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Biological Characterization of Geo-Referenced Soil Samples from Major Rice-Growing Tracts of Southern Kerala

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

A survey was conducted from January to March 2023 to assess the soil biological fertility index across five agro-ecological units in the rice-growing belts of Southern Kerala. These units included Onattukara sandy loam (AEU 3), Kari soil (AEU 4), Pokkali soil (AEU 5), Southern laterites (AEU 8), and Midland laterites (AEU 9). The study was carried out in the College of Agriculture, Vellayani Thiruvananthapuram. The survey aimed to assess microbial activity and organic carbon content, thereby enhancing our understanding of the biological fertility across these diverse soil types. Twenty geo-referenced surface soil samples @ 0- 15 cm were collected in every AEU by prescribed method. The collected samples were subjected to the characterization of soil biological properties such as dehydrogenase activity, organic carbon, microbial biomass C, microbial biomass N and soil respiratory rate following the standard protocol. The findings showed that organic carbon in AEU 5 with the highest mean value (3.03 %), followed by AEU 4 (2.19 %), AEU 9 (2.01 %), AEU 8 (1.01 %), and AEU 3 (0.56 %). Dehydrogenase activity was highest in AEU 5 (682.54 µg TPF g^{-1} hr^{-1}), followed by AEU 4, AEU 9, and AEU 8, and lowest in AEU 3 (76.71 µg TPF g^{-1} hr^{-1}). AEU 5 exhibited the highest microbial biomass carbon and microbial biomass nitrogen activities, while AEU 8 showed the lowest. Soil respiration was highest in AEU 5 and lowest in AEU 3. Based on these soil microbiological indicators, soils were classified into three categories: low, medium, and high fertility. The Biological Fertility Index (BFI) scores indicated favorable conditions in AEU 4 and 5, moderate in AEU 9, and low in AEU 3 and 8. By establishing a comprehensive understanding of soil biological fertility across various agro-ecological units, the research aims to contribute to improved soil health and crop productivity in the region.

Keywords: Agroecological units; soil biological properties biological fertility index; rice; wetland soils.

1. INTRODUCTION

Soil biological characteristics reflect both the direct and indirect impact of the organisms residing within a specific soil. These biological processes involve the cycling of carbon and nutrients, biotic interactions contributing to soil formation, microbial abundance, enzyme functions, as well as microbial biomass of C, N, and S (Azcón-Aguilar et al. 2015). Among the array of enzymes present in soil environments, dehydrogenase holds significant importance as serve as an index of overall microbial activity within the soil. Their significance lies in the fact that they are intracellular and present in all living microbial cells (Moeskops et al. 2010; Yuan et al.2012; Zhao et al. 2010). Furthermore, their function is closely associated with microbial oxidoreduction processes. Soil organic matter (OM), is regarded as a key indicator of soil quality due to its role as a reservoir and contributor of nutrients that can improve soil physical and chemical attributes, while also stimulating biological processes (Salazar et al. 2011;Joseph, 2014). Remarkably, it's not solely the quantity of OM in the soil that holds significance, but primarily its quality, as OM influences the provision of energy for microbial proliferation and the production of enzymes. Manral *et al* (2023) revealed that the microbial biomass within soil significantly influences soil processes like nitrogen mineralization and serves as a bio-indicator of current climatic shifts. Soil microorganism activity in organic matter decomposition governs the mineralization and immobilization of nutrients, influencing nutrient availability in soil crucial for plant growth (Bhupenchandra et al. 2020). Soil microbial biomass functioning as a reservoir and consumer of accessible nutrients, holds significant importance in the transformation of nutrients within the soil. Alterations in microbial biomass have the potential to impact the turnover of soil organic matter. Consequently, soil microbial activity directly influences the stability and fertility of soil. The assessment of microbial biomass serves as a valuable tool for evaluating soil quality (Francaviglia et al., 2017). Soil respiration quantifies the CO_2 emitted by soil, indicating the collective CO_2 release from various living organisms such as bacteria, fungi, earthworms, protists, roots, and more. These measurements serve in carbon balance calculations and serve as an index for soil health (Gao et al. 2020). At the global scale, soil fertility is emerging as a significant concern because changing climatic conditions are leading to declines in soil properties attributed to alterations in soil biota, encompassing both fauna and flora (Gurjar et al. 2017). Kerala state delineated into twenty-three agroecological units has been established considering climate, topography, and soil characteristics. Each AEU corresponds to specific soil and climatic conditions. Therefore, the present study is envisaged to investigate the biological properties of various agroecological units and thematic maps were generated highlighting the biological fertility index of wetland soils of Southern Kerala. To evaluate the spatial distribution of soil biological fertility across different agro-ecological units (AEUs) in Southern Kerala, identifying areas of high and low fertility. This provides valuable insights into the biological fertility index of soils in Southern Kerala, which is crucial for understanding soil health and sustainable agricultural practices in the region. By employing comprehensive parameters such as dehydrogenase activity, microbial biomass carbon, and organic carbon content, the study presents a robust framework for assessing soil quality, potentially influencing future research and policy decisions.

