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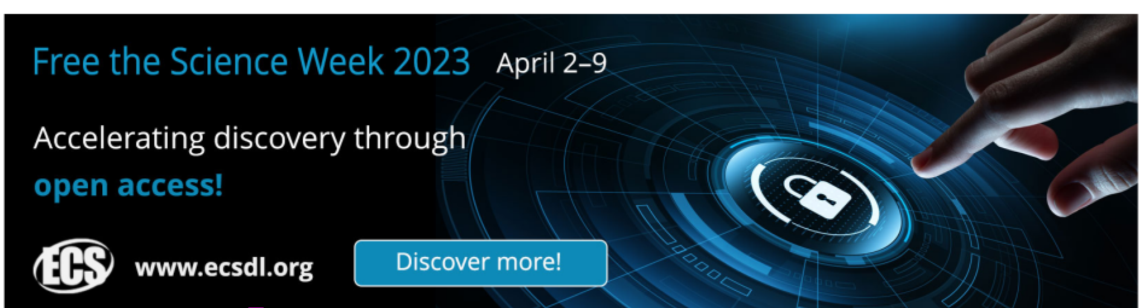

Nematode Community as a Tool to Monitor Ecosystem Health of Kembangkuning Mangrove Forest, Indonesia

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Abstract. Previous studies show that nematode community structure is an efficient bio-tool to detect environmental changes and pollution impacts. Despite their presence and function in an ecosystem, the nematode community is never used to monitor the Kembangkuning mangrove, Segara Anakan. Thus, we intended to collect nematode functional trait data as a baseline to monitor the mangrove ecosystem health. The mangrove is a naturally growing forest dominated by *Rhizophora apiculata* and *Aegiceras corniculatum*, surrounded by settlements and industries. We extracted nematodes from mangrove sediment using the Baermann funnel method. The nematode functional traits were recorded as the baseline data for monitoring purposes. To further examine the nematode community variations, we correlated the nematode functional traits and composition to specific environmental variables. Canonical correspondence analysis revealed a 25.32% accumulated variance with four prominent variables associated with the trait distribution (potassium r = 0.56, water content r = -0.56, salinity r = 0.75, organic carbon r = 0.68). Comparison with published data indicates that the three most abundant nematodes, the non-selective deposit and epistrate feeders of our study site, are also observed in anthropogenically disturbed areas. This finding suggests that our study site requires continual health monitoring to maintain a relatively healthy forest ecosystem.
Keywords: baseline data, early monitoring, ecosystem health, mangrove forest, nematode assemblage

1. Introduction

Nematodes are the most diverse and abundant metazoan that play essential roles in the decomposer food web, mobilizing and transferring nutrients between trophic levels [1]. Their community has served as a bioindicator for environmental changes because of their sensitive response to disturbance. They inhabit numerous conditions of an ecosystem, from pristine to various levels of polluted habitats [2, 3, 4]. This community has been used to monitor the ecosystem health in Europa, such as the estuarine on the Atlantic coast of Portugal [5, 6], the Adriatic sea [7], and the Mediterranean coastal ecosystem [8]. However, to our knowledge, in Indonesia, the free-living nematode community has not been widely used to assess environmental quality, especially the mangrove ecosystem.

The study of nematodes in mangrove primarily focus on community structure. A recent global analysis of mangrove nematodes reports the mangrove communities based on macroecology and the local factors covering 34 ecosystems, including Caribbean-Southwest Atlantic, Western India, Central Indo-Pacific, and Southwest Pacific. It shows that nematode species richness is more significant in the equator than in higher latitudes and concludes that landscape complexity strongly correlates with species richness [9]. In addition, a study in a mangrove forest in the Yen River of northern Vietnam observes 50 species of free-living nematodes [10] and 15 genera in the Kuala Sepetang mangrove in Malaysia [11]. These findings support our purpose to further study the free-living nematode community in Indonesian mangroves.

We focus our study area on the Kembangkuning mangrove. This mangrove is a naturally growing mangrove forest with relatively good condition and lowers human disturbance, such as logging, compared to other mangrove areas of Segara Anakan (Ardli, pers. comm.). Kembangkuning mangrove

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location is unique, secluded in northern Nusakambangan Island, and surrounded by various human activities that put pressure on the mangrove. Our main project includes exploring the nematode community and functional structures to understand the pattern, their relationships, and their response to mangrove environmental gradients. Likewise, ecosystem health monitoring is one of our concerns in the mangrove area due to continual anthropogenic pressures. This article reports our preliminary observation of mangrove nematodes based on their functional structure.

