ASEAN Journal for Science and Engineering in Materials

Journal homepage: https://ejournal.bumipublikasinusantara.id/index.php/ajsem

Hydrological Drainage Effects on Microbial Dynamics in Badas Peatland: Fungal Decline, Bacterial Dominance, and Reduced Microbial Biomass Carbon

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A B S T R A C T

Peatlands are globally important ecosystems for carbon storage and climate regulation, yet hydrological drainage for agriculture and infrastructure has disrupted their ecological balance. This study examined the effects of drainage on soil microbial communities and microbial biomass carbon (MBC) in Badas Peatland, Brunei Darussalam. Soil samples were collected along two transects (Jalan Badas Middle and North) and analyzed using microBIOMETER® and plate count methods. Results revealed that fungal abundance and MBC increased with distance from the drainage canal, whereas bacterial and actinomycete counts decreased. The fungal-to-bacterial ratio showed a strong positive correlation with MBC, indicating that drainage adversely affects fungal populations and soil carbon retention. These findings highlight that fungi are more sensitive to drainage-induced stress than bacteria, reducing the peatland's capacity for carbon sequestration. The study emphasizes the need for sustainable water management strategies to preserve soil microbiology and mitigate carbon loss from tropical peatlands.

A R T I C L E   I N F O

Article History:

Submitted/Received 02 Jul 2025

First Revised 26 Aug 2025

Accepted 22 Oct 2025

First Available online 23 Oct 2025

Publication Date 01 Sep 2026

Keyword:

Drainage,

Microbial biomass carbon,

Microbial communities,

Peatlands,

Soil microbiology.

1. INTRODUCTION

Peatlands are wetland ecosystems characterized by the accumulation of peat and organic matter formed through the slow decomposition of plant materials under water-saturated and anaerobic conditions (Deshmukh *et al*., 2021; Page & Baird, 2016). These ecosystems play a vital role in biodiversity conservation, climate regulation, and human welfare, covering approximately 400 million hectares globally and accounting for nearly one-third of the world's wetlands. Peatlands also serve as one of the largest terrestrial carbon reservoirs, as the rate of primary production exceeds microbial decomposition over millennia, resulting in substantial carbon storage (Urbanová & Bárta, 2016). However, peatland ecosystems are increasingly threatened by anthropogenic activities, particularly by drainage for agricultural expansion and deforestation. Drainage alters the natural hydrological regime, leading to significant changes in soil properties, microbial community structure, and overall ecosystem functions (Holden *et al*., 2004). These alterations enhance soil aeration and decomposition rates while disrupting nutrient dynamics, which collectively accelerate the release of stored carbon into the atmosphere as CO2 and CH4, thereby contributing to global climate change (Hooijer *et al*., 2010; Strack *et al*., 2006; Wilson *et al*., 2009).

Furthermore, the conversion of peat swamp forests to agricultural lands reduces the diversity of fungal communities, including mycorrhizal fungi essential for plant health and organic matter formation, thus impairing critical ecosystem processes (Mishra *et al*., 2021). The fungal-to-bacterial ratio (F:B) in peatlands varies with environmental factors such as drainage and pH. For instance, some researchers (Winsborough & Basiliko, 2010) reported bacterial dominance in acidic peat soils with F:B ratios ranging from 0.31 to 0.68, whereas conifer forest soils (also acidic) exhibited ratios greater than 1, reflecting fungal dominance. This indicates that drainage, by altering moisture and pH, can shift the microbial balance toward bacterial predominance. As a result, anaerobic communities adapted to waterlogged conditions are replaced by aerobic microorganisms, accelerating peat decomposition and altering biogeochemical cycles (Andersen *et al*., 2010; Basiliko *et al*., 2012). Such changes affect carbon balance, soil fertility, and plant-microbe interactions, thereby undermining ecosystem stability (Laiho, 2006; Lin *et al*., 2012). Microbial biomass carbon (MBC), a key indicator of soil microbial activity and health, plays an essential role in nutrient cycling and plant productivity in peatland environments (Croft *et al*., 2001). Drastic differences in MBC between natural and disturbed peatlands reflect how human activities reshape microbial communities and compromise ecosystem resilience (Croft *et al*., 2001).