2. MATERIALS AND METHODS

Initially, a survey was conducted in the five agro-ecological units of rice-growing regions in Southern Kerala by the College of Agriculture, Vellayani, Thiruvananthapuram, during the period from January to March 2023. The aim of this survey was to assess soil biological fertility and related factors across these zones *viz.,* Onattukara sandy loam (AEU 3), – ORARS, pathiyoor, cheppadand,Thattarambalam is located in the kayamkulam municipality of Alappuzha district,Kari soil (AEU 4), Pokkali soil (AEU 5)- Kumbalangal,pallurthy, Kalluchira and Kuzhuppilly of Ernakulam district, Southern laterites (AEU 8)- Vellayani, Chenkal, and Kalliyoor of Thiruvanthapuram district and Midland laterites (AEU 9)-Chenapara, Kariakoam, Kuttikonam and Vettikavala. Wetland soil samples were collected @ 0-15 cm as per the standard procedure. The biological properties including dehydrogenase activity, organic carbon, microbial biomass carbon, microbial biomass N, and soil respiratory rate were estimated. The method for measuring dehydrogenase activity, as outlined by Casida *et al* (1964), involved the utilization of 3% 2, 3, 5-triphenyl tetrazolium chloride (TTC). The concentration of dehydrogenase present in the sample was determined by creating a standard graph employing triphenylformazon (TPF) as a reference. The enzyme activity was then quantified as micrograms of TPF released per gram of soil per hour. The soil organic carbon was analyzed by the protocol stated by Walkley et al. (1934). The microbial biomass carbon was determined by the fumigation-incubation technique described by Jenkinson and Ladd (1981) and is expressed as µg per gram of soil. Brookes *et al.* (1985) outlined the protocol used by estimating soil microbial biomass nitrogen by chloroform fumigation and indicated as µg of nitrogen per gram of soil. Anderson (Anderson et al., 1982) outlined the method for determining soil respiration activity. The respiratory activity of the soil samples was determined by collecting and quantifying the CO_2 evolved from a fixed quantity of incubated soil in standard alkali. According to Brookes (1995), soils were classified into three categories *viz.* low, medium, and high based on indicators of soil biological properties. The biological fertility index was determined by the score assigned to the biological fertility index. Thematic maps, illustrating the soil of five agro-ecological units, were produced using ArcGIS software.

3. GENERATION OF MAPS USING GEOGRAPHIC INFORMATION SYSTEMS

Using Geographic Information Systems (GIS), a geo-referenced thematic map of the biological fertility index for five agroecological units (AEUs) was created in ArcGIS. The map illustrates the spatial distribution of the relative biological fertility index across wetland soils in various AEUs of southern Kerala were presented in Fig. 1.

4. RESULTS AND DISCUSSION

4.1 Organic Carbon

Soil samples were collected from 20 locations in different Agricultural Ecological Units (AEUs). The highest mean value of organic carbon was recorded in AEU 5 (3.03 %), followed by AEU 4 (2.19 %), AEU 9 (2.01%), AEU 8 (1.01 %), and AEU 3 (0.60 %). Organic carbon content ranged from 0.21 to 0.98 in AEU 3 indicating that low to medium organic carbon, 1.00 to 3.14 in AEU 4 was medium to high organic carbon content, 1.00 to 6.22 in AEU 5 showed medium to high organic carbon content, 0.52 to 3.38 in AEU 9, the organic carbon content was from medium to high range, and 0.47 to 1.86 in AEU 8 it was varied

