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Impact of Netted vs Non-Netted Agriculture on Soil Microbial Diversity and Activity in Cameron Highlands

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Abstract: Soil microbial diversity underpins sustainable crop productivity, yet its responsiveness to protected cultivation systems remains poorly understood. This study offers the first comparative insight into how netted structures influence microbial functional traits in highland agroecosystems. Soil samples were analyzed using soil suspension method to isolated microbes based on its characteristic. It was then further analyzed using Biolog EcoPlates to evaluate microbial populations and substrate utilization. Findings revealed that non-netted structures supported a higher abundance of beneficial microbes, including nitrogen-fixers and phosphate-solubilizers, however it was observed that phosphate solubilizer microbes were absent in the netted system. Microbial diversity was greater in non-netted soils with the Shannon Index of 3.389 than in netted soils of just 3.350, alongside enhanced metabolic activity (Average Well Color Development of 1.960 vs. 1.650 respectively for both non-netted and netted structure). Substrate usage differed markedly, with microbes in netted soils primarily metabolizing carboxylic acids, while those in non-netted soils preferred amino acids. These results suggest that netted agricultural structures may suppress microbial populations and their functional potential, likely due to changes in the microclimatic. Optimizing protected cultivation systems to preserve microbial diversity is therefore essential for sustainable soil health.

Keywords: Soil Microbial Diversity, Regenerative Agriculture, Sustainable Soil Health, Protected Cultivation Systems, Functional Metabolic Profiling

1.0 Introduction

Microbial communities form the unseen yet essential backbone of agricultural ecosystems, shaping everything from soil fertility to plant resilience and overall crop productivity (Raven et al., 2024). These communities consisting of bacteria, fungi and other microorganisms differ widely between cultivation systems. Practices such as crop rotation, fertilizer usage and soil tillage play a crucial role in shaping these microbial populations (Khmelevtsova et al., 2022). Understanding of how these factors influence microbial diversity is key to refining land management strategies and promoting sustainable soil health.

Soil itself is a dynamic and living entity. Its dynamic conditions are sustained by nutrient rich humus, which supports a diverse cast of microbial players fungi, bacteria, viruses, actinomycetes, blue-green algae and protozoa (joas et al., 2023). These organisms perform vital ecological roles such as breaking down contaminants, preserving soil structure and cycling biogenic elements that nourish plant roots. The rhizosphere where the plant roots and soil interact are known to be rich in various microbial activities. Root exudates modulate this zone, creating conditions that will affect microbial interactions known as plants microbe's interactions. As observed by Brimecombe et al. (2007), these interactions deeply impact plant nutrition and growth. Moreover, rhizospheric and endophytic bacteria have demonstrated the capacity to enhance plant survival under abiotic stress (Chauhan et al., 2023). Vishwakarma et al. (2020) emphasized that robust microbial diversity fosters disease resistance, mitigates stress, and stimulates development. Beneficial microbes may also help plants to overcome nutrient limitations by boosting antioxidant enzyme production, effectively countering stress-induced reactive oxygen species (ROS) accumulation (Kabir et al., 2020). Beyond their biochemical

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roles, soil microorganisms act as biological indicators of soil health. Their diversity and community structure reflect the vitality of the agricultural ecosystem which will help in guiding decisions on crop and land management (Joas et al., 2023).

This study explores how microbial diversity responds to two distinct farming environments, namely netted and non-netted structures. Understanding how environmental variables sculpt these microbial landscapes holds promise for building resilient and sustainable farming models.

2.0 Materials and methods

2.1 Sample collection

Soil samples were collected from farms situated at Tanah Rata (4.4706ºN, 101.3766ºE) and Habu (4.4401ºN, 101.3913ºE) Cameron Highland, Pahang, Malaysia. Soil samples were collected 15 cm below the rhizosphere of the cabbage and put into a double ziplock bag before being transported back to MARDI headquarters Serdang, Selangor, Malaysia.

2.2 Soil microbial enumeration

Ten grams of each collected soil samples were added into conical flasks containing 100 ml of sterile distilled water (sdH_2O) and agitated for 1 h. After 1 hr, 150 µl of the soil suspension were pipetted onto Nutrient Agar (NA), Potato Dextrose Agar (PDA), Nitrogen fixer Agar (N-fix), Phosphate solubilizer Agar (P-sol.), and Starch Casein Agar (SCA). All the plates were incubated at 28 ± 2ºC for 24 hrs except for PDA and SCA which were incubated for 5 days. Tests were conducted in duplicate dilution of 102-108 were done for each agar plate. Colony forming unit for each plate was calculated and expressed in cfu/g of soil and was average out for each plate.