Understanding these microbial responses is crucial for developing effective peatland management and restoration strategies. This study investigates the effects of drainage on microbial community composition and MBC in the Badas Peatland of Brunei Darussalam, one of the most intact peatland ecosystems in Southeast Asia. The novelty of this research lies in its quantitative comparison of fungal (bacterial dynamics across drainage gradients using both microBIOMETER® and plate count methods) an approach not yet applied in Brunei's peatland studies. The findings provide impactful insights into the biological mechanisms of peatland degradation, offering essential scientific evidence to guide restoration, hydrological management, and climate mitigation strategies in tropical peat ecosystems (Jaatinen *et al*., 2008; Komulainen *et al*., 1998).

2. METHODS

The methodology flowchart is shown in **Figure 1**. Detailed information is explained in the following subsections.

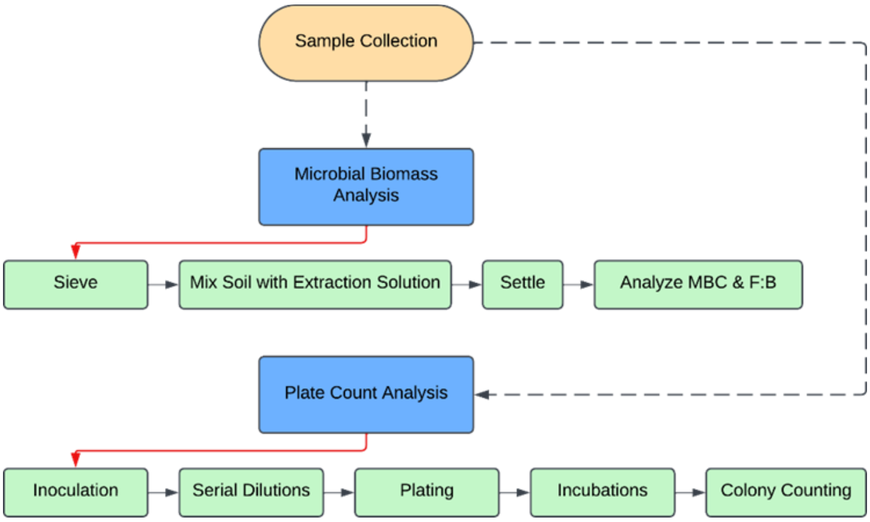

Figure 1. Methodology Flowchart.

2.1. Geographical Overview of Badas Peatland

The Badas Peatland, located in the Belait District of Brunei Darussalam on the island of Borneo in Southeast Asia (Omar *et al.*, 2022), forms part of an extensive peat swamp forest ecosystem that dominates the district's lowland landscape (Suhaili, 2022). It holds significant ecological importance due to its high organic matter accumulation, hydrological balance, and distinctive biodiversity (Page *et al.*, 2011). The region's equatorial monsoonal climate (with consistently high mean temperatures of approximately 28°C and an annual rainfall averaging around 3,000 millimeters) creates the continuously waterlogged conditions essential for peat formation (Koh & Wilcove, 2008; Suhaili, 2022).

While most Southeast Asian peatlands are concentrated in Indonesia (approximately 20.7 million hectares) and Malaysia (about 2.6 million hectares), Brunei Darussalam has retained roughly 16% of its national land area as peatlands (Omar *et al.*, 2022). Unlike the highly degraded peatlands in neighboring countries, Brunei's peatlands remain among the most intact globally and serve as a valuable ecological reference for the restoration and rehabilitation of disturbed peatland ecosystems across the region (Omar *et al.*, 2022).

The formation of the Badas Peatland began approximately 5,000 years ago, following sea-level rise during the Holocene epoch. The peat profile, reaching depths of up to 9.7 meters, primarily comprises slightly decomposed forest debris and organic matter (Becek *et al.*, 2022). The vegetation is typically dominated by *Rubroshorea albida* and kerangas forest species (Omar *et al.*, 2022). However, human activities (such as road construction and water pipeline installation along Jalan Badas and the Seria Bypass) have altered the natural landscape, increasing vulnerability to wildfires and seasonal droughts. Degraded zones within the peatland exhibit reduced groundwater levels and hydraulic conductivity compared to undisturbed areas, with *Rubroshorea albida*-dominated forests showing minimal natural regeneration following disturbance (Becek *et al.*, 2022).