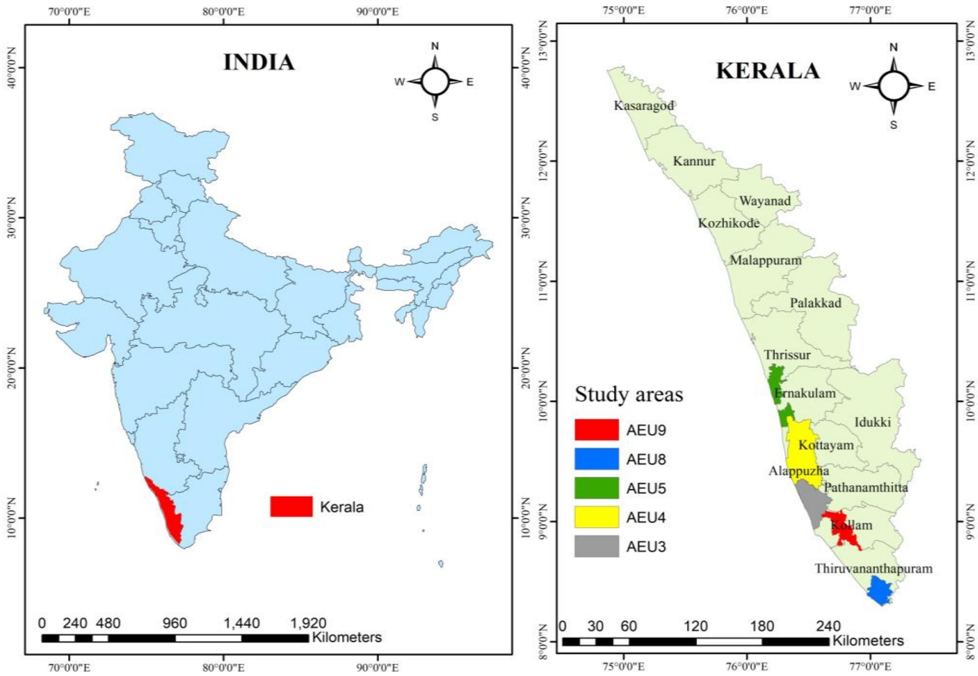

Fig. 1. Location map of study area different AEUs

from low to medium organic carbon content Fig. 2. The low organic carbon content in AEU 3 is attributed to its lateritic soil type and sandy texture. High temperatures and heavy rainfall in this region accelerate the decomposition of organic matter, thereby reducing organic carbon content. Conversely, AEU 4 predominantly features high organic carbon content (>1.5%), largely due to the abundance of partially decomposed fossil woods and roots at various stages of decomposition. The high organic carbon status is also due to the slow decomposition rate of large amounts of organic matter under flooded anaerobic conditions (Unnikrishnan and Sankar, 2021). AEU 5, characterized by Pokkali soils, is known for its high fertility and acid saline conditions, along with high organic carbon content. This is likely due to the presence of partially decomposed roots and other organic materials (Beena et al., 2017). In AEU 9, the accumulation of organic matter is attributed to leaf litter, plant residues, and root biomass, which enrich the soil. The slow decomposition rates in anaerobic conditions further contribute to the high organic matter levels. AEU 8 has medium organic carbon content, with a moderate quantity of dry matter and a range of major and minor nutrients being added to the soil (Aparna et al., 2023; Sheeba et al., 2019).

4.2 Dehydrogenase

Dehydrogenase plays a key role in the biological oxidation of soil organic matter, and its activity is used as an indicator of microbial activity in the soil. The range of dehydrogenase activity was 290.94 to 991.56 in AEU 5 with the highest mean value were (682.54 µg TPF g^{-1} hr^{-1}) followed by a range 121.89 to 888.68 in AEU 4 with a mean value of (593.01 µg TPF g^{-1} hr^{-1}), 177.85 to 355.48 in AEU 9 with mean value (242.93 µg TPF g^{-1} hr^{-1}), 38.75 to 155.25 in AEU 3 with the mean value (95.08 µg TPF g^{-1} hr^{-1}) and the lowest activity was AEU 8 with mean value (76.74 µg TPF g^{-1} hr^{-1}) Fig. 3. Unnikrishnan and Sankar (2021) reported that dehydrogenase activity is highly correlated with the organic carbon content in soil. Higher levels of organic matter can provide enough substrate to support greater microbial biomass, which in turn leads to increased enzyme production (Yuan et al. 2012). Zhang *et al* (2023) found that variations in microbial community composition can significantly influence soil enzyme activities, including dehydrogenase, thereby affecting organic matter decomposition and nutrient cycling. This highlights the need to consider microbial diversity and enzyme activity measurements for a more comprehensive understanding of soil health.