The use of functional structure based on nematode morphological characters as an alternative to the taxonomic approach has been suggested by some authors. It is a simple, effective method to analyze the nematode community [12, 13]. Using a combination of amphid, cuticle, buccal cavity, and tail shape of the nematode morphological character, Semprucci reports that changes in functional trait combination are consistent with changes in the genus composition of the community across the studied ecosystem of the Mediterranean sea. Thus, the method is adaptable to rapidly identify the functional structure of nematodes to assess ecosystem health [13]. Following this recent approach, we intended to collect nematode functional trait data, and use these data as a baseline to monitor the ecosystem health of the Kembangkuning mangrove.

2. Methods

2.1. Study Site

Kembangkuning Mangrove area is a small area of Segara Anakan Mangrove, northeast of Nusakambangan Island, South of Central Java. It includes an area of approximately 136 hectares extended from S-7.717632932, E108.94922627 to S-7.733434595, E108.9791313. The mangrove is a naturally growing forest dominated by *Rhizophora apiculata* and *Aegiceras corniculatum*, surrounded by settlements and industries such as oil refineries, to which oil spills have occurred from several ship accidents. There is a semidiurnal mixed tide with an amplitude reaching 2.3 to 2.57 m [14, 15] and a humid tropical environment (approximate average temperature of 27^{o}C, precipitation of 3400 mm) without seasonal variance in the study site area.

2.2. Sampling

The samples were taken from 14 plots across the study site (Figure 1). The plot direction was set perpendicular to the Segara Anakan Lagoon; thus, four plots were the closest to the lagoon (P1, P6, P8, P12), three plots were on the border with the terrestrial ecosystem (P5, P11, P14), and the remaining plots were in between those mentioned plots. We sampled the mangrove sediment with the ring sampler (5 cm diameter) to 10 cm depth. The sediment samples were kept in hard plastic containers to keep them intact.

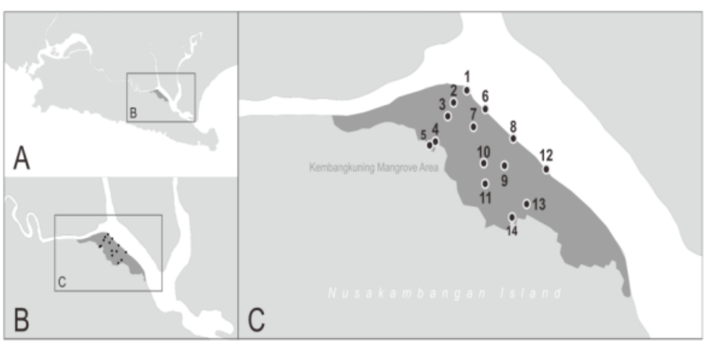

Figure 1. Sampling site from Kembangkuning mangrove area (C), located at the south of Central Java (A), at the northeast of Nusakambangan Island (B).

2.3. Nematode Extraction

We extracted nematodes from mangrove sediment using the Baermann funnel method [16]. The nematodes were collected in conical tubes for preservation. Trait identification was observed from the semi-permanent slide. The nematodes were picked and placed in a glycerin drop on a microscope slide. The selected morphological functional traits to identify the nematode covered body shape, body length, tail shape, feeding type, and life history [12]. The numbering system was used to code the five functional traits of the nematodes [13].

The body shape included three categories (1. stout, 2. slender, and 3. long-thin). The nematode body length was separated into four lengths (1. <1 mm, 2. 1-2 mm, 3. 2-4 mm, and 4. >4 mm). The tail shapes covered five types (1. short-round, 2. elongated, 3. filiform, 4. conical/conico-cylindrical, and 5. clavate). The feeding type includes four categories (1. 1A: selective deposit feeder, 2. 1B: non-selective deposit feeder, 3. 2A: epistrate feeder, and 4. 2B: predator/omnivore. The nematode life history followed Bongers [17] colonizer-persister values (1. cp1, 2. cp2, 3. Cp3, 4. cp4, 5.cp5). Nematodes cp1 have a short life cycle, high reproduction rate and colonization ability, and disturbance tolerance. In contrast, nematodes cp5 have a long-life cycle, low reproductive rate and colonization ability, and are disturbance sensitive.

The nematode functional traits were coded in a series of numbers representing each category of the traits, and taken as the present-absent data. For example, a nematode with a code of 2222 represents a slender nematode with a 1-2 mm long, elongated tail, non-selective deposit feeders (1B), short life, and relatively high reproductive rate, colonization ability, and relatively tolerant to disturbance. The nematode functional traits were recorded as the baseline data for monitoring purposes.