2.2. Peat Soil Sampling

Soil sampling was carried out along two transects, Jalan Badas Middle (JBM) and Jalan Badas North (JBN), located within the Badas Peatland research site (**Figure 2**). Sampling coordinates were precisely recorded using a Garmin GPSMAP 64S to ensure spatial accuracy.

DOI: http://dx.doi.org/10.xxxxx/ijost.v2i2
p- ISSN 2528-1410 e- ISSN 2527-8045

Surface soil samples were collected with a sterilized hand shovel, while subsurface samples, obtained at approximately 50 cm depth, were extracted using a stainless-steel Eijkelkamp auger equipped with a spiral tip.

Immediately after collection, each sample was sealed in sterile ziplock bags to prevent contamination, stored in a cool box to maintain microbial viability during transport, and processed promptly upon arrival at the laboratory. In November 2023, a total of 18 soil samples were collected from nine sampling points along the JBM transect, followed by seven subsurface samples in February 2024, designated for Plate Count Analysis (PCA). For the JBN transect, 10 soil samples were collected from seven points in December 2023, and an additional seven subsurface samples were reserved for PCA in January 2024.

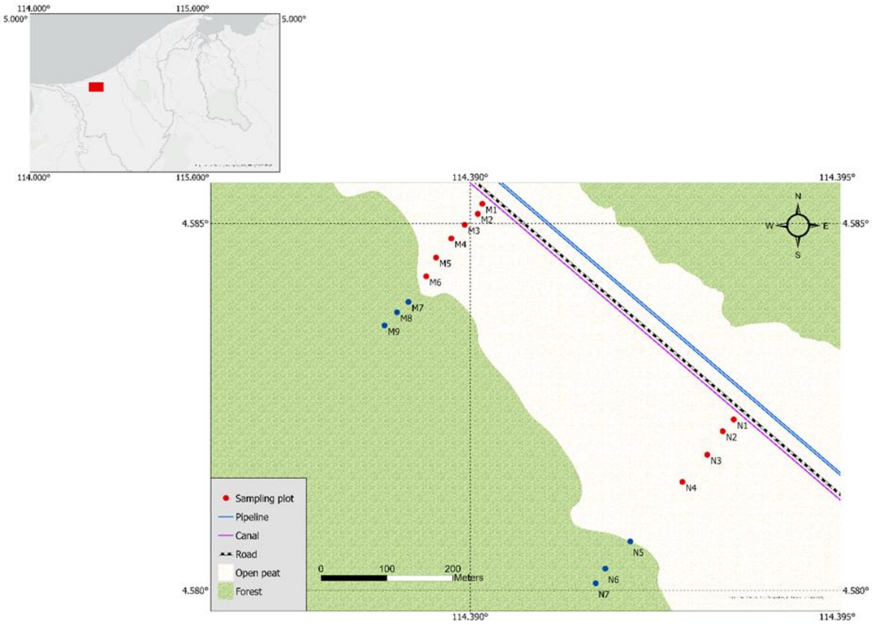

Figure 2. Map showing the location of the study area in Badas Peatland, Brunei Darussalam. Sampling plots are color-coded along two transects: *M* represents Jalan Badas Middle (JBM) and *N* represents Jalan Badas North (JBN). Forest peat samples are indicated by blue dots, while swamp peat samples are marked with red dots (modified after Addly *et al*., 2022).

2.3. Microbial Biomass Analysis

Microbial biomass analysis was conducted using the microBIOMETER® kit (Prolific Earth Sciences, Montgomery, NY, USA), which quantifies microbial biomass carbon (MBC) in soil samples. The procedure followed the protocols (Nouri *et al*., 2021; Pochron *et al*., 2020). Initially, visible plant materials such as roots and leaves were removed using a 3 mm soil sieve. The sieved soil was transferred into a 0.5 mL analysis tube and mixed with 10 mL of a high-salt extraction solution containing diluted sodium chloride (NaCl) and calcium chloride ($CaCl_2$). The mixture was stirred for 30 seconds to detach microbes from soil particles and left undisturbed for 20 minutes to allow sedimentation, leaving the microbial suspension in the supernatant layer.