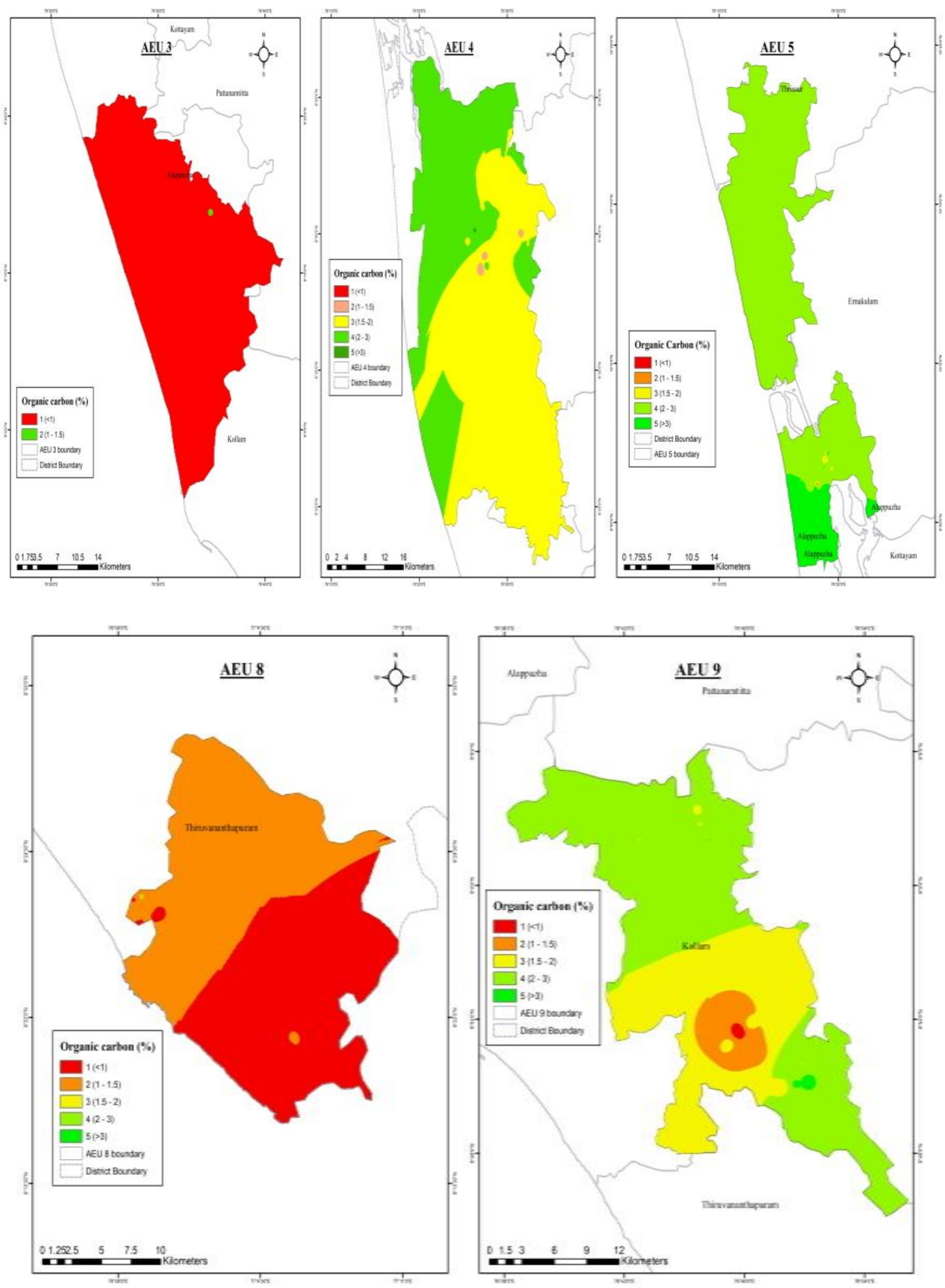

Fig. 2. Spatial distribution of soil organic carbon of AEU 3, 4, 5, 8 and 9.

