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An Assessment of Mangrove Ecosystem Condition by Foliar Stable Nitrogen Isotope Ratio Index in Okinawa, Japan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors collected samples. Authors FZT, KH, YA and ST managed the analyses of the study. Author FZT performed the statistical analysis and wrote the draft of the manuscript. Author YN designed and guided the whole study.

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ABSTRACT

Despite providing valuable ecosystem services, mangroves are endangered under the recent global natural as well as anthropogenic changes. Conservation of mangroves is now one of the pillars of Sustainable Development Goals (SDG) 2030. An assessment of mangrove is essential before initializing any conservation strategy. This paper aimed to assess the ecosystem conditions of mangroves and the relative changes in ecosystem conditions from the natural background under different scales of anthropogenic interruption in Okinawa Prefecture, Japan. To assess mangrove's

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ecosystem condition, foliar stable nitrogen isotope ratio ($\delta^{15}N$) of mangroves is used as ecosystem monitoring indicator. Whereas, a difference index (DI) of mangrove foliar $\delta^{15}N$ is established to compare the relative deviation in ecosystem conditions of anthropogenically impacted mangroves from reference mangroves. Results showed that the mean foliar $\delta^{15}N$ values of *Bruguiera gymnorrhiza, Kandelia obovata*, and *Rhizophora stylosa* on Okinawa Island (Is.) are 5.12 ± 2.32‰, 7.54 ± 2.85‰ and 7.09 ± 3.29‰, respectively, on Iriomote Is., 1.83 ± 1.93‰, 2.01 ± 0.31‰ and 1.04 ± 2.38‰, respectively, and on Ishigaki Is., foliar $\delta^{15}N$ values of *Bruguiera gymnorrhiza* and *Rhizophora stylosa are* 5.23 ± 3.33‰ and 6.00 ± 3.63‰, respectively. A range of negative to positive values from -0.54 to 3.66 of DI indicates different level of changes in ecosystem conditions of the mangroves compared to the reference sites, which is set at zero. A significant negative correlation between DI values and the forest area ratio of the watersheds has been observed. It indicates that the forest cover is the driver of maintaining pristine condition of an ecosystem. Findings of the study recommended that Todoroki and Manko mangrove watersheds in Okinawa, Japan should be taken into consideration for necessary conservation on priority basis.

Keywords: Mangrove; land-use; anthropogenic impact; ecosystem condition; ecosystem changes; foliar stable nitrogen isotope ratio, difference index.

1. INTRODUCTION

Mangroves relatively grown in narrow fringe of the coastal zone of tropical and subtropical countries in the world [1], provide numerous valuable ecosystem services to nature and human livelihoods [2]. However, mangroves are much more sensitive to human impacts [3]. Anthropogenic artifacts through agricultural, industrial and artisanal activities congregate at various levels in coastal areas and change biogeochemistry and ecological characteristics of mangroves [4]. Mangroves are also endangered under the effects of recent global climate change [5]. As a result, to preserve, conserve or restore mangroves is one of the prerequisites for ensuring sustainable use of marine resources, which has been set as one of the pillars of Sustainable Development Goals (SDG 14) [6,7]. Still, actions taken worldwide for conservation and management of mangrove ecosystems is insufficient [2,8-12]. Conservation and management approaches for mangroves should include evidence-based natural systems [11,13], revisiting and monitoring mangroves [14,15], and controlling the land-use [15] and the focus should be placed on high-priority basis on those mangroves, which are at high risk under natural and/or anthropogenic stresses [9]. Moreover, it is not practical that all mangroves will either be compelled by similar level of actions for conservation and management or be required actions to be taken at a time. Therefore, it is urgent to categorize mangroves on necessity basis before initiating management practices. Now, the question is how to categorize mangroves?

Mangroves are difficult to monitor usually by field investigation for a long time due to their distribution on tidal flats in the intertidal zones [8]. As well, a complex setting of ecological and environmental processes [16-19] coupling between land and estuaries have made it difficult to firmly conclude the responses of mangroves to nutrient cycling specifically nitrogen (N) and land use [20-21]. However, being generally oligotrophic in nature and capable of capturing excess N from the environment, mangroves have been using as long-term monitoring tool for eutrophication trends in the ecosystems for decades [22-26]. In contrast, few studies have also found that mangroves are able to add nitrogen (N) to estuaries [27]. Thus, mangroves are highly site-specific ecosystems and each mangrove requires individual study for understanding of its particular ecosystem condition. It must be an advancement if mangrove ecosystem could be assessed through limited sampling time and cost as well as less effort in field investigation.

Excess N from human derived sources stimulates fractionation of N and increase N loss from a system. This phenomenon results in enrichment of stable N isotope (^{15}N) in ecosystem components against the background level of that in a pristine environment [28]. Incorporation of ^{15}N-enriched nitrogenous ions leads to generally higher values of ^{15}N ratio

($\delta^{15}N$) in terrestrial and aquatic plants [25,29-32]. Hence, foliar $\delta^{15}N$ composition of coastal plants have been using as useful indicator for assessing the differences between pristine and anthropogenically impacted ecosystems [22-25,27,29-36]. However, such an assessment is not sufficient to understand the degree of changes in anthropogenically impacted mangroves from the natural condition. Therefore, an index is essential to develop, based on which mangroves would be categorized as 'high-priority' to 'less-priority' for conservation through a limited effort.