Subsequently, the supernatant was analyzed using the provided microBIOMETER® test card, which consists of a membrane enclosed within a heat-laminated envelope with a small observation window. Using a micropipette, three drops of the supernatant were applied onto the membrane, allowing soil microbes and fungal pigments to adhere while excess liquid and low-molecular-weight pigments diffused out. The color intensity retained on the membrane, corresponding to microbial and fungal biomass concentration, was measured using a smartphone camera integrated with the microBIOMETER® mobile application. This optical method has been shown to correlate strongly (r = 0.91) with phospholipid-derived fatty acid (PLFA) analysis, validating its reliability. The app-generated readings provided quantitative estimates of MBC (μg C/g soil) and the F:B ratio.

2.4. Plate Count Analysis

Viable microbial counts for bacteria, fungi, and actinomycetes were determined using the pour plate method (Simarani *et al*., 2018). Specific culture media were used for selective isolation: Plate Count Agar for bacteria, Rose Bengal Agar for fungi, and Actinomycete Isolation Agar for actinomycetes. Soil suspensions were serially diluted up to 10^{-3} and inoculated onto respective agar plates. Incubation was carried out at 28 ± 2 °C for one, five, and seven days, corresponding to bacterial, fungal, and actinomycete growth periods, respectively.

Following incubation, the number of visible colonies was recorded, and microbial concentrations were expressed as colony-forming units per milliliter (CFU/mL) using the standard equation: CFU = number of colonies x dilution factor/volume of sample plated (in mL). This quantitative method enabled a comparative assessment of microbial population densities between transects and distances from the drainage canal, supporting further analysis of microbial community dynamics under varying hydrological conditions.

3. RESULTS AND DISCUSSION

3.1. Microbial Composition and Biomass Carbon Trends Across Transects

The microbial composition at the sampling points closest to the drainage canal along the Jalan Badas Middle (JBM) transect exhibited a slight dominance of fungi over bacteria, comprising 58% fungi and 42% bacteria, with a microbial biomass carbon (MBC) concentration of 729 μg C/g soil (**Figure 3a**). As the distance from the drainage canal increased, a progressive shift in microbial balance was observed. At the furthest sampling point, the fungal proportion increased markedly to 76%, while bacterial presence decreased to 24%, accompanied by a substantial rise in MBC to 1496 μg C/g soil (**Figure 3b**). Statistical analysis revealed a strong positive correlation between distance from the canal and both MBC and F:B ratio, with correlation coefficients (r) of 0.805 and 0.804, respectively.

A similar spatial trend was recorded along the Jalan Badas North (JBN) transect. Microbial composition at sites nearest to the canal comprised 56% fungi and 44% bacteria, with an MBC value of 763 μg C/g soil (**Figure 3c**). At the farthest point, the fungal proportion increased to 70% while bacterial abundance declined to 30%, corresponding to a higher MBC of 1178 μg C/g soil (**Figure 3d**). The correlation analysis indicated a medium-to-strong positive relationship between distance from the canal and both MBC (r = 0.682) and F:B ratio (r = 0.714), confirming a consistent directional pattern across both transects.

These findings demonstrate a clear gradient-driven response of soil microbiota to hydrological disturbance caused by drainage. The increase in fungal dominance and MBC with greater distance from the canal suggests that fungi thrive under less-disturbed, moisture-stable conditions, whereas bacterial abundance is enhanced in aerated and drier soils closer to the canal. This microbial shift reflects a functional transition from anaerobic, carbon-conserving communities to aerobic, carbon-depleting assemblages; a pattern that has been widely associated with drainage-induced peatland degradation. Higher MBC in areas distant from the canal implies a more active and stable microbial ecosystem capable of sustaining nutrient cycling and organic matter turnover, while lower MBC near the canal indicates stress conditions that constrain microbial metabolism.