2.3 Analyzing microbial diversity using BIOLOG Ecoplate

Hundred grams of soil samples were added into 1000mL of sterile distilled water and agitated vigorously at 500 rpm for 1 h. After that, the suspension was filtered using Whatman Filter Paper No 1. A multichannel pipette set at 130 uL was used to pipette the filtrate into each well in the plate. The Biolog Ecoplate was then incubated at room temperature (28 ± 2ºC). The plate was then read using a plate reader at 590 nm for 24, 48, 72, 96 and 120 hrs. The absorbance reading of each well was corrected with the absorbance well A1 (water) to generate the average well colour development (AWCD) of each well. Microbial Richness (S) of the community was calculated based on how many wells change colour during the 120 hrs incubation. While Shannon diversity index (H') was calculated using the equation below: -

$$H' = -\Sigma[pi * \ln(pi)]$$

Where;
H': The Shannon diversity index value
pi : represents the proportion of wells showing positive growth for each substrate utilization

Shannon Evenness index (E) which is the measurement of how evenly the distribution of the microbial communities across the carbon sources was calculated using the equation below:-

$$E=H'/\ln (S)$$

Where ;
H': The Shannon diversity index value
S : Microbial richness

Microbial activity is also known as Average Well Colour Development (AWCD) of the microbial community was calculated using the equation below:-

$$AWCD = \Sigma ODi/n$$

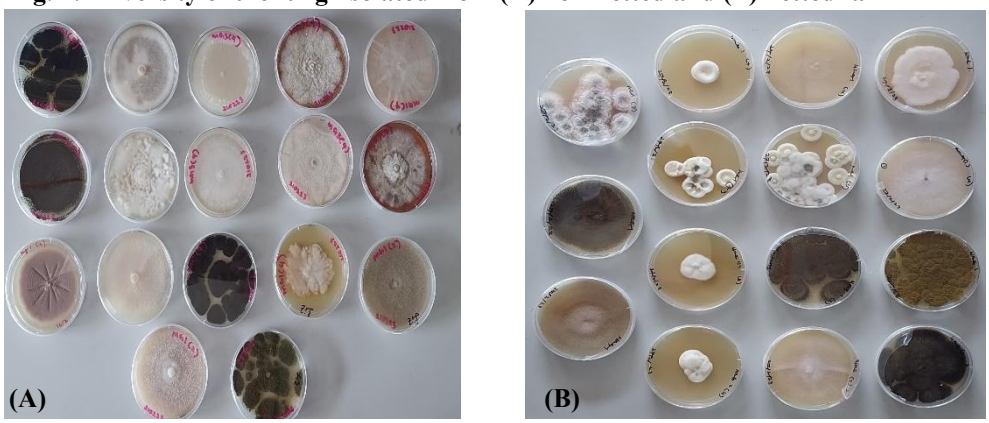
Where:
ODi; Is the absorbance of each well corrected with the control well (Blank)
n: Is the number of wells

3. Results and Discussions

3.1 Soil enumeration and analysis of microbial population in netted structure and non-netted structure condition

The soil microbial community is a vital indicator of overall soil health and biodiversity. Figure 1 showed the diversity of fungus isolated from the non-netted and netted farming clearly indicated that the diversity of non-netted farm having more fungi compare to netted. Based on the results in Table 1, we observed that soils from farms using netted structures had noticeably lower populations of bacteria (4.4×10^6 cfu/g) and nitrogen-fixing microbes (2.85×10^6 cfu/g) compared to those from non-netted farms, which showed higher counts of 5.2×10^6 cfu/g and 1.6×10^7 cfu/g, respectively. This suggests that the netted environment may limit the growth of some beneficial soil microbes. Interestingly, our findings align with those of Lan et al. (2025), who reported that soils under netted structures tend to have more actinomycetes (6.0×10^4 cfu/g), followed by bacteria and fungi at 5.5×10^4 cfu/g and 1.0×10^4 cfu/g, respectively. This pattern indicates that actinomycetes may thrive better than other microbial groups in netted conditions.

Fig. 1: Diversity of the fungi isolated from (A) non-netted and (B) netted farm



One of the most striking findings was the absence of phosphate-solubilizing microbes in soils collected from netted structures. According to Ughamba et al. (2025), the presence of phosphate-solubilizing microbes is influenced by several factors, including soil properties, farming practices, the use of netted systems, and crop type. Supporting this, Xiao et al. (2020) and Zhang et al. (2020) observed that netted structure cultivation can significantly alter the soil environment around plant roots, ultimately impacting the microbial population in the agriculture system. Similarly, Huang et al. (2022), noted a general decline in soil bacterial populations under netted systems compared to open fields. Together, these findings highlight that while netted structures may offer physical protection to crops, they can also impact the diversity and abundance of beneficial soil microbes, which are essential for long-term soil fertility and sustainability.

