QUARTERLY RESEARCH PROGRESS REPORT
QUARTER: 2nd

Research Title: Project EsMaLL: Establishing the Microbial Life Profile of the Leyte Sab-a Basin Peatland in the Philippines

Previous Title: Microbial Community Structure, and Function of Peatsoil and Peatwater in the Leyte Sab-a Basin Peatland: Bioprospecting for Biotechnological and Biomonitoring Applications

I. Program/Project/Study Objectives

This project aims to determine the microbiological profile (composition, diversity, and function) of the Leyte Sab-a Basin Peatland (LSBP). Specifically, this project has the following goals:

1. Identify the surface soil microbial communities in the peatland through 16S rRNA metabarcode sequencing.
2. Isolate peat bacteria with putative potential biological degradation properties; and
3. Assess the composition, abundance, and diversity of testate amoeba in the LSBP

II. Relevance to VSU & College's Thrust and Priorities:

In congruence with the university and college's thrust and priorities, this project addresses the dearth of knowledge concerning microbial communities within the Leyte Sab-a Basin Peatland – a critical wetland ecosystem and one of VSU Alangalang's key research areas. Moreover, the project focuses on isolating soil microbial strains with metabolic properties conducive to biological degradation. This endeavor intends to contribute to developing relevant, sustainable, and innovative technologies. Additionally, the project aims to enhance the capability of the researchers to conduct studies that utilize microbiological, molecular, and advanced DNA sequencing techniques via active participation in various training activities. Thus, this project contributes to the university's implementation of the Agriculture and Fisheries Modernization Act 3 (AFMA) of the government, which now encompasses environmental ecosystems and biotechnology. The project also fosters the university's strong R and D linkages by partnering with institutions locally and abroad.

III. Highlights of accomplishments within the quarter

A. Targets for the quarter

1. Present the complete results and analysis at the American Society (ASM) Microbe 2025 in Los Angeles Convention Center, Los Angeles, California, USA, on June 19-23, 2025.

2. Present project progress at the 2025 RDE Annual In-House Review on the scheduled date at the VSU Main Campus.
3. Process procurement of laboratory materials for component no. 2: Isolation and assessment of bacterial strains from LSBP peat for potential lignocellulolytic activity.
4. Process peat samples for testate amoeba analysis (Study Component Number 3).

B. Highlights of accomplishments

1. Mr. Reynaldo P. Peja Jr., project leader, presented the project results of component study number 1 at the ASM Microbe 2025. This conference was held at the Los Angeles Convention Center, Los Angeles, California, USA. His abstract, titled "The Microbial Perspective on Wetland Health: A Case Study of a Degraded Tropical Peatland in Leyte, Philippines" was presented as a poster. He was also one of the seven presenters in the Rapid Fire Talks session under the Applied and Environmental Science Track, Subtrack: Terrestrial, Extraterrestrial and Extreme Environments Microbiology.

   Mr. Peja's travel to the US for the conference was made possible by the ASM Travel Awards, where he was one of the 14 recipients of the Bill and Melinda Gates Full Travel Grant. The award grants a full-expense paid trip and attendance to the conference.

