

Land-use Imprints on Leaf Nitrogen Stable Isotope Ratio in Ishigaki Mangroves, Japan

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Abstract

Complex ecological setups of mangroves under various environmental factors and land-use form unique ecosystems in tropical and subtropical coasts around the world. This study aimed to investigate the imprints of different levels of land-use ratio on reactive nitrogen (N), reactive phosphorus (P) and stable N isotope ratio ($\delta^{15}\text{N}$) of mangrove leaves on Ishigaki Island (Is.), Japan. Results showed that dissolved inorganic N (DIN), dissolved organic N (DON), soluble reactive phosphorous (SRP) of surface water, and mangrove leaf $\delta^{15}\text{N}$ are significantly influenced by the land-use ratio, mainly the forest area of the watersheds. The mangrove leaf $\delta^{15}\text{N}$ values ranging from 0 to 13‰ indicated different level of human interaction with the ecosystems after land-derived N absorption by mangroves. The circumstance was illustrated through the significant positive correlation of mangrove leaf $\delta^{15}\text{N}$ with surface water DIN, and the negative correlation with the forest area ratio (%). However, a major contribution of DON to the total dissolved N (TDN) of surface water reflected that the concentrations of both DIN and DON might be regulated by the respective land-use in the watersheds, further controlling the mangrove leaf $\delta^{15}\text{N}$ values. The findings are important for taking relative measures for conservation and management of the mangroves on Ishigaki Island, Japan.

Keywords: Land-use; Mangroves; Reactive nitrogen; Soluble reactive phosphorous; Stable nitrogen isotope ratio; Mangrove leaf

1. Introduction

Modern intensive agricultural practices, urbanization, and ensuing damage to ecosystem sustainability are major concerns nowadays. Many ecological services relating to human livelihoods, biodiversity, and blue carbon economies are provided by mangroves having ubiquitous growth in tropical and subtropical coasts. Mangroves are sensitive ecosystems due to the recent loss at an average rate of 0.18% each year (Richards and Friess, 2016). Over the 1990 – 2000 period, it has been

observed that a combination of natural and man-made factors, primarily through deforestation, conversion to aquaculture practices, agricultural fields, and infrastructural development, have changed the biogeochemistry and ecological characteristics of mangrove ecosystems (Thomas *et al.*, 2017). Still, data in the context of ecosystem changes due to the influences of human activities on the biogeochemistry of tropical watersheds are less conclusive than those of the high latitudinal coasts (David *et al.*, 2016).

Watershed land-use and surface and groundwater discharge affect the composition and quantity of N in the environment (Alongi, 2013). Mangroves are oligotrophic (Reis *et al.*, 2017) and respond to nutrient additions from anthropogenic sources (Gritcan *et al.*, 2016), along with the atmospheric deposition and oceanic N input from tidal water (Cohen *et al.*, 1999). Excess N from human activities may enhance N cycling and ^{15}N enrichment in a system due to relative N loss and N fractionation (Hogberg and Johannisson, 1993). Processing and incorporation of ^{15}N -enriched reactive N (NO_2^- - N, NO_3^- - N, and NH_4^+ - N) cause generally higher values of $\delta^{15}\text{N}$ in coastal plants (Costanzo *et al.*, 2005; Cole *et al.*, 2004). Thus, $\delta^{15}\text{N}$ integrates the signature of N sources and isotope fractionations during N transformation reactions (Hogberg, 1997; Robinson, 2001) as well as plant uptake and assimilation. However, no isotope fractionation occurs during root uptake (Mariotti *et al.*, 1982) of ammonium (Evans *et al.*, 1996), and nitrate (Yoneyama *et al.*, 1991) or the fractionation is very negligible ($< 0.3\%$; Nadelhoffer and Fry, 1994). Hence, previous studies have differentiated between naturally formed N and anthropogenically derived N in mangrove ecosystems by determining the mangrove leaf $\delta^{15}\text{N}$ (Gritcan *et al.*, 2016; Tanu *et al.*, 2020). Mangroves on Ishigaki Is., Japan has been reported as ‘moderately affected ecosystems’ compared to those on Iriomote (pristine mangroves) and Okinawa islands (intensively human-affected mangroves) (Tanu *et al.*, 2020). However, a robust understanding of relative degree of changes in mangroves on Ishigaki Is. is yet to be clarified.

