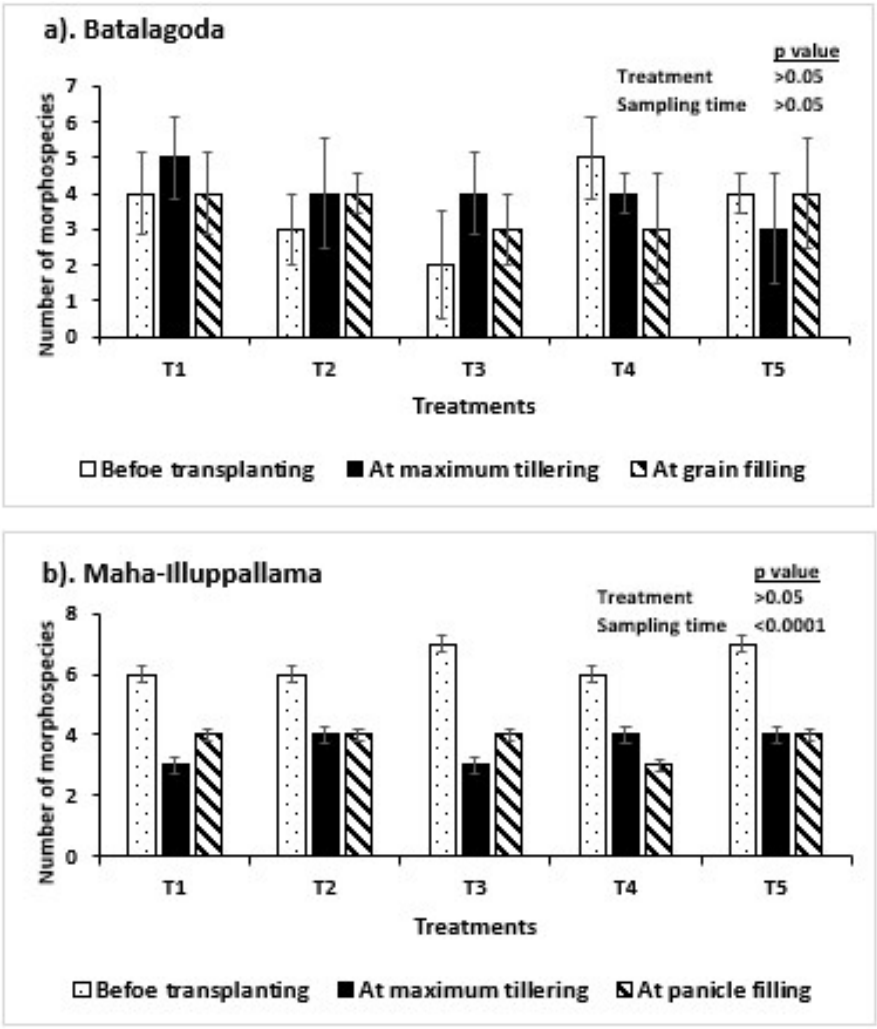

Figure 2. Richness of bacterial morphospecies of the soils collected from fields subjected to five nutrient management regimes (T1-T5) from Batalagoda (a) and Maha-Illuppallama (b) at three sampling stages as observed on nutrient agar medium at 10^{-4} dilution level in spread plating with serial dilution technique. 'p' value indicates the significance of treatment effects on richness of morphospecies in ANOVA. T1: No application of N or P sources (negative control), T2: N as Urea and P as TSP were applied according to DoA recommendation (positive control), T3: N as Urea and P as TSP were applied as per the site-specific requirement determined by NESL software, T4: 65% of N and P decided by the NESL software as site specific requirement were applied using SRF and TSP respectively and T5: 65% of the site-specific requirement of N and P decided by the NESL software was applied using SRF and TSP. The remaining P and N requirement was supplemented through Biofertilizers containing CB and PSB.

