Original Research Article

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

.

ABSTRACT

This paper aimed toassess 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 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 therelative deviationin ecosystem conditions of anthropogenically impacted mangroves from reference mangroves. Results showed that the mean foliar $\delta^{15}N$ values of *Bruguieragymnorrhiza, Kandeliaobovata*, 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, andon Ishigaki Is., foliar $\delta^{15}N$ values of *Bruguieragymnorrhiza*and *Rhizophora stylosaare* 5.23 ± 3.33‰ and6.00 ± 3.63‰, respectively. A range of negative to positive values from -0.54 to 3.66 of DI value 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.A reduction in forest cover due to anthropogenic interruption may cause an increase in foliar $\delta^{15}N$ values of the mangroves. This study is vital before any management planning for conservation of the mangroves.

Keywords: Mangrove; Land-use; Anthropogenic impact; Ecosystem condition; Ecosystem changes; Foliar stable nitrogen isotope ratio, Difference index.

1. INTRODUCTION

Mangroves grow as a relativelynarrow fringe of the coastal zone of tropical and subtropical countries in the world. They providenumerous ecosystem services to human livelihoods.Despite having significant importance, mangroves are vulnerable ecosystems due to a huge change [1] and loss at a rate of 1% per year [2]. Anthropogenic artifacts through agricultural, industrial and artisanal activities congregate at various levels in coastal areas and change biogeochemistry and ecological characteristics of mangroves [3].In general, mangroves are oligotrophic in nature and capable to capture excess nutrients from the environment.Hence, mangroves are using as long- term monitoring tool for eutrophication trends in the ecosystems for decades [4-8].However, few studies have found that mangroves addnitrogen (N) to estuaries [9,10]). A complex setting of ecological and environmental processes [11-16] coupling between land and estuaries have made it difficult to firmly conclude the responses of mangroves to N cycling and land use [8,17-19].

Excess N from human derived sourcesstimulatesfractionation of N and increase N loss from a system, resulting inenrichment in ^{15}N of the ecosystem components against the background isotopic levelbelonging to pristine environment[20].Incorporation of ^{15}N-enriched nitrogenous ions leads to generallyhigher values of $\delta^{15}N$ in coastal plants [21-27]. Accordingly, spatial variation in foliar stable Nisotope ratios ($\delta^{15}N$) integrates the imprints of N sources andisotope fractionations during N transformation processesin N cycle[28,29]. Hence, foliar $\delta^{15}N$ composition of coastal plants have been using for decades as useful indicator for assessing the differences between pristine and anthropogenically impacted mangrove ecosystems[6-8,30-33].Mangroves on three islands named Okinawa, Ishigaki and Iriomotein Japan have previously been reported as intensively anthropogenically impacted, moderately anthropogenically impacted and pristine mangroves, respectively [8], based on the foliar$\delta^{15}N$ values. However, a gap still remains to clearly understand the relative degree of ecosystem changes in anthropogenically impacted mangroves, while comparing to the pristine mangrove sites. In previous studies, mangrove stands having foliar $\delta^{15}N$ below 3‰ have been considered asnatural mangrove sites[6-8,34,35].

Therefore, this study aims to develop an index of foliar $\delta^{15}N$for the assessment ofrelative degree of ecosystem conditionof different mangroves on Okinawa, Ishigaki and Iriomote islands in Okinawa Prefecture, Japan. A difference index (DI) of foliar $\delta^{15}N$ has been established to assess the relative deviation in ecosystem changes in anthropogenically impacted mangroves in comparison to the background level of foliar $\delta^{15}N$ belonging to pristine mangroves. In addition, species-specific responses to upholdfoliar $\delta^{15}N$ is also observed for an understanding of which species better act as ecological indicator for monitoring mangrove ecosystem condition. Insight of this studyis expected to contribute in planning effective management and conservation strategies for the betterment of site-specific mangrove ecosystem internationally.

2. METHODOLOGY

2.1 StudySites

A total of 10 major mangrove watersheds named Kesaji, Okukubi, Oura and Manko on Okinawa Is., Fukido,Miyara,Nagura, Hirakubo, and Todoroki on Ishigaki Is. and Urauchion Iriomote Is. were the study sites. Mangrove watersheds on Okinawa Is. associated with various degrees of land-use by intensive agricultural activities, animal husbandry, local construction, and tourism [8,36,37], whereasthose on Ishigaki Is.associated with relatively large forest area besides agriculture and animal husbandry [8]. On the other hand, the Urauchi mangrove isthe vast National Reserved Forest Park comprising 40 % of theIriomote island [38], whichwaspondered as reference site [8]. The land-use of the Urauchi watershed iscomprised of 97% by forest area and2.75% by a minimal human activity by agriculture [8]. The land-use of the studied mangrove watersheds presented in Table 1 wasadopted from previousstudies by Tanu et al. [8].Three mangrove species *Bruguieragymnorrhiza*, *KandeliaObovata*, and*Rhizophora stylosa*were found as dominant speciesin the selected mangrove watersheds on Okinawa and Iriomote islands, whereas only B. *gymnorrhiza* and R. *stylosa* were available on the selected watersheds on Ishigaki Is.