2.4. Data Analysis

To examine the nematode community variations, we correlated the nematode functional traits and composition to selected environmental variables. The nematode functional traits were represented in the codes of serial numbers. The composition of nematodes consisted of a group of nematode codes. The environmental variables included organic carbon, nitrogen, phosphorous, and potassium. We applied Canonical Correspondence Analysis (CCA) as the multivariate ordination analysis to investigate these relationships [18, 19]. We used the multivariate analysis software Canoco version 5 [19] to perform the analysis.

3. Results

We observed 39 trait combinations of the nematodes. Two combinations of the traits coded 22322 and 22222 were the most common combination distributed up to 78.57% of the studied plots on the site. Four trait combinations were moderately distributed (42.85%) in the area, whereas eight combinations reached 25-40% (low distribution). The remaining trait combination (25) was distributed to less than 25% of the plots in the studied area (Table 1).

The functional trait-combination of the nematode community was between 3 and 20 within each plot. No clear pattern of the trait combination number was observed across the study site. Plot 5 was populated with various trait combinations (20). In Plot 13 and 8, it reached 19 and 16 trait combinations, respectively. Other plots were occupied by the nematode community with 3 up to 10 different trait combinations (Figures 2 and 3). However, our observation also revealed specific trait combinations that correspond to particular plots (6 out of 14 plots, 42.85%) in the site. The greatest variation of specific functional trait-combination occurred in Plot 5, with five specific trait combinations of the nematodes, contributing to 25% of the total (Table 2). Overall, the contribution of the specific trait combinations to the total functional trait-combination ranged from 12.5 to 25%.

Table 1. The plot coverage of nematode community according to their functional traits observed in the sediment of the Kembangkuning Mangrove Area

Code	Nematode Functional Traits					Percent Plot Coverage			
	Body Shape	Body Length	Tail shape	Feeding Type	Life History	> 75%	40-75 %	25-40 %	< 25 %
22322	slender	1-2 mm	filiform	1B	cp2	√			
22222	slender	1-2 mm	elongated	1B	cp2	√			
23433	slender	2-4 mm	conical	2A	cp3		√		
22312	slender	1-2 mm	filiform	1A	cp2		√		
11443	stout	< 1 mm	conical	2B	cp3		√		
33233	long-thin	2-4 mm	elongated	2A	cp3		√		
22422	slender	1-2 mm	conical	1B	cp2			√	
22113	slender	1-2 mm	short-round	1A	cp3			√	
23313	slender	2-4 mm	filiform	1A	cp3			√	
21112	slender	< 1 mm	short-round	1A	cp2			√	
22543	slender	1-2 mm	clavate	2B	cp3			√	
22343	slender	1-2 mm	filiform	2B	cp3			√	
12243	stout	1-2 mm	elongated	2B	cp3			√	
22243	slender	1-2 mm	elongated	2B	cp3			√	
25 others	varied	varied	varied	varied	varied				√

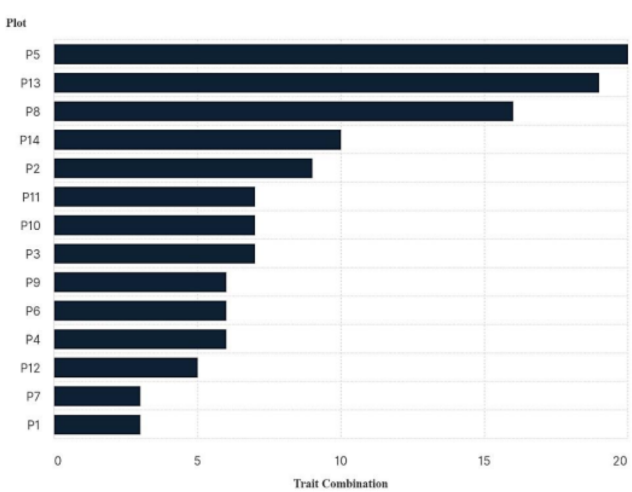

Figure 2. The number of combination traits observed from the nematode community of the mangrove sediment.

