The coral $\delta^{15}N$ record of terrestrial nitrate loading varies with river catchment land use

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Abstract

We analysed the nitrogen isotopes in two coral cores ($\delta^{15}N_{coral}$) from the mouth of the Todoroki River, Ishigaki Island, Japan to examine whether the $\delta^{15}N_{coral}$ reflects the runoff of nitrate related to the land use in the river catchment. The two coral cores were used to examine the seasonal variation in $\delta^{15}N_{coral}$ for 14 years (CORE1; 1993–2007) and the annual variation of $\delta^{15}N_{coral}$ for 52 years (CORE2; 1958–2010). $\delta^{15}N_{coral}$ In CORE1, the 5–month running mean of $\delta^{15}N_{coral}$ was positively correlated with that of monthly precipitation, excluding all strong precipitation events (>150 mm day^{-1}). In CORE2, the $\delta^{15}N_{coral}$ mean in the earlier period (1958–1980) was 1.0‰ greater than that in the later period (1981–2010). The annual averages of $\delta^{15}N_{coral}$ are positively correlated with the total precipitation in the rainy season (May–June) for both time periods. The difference in the $\delta^{15}N_{coral}$ between the earlier and later periods would be caused by the land use changed from paddy fields with ^{15}N-rich manure to sugar cane fields in the early 1980s. Although some uncertainties still remain regarding the precision of $\delta^{15}N$ coral proxy records, this study emphasises the clear potential for their use in reconstructing terrestrial nitrate discharge records from corals.

Keywords: *coral skeletons, nitrogen isotopes, nitrate, land-use, river discharge, coral reefs*

Introduction

Coral reef degradation is rapidly increasing throughout the world due to local and global stresses such as pollution and climate change (Bellwood et al. 2004; Hoegh–Guldberg et al. 2007; De'ath et al. 2012). With anthropogenic development of the areas surrounding coastal reefs, terrestrial runoff into the coral reefs is impacting reef water quality and ecosystem functioning (Fabricius 2005). The detection of nutrient sources is useful to assess reef environments. The nitrogen isotope compositions of seawater, macroalgae, sediments and coral tissue have been used to trace the sources of nitrogen in coral reefs (e.g., Heikoop et al. 2000; Miyajima et al. 2001; Umezawa et al. 2002a, 2008; Yamamuro et al. 2003). Coral skeletons have been widely used as palaeo-environmental archives in coral reefs and communities (e.g., reviewed by Druffel et al. 1997; Gagan et al. 2000; Grottoli and Eakin 2007). Marion et al. (2005) suggested that the nitrogen isotopic signature in the organic matrix of coral skeletons ($\delta^{15}N_{coral}$) records the history of nutrient loading into coral reefs. High-resolution analysis of $\delta^{15}N_{coral}$ has also suggested that $\delta^{15}N_{coral}$ records the origins of nitrogen in coral reefs at the seasonal scale (Uchida et al. 2008; Yamazaki et al. 2011a). $\delta^{15}N_{coral}$ could be a useful proxy to reconstruct nitrogen loading into coral reefs.

The Todoroki River flows into the Shiraho coral reef at the southeast end of Ishigaki Island, and is one of the major nitrate sources for the sites in this study (Blanco et al. 2010). Seasonal variation in the nitrogen isotopic signature of nitrate ($\delta^{15}N_{nitrate}$) at the mouth of the Todoroki River suggests that the nitrate inflow from the Todoroki River is triggered by the rainy season and typhoons (Electronic Supplemental Material, ESM Fig. S1). Another form of dissolved inorganic nitrogen, ammonium, is primarily emitted from reef sediments and sea grass beds, not from the Todoroki River (Miyajima et al. 2001; Blanco et al. 2008).

Yamazaki et al. (2011b) observed a correspondence among the intra reef distribution of $\delta^{15}N_{coral}$ in *Porites*, $\delta^{15}N_{nitrate}$, and $\delta^{15}N$ in macro algae (dissolved inorganic nitrogen (DIN) consumers, reported by Umezawa et al. 2002a).

These results suggested that $\delta^{15}N_{coral}$ in the Shiraho coral reef records the variation of $\delta^{15}N_{nitrate}$. Ammonium could also be a source of DIN for corals. However, as nitrogen sources, the volume of nitrate at the mouth of the Todoroki River is much larger than that of ammonium. The concentrations of ammonium are less than 1% of those of nitrate in this study site (Blanco et al. 2008).

In this study, we examined $\delta^{15}N_{coral}$ as a proxy to reconstruct nitrate loading from terrestrial origins into Shiraho coral reef, Ishigaki Island, Japan. Yamazaki et al. (2011b) reported that a zonal distribution of $\delta^{15}N_{coral}$ was found within the Shiraho coral reef, which corresponded with the $\delta^{15}N$ of seawater nitrate. The distribution of $\delta^{15}N_{nitrate}$ was from two nitrate sources: the Todoroki River flowing into the reef and slight nitrate (<1 μM) in water from the outer reef ocean.