Based on the foliar $\delta^{15}N$ values mangroves on three islands named Okinawa, Ishigaki and Iriomote in Japan have previously been reported as intensively anthropogenically impacted, moderately anthropogenically impacted and pristine mangroves, respectively [23]. Furthermore, the land-use showed distinct influence on leaf and water N in mangrove watersheds on Ishigaki Is. [22]. However, the degree of such kind of anthropogenic impacts in each mangrove watershed on those islands was not clearly explained. Hence, to clarify the degree of impacts is essential to undertake conservation actions in those mangrove watersheds on priority basis. In previous studies, mangrove stands (regardless of species variation) having an average foliar $\delta^{15}N$ up to 3‰ have been considered as natural mangrove sites [22-26,37,38]. Henceforth, this value could be the base of reference site to which other mangroves would be compared. In addition, to analyze mangrove species-specific responses to the anthropogenic stresses in watersheds is essential for understanding of which species better act as ecological indicator for monitoring mangrove's ecosystem condition.

Therefore, the specific objectives of this research were – i) to analyze foliar $\delta^{15}N$ of predominant mangrove species in the study sites; ii) to examine the species-specific responses to the anthropogenic stresses in watersheds by the analysis of relationship between foliar $\delta^{15}N$ and land-use ratio of the watersheds; iii) to develop a 'different index' (DI) based on foliar $\delta^{15}N$ values for the assessment of relative degree of ecosystem conditions of mangroves; iv) to investigate whether there was any effect of land-use on DI values; v) to categorize the mangroves as 'high-priority' to 'less-priority' for conservation based on the corresponding higher to lower values of DI and recommend which watershed should come first under conservation practices. The findings of this study are expected to contribute in planning effective conservation and management strategies for the betterment of site-specific mangrove ecosystems in national to global level.

2. METHODOLOGY

2.1 Study Sites

A total of 10 major mangrove watersheds named Kesaji, Okukubi, Oura and Manko on Okinawa Is. (Fig. 1), Fukido, Miyara, Nagura, Hirakubo, and Todoroki on Ishigaki Is. (Fig. 2) and Urauchi on Iriomote Is. (Fig. 3) were the study sites.

A part of the land-use maps of the watersheds, presented in Fig. 1 to 3, was adopted from the supplementary data in Tanu et al. [23] and a part of those was outlined by using ArcMap Geographic Information Services (GIS) software (ArC Map 10.4.1). The source data for the land-use were collected from the National Land Policy Bureau, Japan Ministry of Land, Infrastructure, Transport, and Tourism. The baseline of the coastlines, waterbodies and watershed areas were obtained from the land conservation maps (1:200,000) from the Geospatial Information Authority of Japan (GSI).

Mangrove watersheds on Okinawa Is. associated with various degrees of land-use by intensive agricultural activities, animal husbandry, local construction, and tourism, whereas those on Ishigaki Is. associated with relatively large forest area besides agriculture and animal husbandry [22,23,39,40]. On the other hand, the Urauchi mangrove is the vast National Reserved Forest Park comprising 40 % of the Iriomote island [41], which was pondered as reference site [23]. In Table 1, the land-use ratios in Urauchi and Kesaji watersheds were obtained from Tanu et al. [23]; those of five watersheds on Ishigaki Is. were obtained from Tanu et al. [22]; those of Okukubi, Oura and Manko watersheds were delineated by using the ArcGIS software as mentioned above.

The commonly existing mangrove species on Iriomote Is. are *Bruguiera gymnorrhiza* Lamk. (L.), *Kandelia Obovata* Sheue, Liu & Yong, and *Rhizophora stylosa* Griff [42,43]. Among these three species, K. *obovata* and B. *gymnorrhiza* are dominantly found on Okinawa Is. [44-46] and

B. *gymnorrhiza* and R. *stylosa* are dominantly found on Ishigaki Is. [47].

2.2 Sample Collection, Processing and Analysis

Mangrove leaves were collected in the years from 2015 to 2019 alongside the water creeks from the up-, middle- and downstream areas. All sampling points were in an area of not more than 500 m from the creek mouth. Wherever available five trees of each mangrove species of B. *gymnorrhiza*, K. *obovata* and R. *stylosa* were selected as sample trees for collection of five mature leaves from the middle-stage branch from each tree. The leaf samples were dried in an oven at 60 °C (DNE 910, Yamato) for 48 hours after washing. Then, samples were ground by a wonder blender and analyzed for foliar δ^{15}N by using an on-line isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific, Waltham, MA, USA) linked to continuous flow interface (Temperature Conversion/Elemental Analyzer). The detailed data processing and calibration methods for IRMS for the determination of foliar δ^{15}N values have been described in Tanu et al. [23].