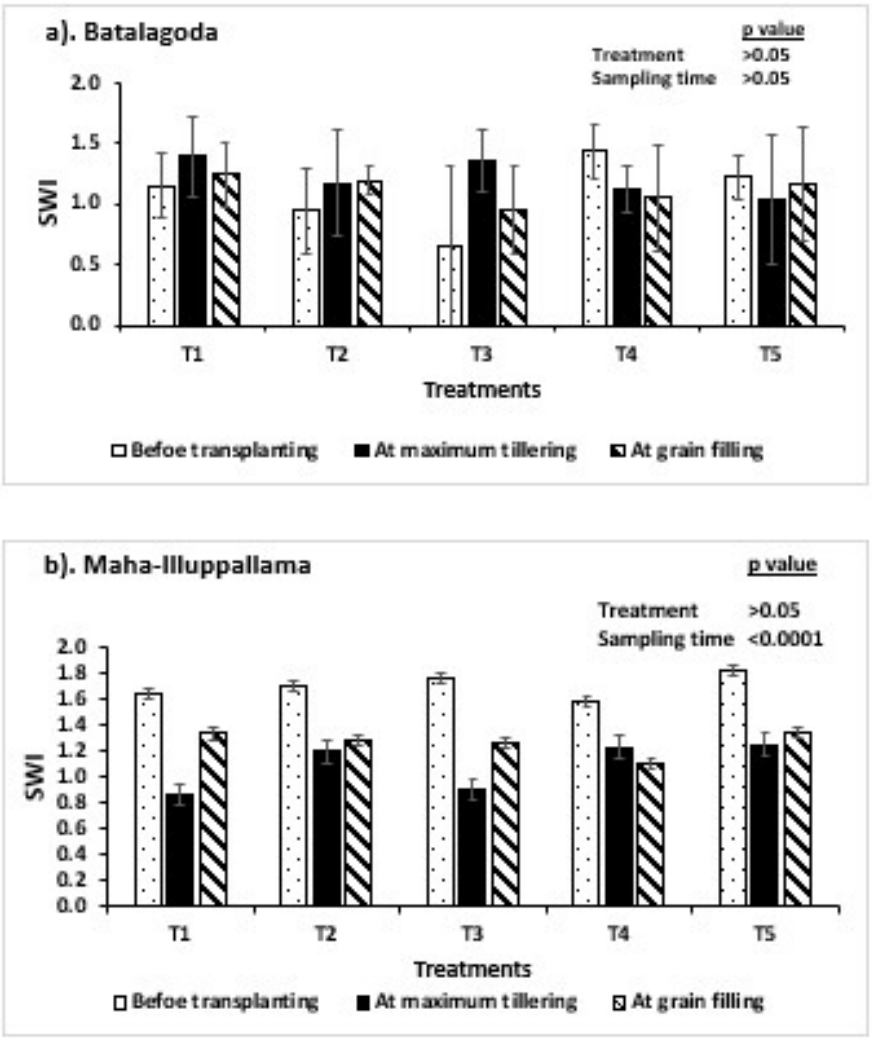

Figure 3. Diversity of bacterial morphospecies estimated with Shannon Wiener Index (SWI) in the soils collected from fields subjected to five nutrient management regimes (T1-T5) from Batalagoda (a) and Maha-Illuppallama (b) at three sampling stages as observed on nutrient agar medium at 10^{-4} dilution level in spread plating with serial dilution technique. 'p' value indicates the significance of treatment effects on SWI in ANOVA. T1: No application of N or P sources (negative control), T2: N as Urea and P as TSP were applied according to DoA recommendation (positive control), T3: N as Urea and P as TSP were applied as per the site-specific requirement determined by NESL software, T4: 65% of N and P decided by the NESL software as site specific requirement were applied using SRF and TSP respectively and T5: 65% of the site-specific requirement of N and P decided by the NESL software was applied using SRF and TSP. The remaining P and N requirement was supplemented through Biofertilizers containing CB and PSB.