2.2Sample Collection, Processing and Analysis

Mangrove leaves were collectedinthe 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 hoursafter washing.

Then, samples were ground by a wonder blender analyzed for foliar $\delta^{15}N$ by using an on-line isotope ratio mass spectrometer (IRMS, Thermo Fisher Scientific, Waltham, MA, USA)inked to continuous flow interface (Temperature Conversion/Elemental Analyzer). The detailed data processing and calibration methods for the determination of foliar $\delta^{15}N$ values have been described in Tanu et al. [8].

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	78	22	0.00
	Okukubi	17	48	45	7
	Oura	5.6	nd	nd	nd
	Manko	38	14	56	29
Ishigaki	Fukido	3	85.5	14	0.5
	Miyara	36	50	46	0.5
	Nagura	24	72	27	0.2
	Hirakubo	3	63.4	38	0.2
	Todoroki	12	33.5	65.5	0.00
Iriomote	Urauchi	68	97	2.75	0.01

Source:The land-use ratio in watersheds, except Oura, was obtained from Tanu et al. [8]; the watershed area of Oura was obtained from Okinawa Prefecture[39]; the abbreviation 'nd' indicates that data was not available.

2.3Determination of Difference Index (DI) by Foliar $\delta^{15}N$

An equation for the calculation of a difference index (DI) of foliar $\delta^{15}N$ was developed by modifying the model used in Fry and Cormier [40] for the determination ofcomparative ecosystem conditionsin mangrove watersheds. The equation is as follows-

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

The equation was derived based on the consideration that thefoliar $\delta^{15}N$ in Urauchi and Fukidobelong to natural condition. Hence, foliar $\delta^{15}N$ found in Urauchi and Fukido watersheds was used for 'reference site average' calculation, which was standardizedto zero.Thus, the DI values of each anthropogenically impacted mangrove ecosystem gave arelative deviation ofthe 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 conditionscompared to the reference sites and thenegative values indicated nochanges in ecosystem conditions. DI valuethus could resemblethe condition of the anthropogenically impactedmangrove ecosystems compared to the pristine mangroves.

2.4Mangrove 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 performance as ecological indicator through the correlation analysis between foliar $\delta^{15}N$ and anthropogenically impacted area ratio of the corresponding watersheds. Due to unavailability of land-use data, Oura watershed was excluded in this analysis.

2.5Statistical 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.

3. RESULTS AND DISCUSSION

3.1 Distribution of Foliar δ^{15}N of Mangroves across the watersheds

The comparative distribution of foliar δ^{15}N of three mangroves in ten watersheds on three islands has been shown in the boxplots (Figure 1). The highest mean foliar δ^{15}N (11.4 ± 2.6‰) of three mangroves 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 δ^{15}N of 3‰[34], it appears that both Urauchi and Fukidowatersheds could be used as reference sites for further comparative studies of mangroves' ecological processes in Japan.