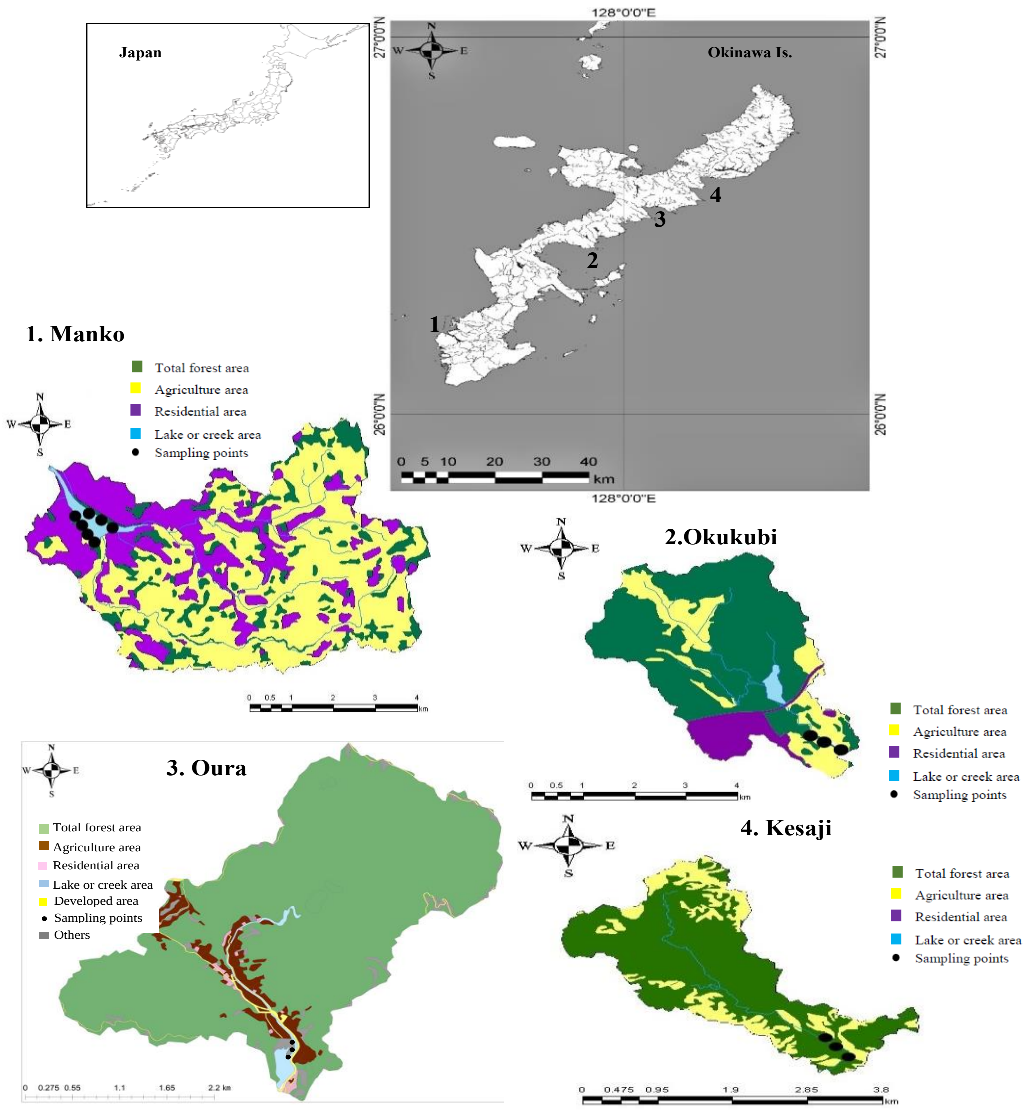

Fig. 1. Sampling sites and relative land use area of mangrove watersheds on Okinawa Is.