Table 1: Microbial population from netted and non-netted structure

	Colony forming unit per gram soil (cfu/g)	
	Netted structure	**Non-netted structure**
Bacteria	4.4×10^6	5.2×10^6
Fungi	7.5×10^4	1.25×10^8
Actinomycetes	1.35×10^8	8.3×10^4
Nitrogen fixer	2.85×10^6	1.6×10^7
Phosphate solubilizer	0	4.1×10^6

3.2 Microbial diversity, richness, evenness and activity in both netted structure and non-netted condition

Figure 2 (A) illustrates that microbial diversity, as measured by the Shannon diversity index, is consistently lower under netted structures compared to non-netted fields. In this study, the Shannon diversity index values for netted systems

ranged from 3.345 to 3.350, while non-netted fields exhibited slightly higher values between 3.385 and 3.389. These findings align with observations by Liao et al. (2018), who reported greater microbial diversity in open-field conditions compared to netted farming practices. Figure 2 (C) and (D) also showed that under netted structure the evenness of microbial distribution and microbial activity was also low compared to non-netted condition.

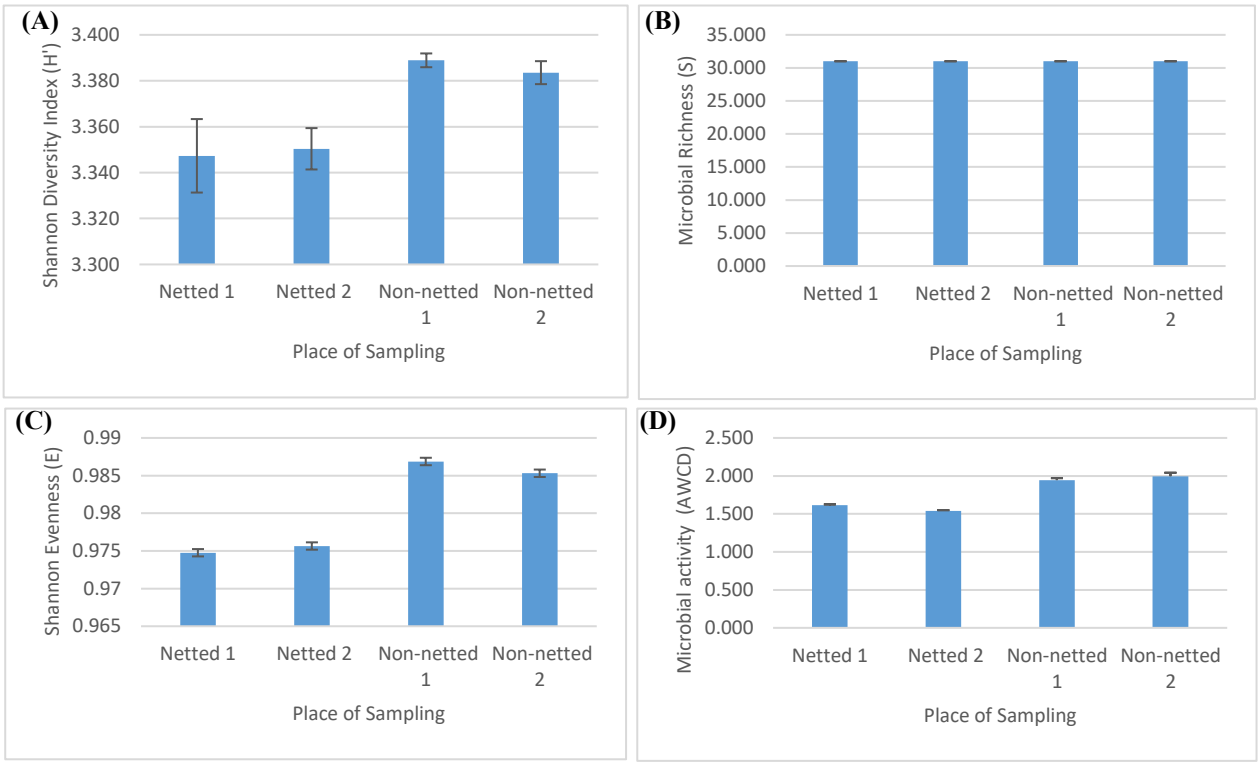


Fig. 2: (A) Shannon Diversity Index, (B) Microbial Richness and (C) Shannon Evenness Index and (D) Microbial activity in netted non-netted structure farms

The observed differences in microbial diversity between netted and non-netted farming systems may stem from variations in environmental exposure. Netted structures by design create a semi-closed habitat that likely limits airborne microbial influx and dampens microclimatic fluctuations that typically support microbial diversity. In contrast, non-netted environments remain fully open to the surrounding ecosystem, encouraging higher microbial exchange and colonization through natural interactions. It's also essential to know that microbial diversity in agricultural soils is shaped by a web of interconnected factors. Crop species, prevailing environmental conditions, irrigation methods and soil amendment practices all contribute to the complexity of microbial ecology (Majumdar et al., 2024). In this context, the shifts observed in the Shannon diversity index between farming structures provide more than just numerical data, but they also offer an accessible tool for gauging soil health and the ecological resilience of cultivated land.