Table 2. The nematode functional trait combination that specifically occurred in the particular plots of the study site (the numbers in the code correspond to body shape, body length, tail shape, feeding type, and life history, respectively)

Plot	P3	P4	P5	P8	P13	P14
Code (Functional Trait Combination)	**22232** slender, 1-2mm, elongated, 2A, cp2	**33614** long-thin, 2-4mm, clavate, 1A, cp4	**22542** slender, 1-2mm, conical, 2B, cp2 **23232** slender, 2-4mm, elongated, 2B, cp2 **23233** slender, 2-4mm, elongated, 2A, cp3 **23432** slender, 24mm, con-cylindrical, 2A, cp2 **13243** stout, 2-4mm, elongated, 2B, cp3	**23412** slender, 24mm, con-cylindrical, 1A, cp2 **22333** slender, 1-2mm, filiform, 2A, cp3	**34112** long-thin, >4mm, short-round, 1A, cp2 **21133** slender, <1mm, short-round, 2A, cp2 **11233** stout, <1mm, elongated, 2A, cp3	**22412** slender, 12mm, con-cylindrical, 1A, cp2 **23522** slender, 2-4mm, conical, 1B, cp2

Canonical correspondence analysis revealed a 25.32% accumulated variance with four prominent variables associated with the trait distribution (potassium r = 0.56, water content r = -0.56, salinity r = 0.75, organic carbon r = -0.68). Axis-01 is mainly associated with potassium and water content gradients, whereas axis-02 has salinity and organic carbon (Figures 3 and 4). The range and average values of the environmental variables are available in Figure 5.

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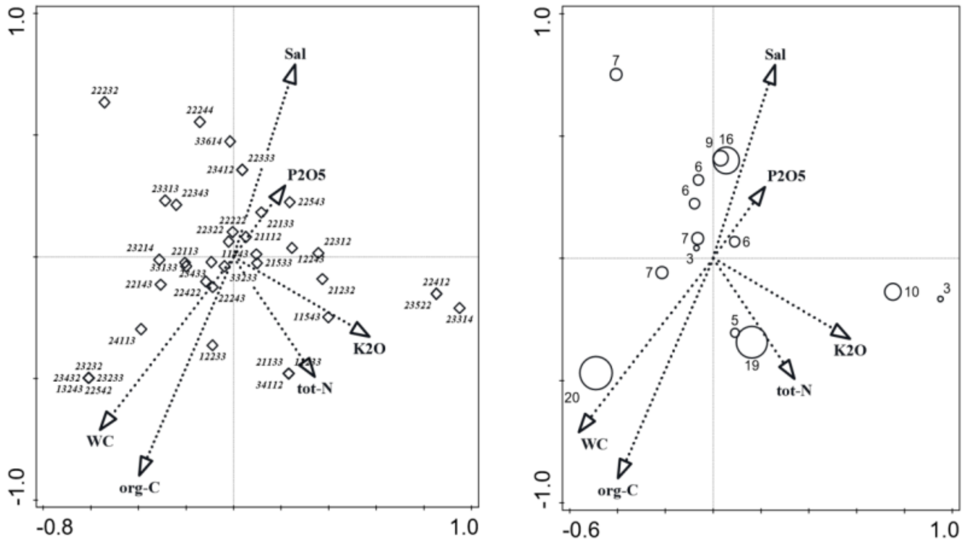

Figure 3. CCA biplots reveal the correlation between the distribution of nematode functional traitcombinations (left), the numbers of trait-combinations (right), and environmental variables of the mangrove sediment (eigenvalues axis-1: 0.4575, axis-2: 0.2692, cumulative explained variation: 25,32%, cumulative explained fitted variation: 55.21%, *p* axis-1: 0.002) (Sal: salinity in the sediment, WC: water content in the sediment, org-C: organic carbon, tot-N: total nitrogen).

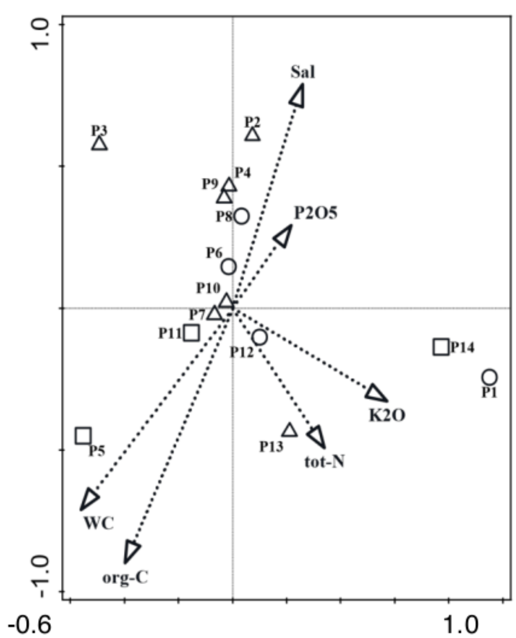

Figure 4. CCA biplots show the correlation between the composition of trait-combinations in each plot and environmental variables of the mangrove sediment (eigenvalues axis-1: 0.4575, axis-2: 0.2692, cumulative explained variation: 25,32%, cumulative explained fitted variation: 55.21%, *p* axis-1: 0.002) (Sal: salinity in the sediment, WC: water content in the sediment, org-C: organic carbon, tot-N: total nitrogen)