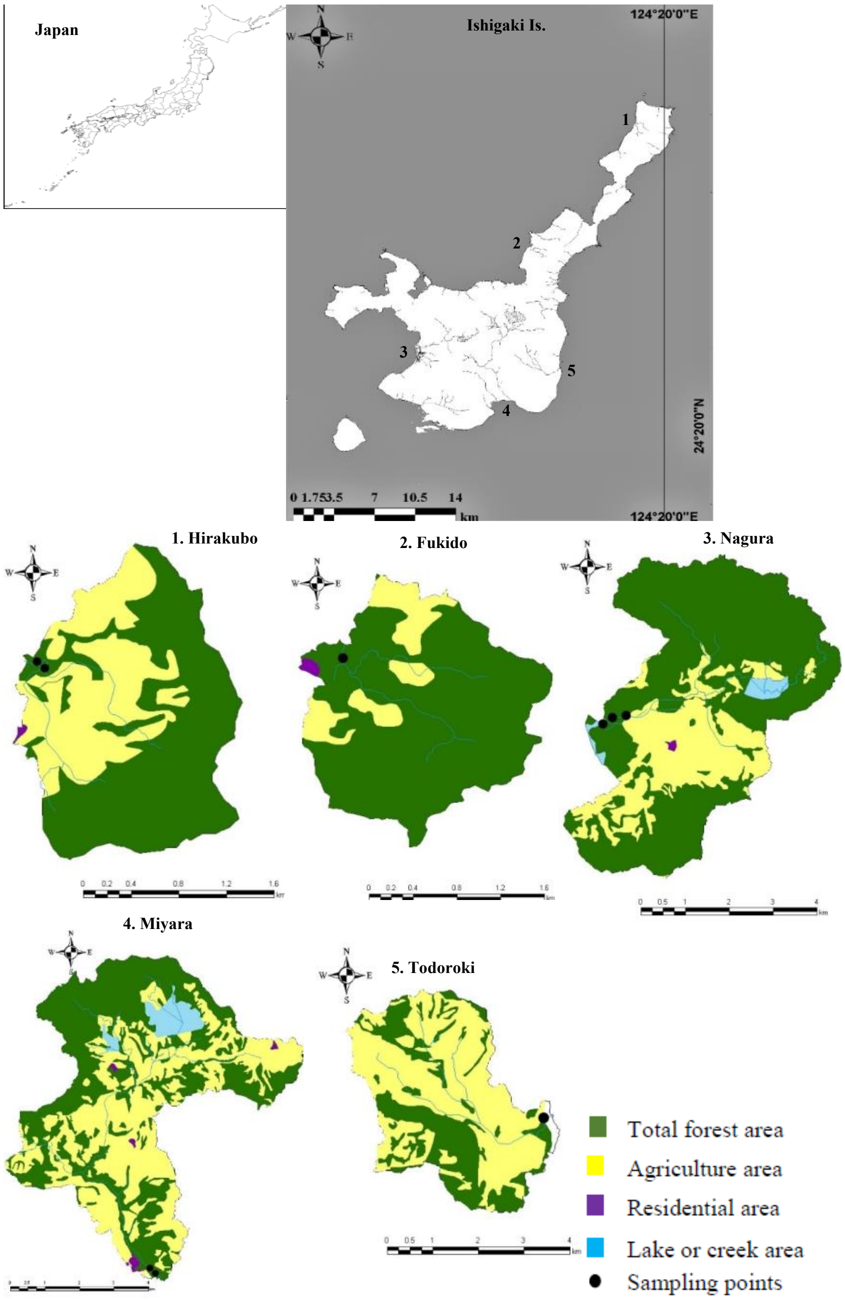

Fig. 2. Sampling sites and relative land use area of mangrove watersheds on Ishigaki Is.

2.3 Determination of Difference Index (DI) by Foliar δ^{15}N

An equation for the calculation of a difference index (DI) of foliar δ^{15}N was developed by modifying the model used in Fry and Cormier [48] for the determination of comparative ecosystem conditions in mangrove watersheds. The equation is as follows-

$$DI = \frac{\text{Human-affected Site Average} - \text{Reference Site Average}}{\text{Reference Site Standard Deviation}}$$

The equation was derived based on the consideration that the foliar δ^{15}N in Urauchi and Fukido belong to natural condition. Hence, foliar δ^{15}N found in Urauchi and Fukido watersheds was used for the calculation of ‘reference site average', which was standardized at zero. Thus, the DI values of each anthropogenically impacted mangrove ecosystem gave a relative deviation of the foliar $\delta^{15}N$ values versus the reference sites. DI ranged from negative to positive values, where the positive values indicated more changes in ecosystem conditions compared to the reference sites and the negative values indicated no changes in ecosystem conditions. DI value thus could resemble the condition of the anthropogenically impacted mangrove ecosystems compared to the pristine mangroves.

2.4 Mangrove Species as Ecological Indicator

Two mangrove species i.e., B. *gymnorrhiza* and R. *stylosa* were available in all studied watersheds and were tested for their performances as ecological indicator through the correlation analysis between foliar $\delta^{15}N$ and anthropogenically impacted area ratio (area ratios of agricultural activities plus development activities) of the corresponding watersheds.

2.5 Statistical Analysis

Normality of the foliar data sets was tested. Significant differences among mean values were investigated by Welch's t-tests. Data processing, statistical analysis, and presentation of graphs were done by using Excel 2010 (Microsoft, Redmond, WA, USA) and SPSS v24.0. The significance level was considered at P = .05.