Table 2. Percentages of functional groups of culturable bacteria present at the three sampling stages from the two locations. Means within a row and location followed by the same letter are not significantly different (p>0.05).

Group of bacteria	Batalagoda			Maha-illuppallama		
	Before transplanting	At maximum tillering stage	At grain filling stage	Before transplanting	At maximum tillering stage	At grain filling stage
Phosphate solubilizing bacteria	11.66[a]	17.00[a]	21.11[a]	12.39[b]	46.22[a]	33.11[a]
N-fixing bacteria	49.55[a]	26.55[b]	4.66[c]	4.45[b]	10.48[a]	11.12[a]
Gram-positive bacteria	87.17[a]	62.39[b]	59.11[b]	75.92[a]	69.07[a]	67.54[a]

N-fixing bacteria also differed between the locations ($p< 0.0001$) and between the sampling stages ($p<0.0005$).

The two locations demonstrated clear differences in the percentage of morphospecies capable of phosphate solubilization and nitrogen fixation (Table 2). In BG fields, the percentage of PSB did not differ significantly among the three sampling stages, whereas N fixers declined significantly as the cropping season progressed (Table 2). In contrast, at the MI field, significantly higher percentages of PSB and N-fixing morphospecies were reported at the latter two sampling stages compared to the initial stage (before transplanting). The percentage of Gram-positive bacteria differed among the sampling stages only at BG and not at MI. Solubilization index (SI) differed significantly ($p<0.05$) among the PSB morphospecies, with values ranging from 1.115 to 1.514. Among the 20 bacterial morphospecies, six demonstrated a high SI (greater than 1.387), all isolated from either the grain-filling or maximum tillering stages. Notably, all six high-performing PSB were identified as Gram-positive bacteria. All of them were found in the T5 treatment.

Soil bacterial community parameters, both structural and functional are key indicators of the soil health towards a healthy and vigorous crop growth. Findings of the present study revealed that the bacterial community parameters, both on structural composition and functional groups studied by culture-dependent approach did not differ by the five different nutrient management regimes. But they varied significantly between the two locations and among the sampling stages. Therefore, the abundance, richness and diversity of culturable aerobic bacteria that were selective on nutrient agar medium seems to be more affected by the soil edaphic factors decided by the soil type and agro-climatology, and the crop growth stage than the soil nutrient management regime. The same trend was observed for the percentages of PSB and N fixing bacteria among the morphospecies isolated from the BG and MI fields at three sampling stages. The significantly high abundance of aerobic culturable population in soil before transplanting of rice compared to the conditions after crop establishment may be mainly due to changes in soil condition. Soil was not submerged at the time of sampling before transplanting, and therefore aeration should be higher than when the soil is being submerged supporting the growth of aerobic microorganisms. This may explain the drastic reduction of aerobic bacteria abundance from before transplanting to the measurements taken after transplanting rice (Fig. 1). In rice paddies not only does the soil surface contain oxygen but due to diffusive transport of oxygen through the aerenchyma of rice roots the rhizosphere also performs as an oxic zone. In addition, the upper 2 mm directly beneath the floodwater-soil boundary layer is an oxic zone (Liesack *et al.* 2000). Therefore, the samples

we collected have sufficient provision to capture a representative profile of aerobic bacteria. Thus, although the soil was submerged with a standing water layer at the maximal tillering and grain-filling stages of the crop, aerobic bacteria was observed in the rice rhizosphere. Soil moisture content is an important factor determining the microbial activity as it affects profoundly on soil microbial respiration and soil organic matter mineralization. In general, aerobic microbial respiration is optimal when the soil moisture content is around 60% water holding capacity (WHC) (Das *et al.* 2019). Furthermore, when soil moisture content is above 70% WHC, diffusion of O_2 reduces and forms a flooded anaerobic condition while inhibiting the activity of aerobic microbes and various oxidative enzymes, affecting directly the activities and population of aerobic microbes (Schjønning *et al.* 2003). Therefore, at both locations of our study, before the transplanting stage which had an ideal water holding capacity (around 60%) resulted in the highest bacterial population compared to the other two stages.