Therefore, this study aimed to measure the typical ecosystem indicators such as dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP) and the mangrove leaf $\delta^{15}\text{N}$ to understand the relative degree of ecosystem conditions in the mangroves under the effects of different level of land-use ratios. Before beginning any management actions for a specific mangrove, it is essential to disclose the level of human interaction with the investigated mangroves. The findings of this study are expected to disclose the level of human interaction with

the ecosystems through N dynamics and perseverance of the mangroves in priority basis. To improve the status of mangroves, ecologists, environmentalists, social workers, and other stakeholders are urged to establish the essential regulations for mangroves.

2. Methodology

2.1 Study sites

Five mangrove watersheds associated with five creeks named Fukido, Nagura, Hirakubo, Miyara, and Todoroki on Ishigaki Is., Japan were selected as the study sites based on different levels of human interaction with ecosystems by agriculture, livestock farming, and municipal development as well as the accessibility into mangroves. The map of the study watersheds (Figure 1) was prepared by using ArcMap Geographic Information Services (GIS) software (ArC Map 10.4.1). The coordinates of the sampling points in the watersheds are available in Table 1.

2.2 Delineation of the land-use ratio of watersheds

According to the study by Tanu *et al.* (2020), the order of the largest human-influenced area ratio of the studied watersheds was Todoroki > Miyara > Nagura > Hirakubo > Fukido. However, the land-use ratio, the creek length and corresponding stream area of those watersheds were re-estimated. The source data for the delineation of the land-use of watersheds and the border line were used from the National Land Policy Bureau, Japan Ministry of Land, Infrastructure, Transport, and Tourism. The land conservation maps (1:200,000) and baseline data of coastlines, swamps and creeks was used from the Geospatial Information Authority of Japan (GSI). The land-use was divided into agricultural area, developed area and livestock area, as shown in Table 1. Fukido watershed is considered as a pristine forested watershed due to a high ratio of forested area (87 %) with minimal levels of human activities, whereas, other watersheds have different level of human-influenced area ratio (sum of agriculture, livestock farming, and other developed area) ranging from around 20 to 70% (Table 1).