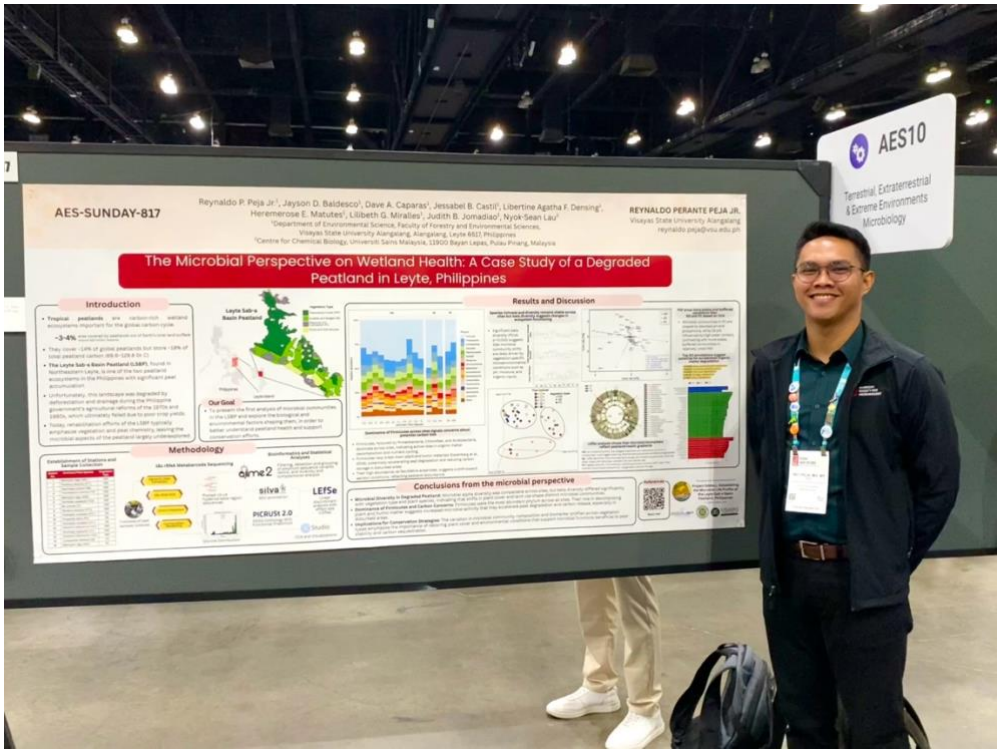

Figure 1. Mr. Reynaldo P. Peja Jr., project leader, in front of his poster, titled "The Microbial Perspective on Wetland Health: A Case Study of a Degraded Peatland in Leyte, Philippines."

Figure 2. Mr. Reynaldo P. Peja Jr., project leader, presenting at the Rapid Fire Talk in the Applied and Environmental Science Track, Subtrack: Terrestrial, Extraterrestrial, and Extraterrestrial Environments Microbiology.

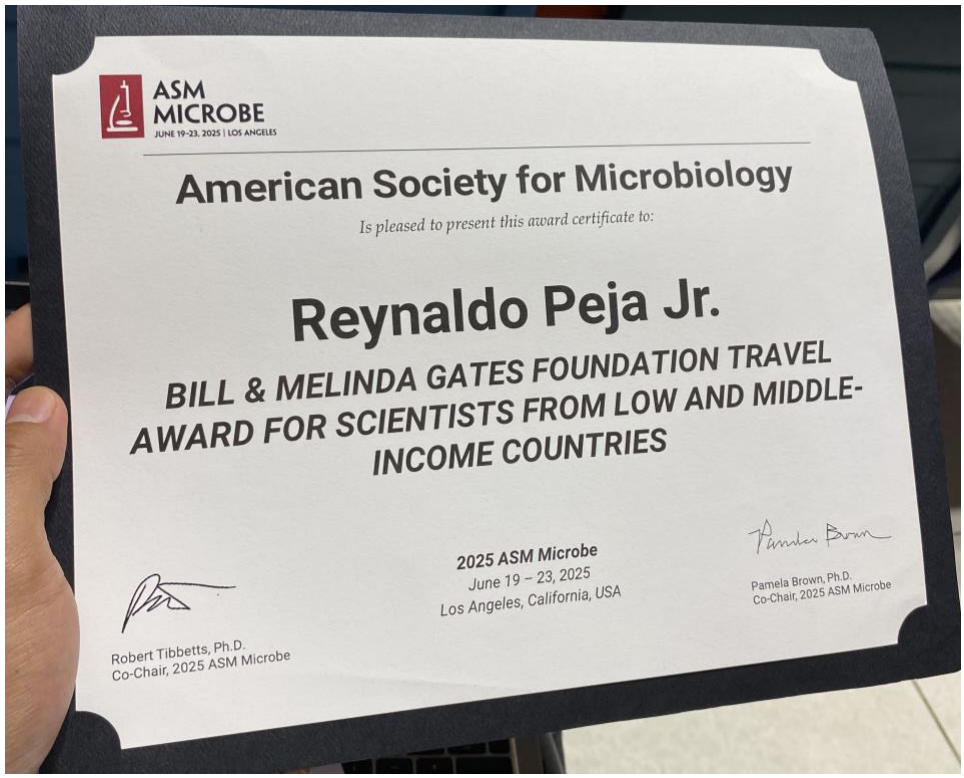

Figure 3. Award Certificate of Mr. Reynaldo P. Peja Jr. as recipient of the Bill and Melinda Gates Foundation Travel Award for Scientists from Low and Middle-Income Countries.