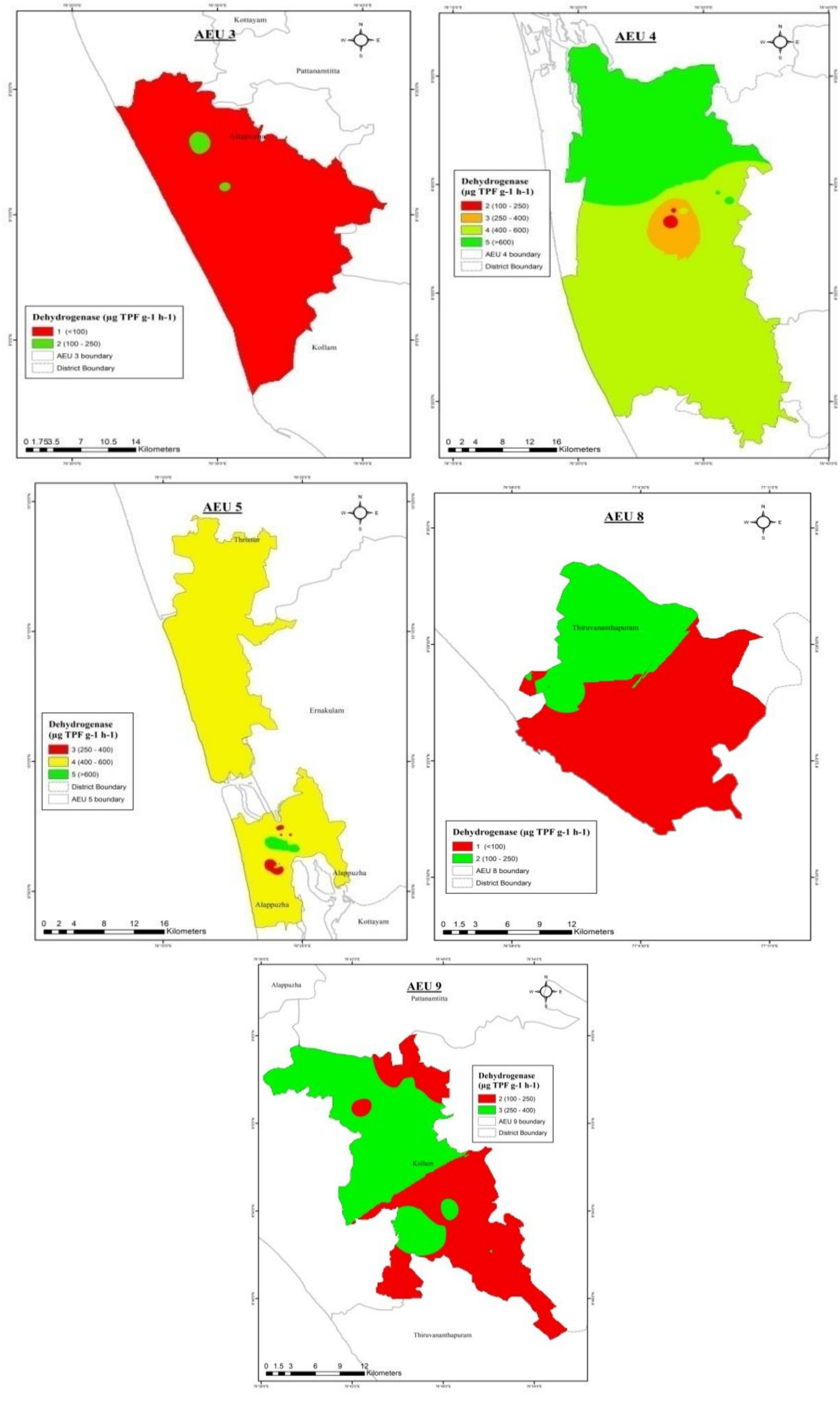

Fig. 3. Spatial distribution of soil dehydrogenase activity of AEU 3, 4, 5, 8 and 9

4.3 Microbial Biomass Carbon

The mean value of microbial biomass carbon (MBC) was highest in AEU 5 (628.57µg $g^{-1}$of soil), followed by AEU 4 (429.49 µg $g^{-1}$of soil), AEU 9 (435.79 µg $g^{-1}$of soil), AEU 3 (208.66 µg $g^{-1}$of soil), and lowest in AEU 8 (163.65µg $g^{-1}$of soil). The MBC values ranged from 102.54 to 280.26 in AEU 3, 255.25 to 699.15 in AEU 4, 251.36 to 976.38 in AEU 5, 103.14 to 675.28 in AEU 9, and 33.16 to 384.49 in AEU 8 Table 1. Joseph (2014) reported that microbial biomass carbon in Pokkali soil varied from 50.12 mg kg^{-1} to 658.52 mg kg^{-1}.Soil microbial biomass is highly sensitive to even slight changes in the organic matter content, which directly serves as an energy source. There is a positive correlation between organic carbon and microbial biomass carbon (Haripal and Sahoo, 2014). Liao *et al* (2024) found that MBC increases with organic inputs, especially in soils under agricultural use. Sahoo *et al* (2019) confirmed that organic amendments improve microbial biomass, enhancing long-term soil productivity and resilience.