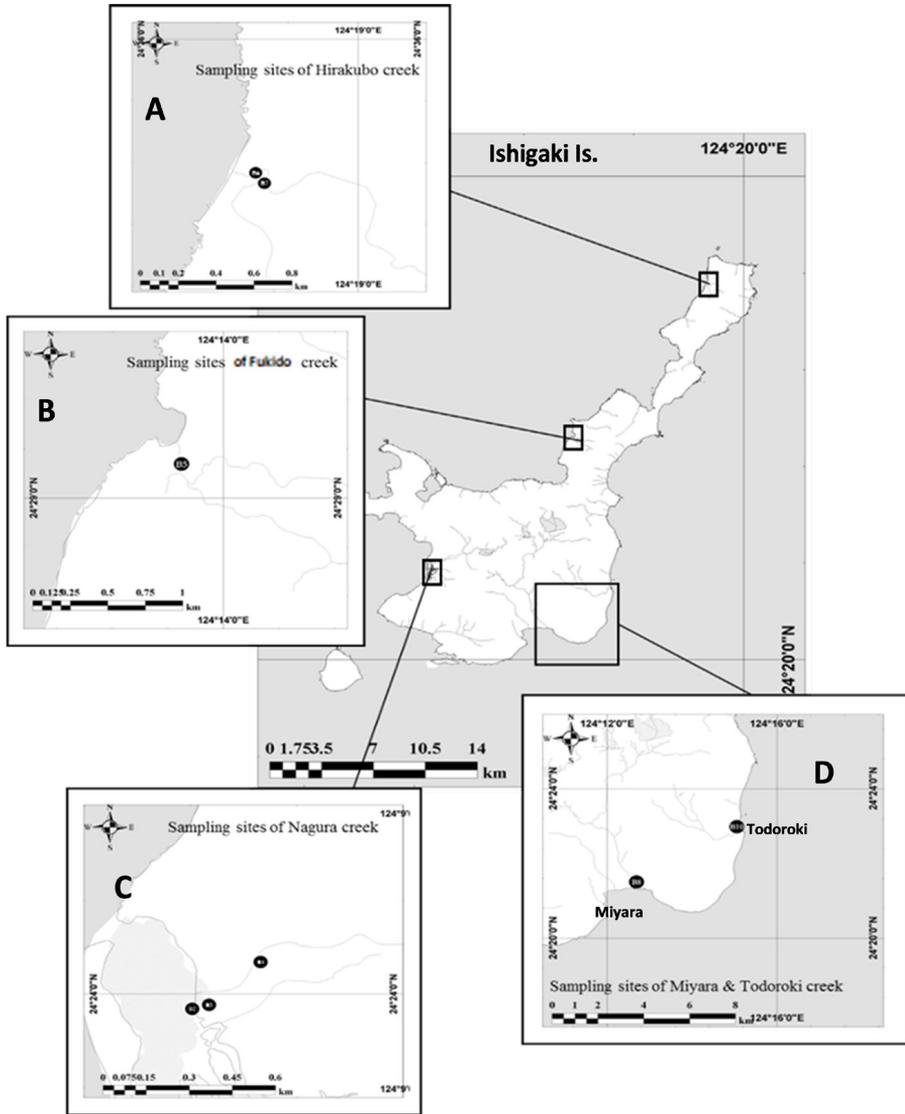


Figure 1. Location of five mangrove watersheds on Ishigaki Is., Japan, and sampling points (black dots) in watersheds: Hirakubo (A), Fukido (B), Nagura (C), Miyara and Todoroki (D)

Table 1. The land-use area ratio of five mangrove watersheds on Ishigaki Is., Japan and coordinates of the sampling points.

Island	Watershed	Creek length (Km)	Land-use				Sampling coordinates	Stream area
			Total Forested area (%)	Agricultural area (%)	Developed Area (%)	Livestock area (%)		
Ishigaki	Fukido	0.70	87.0	9.73	2.07	Absent	N 24°29'07.34" E 124°13'50.30"	Middle
	Nagura	4.55	73.8	15.7	4.83	0.16	N 24°24'04.05" E 124°08'43.03"	Up
							N 24°23'58.87" E 124°08'39.83"	Middle
	Hirakubo	na*	84.5	10.2	4.02	0.26	N 24°23'59.01" E 124°08'36.07"	Down
							N 24°35'34.58" E 124°18'44.75"	Middle
	Miyara	12.0	59.8	24.5	7.69	0.45	N 24°35'33.50" E 124°18'40.70"	Down
Todoroki	3.10	29.5	45.2	7.96	1.17	N 24°21'24.74" E 124°12'46.66"	Down	
						N 24°22'58.72" E 124°15'30.94"	Down	

*The abbreviation 'na' indicates that data was not available

2.3 Sampling and analysis

Surface water samples (collected during ebb tide) and co-located mangrove leaves in upstream, middle stream and downstream area of the creeks upon the availability of mangroves were collected within an area of approx. 500 m from the creek mouth in the year of 2018. The landward end of the natural vegetation and/or mangrove distribution was selected as the upstream point, whereas the creek mouth associated with mangroves was selected as the downstream point. The middle stream was considered as the area in between up- and downstream where well-distributed mangroves were found. Therefore, all three stream areas (up, middle and down) of five creeks were not sampled due to the unavailability of mangroves.

An amount of 1L surface water samples with replication were collected from eight sampling points in five creeks. Physicochemical parameters of water samples, such as temperature, pH, oxidation-reduction potential (ORP), and electrical conductivity (EC), were measured on sites using portable meters (Model-HI-98121 for Temp. and pH; Model YSI for Electrical Conductivity). All water samples were treated with sodium azide to prevent biological activities. They were stored in cooler boxes while transporting to the laboratory and refrigerated at 4 °C until analysis.

Water samples were filtered through 0.45 - μm membrane filters (MCE; Merck Millipore Ltd, Dublin, Ireland) and used for the analysis of NO_3^- -N and NO_2^- -N by using a flow injection analysis (FIA; autosampler-AS-55F, flow injector-PD-4000, thermostat reactor-521, spectrometric detector-S3250, Suma data recorder-C-R6A Chromatopac, Shimadzu, OGAWA & Co. Ltd, Tokyo, Japan). The concentrations of NH_4^+ -N and soluble reactive phosphorus (SRP, PO_4^{2-}) were determined by the colorimetric method (Strickland and Parsons, 1972). DIN is calculated as the sum of NO_3^- -N, NO_2^- -N, and NH_4^+ -N. Total dissolved nitrogen (TDN; the sum of DIN and DON) was determined by using the persulphate oxidation method (Solorzano and Sharp, 1980). Then, dissolved nitrogenous compounds were

measured as NO_3^- -N by FIA. DON is calculated by subtracting the value of DIN from TDN. If either NO_3^- -N or NH_4^+ -N was undetectable, the value for the other N species was considered to be the total measurable DIN or TDN. The DIN/SRP ratio was calculated when both components were above the detection limit.