2. Progress reports for the three study components of Project EsMaLL were presented during the 2025 RDE In-House Review under the Forestry, Agroforestry, and Biodiversity session, held on June 30, 2025, at Breakout Room 1, RDE Hall, VSU Main, Baybay City, Leyte. Mr. Reynaldo P. Peja reported that the analysis of metabarcode sequencing results for Component Study 1 has been completed, and the manuscript is being finalized for publication. He also noted that Component Study 2 has experienced delays due to equipment breakdown and procurement issues with bacterial isolation reagents and materials. Despite this, the initial optimization of the microbial assay has been improved: serial dilution at 10^4-10^5 provided better separation of bacterial colonies on carboxymethylcellulose agar (CMCA), and cellulolytic microbes grow when incubated for 15-30 days at 40°C. Meanwhile, Component Study 3, the isolation of testate amoeba, is ongoing, with 60% of the total 48 samples already processed and examined using microscopy. These will be forwarded for identification and downstream analysis.

Figure 4. Mr. Reynaldo P. Peja Jr., reporting the progress of Project EsMaLL during the 2025 RDE Annual In-House Review held on June 30, 2025, at Breakout Room 1, RDE Hall, VSU Main, Baybay City, Leyte.

3. Procurement of microbiological materials and reagents from the previous quarter is continuing up to this last quarter of the year. Items amounting to (amount) have been awarded to suppliers, subject to procurement rules and guidelines. Still, the same items that are needed in the optimization of bacterial isolation have yet to be received.

Optimization of bacterial isolation is in progress. Sample concentrations from serial dilutions 10^5, 10^6, and 10^7 were successfully plated in carboxymethylcellulose agar (CMCA) using Congo red as an indicator of cellulase activity (Figure 17). Several bacterial colonies with cellulase activity have been observed after prolonged incubation (>30 days) at 40°C. These results significantly advance the project in its efforts to isolate bacterial strains with cellulolytic activity. These colonies will be cultured in pure isolates and will be validated for cellulolytic activity in a separate batch of CMCA.

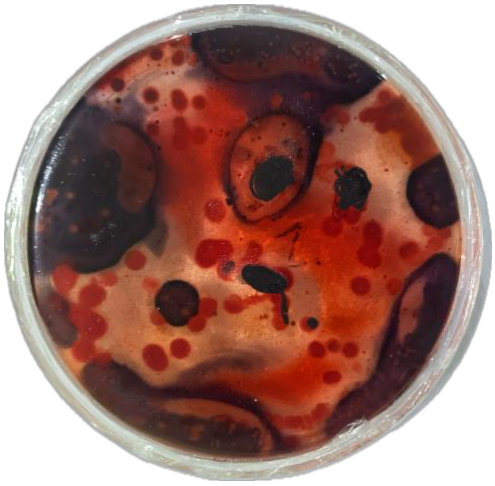

Figure 5. Bacterial colonies growing on carboxymethylcellulose agar (CMCA) show cellulolytic activity indicated by the clearing of Congo Red stain after prolonged incubation at 40°C for 30 days.

4. About 63% of the peat samples for testate amoeba have been processed (n=30) as of this reporting period. The study commenced with the optimization of the isolation process, given the inherent difficulty of working with organic-rich peat samples. Initially, a modified method of Booth et al. (2010) was adopted, which includes multiple peat heating cycles. However, the high organic content and density of the peat continued to hinder clear visualization under the microscope. The method also involves sequential sieving starting with 500 µm, followed by 300 µm, which may contribute to the loss of rare but important taxa.