4.4 Microbial Biomass Nitrogen

The microbial biomass nitrogen was found to be highest mean value at AEU 5 (197.30µg $g^{-1}$of soil) and varied from 116.24 to 330.04 followed by AEU 9 (160.50µg $g^{-1}$of soil) and the range from 57.36 to 270.11, AEU 4 (146.07µg $g^{-1}$of soil) varied from 79.61 to 236.08, AEU 3 (87.30 µg $g^{-1}$of soil) from 48.24 to 175.65 and lowest MBN was noticed in AEU 8 (57.17µg $g^{-1}$of soil) varied from 35.58 to 89.22 Table1. These results align with recent studies showing that factors like nitrogen addition and organic carbon content significantly affect microbial biomass nitrogen, which is crucial for soil fertility and plant productivity (Yuan et al. 2012). This variability in microbial biomass nitrogen values guide specific soil management practices to enhance fertility in less productive zones like AEU 8. Introducing specific soil management practices will significantly improve fertility in zones like AEU 8. By adjusting inputs such as organic amendments, nitrogen sources, and microbial inoculants, it is possible to enhance microbial biomass and nutrient cycling, enhancing soil health and productivity.

4.5 Soil Respiration

Soil respiration, which refers to the CO_2 produced by the biological activity of soil organisms, is a significant component of the global carbon cycle (Phillips *et al.,* 2015). The highest mean value of soil respiration was observed in AEU 5 (7.76 mg g^{-1} of soil), with a range from 5.28 to 11.01. This was followed by AEU 4 (4.81 mg g^{-1} of soil) with a range from 2.88 to 6.78, AEU 9 (3.51 mg g^{-1} of soil) ranging from 1.87 to 5.71, AEU 3 (1.14 mg g^{-1} of soil) ranging from 0.14 to 2.26, and the lowest was recorded in AEU 8 (1.61 mg g^{-1} of soil) with a range from 1.06 to 2.78. Soil respiration rates typically increase with ambient temperature, as higher temperatures accelerate carbon cycling processes (Tang et al. 2019). The higher soil respiration rates in AEU 5 can be attributed to potentially warmer temperatures and sufficient moisture, providing a favorable environment for microbial growth and activity. Conversely, the lower rates in AEUs 3 and 8 may reflect suboptimal conditions, possibly linked to soil compaction, nutrient deficiencies, or inadequate organic matter. These results were supported by the findings (Liu et al. (2023).

5. BIOLOGICAL FERTILITY INDEX

The Biological Fertility Index (BFI) is a metric used to evaluate the potential fertility of soil. It takes into account various biological factors that contribute to soil fertility, including the presence and activity of soil microorganisms, organic matter content, and the soil's ability to support plant growth (Casida et al. 1964)**.** The Biological Fertility Index (BFI) is an evaluation system that measures soil health and fertility by considering several key parameters: soil organic carbon, soil respiration, dehydrogenase, soil microbial biomass carbon and soil microbial biomass nitrogen. Each parameter is divided into five intervals, with each interval assigned a score from 1 to 5. These scores are based on findings from previous research (Brookes et al., 1995; Vance et al. 1987). The overall biological fertility is determined by summing the scores of all parameters, which then classifies the soil into different levels of biological fertility, as illustrated in Tables 2 and 3.

Table 1. Mean value of microbiological parameters in various agroecological units

	OC (%)	Range	Dehydrogenase (μgTPF g^{-1} hr^{-1})	Range	Microbial biomass carbon (μg g^{-1} of soil)	Range	Microbial biomass nitrogen (μg g^{-1} of soil)	Range	Soil respiration (mg g^{-1} of soil)	Range
AEU 3	0.56	0.21 to 0.98	76.74	38.75 to 155.25	208.66	102.54 to 280.26	87.30	48.24 to 175.65	1.14	0.14 to 2.26
AEU 4	2.19	1.00 to 3.14	593.01	121.89 to 888.68	429.49	255.25 to 699.15	146.07	79.61 to 236.08	4.81	2.88 to 6.78
AEU 5	3.03	1.86 to 6.22	682.54	290.94 to 991.56	628.57	251.36 to 976.38	197.30	116.24 to 330.04	7.76	5.28 to 11.01
AEU 8	1.01	0.47 to 1.86	95.08	44.25to 157.26	163.65	33.16 to 384.49	57.17	35.58 to 89.22	1.61	1.06 to 2.78
AEU 9	2.01	0.52 to 3.38	242.93	177.85 to 355.48	435.79	103.14 to 675.28	160.50	57.36to 270.11	3.51	1.87 to 5.71

Based on the Biological Fertility Index (BFI) scores, the selected agroecological zones (AEUs) in the study were classified as follows: AEU 3 had a BFI score of 10, placing it in the Low BFI class. AEU 4 had a BFI score of 19, classifying it as Good biological fertility. AEU 5 had a BFI score of 21, indicating Very Good biological fertility. AEU 8, with a BFI score of 8, was categorized as Low. AEU 9, with a BFI score of 17, placed into the Medium fertility class Fig. 4 and Fig. 5. The highest BFI scores were attributed to the high organic matter content and microbial activity in the soil.