For leaf sampling, five trees of each dominant mangrove species of *Rhizophora stylosa* and *Bruguiera gymnorrhiza* were chosen and a total of five sun-facing mature leaves per individual tree were collected within an area of approx. 10 m² in each point. They were washed, wiped, and dried in an oven at 60 °C (DNE 910, Yamato) for 48 hrs and ground into fine powder by a wonder blender. Leaf total N (TN; % of dry wt.) and carbon (TC % of dry wt.) of powdered leaf samples were measured using an NCH (Nitrogen, Carbon, Hydrogen) analyzer (SUMIGRAPH; SCAS), and C/N ratio was calculated. The samples were stored in a desiccator until the analysis of $\delta^{15}\text{N}$ by TC/EA-IRMS.

The leaf $\delta^{15}\text{N}$ values are expressed by the conventional δ -notation using the following equation (Coplen, 2011) automatically calculated in the Lab Solution software (Thermo Fisher Scientific):

$$\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{reference}}) - 1]$$

where R_{sample} and $R_{\text{reference}}$ are the ratios of heavier to lighter N isotopes in the samples and the working reference materials, respectively. Calibration of $\delta^{15}\text{N}$ followed the process as mentioned in Tanu et al. (2020).

2.4 Statistical analysis and data presentation

Data were examined for normal distribution. Student-t tests were done to check significant differences of leaf TN, leaf C/N, and leaf $\delta^{15}\text{N}$ between two mangrove species *R. stylosa* and *B. gymnorrhiza* in each watershed. Since the foliar traits of two mangroves varied nonsignificantly, the leaf $\delta^{15}\text{N}$ values of two mangrove species were collectively used as a single data set for further statistical analysis. One-way Welch's analysis of variance (ANOVA) was

performed for comparison of the variables among watersheds. Best fitted multiple regression models were used for investigation of relationships among mangrove leaf $\delta^{15}\text{N}$, nutrient concentrations and land-use ratios. The statistically significant ($p < 0.05$) or nearly significant ($0.05 < p < 0.1$) relationships of the variables were graphically presented. All statistical analyses were done using SPSS v24 (IBM Corp., Armonk, N.Y., USA). Graphical presentations were made in Excel 2010 (Microsoft, Redmond, WA, USA) and SPSS v24.

3. Results and discussion

3.1 Reactive N and P concentrations of surface water

The surface water samples showed temperature ranging from 27.7 to 28.8 °C, pH from 7.1 to 7.9, ORP from 75 to 161 mV and EC from 2 to 28 dSm^{-1} . The highest concentration of DIN (170 μM) was found in Todoroki downstream, whereas it ranged from about 6 to 34.4 μM in the rest sampling points. The $\text{NO}_3^- \text{-N}$ was significantly higher in

downstream of Miyara (73 μM) and Todoroki (168 μM) creeks compared to those of other creeks (ranging from 3 to 34 μM). The $\text{NH}_4^+ \text{-N}$ ranged from < 1 μM to 23 μM , and the $\text{NO}_2^- \text{-N}$ ranged from ‘not detected’ to 3 μM . The highest concentration of both $\text{NH}_4^+ \text{-N}$ and $\text{NO}_2^- \text{-N}$ were detected in Hirakubo creek. The concentration of DON ranged from 9 to 37 μM in the samples.

The relative proportion of $\text{NO}_3^- \text{-N}$, $\text{NO}_2^- \text{-N}$, $\text{NH}_4^+ \text{-N}$, and DON to TDN demonstrated that DON was a dominant form of N in all the creeks except Miyara and Todoroki. The highest proportion of $\text{NO}_3^- \text{-N}$ was found in Todoroki creek followed by that in Miyara creek (Figure 2). The proportion of DON to TDN ranged from 10 to 79 %, which is similar to the observations of an estuarine river in West Bengal, India (Fischer *et al.*, 2016) and in some tropical watersheds (Taylor *et al.*, 2015; Valiela *et al.*, 2018). The concentration of SRP was very low in the samples and ranged from ‘not detected’ to 3 μM . The DIN/SRP in Fukido and Nagura creek was less than 20, whereas those in Hirakubo, Miyara and Todoroki creeks were greater than 50.