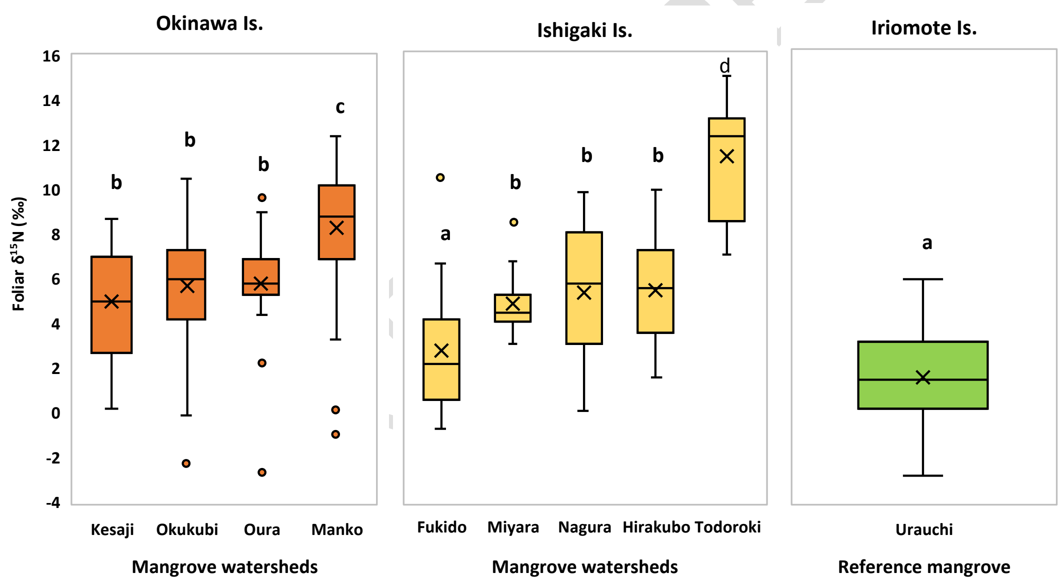

Figure 1. Distribution of foliar δ^{15}N of mangroves in ten watersheds on three islands in Okinawa Prefecture, Japan. Boxplots denote the summary of data as a minimum, first quartile, median, third quartile, and a maximum. The cross sign in each box symbolizes the mean foliar δ^{15}Nof the data set. 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.

In contrast, Todoroki and Manko watersheds showed the level of foliar δ^{15}N derived from animal or human originated N [6-8,41] and could be considered as intensively anthropogenically impacted mangroves.The rest of the watersheds were moderately anthropogenically impacted while showing moderately elevated foliar δ^{15}N.Such a grouping of ten watersheds in respect of ecosystem conditions is also distinct in Figure 1, based on significant differences among foliarδ^{15}N. Similar paradigm was shown in temperate mangroves in New Zealand by Gritcan et al. [7]. It reported on that the mangrove could be used as monitoring tool to assess heavily impacted coastal ecosystem and categorized Mangawhaimangrove as mildly impacted at 5.2‰ level of foliar δ^{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 δ^{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 andtourism [8,36,37,39,42-44]. Japan is the secondlargestcountry in the OECD countries to use nitrogenous fertilizer in agriculture [45].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 causeincreased isotope fractionation either via microbial denitrification[46] or by ammonia volatilisation[24]. Previous studies have confirmed that canal water draining agricultural lands suppliedhigh concentration of nitrates to fringing mangrovesand resulted in elevated foliar δ^{15}N (11 to 16‰) in *Rhizophora* mangroves in Florida, whereas pristine mangroves had foliar δ^{15}N of -5 to 2‰ [35]. On the other hand, mangrove stands of *Avicinia marina*also hadelevated δ^{15}N valuedue to N uptake from human sewage sources [4].

3.2Species variation in Foliar δ^{15}N of Mangroves

Species variation in foliar δ^{15}N of three mangroves showed a wide range across thestudied watersheds. The species distribution of foliar δ^{15}Nin ten watersheds on three islands are presented in Table 2.The lowest means of foliar δ^{15}N (below 3‰) of three mangrovesB. *gymnorrhiza,* K. *obovata*, and R. *stylosa*were found in pristineUrauchiand Fukido watersheds. No pollutants in Urauchi creek water were apparent under a negligible human activity by scientific research and small-scale tourism [47]. However, Fukido watershed was associated with a limited degree of anthropogenic impacts covering 14% 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 foliarδ^{15}N of K. *obovata*(around 8‰) was measured in Manko watershed.The highest foliar δ^{15}Nin Todoroki watershedwas observed due to the fractionation of N sources from livestock [8] throughthe process of ammonia volatilization [24]. Whereas, the second highest foliar δ^{15}N was determined in Manko watershed due to relative fractionation of N sources from urbanization and agricultural practices (Table 1, [8]).Previously, theδ^{15}N was recorded in between 10‰ and 30‰ when N sources were livestock wastes, domestic wastewater [48] and wastewater treatment plant effluents[49, 50].

Species variation in foliar traits is generally found due to variation in N physiology of mangrove species,various leaf longevity of mangroves [51, 52], as well as the microclimatic variation [53] across the watersheds. Also,the distances of waterways from N sources to sinks [4, 53] could be the factors of corresponding changes in δ^{15}N of marine plant tissues[4]. However, foliar δ^{15}N of all the species showed clear correspondence to the degree of anthropogenic impact on mangrove ecosystems.

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)	5.0 ± 2.7^{b}
	Hirakubo	B.*gymnorrhiza*(n = 10)	5.0 ± 1.9^{b}
		R. stylosa (n = 7)	6.2 ± 2.7^{b}
	Todoroki	B.*gymnorrhiza*(n = 5)	10.0 ± 2.8^{c}
		R. stylosa(n = 5)	12.8 ± 1.3^{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.