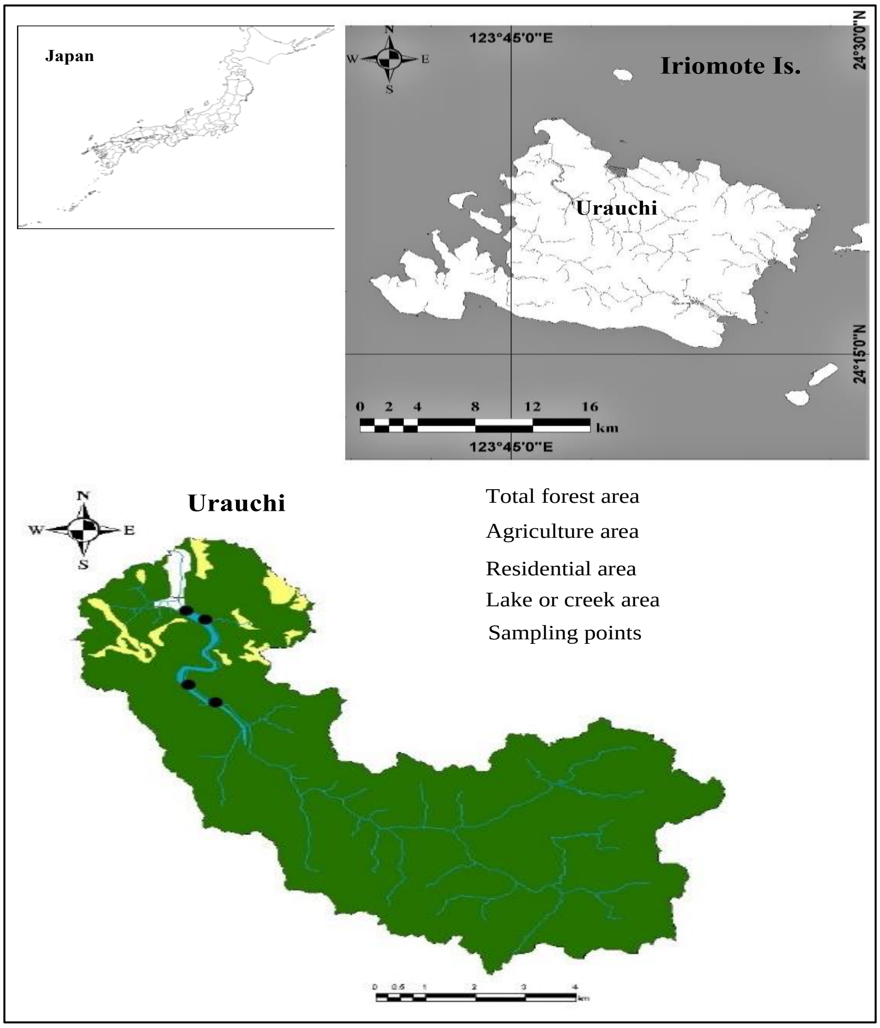

Fig. 3. Sampling site and relative land use area of mangrove watershed on Iriomote Is.

3. RESULTS AND DISCUSSION

3.1 Distribution of Foliar $\delta^{15}N$ of Mangroves across the Watersheds

The comparative distribution of foliar $\delta^{15}N$ of three mangroves in ten watersheds on three islands has been shown in the boxplots (Fig. 4). The highest mean foliar $\delta^{15}N$ (11.4 ± 2.6‰) were measured in Todoroki watershed on Ishigaki Is., followed by that (8.3 ± 2.7‰) in Manko watershed on Okinawa Is. In contrast, the lowest mean (1.5 ± 2.1‰) was detected in Urauchi watershed on Iriomote Is., followed by that (2.7 ± 3.1‰) in Fukido watershed on Ishigaki Is.

Based on the pristine range of foliar $\delta^{15}N$ upto 3‰ [37], it appears that both Urauchi and Fukido watersheds could be used as reference sites for further comparative studies of ecological processes of mangroves in Japan. In contrast, Todoroki and Manko watersheds showed the level of foliar $\delta^{15}N$ derived from animal or human originated N [23-26] and could be considered as intensively anthropogenically impacted mangroves. The rest of the watersheds were moderately anthropogenically impacted while showing moderately elevated foliar $\delta^{15}N$. Such a grouping of ten watersheds in respect of ecosystem conditions is distinct in Fig. 4, based on significant differences among foliar $\delta^{15}N$. Similar paradigm was shown in temperate mangroves in New Zealand by Gritcan et al. [24]. It reported on that the mangrove could be used as monitoring tool to assess heavily impacted coastal ecosystem and categorized Mangawhai mangrove as mildly impacted at 5.2‰ level of foliar $\delta^{15}N$ and Manukau mangrove as strongly impacted at 9.9‰ level. Additionally, Waitemata Harbour was grouped in between the mild and strong level of foliar $\delta^{15}N$ at 6.4‰.

In Japan, both Okinawa and Ishigaki islands are under different level of environmental stresses like upland soil erosion, urbanization, agricultural inputs, livestock, and tourism [22,23,39,40,49,50]. Japan is the second largest country in the OECD countries to use nitrogenous fertilizer in agriculture [51]. Excess application of nitrogen fertilizer on land, urine deposition in ground from livestock and other nitrogenous contaminants and pollutants coming into water ways from inland runoff may cause increased isotope fractionation either via microbial denitrification [52] or by ammonia volatilization [53]. Previous studies have confirmed that canal water draining agricultural lands supplied high concentration of nitrates to fringing mangroves and resulted in elevated foliar $\delta^{15}N$ (11 to 16‰) in *Rhizophora* mangroves in Florida, whereas pristine mangroves had foliar $\delta^{15}N$ of -5 to 2‰ [54].

3.2 Species Variation in Foliar $\delta^{15}N$ of Mangroves

Species variation in foliar $\delta^{15}N$ of three mangroves showed a wide range across the studied watersheds. The species distribution of foliar $\delta^{15}N$ in ten watersheds on three islands are presented in Table 2. The lowest means of foliar $\delta^{15}N$ (below 3‰) of three mangroves B. *gymnorrhiza,* K. *obovata*, and R. *stylosa* were found in pristine Urauchi and Fukido watersheds. No pollutants in Urauchi creek water were apparent under a negligible human activity by scientific research and small-scale tourism [55]. However, Fukido watershed was associated with a limited degree of anthropogenic impacts covering approx. 12% of the watershed area (Table 1).

Conversely, the highest means of B. *gymnorrhiza* and R. *stylosa* varied between 10 and 13‰ in Todoroki watershed and mean foliar $\delta^{15}N$ of K. *obovata* (around 8‰) was measured in Manko watershed. The highest foliar $\delta^{15}N$ in Todoroki watershed was observed due to the fractionation of N sources from livestock [22,23] through the process of ammonia volatilization [53]. Besides, the second highest foliar $\delta^{15}N$ was determined in Manko watershed due to relative fractionation of N sources from urbanization and agricultural practices [8]. Previously, the $\delta^{15}N$ was recorded in between 10‰ and 30‰ when N sources were livestock wastes, domestic wastewater [56] and wastewater treatment plant effluents [57,58].