The strong F:B correlation with MBC also reinforces the role of fungal biomass as a major contributor to peat carbon stabilization. Fungal dominance enhances carbon sequestration through slow decomposition rates and complex organic compound formation, whereas bacterial predominance tends to accelerate carbon mineralization and CO_2 release. Therefore, the observed pattern not only confirms the sensitivity of fungi to hydrological stress but also highlights the ecological significance of maintaining waterlogged conditions to preserve peatland microbial integrity and carbon storage capacity.

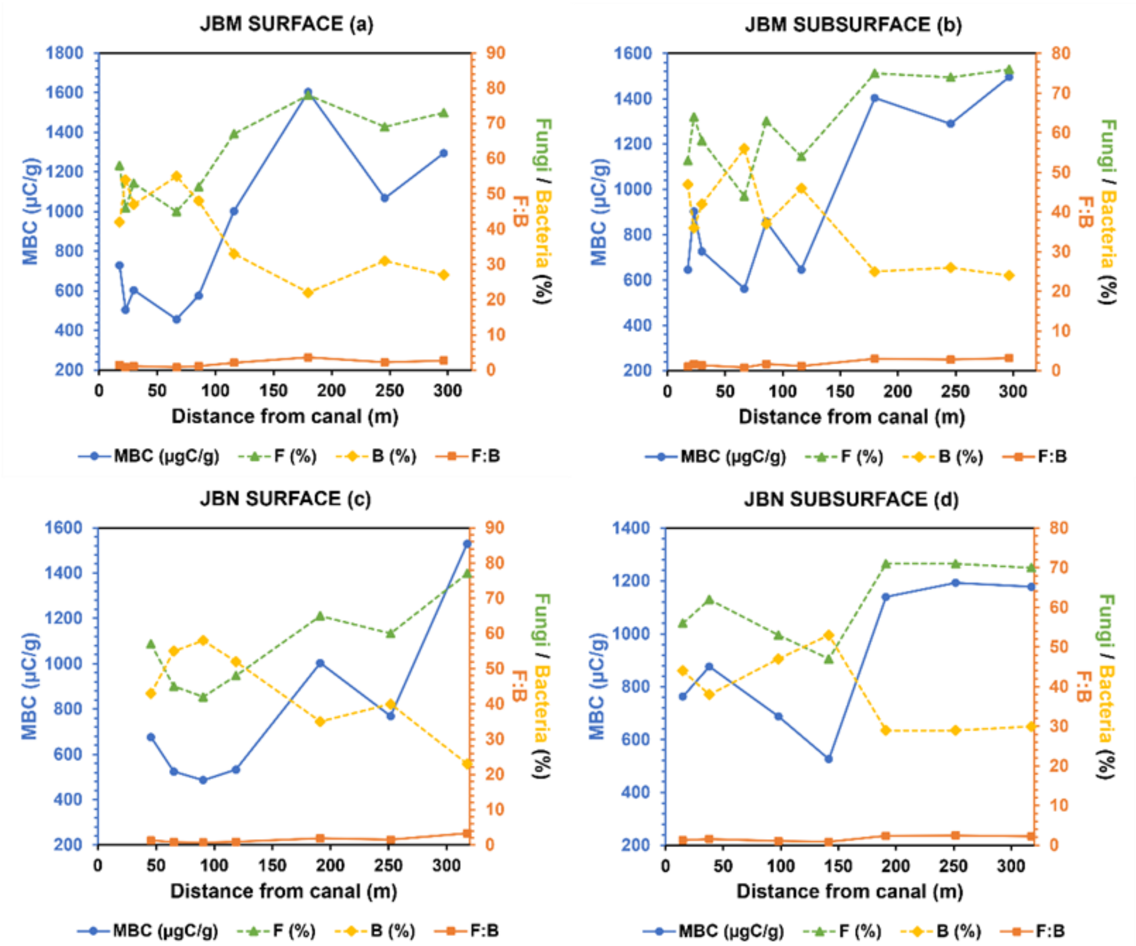

Figure 3. Soil Microbe Test results showing microbial biomass carbon (MBC), fungal and bacterial percentages, and fungal-to-bacterial (F:B) ratios along two transects in the Badas Peatland, Brunei Darussalam. (a) MBC and relative abundance of fungi and bacteria at sampling points along the Jalan Badas Middle (JBM) transect. (b) Fungal-to-bacterial ratio and its correlation with distance from the drainage canal along the JBM transect. (c) MBC and relative abundance of fungi and bacteria at sampling points along the Jalan Badas North (JBN) transect. (d) Fungal-to-bacterial ratio and its correlation with distance from the drainage canal along the JBN transect. The combined results demonstrate that fungal dominance and MBC increase with distance from the canal, whereas bacterial dominance is stronger near the canal, indicating that hydrological disturbance influences microbial balance and soil carbon stability.