3.3Performance of Mangrove Species as Ecological Indicator

Mangroves exhibit species-specific variation in foliar physiological functions in response to environmental condition including availability of nutrients[54]. In this study, foliar $\delta^{15}N$ of both B. *gymnorrhiza* and R. *stylosa*were significantly correlated to the anthropogenically impacted area ratio of the studied watersheds (Figure 2). 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 probableinput of N in ecosystems through anthropogenic activities significantly (P = 0.01) increase the foliar $\delta^{15}N$regardless of plant species [8].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) (Figure 2). It suggested that the foliar $\delta^{15}N$ of R. *stylosa* wasslightly more sensitive to N input in ecosystemsof the sampling sites, though the difference of such sensitivity forR. *stylosa*was not significantly different from B. *gymnorrhiza*.It was also previously observed that species-specific variation in foliar $\delta^{15}N$values were relatively small [6].

B. *gymnorrhiz typically* showed a slow mean growth rate in response to N supply compared to other mangroves such as *Rhizophora apiculate,Avicennia marina, Xylocarpusmoluccensis, Xylocarpusgranatum and Ceriops tagal*[55]. Such a kind of intrinsic difference in growth pattern among species is considered as one of the possible causes of species-specific variation inrelative habitats[55].Comparatively a high nutrient retention strategy(>70%) with a high tannin content [56] and a low foliar nutrient loss with an average leaf longevity of 16 months of R. *stylosa*[57,58] might be the factors for better performances in response to N availability in ecosystems.

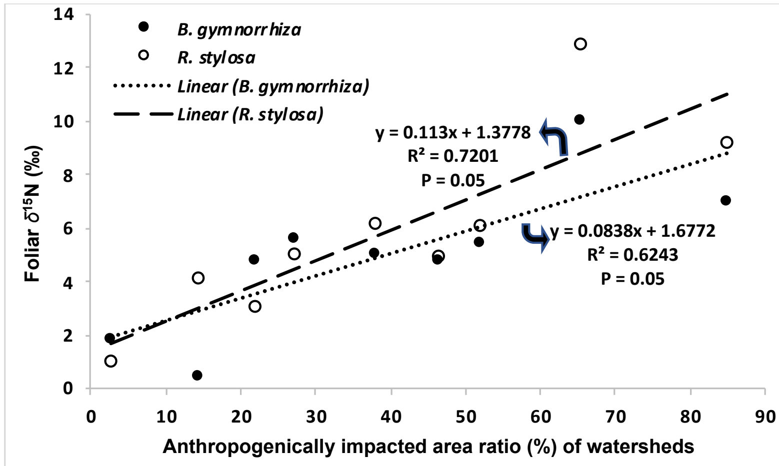

Figure 2. The relationship of foliar δ^{15}N of B. *gymnorrhiza*and R. *stylosa* with anthropogenically impacted area ratio of watersheds; the equation of regression analysis forB. *gymnorrhiza*is y = 1.68 + 0.08x, R^2 = 0.62,P = 0.05 and forR. *stylosa*is y = 1.38 + 0.11x, R^2 = 0.72,P = 0.05.

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

The difference index (DI) demonstrated the relative degree of deviation of foliarδ^{15}Nin the studied watersheds from the reference sites set at zero (Figure3).

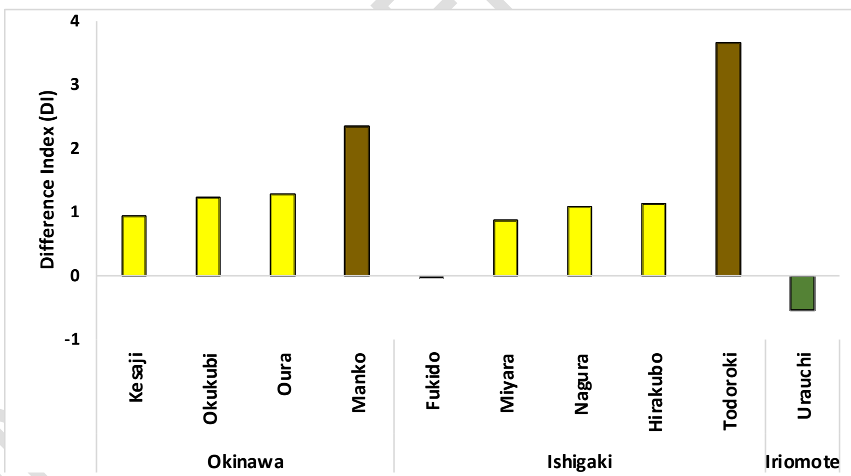

Figure 3. Relative deviation of foliar δ^{15}N of mangroves in anthropogenically impacted watersheds from the average foliar δ^{15}N in reference sites set at zero, indicated by a difference index (DI).