Species variation in foliar traits is generally found due to variation in N physiology of mangrove species, various leaf longevity of mangroves [59,60], as well as the microclimatic variation [61] across the watersheds. Also, the distances of waterways from N sources to sinks could be the factors of corresponding changes in $\delta^{15}N$ of marine plant tissues [61]. However, foliar $\delta^{15}N$ of all the species showed clear correspondence to the degree of anthropogenic impact on mangrove ecosystems.

Table 1. Ten mangrove watersheds with relative area (%) of forest, agriculture and development activities on three islands in Okinawa Prefecture, Japan

Island	Watershed	Area of watershed (ca km^2)	Area of Forests including mangroves (ca %)	Area of agriculture (ca %)	Area of development activities (ca %)
Okinawa	Kesaji	7.0	78.0	22.0	0.00
	Okukubi	17.0	54.5	12.8	19.2
	Oura	9.9	88.6	5.50	1.94
	Manko	38.2	5.84	32.5	48.6
Ishigaki	Fukido	3.0	87.0	9.73	2.07
	Miyara	36.0	59.8	24.5	7.69
	Nagura	24.0	73.8	15.7	0.16
	Hirakubo	3.0	84.5	10.2	4.02
	Todoroki	12.0	29.5	45.2	7.96
Iriomote	Urauchi	68.0	97.0	2.75	0.01

Source: The land-use area ratios in Urauchi and Kesaji watersheds were obtained from Tanu et al. [23]; those of five watersheds on Ishigaki Is. were obtained from Tanu et al. [22]; those of Okukubi, Oura and Manko watersheds were delineated by using the ArcGIS software as mentioned in the section 2.1.

Table 2. Foliar δ^{15}N of mangroves (B. *gymnorrhiza*, K. *obovata*, and R. *stylosa*) in ten watersheds on three islands in Okinawa Prefecture, Japan

Islands	Mangrove Watersheds	Mangrove Species (Sample number, n)	Foliar δ^{15}N (‰) mean ± stdv.
Okinawa	Kesaji	B. *gymnorrhiza* (n = 15)	4.7± 2.5^b
		K. *obovata* (n = 7)	6.8 ± 1.5^b
		R. *stylosa* (n = 5)	3.0 ± 2.5^a
	Okukubi	B. *gymnorrhiza* (n = 31)	5.4 ± 2.1^b
		K. *obovata* (n = 31)	5.8 ± 3.1^b
		R. *stylosa* (n = 31)	6.0 ± 2.2^b
	Oura	B. *gymnorrhiza* (n = 10)	4.5 ± 2.9^b
		K. *obovata* (n = 11)	6.9 ± 1.8^b
	Manko	B. *gymnorrhiza* (n = 3)	7.0 ± 0.7^b
		K. *obovata* (n = 54)	8.1 ± 2.7^c
		R. *stylosa* (n = 15)	9.2 ± 2.6^c
Ishigaki	Fukido	B. *gymnorrhiza* (n = 5)	0.4 ± 1.1^a
		R. stylosa (n = 8)	4.1 ± 3.2^a
	Miyara	B. *gymnorrhiza* (n = 7)	4.8 ± 1.8^b
		R. stylosa (n = 5)	4.9 ± 1.1^b
	Nagura	B. *gymnorrhiza* (n = 16)	5.6 ± 3.1^b
		R. stylosa (n = 15)	5.1 ± 2.7^b
	Hirakubo	B. *gymnorrhiza* (n = 10)	5.0 ± 1.9^b
		R. stylosa (n = 10)	6.2 ± 2.7^b
	Todoroki	B. *gymnorrhiza* (n = 10)	10.1 ± 2.5^c
		R. stylosa (n = 10)	13.0 ± 1.1^d
Iriomote	Urauchi	B. *gymnorrhiza* (n = 20)	1.8 ± 1.9^a
		K. *obovata* (n = 5)	2.0 ± 0.3^a
		R. *stylosa* (n = 19)	1.0 ± 2.4^a

Note: Different alphabets in column indicate significant differences from each other at P = .05.