We collected coral cores at the mouth of Todoroki River to reconstruct the origins of nitrate loading into coral reefs. We (1) investigated the relationship between the seasonal variation of $\delta^{15}N_{coral}$ and precipitation, and (2) compared the 52-year record of the annual $\delta^{15}N_{coral}$ to changes in the land-use history in the Todoroki River catchment.

Materials and Methods

Study site

Coral cores were collected at the mouth of the Todoroki River on Ishigaki Island, southwest of the Ryukyu Islands, Japan (24°21’–31’N, 124°4’–16’E); this site has a subtropical climate. The river catchment area is composed of sugarcane, pasture, pineapple and paddy fields (Fig. 1a). The mouth of Todoroki River crosses the coral reef (Fig. 2a). The average annual precipitation is approximately 2000 mm, 60% of which falls in the rainy season from May to June or is caused by a typhoon in August or September.

To analyse the $\delta^{15}N_{nitrate}$ and the oxygen isotopic signature of nitrate ($\delta^{18}O_{nitrate}$), we collected water samples in March and August 2009 from the Todoroki River (RW1–3), ground water in sugar cane (GW1 and GW2), paddy fields (GW3), and a cattle barn (GW4) from the drainage basin (Fig. 1a). We also collected rainwater samples in August 2009.

Based on the data in Table 1 of Yamazaki et al. (2011b), we compared the nitrate concentrations and $\delta^{15}N_{nitrate}$ of seawater samples collected at 50 m intervals along a sampling line from the mouth of the Todoroki River to offshore (Fig. 2b). $\delta^{15}N_{nitrate}$ was positively correlated ($R=0.85$, $P<0.004$) with the logarithm of nitrate concentrations in the range from +2‰ in the outer ocean nitrate to +9‰ in the nitrate from the Todoroki River. Time series analyses of $\delta^{15}N_{coral}$ at the mouth of the Todoroki River demonstrated the $\delta^{15}N_{nitrate}$ changes with terrestrial nitrate discharges.

Coral core sampling

Coral drilling was performed at the mouth of Todoroki River using an air drill (Adachi and Abe 2003). On 2 September 2007, we vertically drilled a 60-cm length core from the top of a live *Porites* coral colony at 272 m offshore from the shoreline (CORE1; project ID: SPc070902, sampled from N24°23'12.40", E124°15'20.56", ~2 m depth). The record distance of CORE1 was 14 years (1993–2007), which was determined in monthly resolution by an age model using carbonate Sr/Ca ratios and oxygen isotopes (K. Ohmori, pers. comm.). The annual growth rate of CORE1 was approximately 20 mm year^{-1} (ESM Fig.S2). Microsampling was performed at 3-mm (~2 month) intervals along the major growth axis to obtain 30–40 mg of coral powder (~100 μmolN). We reconstructed the seasonal variation in the $\delta^{15}N_{coral}$ in CORE1 for the past 14 years. To sample a longer core, we also performed coral micro–atoll boring on 2 September 2010 (CORE2; project ID: IStr-p100902-2, sampled from N24°23'04.6", E124°15'21.5"), 299 m offshore from the shoreline. CORE2, which was 1.2 in length, was horizontally drilled from the side of a micro-atoll with a depth of less than 2 m. The age model of CORE2 was also determined by fitting the density bands of X-radiographs and Sr/Ca ratios and oxygen isotopes (T. Miyaji, personal communication), and its record distance was the sub-annual variation in $\delta^{15}N_{coral.}$ The age models and X-radiographs of the two cores (CORE1 and CORE2) were described in Inoue et al. (2014) and ESM Figure S2.

$\delta^{15}N_{coral}$ analysis

The sampled powder was treated with NaOH (2N, 60°C) for 3 h and rinsed using Milli-Q water to remove the extra organic matter (e.g., algal and fungal bioeroders) in the coral powder according to Yamazaki et al. (2013), who suggested that this cleaning process provides the same values of $\delta^{15}N$ in intra-crystal aragonite as determined by stepwise heating methods. The tissue layer in the core top (< 4 mm depth from the top of the core) was not used for nitrogen isotope analysis.