Here, DI extended from negative to positive values. The largest positive value of DI was in Todoroki (3.7), followed bythat in Manko(2.3), whereas two negative values of DI were in Fukido (-0.04) and Urauchi (-0.5).Having DI values more than 2, Todoroki and Manko watersheds confirmed the status of intensively anthropogenically impacted mangroves in this study. In contrast, the negative DI values indicated no change in ecosystem condition due to anthropogenic perturbation. However, more negative value found in Urauchi than

Fukidodenoted that the Urauchi is more pristine in ecosystem condition than Fukido. The finding was supported by the relative forest area ratio in these two watersheds (Table 1).

Additionally, Kesaji, Okukubi, Oura, Miyara, Nagura and Hirakubo watersheds, associated with the positive DI values less than 2, were confirmed their ecosystem condition as mildly anthropogenically impacted mangroves. Thus, DI values successfully unveiled relative ecosystem conditions of the studied watersheds, which was further reinforced by the strong negative linear relationship between DI and the forest area ratio (%) of the watersheds (Figure 4).

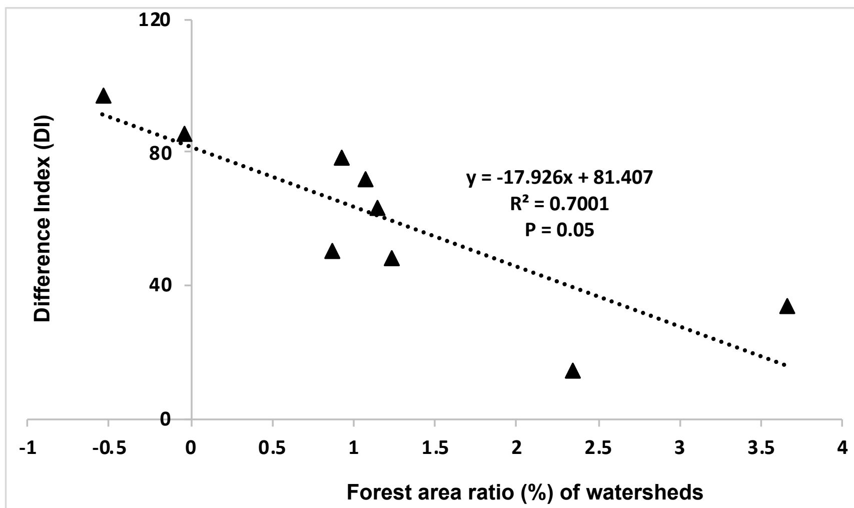

Figure 4. The relationship between forest area ratio (%) of watersheds and difference index (DI) of foliar δ^{15}N (‰; y = 81.41 - 17.93x; R^2 = 0.7; P = 0.05) determined at ten mangrove watersheds on three islands in Okinawa Prefecture, Japan.

The result revealed the importance of forestcover in maintaining the natural ecosystem of a watershed as well as reducing the deviation of isotopic imprint in impacted watersheds from the natural level.The forest areain watersheds having the capacity to capture excess N from anthropogenic perturbation is considered the main driving force to control nutrient dynamics, resulting in distinguishable foliar δ^{15}Nin the ecosystems.

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 occursin this study. Nitrogen inputs from anthropogenic sources potentially cause higher foliar □^{15}N of three mangroves in anthropogenically impacted mangrove**S** compared to the natural mangroves. A newly modeled difference index (DI) in this study further unveiled the relative deviation in foliar □^{15}N in the anthropogenically impacted mangrove watersheds from the background level of natural mangroves. A strong negative relationship between the distribution of DI and the forest area ratio of the watersheds efficiently indicated that a large forest cover ensured ecosystem's prissiness and any change or reduction of forest cover due to anthropogenic interruption might induce the DI value. It is expected that the insight of relative changes inecosystem conditions in anthropogenically impacted mangrove watershedsin Okinawa Prefecture may help the ecologist, environmentalists, and policy makers to take the necessary conservation and management steps in priority basis.

REFERENCE

1. Thomas N, Lucas R, Bunting P, Hardy A, Rosenqvist A, Simard M. Distribution and drivers of global mangrove forest change, 1996– 2010. PLoS ONE.2017;12(6):e0179302. https://doi.org/ 10.1371/journal.pone.0179302

2.Mayaux P, Holmgren P, Achard F, Eva H, Stibig HJ, Branthomme A. Tropical forest cover change in the 1990s and options for future monitoring. Philosophical Transactions of the Royal Society B: Biological Sciences. 2005;360(1454):373-384. https://doi.org/10.1098/rstb.2004.1590.

3. Erazo NG,Bowman JS. Sensitivity of the mangrove-estuarine microbial community to aquaculture effluent. iScience.2021;24:1–16. https://doi.org/10.1016/j. isci.2021.102204.