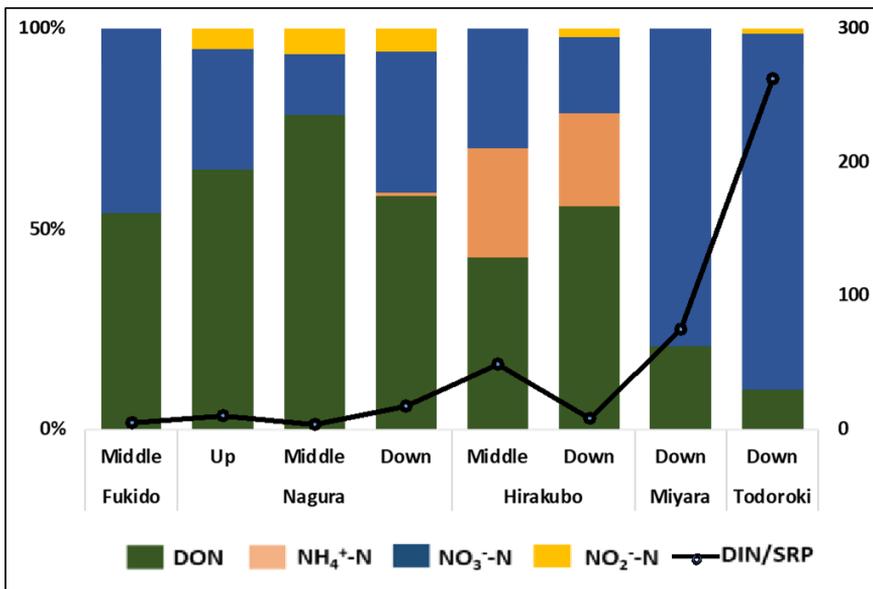


Figure 2. Relative proportion (%) of DON, $\text{NH}_4^+ \text{-N}$, $\text{NO}_3^- \text{-N}$, and $\text{NO}_2^- \text{-N}$ to TDN (μM) and DIN/SRP of surface water collected from five mangrove creeks on Ishigaki Is., Japan

3.2 Imprints of land-use ratios on reactive N and P

Todoroki and Miyara are the two most human-influenced watersheds along with a low range of forest cover. However, the higher values of DIN/SRP indicated that the human-influenced watersheds are not readily N limiting. Human activities and modern agricultural practices are attributed to greater amounts of inorganic N input in the creeks and streams regardless of the time they started (Harris, 2001; Turner et al., 2003). Significant positive relationships between the concentrations of different forms of N and land-use ratios of agricultural practices, developed areas and livestock are presented in Figure 3. Agricultural activities have significantly increased TDN and DIN/SRP in the watersheds (Figure 3-A). TDN, NO_3^- -N, and DIN/SRP have also been increased with the ratio of developed areas (Figure 3-B). Likewise, the increased area ratio of livestock has significantly enhanced TDN, NO_3^- -N, DIN, and DIN/SRP (Figure 3-C).

In contrast, the forest area ratio (including mangroves and natural vegetation) has shown significant negative correlations with TDN, NO_3^- -N, DIN, and DIN/SRP (Figure 3-D). The forest covers may significantly control N concentrations and availability through an intensive microbial function of nitrification-denitrification, absorption, and assimilation (White et al., 2018; Wong et al., 2018). The present results coincided a meta-scale analysis on Panamanian estuaries, reporting on a significant linear relationship between N-interception and forest covers in watersheds (Valiela et al., 2013; Valiela et al., 2018). The significant negative relationship between DIN/SRP and the forest area ratio indicated that the imprints of excess N inputs in human-influenced watersheds may be disappeared under a large extent of forest cover.

Human activities have not only been linked to increased DIN (Jordan et al., 1997; Boyer et al., 2002;) but also DON (Westerhoff and Mash, 2002). Wastewater discharge and fertilizer use can be significant sources of DON in ecosystems (Pregitzer et al., 2004, Pellerin et al., 2006). However, 32 % of forest area in watersheds could influence

DON of surface water, whereas 0 to 92 % area of urban development and agricultural practices could be a poor predictor of DON variability (Pellerin et al., 2004). In current study, both human-influenced area ratio and forested area ratio overlap as major contributors to the concentration of DON and bring different level of changes in mangrove ecosystems.

The highest concentration of DON (> 50 % of TDN) was detected in Nagura watershed associated with 73.8 % forest area and 20.7 % human-influenced area. Fertilizer use and animal farming may stimulate plant roots and soil microbes to produce N-rich organic products over a relatively short time-scale and influence existing biotic and abiotic mechanisms of a forested watershed (Murphy et al., 2000; Pellerin et al., 2006). The vegetation types including turf, shrub and trees other than mangroves may also differ in increasing different forms of N (Stanley and Maxted, 2008) and interception of it (Pocewicz et al., 2007).