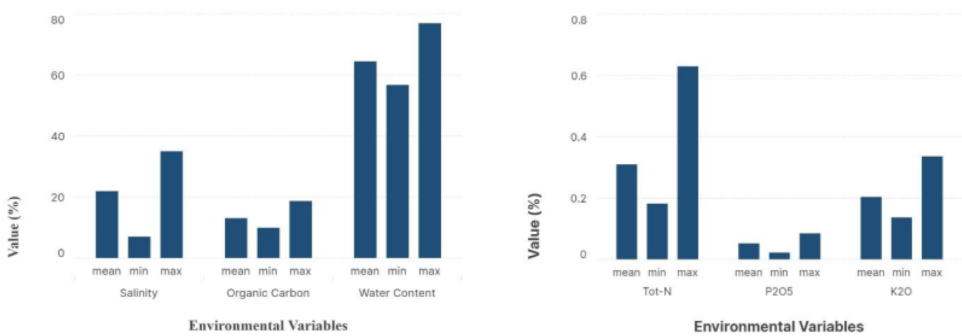

Figure 5. The range and average values of the selected environmental variables measured from the mangrove sediment (min: minimum values, max: maximum value, mean: average value).

4. Discussion and Conclusion

This preliminary research to create a baseline in the Kembangkuning Mangrove ecosystem demonstrates that the area was home to 39 trait-combination of nematodes. Nematodes with slender bodies, 1-2 mm, filiform or elongated tails, living as non-selective deposit feeders (1B) with a relatively high reproduction rate (cp2), were the most common nematodes in the area. Using two different traits (amphid types and cuticle types instead of body shape, body length, and life history as in our research), Semprucci [13] reports 47 trait-combinations of nematodes from the coastal areas of the Mediterranean Sea affected by human activities such as oil transportation.

Nematode observation in the Arabian Sea covering various depths up to 2546 m, which appears to be less influenced by human pressure, shows much-varied nematode trait-combination reaching three times that in our study site [12]. The nematode traits they applied are similar to ours. Therefore, it indicates that human pressure in our study site significantly impacts the ecosystem, as indicated by the low variation of nematode functional traits. Human settlement, fishery, limestone quarry, coal power plant, and oil terminal surround the Kembangkuning Mangrove, which most likely disposes of waste or oil spill has drawn into the study site, contaminating the sediment as the nematode habitat. The contaminants probably selected for the nematode adapted to the situation. The most common nematodes are those of non-selective deposit-feeding and reproducing quickly. These two traits appear to be advantageous.

We observed specific trait-combinations in a particular area of the study site. Nematodes with clavate tails that are prevalent in the stressful ecosystem [13] occurred in a particular spot (P4) of the studied mangrove. The nematode has a long-thin body shape, a selective deposit feeder (1A) whose generation time is quite long (cp4). The cp4 nematode is usually sensitive to pollutants and actively searches for prey [20, 21]. Singh and Ingole's study shows that approximately 50% of the clavate tail nematode occurs in the Arabian Sea [12]; thus, their presence in our study site might be temporary, drifting by the tide current.

Other specific nematode trait-combinations are present in the other five specific sites of the study site (Table 2). Most of them belong to the selective epistrate feeder (2A) or predators/omnivores (2B). Their occurrence was related to their microhabitat condition. P5 was the plot closest to the terrestrial ecosystem, in which the water content and organic carbon were high, but salinity and P_2O_5 were low (Figure 2). In this plot, five trait-combinations nematodes (out of 20) inhabited that are absent anywhere else on the site. High organic carbon might have provided rich resources for the nematode preys, providing sufficient food for the nematodes (2A and 2B). Plots with high nitrogen and K_2O provide a suitable microenvironment for the other five (out of 29) particular trait-combinations (P13 and P14).

Our findings suggest the importance of microhabitat environments for the nematodes. By applying the morpho-trait approach, it is shown that the changes in nematode specific combinations are consistent with microhabitat change. The collected data on nematode provides the baseline for future monitoring programs in the Kembangkuning mangrove ecosystem health, as this ecosystem is subject to disturbance pressure.

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Acknowledgments

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