We did not observe a significant change in abundance and diversity of aerobic bacteria in the rhizosphere between maximum tillering and grain-filling stages of the crop (Fig. 1 to Fig. 3). A significant influence on the rice rhizosphere bacterial community (i.e. diversity, abundance and bacterial groups) at different growth stages (phenological stages) has been reported by Edwards *et al.* (2015). Therefore, the group of rhizosphere bacteria selected by the nutrient agar culture medium and the culturing conditions may be not very sensitive to the changes happen in the rhizosphere with crop growth stage. However, interestingly, the percentage of N fixers among the morphospecies isolated from BG soil declined from maximum tillering to grain-filling stages of the crop. Both PSB and N fixing bacteria among the morphospecies increased in soils collected from MI fields from before transplanting stage to maximum tillering stage, suggesting the influence of plant for selecting beneficial microorganisms in the rhizosphere. It was interesting to note that similar trend was not observed in soils from BG, indicating intricate nature of soil-plant-microbial interactions shaping the community composition and structure in the rhizosphere. Plant exudates shape the rhizosphere microbial community and the changes of exudation profiles of a plant during the different growth stages have been reported by Chaparro *et al.* (2014), hence changes in rhizosphere microbial community profiles over the growth stages is a possibility. According to Tahir *et al.* (2018), an increasing trend of PSB population from tillering to booting stage and a decreasing trend towards grain filling stage has been observed in bread wheat grown under several fertilizer regimes including recommended and half-dose rates of N and P fertilizers.

Our findings did not demonstrate a clear variation of the bacterial community parameters or functional groups of bacteria among the different fertilizer treatments (regimes) used, though the tested soils were under the different fertilizer regimes over a one-year period. This could be partly due to the short-term exposure of the soils to different fertilizer treatments and the time of exposure might not have been sufficient to make a difference on community parameters or functional groups of bacteria. Based on previous research findings, it has been demonstrated that at least two years of exposure to a given fertilization treatment is required to observe a significant change in soil microbial community parameters (Wu *et al.* 2020, You *et al.* 2022, Dondjou *et al.* 2023). Dondjou *et al.* (2023) have reported a significant change in both rhizosphere bacterial and archaeal community compositions in response to 27 years long exposure to N and NPK-fertilization treatments. Wu *et al.* (2020) have observed bacterial community changes and soil property changes in grape rhizosphere soil subjected to organic fertilization and reduced inorganic fertilization treatments for two-year period. You *et al.* (2022) have studied the sole effect of organic fertilizer on functional microbial communities associated with greenhouse gas emissions in paddy soils based on continuous application of treatments over a five-year period.

Partiality of nutrient agar medium for specific group of heterotrophic bacteria could be another contributing factor for not observing the effect of nutrient management regimes on microbial

community (Pan & Umbreit 1972, Manning 1975). Because the experimental approach we have used does not capture the full scale of changes that happen to the entire microbial community in the rhizosphere. However, this culture-based approach has demonstrated the effect of soil type and stage of the cropping season on aerobic bacteria populations. The differences in the determined diversity parameters of the bacterial communities in soil from BG and MI could be attributed to differences in soil characteristics mainly. Soil pH has been identified as the primary determinant of the diversity of bacterial community in soil (Wu *et al.* 2017). We have observed that pH of soil in BG field is more acidic than the soil of MI field, which was closer to neutral (data was not shown). Bacterial diversity is higher in neutral soils than that in acidic soils (Wu *et al.* 2017) and a similar trend has been observed with reference to the richness and diversity and abundance of soil bacteria in MI field, which reported a neutral pH (6.78±0.225)).