The cause of a high concentrations of NO_3^- -N in both Miyara and Todoroki creeks is likely a comparatively large area of agricultural practices by sugarcane, pineapple, paddy cultivation, development activities, and livestock farming. The Todoroki watershed previously experienced a heavy N input in the form of NO_3^- through groundwater seepage along the beach (Umezawa et al., 2002). In Todoroki, an annual discharge of about 71.5 tons of N was reported (Nakasone et al., 2001) through baseflow (Blanco et al., 2010), resulting in harmful consequences on adjacent coral reefs.

Furthermore, oceanic inputs and atmospheric deposition (McDowell et al., 2004; Pregitzer et al., 2004) typically link to increased concentration of inorganic N (Boyer et al., 2002; Valiela et al., 2018). However, contribution of precipitation to the concentration of NO_3^- -N in Todoroki is minimal (< 3.6 μM ; Umezawa et al., 2002). Moreover, oceanic N input in Todoroki and Miyara watersheds was not significant during ebb tide (measured at laboratory but data is not shown). So, there is a little chance of NO_3^- -N export through tidal flushing in Miyara downstream (Shimoda and Fukuoka, 2013).

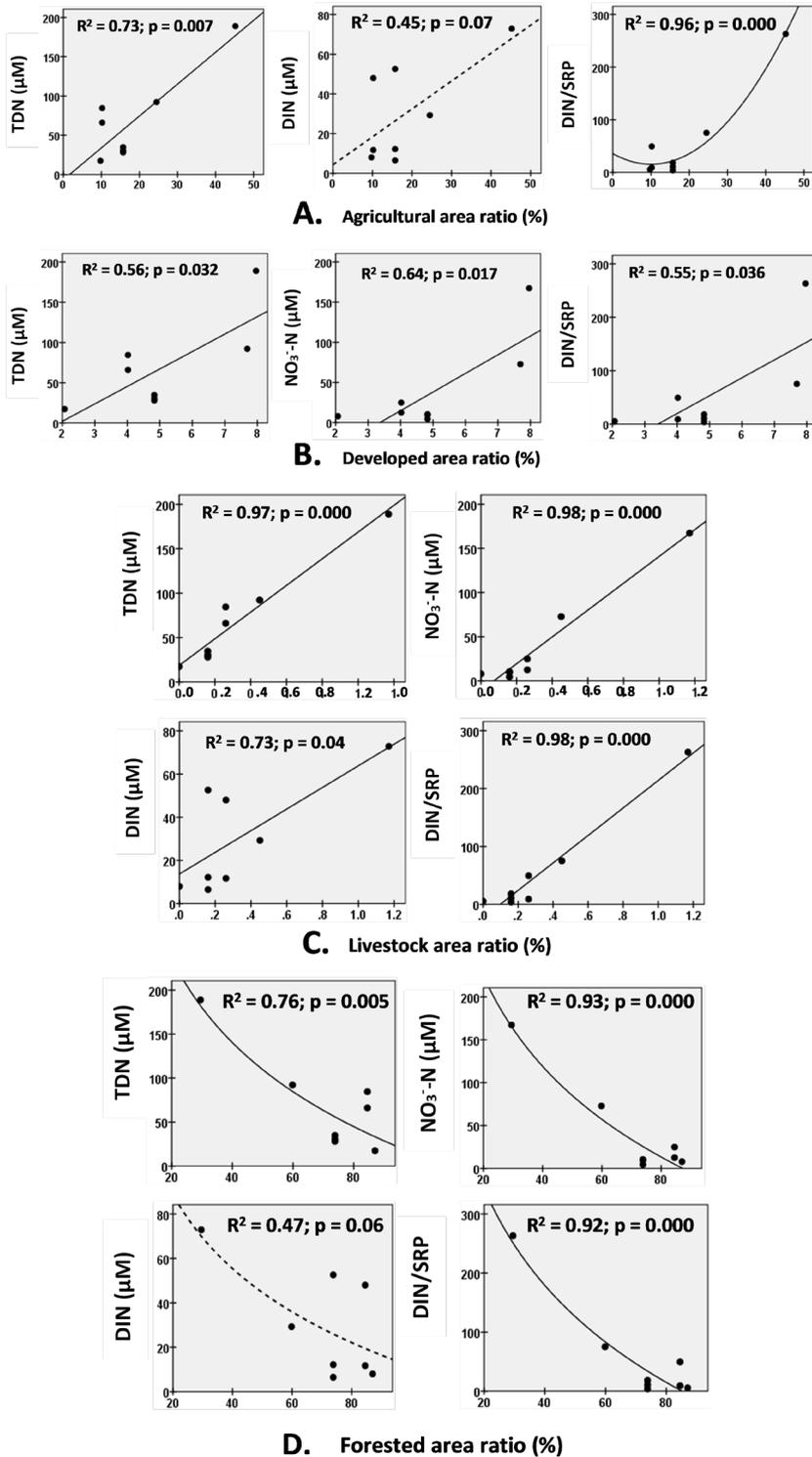


Figure 3. Relationships of the concentrations (μM) of different forms of N with agricultural area ratio (A), with developed area ratio (B), with livestock area ratio (C) and with forested area ratio in five mangrove watersheds on Ishigaki Is., Japan. The dashed line represented a weak relationship ($0.05 < p < 0.1$)

In contrast, the pristine environment Fukido, having 87 % forest area and a limited extent of human activities, showed a fairly high concentration of $\text{NO}_3^- - \text{N}$ and DON . Besides, the Hirakubo creek, showing a high concentration of $\text{NH}_4^+ - \text{N}$ (Figure 2), differed from the other watersheds. The reason is not possibly the oceanic N input because the concentration of $\text{NH}_4^+ - \text{N}$ in the seawater sample (0.15 μM) was negligible. The investigation of microbial contribution to the N pool might disclose the reason of the high $\text{NH}_4^+ - \text{N}$ concentration.

3.3 Imprints of land-use ratios on leaf $\delta^{15}\text{N}$