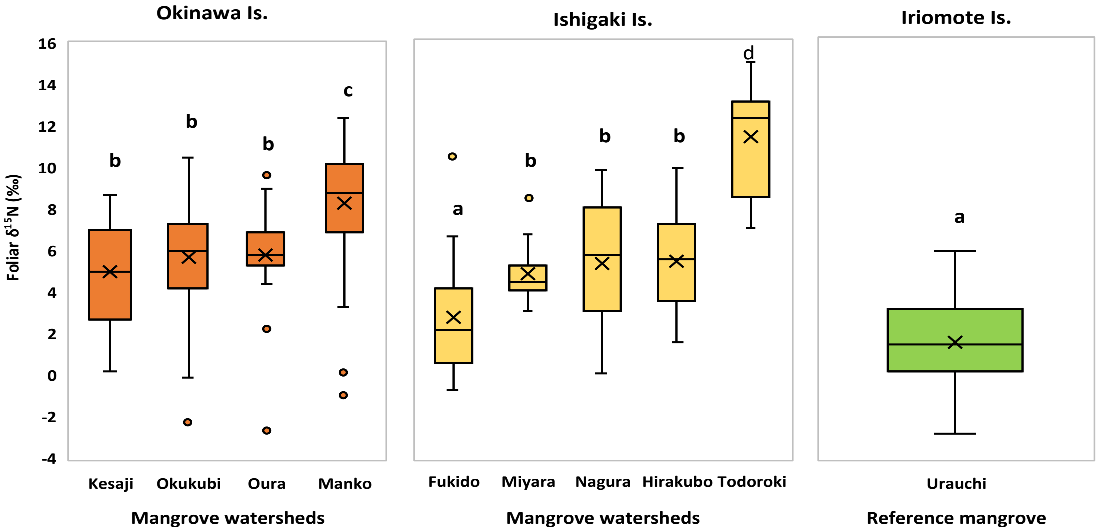

Fig. 4. Distribution of foliar δ^{15}N of mangroves in ten watersheds on three islands in Okinawa Prefecture, Japan. Boxplots denote the summary of data as a minimum, first quartile, median, third quartile, and a maximum. The cross sign in each box symbolizes the mean foliar δ^{15}N of the data set. The whiskers are extended to the minimum and the maximum values from the first and the third quartiles, respectively. The outliers beyond the inner fence and the outer fence characterize the extreme values that are outside of the summarized data. Different alphabets upon each box indicate the significant differences from each other at significant level P = 0.05

3.3 Performance of Mangrove Species as Ecological Indicator

Mangroves exhibit species-specific variation in foliar physiological functions in response to environmental condition including availability of nutrients [62]. In this study, foliar δ^{15}N of both B. *gymnorrhiza* and R. *stylosa* were significantly correlated to the anthropogenically impacted area ratio of the studied watersheds (Fig. 5). The findings indicated that both species performed well as ecosystem monitoring tool under different degree of anthropogenic stresses.

The equations of regression analysis for both B. *gymnorrhiza* and R. *stylosa* elucidated that probable input of N in ecosystems through anthropogenic activities significantly (P = 0.01) increased the foliar δ^{15}N regardless of plant species [22,23]. However, the slope of the regression line of R. *stylosa* (y = 1.38 + 0.113x) were slightly steeper than that of B. *gymnorrhiza* (y = 1.68 + 0.084x) (Fig. 5). It suggested that the foliar δ^{15}N of R. *stylosa* was slightly more sensitive to N input in ecosystems of the sampling sites, though the sensitivity of R. *stylosa* was not significantly different from that of B. *gymnorrhiza*. It was also previously observed that species-specific variation in foliar δ^{15}N values were relatively small [25].

B. *gymnorrhiz typically* showed a slow mean growth rate in response to N supply compared to other mangroves such as *Rhizophora apiculate, Avicennia marina, Xylocarpus moluccensis, Xylocarpus granatum and Ceriops tagal* [63]. Such a kind of intrinsic difference in growth pattern among species is considered as one of the possible causes of species-specific variation in relative habitats [63]. Comparatively a high nutrient retention strategy (>70%) with a high tannin content [64] and a low foliar nutrient loss with an average leaf longevity of 16 months of R. *stylosa* [65,66] might be the factors for better performances in response to N availability in ecosystems.

3.4 Degree of Changes in Ecosystem Condition by Using Difference Index (DI)

The difference index (DI) demonstrated the relative degree of deviation of foliar δ^{15}N in the studied watersheds from the reference sites set at zero (Fig. 6).

Here, DI extended from negative to positive values. The largest positive value of DI was in Todoroki (3.7), followed by that in Manko (2.3), whereas two negative values of DI were in Fukido (-0.04) and Urauchi (-0.5). Here the negative DI values indicated no change in ecosystem condition due to anthropogenic perturbation. However, more negative value found in Urauchi than Fukido denoted that the Urauchi is more pristine in ecosystem condition than Fukido. In contrast, different levels of positive DI values indicated that the ecosystem conditions of those mangroves have been changed at different degrees from the natural condition. Having DI values more than 2, Todoroki and Manko watersheds are 'high priority' mangroves which are intensively anthropogenically impacted, whereas those less than 2, subsequently indicated Kesaji, Okukubi, Oura, Miyara, Nagura and Hirakubo watersheds as 'less priority' mangroves for conservation in this study. Therefore, Todoroki having the highest DI must require intensified awareness for conservation actions to be started. The conservation strategy may include to change land-use of the watersheds. The strong negative linear relationship between DI and the forest area ratio (%) of the watersheds (Fig. 7) reinforced the finding.Thus, DI values successfully unveiled relative ecosystem conditions of the studied watersheds.