The study on the biological characterization of geo-referenced soil samples from Southern Kerala provides crucial insights into the soil microbiological health across various agroecological units (AEUs) in a rice-growing region. By measuring parameters like dehydrogenase activity, organic carbon content, microbial biomass, and soil respiration, the research offers a detailed understanding of soil fertility. Notably, AEU 5 (Pokkali soil) exhibited the highest biological fertility, followed by AEU 4 (Kari soil), underscoring the significance of organic carbon and microbial activities in enhancing soil productivity. These findings are essential for improving rice cultivation practices and tailoring soil management strategies specific to each AEU, aiming to sustain productivity in ecologically varied zones (Olivares et al. 2017).

Table 2. Biological fertility index score for the interval value of different parameters

Parameter	Scores				
	1	2	3	4	5
Organic carbon	<1.0	>1.0 to <1.5	>1.5 to >2.0	>2.0 to <3.0	>3.0
Dehydrogenase	<100	>100 to <250	>250 to <400	>400 to <600	>600
Microbial biomass carbon	<100	>100 to <200	>200 to <300	>300 to <400	>400
Microbial biomass nitrogen	<50	>50 to <100	>100 to <150	>150 to <200	>200
Soil respiration	<5	>5 to <10	>10 to <15	>15 to <20	>20

Table 3. Categories of Biological Fertility Index

Biological fertility class	Low	Medium	Good	Very good
Biological fertility scores	5-12	13-18	19-25	>25

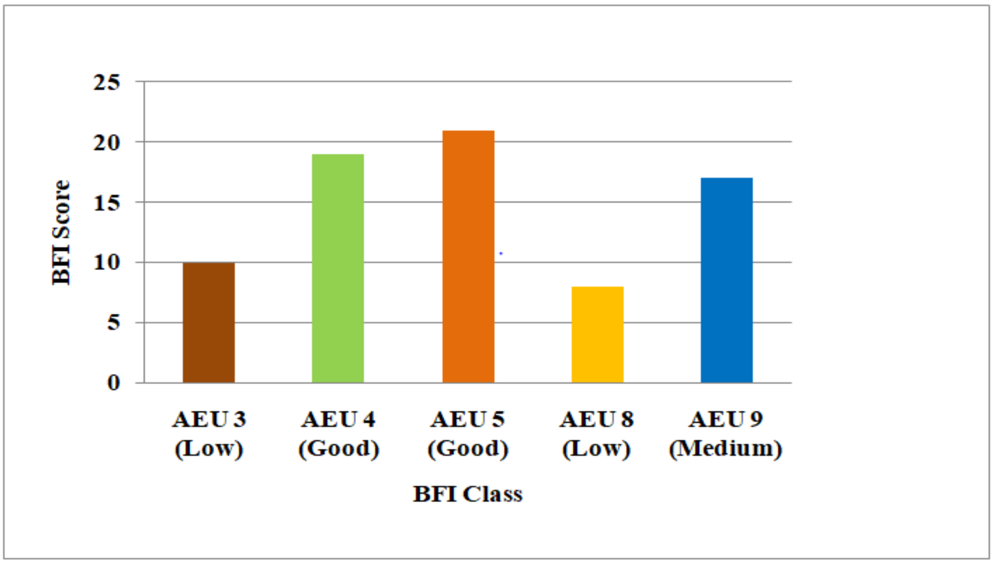

Fig. 4. Biological fertility index (BFI) scores and classifications for various agro-ecological units