The leaf $\delta^{15}\text{N}$ of two mangrove species, *R. stylosa* (Rs) and *B. gymnorrhiza* (Bg), varied within a range from 0.43 to 13.0 ‰ (Table 2). The highest value of mangrove leaf $\delta^{15}\text{N}$ was found in Todoroki watershed. The values of leaf TN and C/N ranged from 0.77 to 1.29 % (of dry wt.) and from 40 to 64, respectively (Table 2) and were not significantly different among the watersheds.

The results of Welch t-test analyses showed that mangrove leaf $\delta^{15}\text{N}$ were significantly varied ($p < 0.05$) among the watersheds, and mangrove in Todoroki showed the highest value of leaf $\delta^{15}\text{N}$ (Table 2). The leaf $\delta^{15}\text{N}$ value is conservative and the fractionation of N isotope during uptake of NH_4^+ and NO_3^- by roots is absent (Evans et al., 1996) or very negligible ($< 0.3\%$; Nadelhoffer and Fry, 1994).

Henceforth, the range of mangrove leaf $\delta^{15}\text{N}$ values help understanding of the potential N sources in the ecosystems. Mangrove leaf $\delta^{15}\text{N}$ in Todoroki (up to 13 ‰) resembled the range of animal-derived N sources (10 to 22 ‰; Letolle, 1980; Hoffman et al., 2012). However, mangrove leaf $\delta^{15}\text{N}$ values in Nagura, Hirakubo, and Miyara watersheds (4 to 9 ‰) resembled the elevated range of naturally fixed (up to 3‰; Peterson and Fry, 1987) or atmospherically deposited N sources (Letolle, 1980; Hoffman et al., 2012). Moreover, fertilizers have $\delta^{15}\text{N}$ values ranging from - 3.0 to 1.5 ‰ in NH_4^+ , and 2 to 3.5 ‰ in NO_3^- (Han et al., 2018). Different fractionation levels of N isotope from fertilizer or animal manure whether through microbial or chemical transformation processes could indicate the imprints of land-use ratio and reactive N on mangrove leaf $\delta^{15}\text{N}$ (Gritcan et al., 2016). Cow manure is commonly used fertilizer in agricultural plots in watersheds where the livestock is available. Therefore, mangrove watersheds except Fukido are probably incorporating N from land-derived sources after different level of transformation and fractionation processes. Fukido watershed is nearly pristine and no human-used substances were found flowing into the creek (Kida et al., 2017). Naturally fixed N could be considered as the major source of N input in the pristine watershed (Valiela et al., 2018), which is why a lower mangrove leaf $\delta^{15}\text{N}$ value is recorded in Fukido.