Plate count data from the Jalan Badas Middle (JBM) transect revealed distinct spatial variations in microbial populations relative to proximity to the drainage canal. Near the canal, bacterial and actinomycete populations were predominant, with mean counts of 1.17×10^4 and 8.17×10^4 CFU/mL, respectively, while fungal populations were comparatively lower at 2.67×10^3 CFU/mL (**Figure 4a**). At the furthest sampling point, bacterial and actinomycete counts declined to 5.73×10^4 and 3.97×10^4 CFU/mL, respectively, whereas fungal populations increased slightly to 3.67×10^3 CFU/mL (**Figure 4b**).

A similar distribution pattern was observed along the Jalan Badas North (JBN) transect. At sites nearest to the canal, bacterial and actinomycete populations averaged 1.74×10^5 and 1.53×10^5 CFU/mL, respectively, with fungi showing the lowest abundance at 6.67×10^2 CFU/mL (**Figure 4c**). In contrast, at the farthest point from the canal, bacterial and actinomycete counts decreased to 1.17×10^5 and 1.08×10^5 CFU/mL, while fungal abundance increased markedly to 2.67×10^3 CFU/mL (**Figure 4d**).

These quantitative trends confirm a consistent inverse relationship between drainage proximity and fungal abundance, while bacterial and actinomycete populations exhibit a decline with increasing distance. The elevated bacterial and actinomycete counts near the canal likely reflect the aerobic and nutrient-enriched microenvironment created by artificial drainage. Increased oxygen diffusion and periodic drying favor fast-growing bacterial taxa and filamentous actinomycetes that can rapidly colonize exposed substrates and utilize labile carbon compounds. Conversely, the relatively low fungal counts in these regions indicate that filamentous fungi, which rely on stable and moisture-saturated environments, are more sensitive to hydrological disturbances.

In contrast, the gradual increase in fungal populations and corresponding reduction in bacterial counts at greater distances suggest a microbial community recovery towards anaerobic equilibrium, characteristic of undisturbed peat soils. This transition implies that oxygen limitation and consistent water saturation farther from the canal recreate conditions conducive to fungal growth and carbon preservation. The data thereby reinforce the hypothesis that drainage intensity drives a microbial regime shift, favoring bacterial dominance and carbon mineralization in disturbed peat zones while reducing the capacity of fungi-mediated carbon sequestration processes.

Overall, the plate count results corroborate the microBIOMETER® findings, showing that drainage-induced hydrological stress directly alters the microbial balance and soil carbon potential. The increased bacterial activity and reduced fungal abundance near drainage areas signify an ecosystem in metabolic imbalance, where carbon loss via decomposition may exceed long-term sequestration rates. This observation underlines the ecological importance of maintaining high water tables and minimizing artificial drainage to preserve microbial functionality and peatland carbon stability.