The use of serial dilution liquid culture techniques (e.g. Most Probable Number method) has proved to be effective for isolating numerically significant members of the community in anoxic rice paddy soil (Chin *et al.* 1999, Großkopf *et al.* 1998). In the present study, we used a reasonably larger soil sample (i.e. 10 g) and used nutrient agar as the medium of isolation and the isolations were observed at a dilution level of 10^{-4}. According to theory of dilution culturing, microbial isolates developing from the terminal positive tubes of liquid dilution series can be expected as the representatives of species having larger populations in the original soil sample (Chin *et al.* 1999). Therefore, we expected that a wide range of culturable aerobic bacteria could be captured and a reliable insight on the bacterial community parameters would result in. Although serial dilution technique with nutrient agar medium helped to understand the effect of soil-to-soil variability and differences resulting from stages of cropping season on aerobic culturable population, this approach has not captured the effect of nutrient management practices on the same.

Since nutrient agar is a general-purpose medium widely used in culture-based studies targeting heterotrophic bacteria (Manning 1975, Sanghani*et al.* 2023, Cahyani*et al.* 2024), the results should reflect main changes happen in the heterotrophic bacterial populations. Nutrient agar has been used in studying rice rhizosphere microorganisms in previous research (Sudewi *et al.* 2020, Herwati *et al.* 2021, Hossain *et al.* 2021, Cahyani *et al.* 2024). As a key group of bacteria involved in nutrient cycling by mineralizing organic materials in soil, heterotrophs are sensitive to the availability of labile forms of nutrients in soil environment, which is affected by characteristics of the fertilizers applied (Li *et al.* 2017, Chen *et al.* 2018, Enebe & Babalola 2021). A previous study reported that the application of chemical fertilizers lowered the abundance of genes related to N assimilation and increased the abundance of catabolic genes related to carbon cycling (Enebe & Babalola 2021). Urea is 100% water soluble and release N to soil solution rapidly than SRF (Dissanayake *et al.* 2022). Biofertilizers mobilize nutrients in soil and supply plants at a rate slower than chemical fertilizers but modify the microbial community structure (Li *et al.* 2017).

Moreover, the amount of nutrients added with NESL recommendation is different from DOA recommendation (Kulasinghe 2022). Thus, there should be a significant variability of the amount of labile nutrients in soil solution as affected by the five treatments and if there are any drastic effects of the fertilizer regimes on the heterotrophic bacteria, changes should be reflected in in the microbial community parameters. Therefore, not seeing the impact of fertilizer management regime on the measured parameters of microbial communities in our study cannot be summed up into methodological limitations alone. Interestingly, although there was no significant difference in the percentage of PSB among soils with respect to fertilizer management treatments (i.e. regimes), there was a marked difference in the PSI among the PSB based on these treatments. For instance, the PSB with the highest PSI were reported in the T5 treatment, which received external application of PSB. Therefore, in order to rule out methodological limitations, we suggest

further studies with high resolution techniques in functional genomics to isolate the effects of nutrient management regimes on soil microbial populations. If culture-based studies are used, it is suggested to consider specific growth media targeting different groups of bacteria, such as PVK targeting PSB, N-free mineral media targeting N fixers, etc. to obtain a comprehensive picture of the changes in culturable populations of bacteria in response to fertilizer regimes, without limiting to a general-purpose medium like nutrient agar alone.

CONCLUSION

Culturable aerobic bacterial abundance, richness and diversity did not differed among the five fertilizer regimes maintained over a one year period which included zero application and recommended dosage of synthetic N, P, K fertilizers, site-specific rates of synthetic N, P, K fertilizers and two other EFT-fertilizer managements. Percentage PSB and N fixing bacteria among the aerobic bacteria morphospecies obtained using nutrient agar medium was also not differed among the five fertilizer regimes. However, the above bacterial community parameters and functional groups differ between the locations (i.e. BG and MI) and among the sampling stages (i.e. before transplanting, at maximum tillering and grain filling stage). Results demonstrated the culturable aerobic bacterial community in rice rhizosphere studied using nutrient agar as the growth medium does not differ by the short-term exposure of soils to different fertilizer regimes.

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