4. Costanzo SD, DonohueMJOÕ, DennisonWC,Loneragan NR, Thomas M. A new approach for detecting and mapping sewage impacts. Mar. Pollut. Bull. 2001;42:149–156. doi:10.1016/S0025- 326X(00)00125-9.

5. Ansari AA, Gill SS. Eutrophication, **C**auses, **C**onsequences and **C**ontrol. Springer Science and Business Media. Townsville, Qld, Australia. 2014. Available online: https://link.springer.com/book/10.1007/978-94- 007-7814-6 (accessed on 17 October 2010).

6. Hall SJ, Hale RL, Baker MA, Bowling DR, Ehleringer JR, Peters DPC. Riparian plant isotopes reflect anthropogenic nitrogen perturbations: Robust patterns across land-use gradients. Ecosphere.2015;6:200. doi:10.1890/ES15-00319.1.

7. Gritcan I, Duxbury M, Leuzinger S, Alfaro AC. Leaf sable isotope and nutrient status of temperate mangroves as ecological indicators to assess anthropogenic activity and recovery from eutrophication. Front. Plant Sci. 2016;7:1–11. doi:10.3389/fpls.2016.01922.

8. Tanu FZ, Asakura Y, Takahashi S,Hinokidani K, Nakanishi Y. Variation in foliar □^{15}N reflects anthropogenic nitrogen absorption potential of mangrove forests. Forests.2020;11: 133. doi:10.3390/f11020133

9. Talane-McManus L, Kremer HH, Crossland JIM. Biogeochemical and **H**uman **D**imensions of **C**oastal **F**unctioning and **C**hange in **S**outheast **A**sia. Final Report of the SARC/WOTRO//LOICZ Project 1996–1999. 2001;LOICZ Reports and Studies No. 17:II-277p.

10. Mukhopadhyay SK, Biswas H, De TK, Jana TK. Fluxes of nutrients from the tropical river Hooghly at the land-ocean boundary of Sundarbans, NE Coast of Bay of Bengal, India. J. Mar. Syst. 2006;62:9–21.

11.Alongi DM. The Energetics of Mangrove Forests, Springer, Dordrechr, Netherlands.2009.

12. Voss M, Dippner JW, Humborg C, Hurdler J, Korth F, Neumann T, et al. History and scenarios of future development of Baltic Sea eutrophication. Estuar. Coast. Shelf Sci. 2011;92:307–322.

13. Jorgensen L, Markager S, Maar M. On the importance of quantifying bioavailable nitrogen instead of total nitrogen. Biogeochemistry.2014;117:455–472.

14. Bryhn AC, Dimberg PH, Bergstrom L, Fredriksson RE, Mattila J, Bergstrom U. External nutrient loading from land, sea and atmosphere to all 656 Swedish coastal water bodies. Marine Pollution Bulletin.2017;114:664–670.

15.Valiela I, Elmstrom E, Lloret J, Stone T, Camil L. Tropical land-sea couplings: Role of watershed deforestation, mangrove estuary processing, and marine inputs on N fluxes in coastal Pacific Panama. Science of the Total Environment.2018:630:126–140.

16.Dewiyanti I, Darmawi D, Muchlisin ZA, Helmi TZ, Imelda I, Defira CN. Physical and chemical characteristics of soil in mangrove ecosystem based on differences habitat in Banda Aceh and Aceh Besar. IOP Conf Ser: Earth Environ Sci.2021;674:012092. DOI: 10.1088/1755-1315/674/1/012092.

17.Boyer EW, Goodale CL, Jaworski NA, Howarth RW. Anthropogenic nitrogen sources and relationships to riverine nitrogen export in the northeastern USA. Biogeochemistry.2002:57/58:137–169.
https://link.springer.com/content/pdf/10.1023/A:1015709302073.pdf

18**.** McDowell WH, Magill AH, Aitkenhead-Peterson JA, Aber JD, Merriam JL, Kaushal SS**.**Effects of chronic nitrogen amendment on dissolved organic matter and inorganic nitrogen in soil solution. For Ecol Manage**.**2004**;**196**:**29–41.

19**.** David SE, Jennerjahn TC. Extreme dissolved organic nitrogen fluxes in the human impacted Pamba River, Kerala, India. Biogeosciences Discussions**.**2013**;**10**:**16137–16171. doi:10.5194/bgd-10-16137-2013.

20**.** Hogberg P, Johannisson C. ^{15}N abundance of forests is correlated with losses of nitrogen. Plant Soil**.**1993**;**157**:**147-150.

21**.** McClelland JW, Valiela I. Linking nitrogen in estuarine producers to land-derived sources. Limnol. Oceanogr. 1998**;**43**:**577–585, doi:10.4319/lo.1998. 43.4.0577.