DOI: http://dx.doi.org/10.xxxxx/ijost.v2i2
p- ISSN 2528-1410 e- ISSN 2527-8045

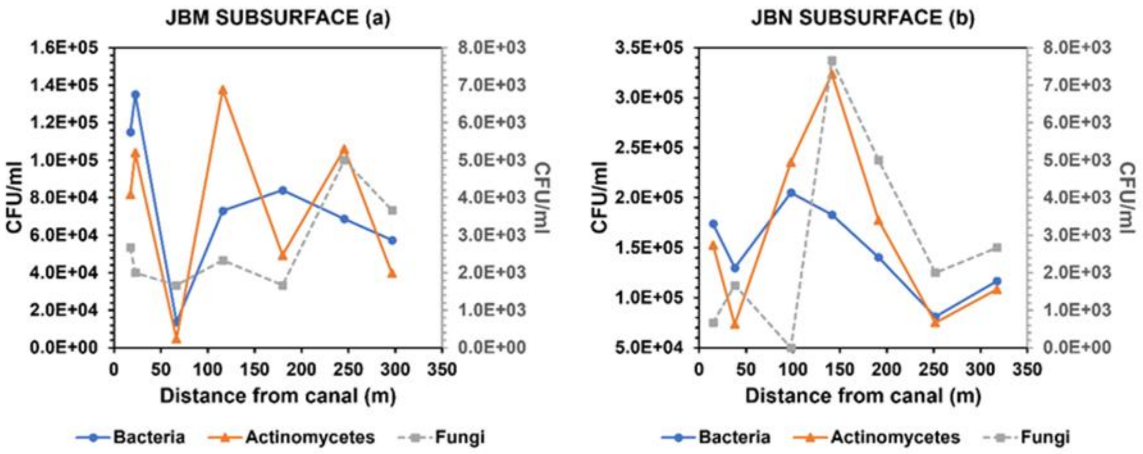

Figure 4. Plate Count Analysis showing variations in bacterial, actinomycete, and fungal populations along two transects in the Badas Peatland, Brunei Darussalam. (a) Bacterial colony-forming unit (CFU/mL) counts at different sampling distances along the Jalan Badas Middle (JBM) transect. (b) Actinomycete CFU/mL counts at different sampling distances along the JBM transect.(c) Bacterial and actinomycete CFU/mL counts at different sampling distances along the Jalan Badas North (JBN) transect. (d) Fungal CFU/mL counts at different sampling distances along the JBN transect. The results across all panels demonstrate a consistent pattern of decreasing bacterial and actinomycete abundance with increasing distance from the drainage canal, while fungal populations show an opposite trend, indicating that hydrological stability and higher soil moisture farther from the canal promote fungal growth and enhance overall microbial balance in the peatland ecosystem.

3.2. Impact of Drainage Canal on Microbial Populations

The findings across both transects reveal a consistent microbial pattern in relation to drainage proximity. There is a clear inverse relationship between the distance from the canal and bacterial abundance, while fungal abundance and microbial biomass carbon (MBC) increase with distance. This trend indicates that fungi are more negatively affected by drainage than bacteria within the peatland ecosystem. The observed shift toward a fungal-dominated community farther from the canal suggests that soil environments with greater moisture stability promote fungal proliferation or inhibit bacterial dominance. The simultaneous rise in MBC in these regions implies enhanced microbial activity and ecosystem stability, reflecting improved soil structure, nutrient retention, and reduced physical or chemical stress.

Conversely, the lower MBC and higher bacterial counts near the canal correspond to increased soil aeration and physical disturbance resulting from drainage, which accelerates organic matter decomposition and carbon loss. Such conditions are typical of degraded peatlands where hydrological alteration disrupts anaerobic balance. These findings align with previous studies (Mastný *et al*., 2021), which reported bacterial predominance in waterlogged peatlands with extremely low fungal-to-bacterial ratios (1.15×10^{-5} to 0.026). The comparison validates the hypothesis that bacterial populations thrive near drainage canals, where fungal decline limits carbon stabilization and lowers MBC, thereby reducing long-term soil carbon retention capacity.

The ecological implication of these results highlights that drainage not only modifies microbial composition but also reshapes carbon cycling pathways. As fungal biomass is closely associated with recalcitrant carbon storage, its suppression near canals may shift peatlands from net carbon sinks to potential carbon sources, amplifying greenhouse gas emissions.

Therefore, maintaining the hydrological equilibrium of peatlands is crucial to sustaining fungal diversity and preserving microbial-mediated carbon sequestration functions.

3.3. Bacterial Adaptation and Resilience

Bacteria exhibit remarkable physiological plasticity, allowing them to adapt effectively to environmental stressors imposed by drainage. Bacterial populations can undergo rapid genetic and metabolic adjustments to survive nutrient scarcity, enabling persistence under fluctuating soil moisture and aeration (Hottes *et al*., 2013). Near drainage canals, where soil desiccation and oxygen availability fluctuate, this adaptability gives bacteria a competitive advantage over fungi.