Interestingly, despite these structural differences, carbon source utilization patterns were broadly similar between the two systems. However, we are still able to observe some differences such as netted farms showed a higher utilization of carboxylic acids, while amino acid utilization was more distinct in non-netted environments. These patterns suggest that while overall metabolic functions may be stable, certain biochemical pathways could be selectively influenced by cultivation structure. These findings underscore the delicate interplay between environmental design and microbial dynamics. They highlight the importance of considering both structural configuration and ecological inputs when evaluating soil functionality and planning for sustainable farming systems.

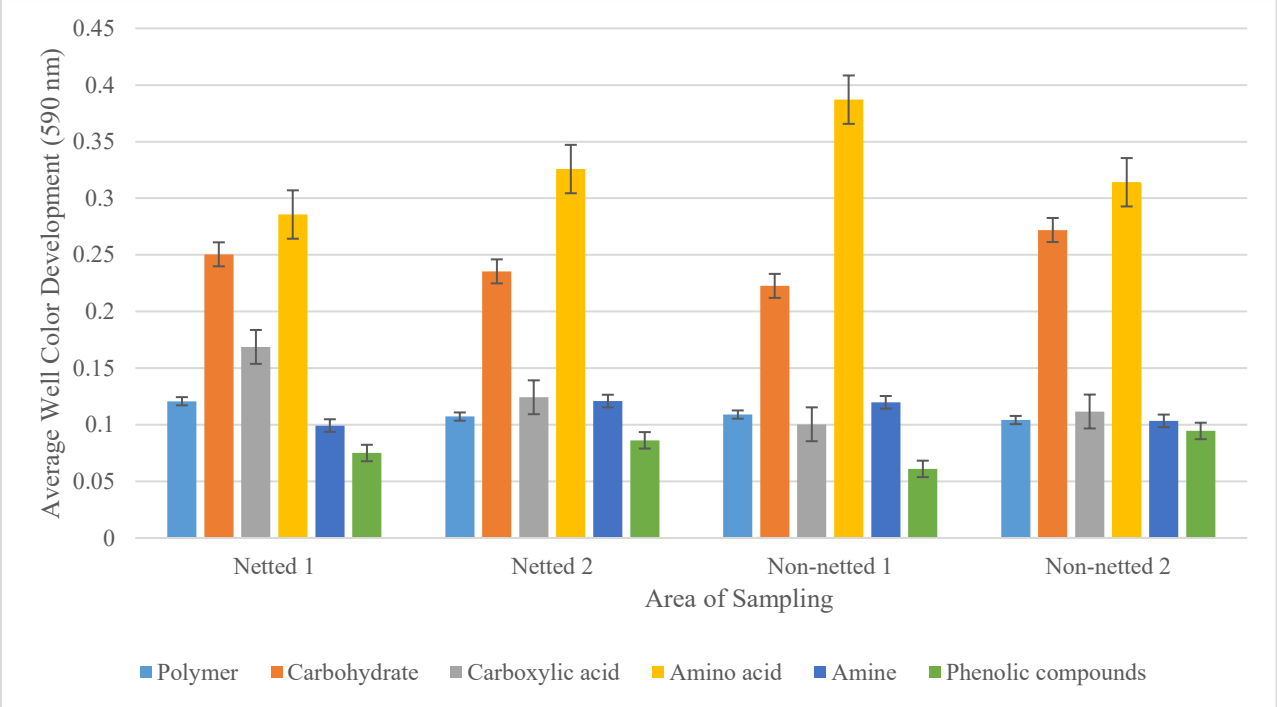


Fig. 3: Carbon sources used by microbes in netted and non-netted structure farms

4.0 Conclusion

This study underscores the influence of cultivation environment on microbial diversity. Microbial populations were found to be more diverse in non-netted farms compared to netted systems, suggesting that enclosed environments may constrain microbial proliferation by limiting exposure to external ecological inputs. Despite this, patterns of microbial carbon source utilization remained comparable between both farming approaches, indicating that core metabolic functions of the microbial communities were largely maintained regardless of structural enclosure. These findings provide important insights for farmers when designing their agricultural plot, highlighting that while physical barriers may reduce microbial diversity, they do not necessarily compromise microbial functionality. Such information is vital for balancing productivity and sustainability when designing cultivation systems in variable ecological settings.

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Conflict of Interest

The authors declare no conflicts of interest.

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