22. McKee KL, Feller IC, Popp M, Wanek W. Mangrove isotopic fractionation across a nitrogen vs phosphorus limitation gradient. Ecology.2002;83:1065-1075.

23. Costanzo SD, O'Donohue MJ, Dennison WC. Assessing the seasonal influence of sewage and agricultural nutrient inputs in a subtropical river estuary. Estuaries.2003;26:857–865.

24. Fry B, Gace A, McClelland JW. Chemical indicators of anthropogenic nitrogen loading in four Pacific estuaries. Pac. Sci. 2003;57:77–101.doi: 10.1353/psc.2003.0004.

25. Wooller M, Smallwood B, Scharlerc U, Jacobsonb M, Fogel M. A taphonomic study of ^{13}C and ^{15}N values in *Rhizophora mangle* leaves for a multi-proxy approach to mangrove palaeoecology. Org Geochem.2003;34:1259–1275. doi:10.1016/S0146-6380(03)00116-5.

26. Cole ML, Valiela I, Kroeger KD, Tomasky GL, Cebrian J, Wigand C, et al. Assessment of a □^{15}N isotopic method to indicate anthropogenic eutrophication in aquatic ecosystems. J. Environ. Qual. 2004;33:124–132.doi: 10.2134/jeq2004.1240.

27. Costanzo SD, Udy J, Longstaff B, Jones A. Using nitrogen stable isotope ratios (□^{15}N) of macroalgae to monitor changes in the extent of sewage impacts in Moreton Bay. Marine Pollution Bulletin.2005;51:212–217.

28. Hogberg P. Tansley review no 95: ^{15}N natural abundance in soil-plant systems. New Phytol.1997;137:179–203. doi:10.1046/j.1469-8137.1997.00808.x.

29.Robinson D. δ^{15}N as an integrator of the nitrogen cycle. Trends Ecol. Evol.2001;16:153–162, https://doi.org/10.1016/S0169- 5347(00)02098-X.

30. Atkinson CL, Christian AD,Spooner DE,Vaughn CC. Long-lived organisms provide an integrative footprint of agricultural land use. Ecological Applications.2013;24:375–384.

31.Lugendo BR, Kimirei IA. Anthropogenic nitrogen pollution in mangrove ecosystems along Dares Salaam and Bagamoyo coasts in Tanzania. Marine Pollution Bulletin.2021;168:112415. https://doi.org/10.1016/j. marpolbul.2021.112415.

32. Medina-Contreras D, Cantera J,Sánchez A. Trophic structure of fish communities in mangrove systems subject to different levels of anthropogenic intervention, tropical eastern pacific, Colombia. Environmental Science and Pollution Research Springer, Berlin Heidelberg. 2021. https:// doi.org/10.1007/s11356-021-16814-x.

33. Medina-Contreras D, Arenas F. Are stable isotopes an efficient tool for tracking the effect of anthropogenic activities on mangrove food web structure and function? Hydrobiologia.2023;850:1237–1249.https://doi.org/10.1007/s10750-022-05086-2

34. Peterson BJ, Fry B. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Sys. 1987;18:293–320. Available online: http://www.jstor.org/stable/2097134 (accessed on).

35**.** Fry B**,** Bern AL**,** Ross MS**,** Meeder JF. δ^{15}N studies of nitrogen use by the red mangrove, *Rhizophora mangle* L. in south Florida. Estuar. Coast. Shelf Sci. 2000**;**50**:**291–296**.** doi:10.1006/ecss.1999.0558.

36. Higashi T, Katayama TC, Shinagawa A. Land development works and soil erosion in Okinawa Prefecture. Mem. Kagoshima Univ. Res. Center S. Pac. 1985;6(1):26-36. http://hdl.handle.net/10232/15659

37. Hasegawa H. The decline of coral reef conditions caused by the extensive land modification: A case study of the Shiraho Area on Ishigaki Island, Okinawa Prefecture, Japan. J. Remote Sens. Soc. Jpn. 2011;31:73–86. (In Japanese with English abstract)

38. Fujimura N,Fukamachi K, Shibata S. The history of land use in Iriomote Island from the 1960s and its sociocultural background. J. Jpn. Ins. Landsc. Arch. 2017;80:713–718. doi:10.5632/jila.80.713. (In Japanese)

39. Okinawa Prefecture. Primary **P**lanning **O**f**P**rotection **O**f **E**rosion **O**f **R**ed **S**oils **A**nd **O**ther **S**oils; Okinawa, Japan. 2013;63p.

40. Fry B, Cormier N. Chemical ecology of red mangroves, *Rhizophora mangle*, in the Hawaiian Islands. Pacific Sci.2011;65:219–34. http://www.bioone.org/doi/abs/10.2984/65.2.219.