Bacteria also possess simpler cellular structures and the capacity to form spores or other resistant morphotypes, enhancing survival under mechanical disturbance such as soil compaction or machinery movement (Raza *et al*., 2021). In contrast, filamentous fungi rely on extensive mycelial networks for nutrient uptake and are therefore more susceptible to disruption. These physiological differences explain the dominance of bacterial and actinomycete populations in disturbed zones near the canal. Bacterial communities dominate in biofilm systems subjected to environmental perturbations, a pattern consistent with the current findings (Exton *et al*., 2024).

Overall, bacterial resilience underscores their ecological role as opportunistic colonizers in drained peat environments. While this adaptation supports microbial survival under stress, it simultaneously accelerates organic matter oxidation, leading to higher CO_2 fluxes and reduced soil carbon stability. Hence, the proliferation of bacteria near drainage canals may serve as a bioindicator of peatland degradation intensity.

3.4. Microbial Biomass Carbon (MBC) and Environmental Stressors

Microbial biomass carbon (MBC) represents the living fraction of soil organic matter, reflecting the overall metabolic potential and biological health of the soil ecosystem. The lower MBC observed near the drainage canal indicates a decline in microbial diversity and metabolic activity, likely caused by environmental stressors such as fluctuating water levels, increased aeration, and possible chemical leaching. These disturbances reduce the availability of organic substrates and create suboptimal conditions for microbial proliferation. Disturbed peatlands exhibit lower microbial biomass and activity compared to undisturbed counterparts (Könönen *et al*., 2018).

Hydrological imbalance plays a particularly critical role in shaping MBC distribution. Drainage lowers the water table, reducing anaerobic conditions necessary for fungal persistence. As fungi contribute substantially to total microbial biomass, their reduction directly translates to diminished MBC. Some researchers (Qiu *et al*., 2013) observed significant ($p < 0.05$) declines in fungal populations following artificial drainage in waterlogged soils, supporting the current study's inference. Moreover, continuous drainage facilitates nutrient leaching, especially nitrogen and phosphorus, which are vital for microbial metabolism and community stability.

Despite recent canal blockage efforts within the Badas Peatland, residual drainage effects remain evident in microbial indicators, suggesting prolonged ecological sensitivity. Therefore, future studies should integrate hydrological monitoring, nutrient profiling, and seasonal microbial assessments to elucidate the dynamic interactions among soil moisture, nutrient fluxes, and microbial community resilience.

p-ISSN: 2828-920X e-ISSN: 2828-9951

The observed relationship between MBC, microbial diversity, and distance from drainage features underscores the **critical role of hydrological management** in sustaining peatland functionality. Effective conservation practices such as rewetting, buffer zone establishment, and regulated land use are essential to restoring microbial balance, mitigating carbon loss, and preserving the long-term ecological integrity of tropical peat ecosystems.

4. CONCLUSION

This study demonstrated that hydrological drainage in the Badas Peatland of Brunei Darussalam significantly alters soil microbial dynamics, reducing fungal abundance and microbial biomass carbon (MBC) while promoting bacterial dominance near the canal. The observed inverse relationship between drainage proximity and fungal activity indicates that fungi are more sensitive to moisture depletion and physical disturbance. These microbial shifts signify declining carbon stabilization capacity in drained zones. Maintaining peatland hydrological balance is therefore crucial to preserving microbial diversity, enhancing carbon sequestration, and mitigating peat degradation. The findings provide essential insights for sustainable peatland restoration and water management strategies in tropical ecosystems.

5. ACKNOWLEDGMENT

We would like to extend heartfelt thanks to the Forestry Department of Brunei Darussalam for granting the research permit essential to this study. Special appreciation is due to Anis Nadiah Abdillah, Research Assistant, for her invaluable assistance with the Plate Count Analysis. We also wish to thank Aaron Daniel Selavisi and Danish Wa'ie Roslan for their assistance during the soil sampling process. We gratefully acknowledge Universiti Brunei Darussalam and the Ministry of Education, Brunei Darussalam, for the scholarship provided throughout the research study.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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