Table 2. Leaf TN, leaf C/N and leaf $\delta^{15}\text{N}$ of mangroves (*R. stylosa* and *B. gymnorrhiza*) in five watersheds on Ishigaki Is., Japan.

Island	Species	Watershed	Leaf TN (% of dry wt.) mean ± stdv.	Leaf C/N (% of dry wt.) mean ± stdv.	Leaf $\delta^{15}\text{N}$ (‰) mean ± stdv.
Ishigaki	<i>R. stylosa</i>	Fukido	0.77 ± 0.10	64.2 ± 7.74	2.80 ± 2.60 ^a
	<i>B. gymnorrhiza</i>		1.29 ± 0.17	41.5 ± 9.33	0.43 ± 1.13 ^a
	<i>R. stylosa</i>	Nagura	1.05 ± 0.24	45.4 ± 6.27	5.03 ± 2.74 ^b
	<i>B. gymnorrhiza</i>		0.93 ± 0.14	50.6 ± 7.78	5.98 ± 2.82 ^b
	<i>R. stylosa</i>	Hirakubo	1.24 ± 0.15	40.3 ± 6.18	6.16 ± 2.72 ^b
	<i>B. gymnorrhiza</i>		1.26 ± 0.20	40.2 ± 6.58	4.97 ± 1.86 ^b
	<i>R. stylosa</i>	Miyara	1.17 ± 0.13	41.8 ± 4.75	4.92 ± 1.09 ^b
	<i>B. gymnorrhiza</i>		0.95 ± 0.16	50.8 ± 8.92	4.16 ± 0.91 ^b
	<i>R. stylosa</i>	Todoroki	1.23 ± 0.14	40.1 ± 4.61	12.8 ± 1.34 ^c
	<i>B. gymnorrhiza</i>		1.16 ± 0.11	45.5 ± 4.40	10.0 ± 2.82 ^c

Note: For each species on each watershed, n = 5; Different alphabets in a column indicate the significant differences from each other.

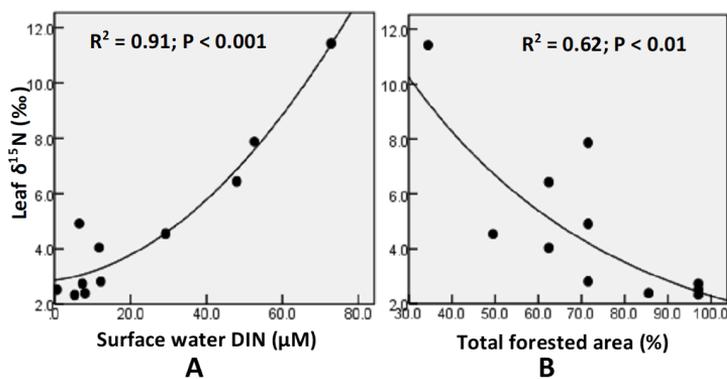


Figure 4. Relationships between mangrove leaf $\delta^{15}\text{N}$ and surface water DIN concentrations (μM) (A), and between mangrove leaf $\delta^{15}\text{N}$ (‰) and the forested area ratio in five mangrove watersheds on Ishigaki Is., Japan

Furthermore, a significant positive correlation was established between mangrove leaf $\delta^{15}\text{N}$ and surface water DIN, which indicated that land-derived reactive nitrogen strongly influenced the mangrove leaf $\delta^{15}\text{N}$ (Figure 4). Despite considerable impacts of human activities on reactive N, the forest covers of the watersheds were the principal driver of the distinguishable imprints of mangrove leaf $\delta^{15}\text{N}$ values, which was reflected by the significant negative relationship between the mangrove leaf $\delta^{15}\text{N}$ and forest area ratio (Figure 4).

4. Conclusion

Modern agricultural methods and human activities may influence external N inputs and control the levels of reactive N. The distinct values of mangrove leaf $\delta^{15}\text{N}$ in five mangrove watersheds reflect different level of land-use and respective load of reactive N. The higher values of mangrove leaf $\delta^{15}\text{N}$ ranging from 4 to 13‰ in the human-influenced watersheds and the lower values of those ranging from 0 to 3‰ in forested watershed differentiated between these two groups of ecosystems. The findings of this study have revealed that the watersheds having the largest value of mangrove leaf $\delta^{15}\text{N}$ might require more attention compared to others for improving ecosystem conditions. Stakeholders, ecologists, environmentalists, and policymakers may take the appropriate conservation and management plan by prohibiting or limiting human interaction as necessary with the mangrove ecosystem.

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