41.Nadelhoffer KJ, Fry B. Nitrogen isotope studies in forest ecosystems.**I**n: Lajtha K, Mitchener RH, **EDITORS.** Stable **I**sotopes in **E**cology and **E**nvironmental Science. Oxford, UK: Blackwell Scientific Publications; 1994.pp. 22– 44.

42. Onaga K,Komesu R, Arakaki A. The outline of red soil loss and erosion control measure in Okinawa. Sci. Bull. College Agri. Univ. Ryukyus.1999;46:71–82.

43. Banzai K, Nakamura K. Red **S**oil **R**unoff **F**rom**T**he **M**iyara **R**iver, **A**nd **A**n **E**nvironmental **P**roblem on Ishigaki Island. Japan International Research Center for Agricultural Sciences: Tsukuba, Japan. 2005;pp.97–102.https://www.jircas.go.jp/en/publication/intlsymp/13/97

44.Ishiga H, Diallo IMB. Geochemical evaluation of present mangrove soil in Okinawa Island, Japan. Earth Sci. (Chikyu Kagaku).2016;70:119–128.https://www.jstage.jst.go.jp/article/agcjchikyukagaku/70/4/70_119/_pdf.

45. OECD. Environment at a Glance 2015: OECD Indicators. Paris: OECD Publishing. 2015doi: 10.1787/19964064

46.Harrington RR, Kennedy BP, Chamberlain CP, Blum JD,FoltCL. ^{15}N enrichment in agricultural catchments: field patterns and applications to tracking Atlantic salmon (Salmo salar). Chem. Geol. 1998;147:281–294. doi: 10.1016/S0009-2541(98)00018-7

47.Ishiki M, Sakihama H, Agata S, Tokuyama A. Chemistry of the Urauchi river water in Iriomote Island national park, Okinawa, Japan. Water Air Soil Pollut. 2009;198:111–124. doi:10.1007/s11270-008-9830-9

48. Kendall C, Elliott EM, Wankel SD. Tracing anthropogenic inputs of nitrogen to ecosystems. In:Michener R, Lajtha K, **EDITORS.** Stable **I**sotopes **I**n **E**cology **A**nd **E**nvironmental **S**cience.Hoboken, NJ, USA:Blackwell publishing. 2007. pp.375–449.

49. Savage C, Elmgren R. Macroalgal (Fucus vesiculosus) δ^{15}N values trace decrease in sewage influence. Ecol. Appl. 2004;14:517–526. doi:10.1890/02-5396.

50. Fair JM,Heikoop JM. Stable isotope dynamics of nitrogen sewage effluent uptake in a semi-arid wetland. Environ. Poll. 2006;140:500–505. doi:10.1016/j.envpol.2005.08.005.

51. Gartner A, Lavery P, Smit A. Use of δ^{15}N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Mar. Ecol. Prog. Ser. 2002;235:63– 73. doi:10.3354/meps235063.

52. Deutsch B, Voss M. Anthropogenic nitrogen input traced by means of □^{15}N values in macroalgae: results from in-situ incubation experiments. Sci. Total Env. 2006;366:799-808. doi:10.1016/j.scitotenv.2005.10.013.

53. García-Sanz T, Ruiz JM, Pérez M, Ruiz M. Assessment of dissolved nutrients dispersal derived from offshore fish-farm using nitrogen stable isotope ratios (δ^{15}N) in macroalgal bioassays. Estuar. Coast. Shelf Sci. 2011;91:361–370. doi:10.1016/j.ecss.2010.10.025.

54. Wintah, Kiswanto, Hilmi E,Sastranegara MH. Mangrove diversity and its relationships with environmental conditions in Kuala Bubon Village, West Aceh, Indonesia. Biodiversitas. 2023;24(8):4599-4605. doi:10.13057/BIODIV/D240864

55. Alongi DM. Early growth responses of mangroves to different rates of nitrogen and phosphorus supply.Journal of Experimental Marine Biology and Ecology.2011;397:85–93.doi:10.1016/j.jembe.2010.11.021

56. Lin Y, Liu X, Zhang H, Fan H, Lin G. Nutrient conservation strategies of a mangrove species *Rhizophora stylosa* under nutrient limitation. Plant Soil.2010;326:469–479. doi:10.1007/s11104-009-0026-x.

57. Saenger P. Mangrove ecology, silviculture and conservation. Kluwer: Dordrecht, The Netherlands, 2002.

58. Alongi DM, Clough BF, Robertson AI. Nutrient-use efficiency in arid-zone forests of the mangroves *Rhizophora stylosa* and *Avicennia marina*. Aquat. Bot. 2005;82:121–131. doi:10.1016/j.aquabot.2005.04.005