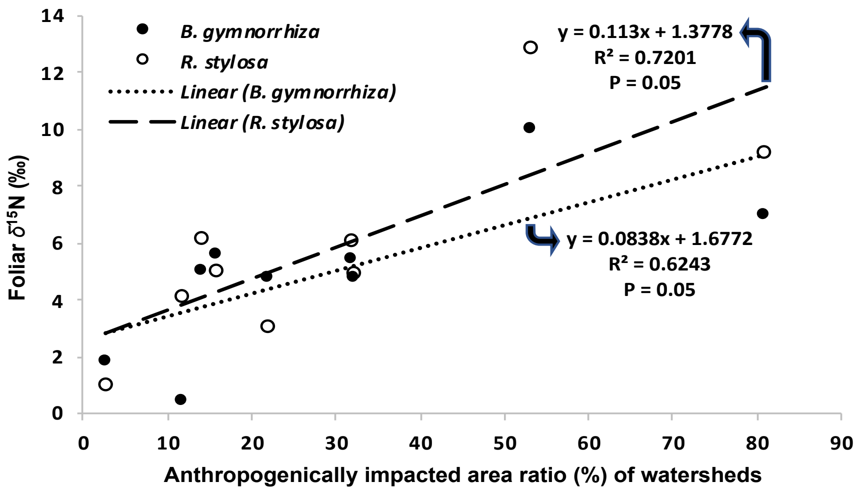

Fig. 5. The relationship of foliar $\delta^{15}N$ of B. *gymnorrhiza* and R. *stylosa* with anthropogenically impacted area ratio of watersheds; the equation of regression analysis for B. *gymnorrhiza* is y = 1.68 + 0.08x, R^2 = 0.62, P = 0.05 and for R. *stylosa* is y = 1.38 + 0.11x, R^2 = 0.72, P = 0.05

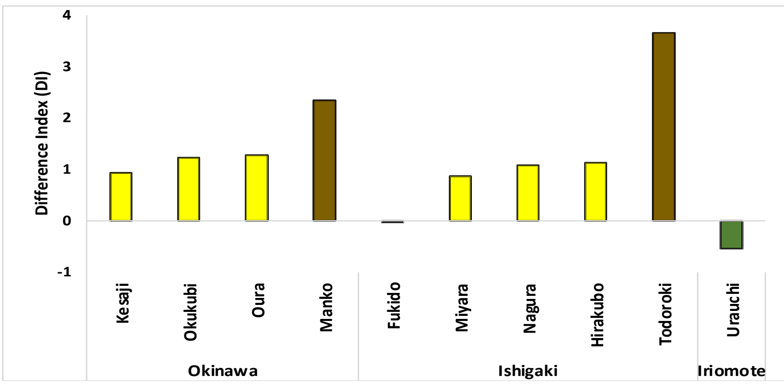

Fig. 6. Relative deviation of foliar $\delta^{15}N$ of mangroves in anthropogenically impacted watersheds from the average foliar $\delta^{15}N$ in reference sites set at zero, indicated by a difference index (DI)

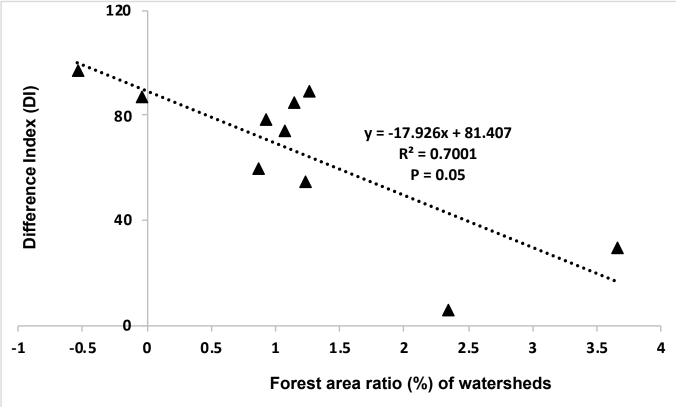

Fig. 7. The relationship between forest area ratio (%) of watersheds and difference index (DI) of foliar δ^{15}N (‰; y = 81.41 - 17.93x; R^2 = 0.7; P = 0.05) determined across ten mangrove watersheds on three islands in Okinawa Prefecture, Japan

4. CONCLUSION

Mangrove species are successfully being used as indicators for monitoring ecosystem conditions under various degrees of anthropogenic stresses for decades. No exceptional case occurs in this study. Nitrogen inputs from anthropogenic sources potentially instigate higher foliar δ^{15}N of three mangrove species *Bruguiera gymnorrhiza, Kandelia obovata*, and *Rhizophora stylosa* in anthropogenically impacted mangroves compared to the natural mangroves. A newly modeled difference index (DI) in this study unveiled the relative deviation in foliar δ^{15}N in the anthropogenically impacted mangroves from the background level of natural mangroves. The highest positive DI value corresponds to the 'high-priority' mangroves as Todoroki watershed, which requires intensive conservation to maintain the usual ecological processes. A strong negative relationship between DI values and the forest area ratio of the watersheds efficiently indicated that a large forest cover ensured ecosystem's prissiness. According to this study, to change the land-use or to increase the forest cover of the watersheds might help to keep the ecological processes naturally functionable. However, the index must be limited to the mangrove species and a small distribution of mangroves as used in this study. Also, the performance of this index should be examined in a broad mangrove distribution with various mangrove species grown across the tropical, sub-tropical and temperate zone under different scale of anthropogenic stresses for validation. It is expected that the insight of relative changes in ecosystem conditions in anthropogenically impacted mangrove watersheds in Okinawa Prefecture may help the ecologist, environmentalists, and policy makers to take the necessary conservation and management strategies in priority basis.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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