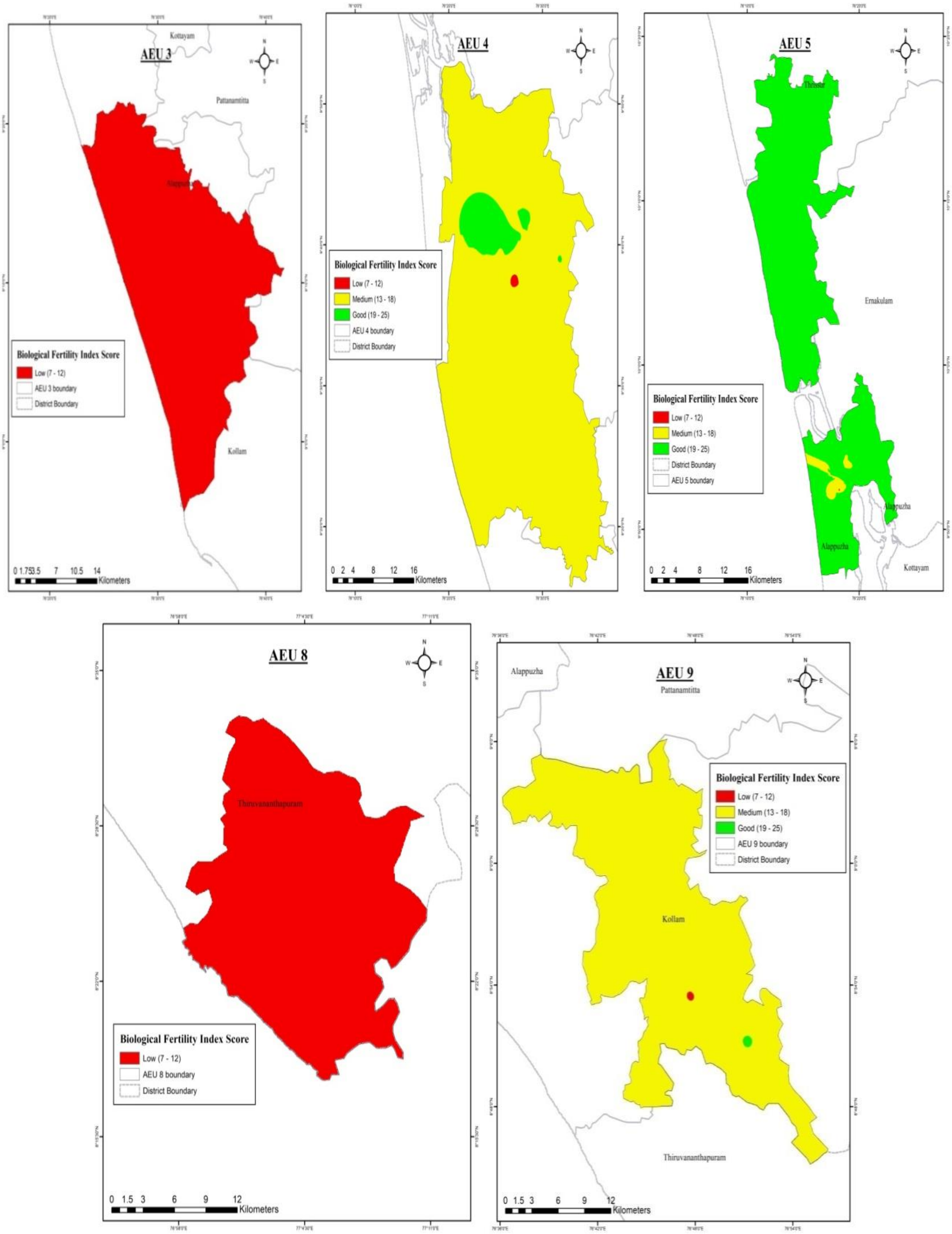

Fig. 5. Spatial distribution of soil dehydrogenase of AEU 3, 4, 5, 8 and 9

Fig. 6. Pearson correlation correlogram of various microbiological parameters across Agro-Ecological Units

6. CORRELATION ANALYSIS

Pearson correlation analysis across Agroecological units shows a strong positive correlation between dehydrogenase activity, microbial biomass carbon, microbial biomass nitrogen, and soil respiration with organic carbon content. As organic carbon increases, these microbiological parameters also rise, emphasizing its role in soil health and microbial activity. Blue circles represent positive correlations, brown circles indicate negative ones, and circle size reflects correlation strength (r), with significant correlations at ($p \leq 0.5$). Fig. 6.

7. CONCLUSION

The present study concluded that Agro-ecological Zones AEU 5 was the highest biological fertility index, followed by AEU 4, AEU 9, AEU 3, and AEU 8. Soil biological properties can be improved through various practices that enhance the activity and diversity of soil microorganisms, promote healthy plant growth, and maintain soil structure. Some of the effective strategies like using organic amendments like manure application, crop management practices like crop rotation, and cover cropping, nutrient management like balanced fertilization, green manuring, etc.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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