   To address this, the decantation and multiple-settling method of Mazei and Chrnyshov (2011), with further modications. This approach allowed for gradual separation and concentration of the tests, which minimizies organic debris while preserving smaller taxa that could be lost in more aggressive sieving protocols. The sieving was limited to a single 0.50 mm mesh size to reduce the risk of losing rare and smaller-sized taxa, which are often critical for detecting ecological shifts. For each sample, a minimum of 150 individual tests were counted from a 2 mL aliquot of the final 10 mL concentrate. Setting this minimum count was important to

ensure statistical reliability in estimating community composition and diversity, as recommended in standard ecological protocols for microfaunal analysis (e.g., Charman et al., 2000; Lamentowicz et al., 2007; Mazei and Chernyshov, 2011). This sample size also provides sufficient representation of both common and rare species which increases confidence in the observed patterns of alpha and beta diversity across the vegetation types studied. Identification was made based on

IV. Physical Report of Operation

A. Research Program

	Particulars/Name and Brief Description of Utilized/ Commercialized Technologies	Number
Outcome Indicator		
1. Number of research outputs utilized by the industry or by other beneficiaries	-	
Output Indicator		
1. Number of research outputs completed within the year	-	
2. Percentage of research outputs published in internationally-referred or CHED recognized journal within the year	-	

B. Technologies/Information patented and commercialized

Technology Invention(s) New Information	Invention Patent Number	Date of Issue	Utilization of Invention		Name of Commercial Product
			Development	Service	
A. Technology Invention(s)					
	-				
B. New Information	-				

C. Research papers published *(Identify if articles were for Research, Extension, Innovation or MSc/ PhD Studies)*

	Title	Author (s)	Date/Year/Publication/ Publisher	Remarks *(if Research, Extension, Innovation, Thesis, MSc/PhD*
a. Refereed Journal				
Institutional	-			
National	-			
International	-			
b. Semi-popular publ'n (newsletter, etc.)	-			
c. Popularized pub'ln	-			

(technoguides, etc.)				
d. Book Chapter/s	-			
e. Books	-			

D. Citation

Research Output as Cited by Other Researcher(s) in Journal Activities									
Title of Research Output/ Published Journal Articles/ Book	Title of Journal & Vol. Issue/ Year	Keywords	Researcher (s)	Citation Details					
				Author(s) Who Cited the Research Output	Title of Article Where the Research Output Was Cited	Title of Journal	Vol. / Issue / Page No.	City/ Year Published	Publisher
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V. Issues, Problems, and Recommendations

Several bottlenecks were encountered in the implementation of Year 3 activities of the project. For bioinformatics analysis, we used the supercomputers of the Centre for Chemical Biology, Universiti Sains Malaysia (USM). However, we lost access to the server for about a month after the RAM containing our project files was accidentally removed during maintenance. Upon regaining access with the assistance of our collaborator, Dr. Nyok-Sean Lau, it was discovered that all project files were missing, along with the packages and environments previously used in the analysis. As a result, all raw files had to be re-uploaded and the entire analysis re-run. Nonetheless, this setback provided an opportunity to review and validate all steps in the analysis pipeline. During the remaining period of project implementation and in future projects, backup copies of scripts, environments, and raw data should be maintained both locally and on secure cloud storage.

In the laboratory, the equipment used for measuring physicochemical parameters broke down. Fortunately, the project team was able to continue drying the samples using the oven at the Analytical Laboratory of the Department of Agricultural Sciences, VSU Alangalang.

Procurement delays also persisted. Several items were not procured due to the lack of suppliers submitting quotations, non-responsiveness of those who initially quoted during post-qualification, and quotations exceeding the Approved Budget for the Contract (ABC). To address this, market research efforts will be strengthened to expand supplier coverage and anticipate budget adjustments in future PPMP submissions.

Initial difficulties in isolating cellulolytic bacteria with enzymatic activity were also expected due to limited resources. Despite this, bacterial colonies showing cellulolytic activity were successfully recovered after prolonged incubation for over 30 days at 40°C. This development fills the gap in the previous reports where no isolates had yet been confirmed.

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Received by OVPREI-RPO: _____

Date Received :**_____**