Nitrogen isotope values of organic nitrogen in coral skeletons were analysed using the method developed by Tsunogai et al. (2008) and Yamazaki et al. (2011b). This method involves oxidation/reduction methods such as the oxidation of organic nitrogen to nitrate using persulphate (Knapp et al. 2005; Tsunogai et al. 2008), reduction of nitrate to nitrite using spongy cadmium, and further reduction of nitrite to nitrous oxide using sodium azide. Wet conditions were maintained throughout the chemical treatments to avoid the evaporation of dissolved organic nitrogen, which would affect the $\delta^{15}N$ values obtained after the re-drying process after acid treatment and to recover $\delta^{15}N$ in coral skeletons without isotope fractionation.

First, organic nitrogen in the coral skeletons was oxidised to NO_3^- using persulphate under alkaline conditions. The coral skeletal powder (28 mg) was decalcified with 0.6 ml of 1 N HCl in 30-ml Teflon bottles for 2 h. Then, 0.4 ml of deionised water (DIW) and 50 μl of oxidising reagent (peroxodisulphate; Tsunogai et al. 2008) were added. The Teflon bottles were capped tightly with Teflon screw caps and autoclaved for 1 h at 121°C. After the samples were cooled for 8 hours, needle crystals of $CaSO_4$ were deposited. A 1-ml volume of the sample solution, excluding the $CaSO_4$ crystals, and 9 ml of DIW were pipetted into 10-ml vials with butyl rubber caps. For two coral samples, we prepared internal standards, including L-alanine ($\delta^{15}N$=+1.78±0.06%AIR), L-histidine ($\delta^{15}N$=-7.96±0.05%AIR), and tuna flakes ($\delta^{15}N$=+12.55±0.06%AIR). Organic nitrogen standards diluted with DIW (400 μM-N) were oxidised to NO_3^- using the same methods. The internal standard samples contained the organic material of the coral skeletons (1 ml), 400 μM-N (1 ml), and 8 ml of DIW in 10-ml vials. Next, NO_3^- was reduced to NO_2^- by adding 0.5 g of spongy cadmium to each vial, followed by 0.3 g of NaCl and 0.1 ml of a 1 M $NaHCO_3$ solution to yield a final pH of approximately 8.5. The samples were then shaken for 5 h on a horizontal shaker at a rate of 2 cycles sec^{-1} Subsequently, NO_2^- was reduced to N_2O using sodium azide. Then, 10 ml of the samples was decanted into a clean 20-ml vial and capped tightly with a butyl rubber cap. After purging with helium to evacuate the air from the headspace and the sample solution for 2 min, 0.4 ml of azide/acetic acid buffer was added to each vial via a syringe, and the mixture was shaken. After 2 h, the solution was made basic by adding 0.2 ml of 8 M NaOH with a syringe and shaking to prevent residual HN_3 from escaping into the laboratory during the subsequent isotopic analysis. These chemical treatments were performed under wet conditions to prevent the evaporation of dissolved organic nitrogen, which would affect the $\delta^{15}N$ values obtained after the re-drying process after acid treatment and to recover all of the nitrogen in the skeletons.

The stable isotopic N_2O composition was determined using our Continuous-Flow Isotope Ratio Mass-Spectrometry (CF-IRMS) system (Tsunogai et al. 2008, Konno et al. 2010; Hirota et al. 2010), which consists of an initial helium purge and trap line, a gas chromatograph (Agilent 6890), and a Finnigan MAT 252 (Thermo Fisher Scientific, Waltham, MA, USA) with a modified Combustion III interface. $\delta^{15}N$ values were determined relative to $\delta^{15}N$ of air. The standard deviation of the coral sample measurements was less than 0.2‰ (σ, n=4) (Yamazaki et al. 2013).

Environmental data

Daily precipitation data for 1958–2010 on Ishigaki Island were available at the website of the Japan Meteorological Agency (http://www.jma.go.jp). Monthly and annual precipitation averages were calculated from the daily data. The 52-year $\delta^{15}N_{coral}$ record was compared with the land-use changes revealed by historic aerial photographs and satellite images from Hasegawa (2011) and Ishihara et al. (2014). Five land-use classes (forest, pasture, pineapple, paddy field and sugarcane) were assigned. Aerial photographs from 1962, 1972, 1977/78, 1986, 1989, 1991, and 1995 were classified by visual interpretation based on the texture of the image (Hasegawa 2011), and satellite images from 2006 to 2008 were classified using the decision tree method (e.g., Pal and Mather 2003) based on a crop calendar as each category has specific seasonal changes in vegetation cover (Ishihara et al. 2014).

Results

Isotope composition of nitrate in the river water

The groundwater (GW1, GW2, GW3, and GW4 in Fig.1a) had higher nitrate concentrations (>450 μM) than the river water. $\delta^{15}N_{nitrate}$ in the river water increased from +5.5–+6.4‰ in the upstream area to +8.5‰ at the mouth. The linear relationship (R=0.91, P=0.01) between $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ suggested that the river water nitrate was a mixture of nitrate from spring water, field drainage, and rainwater (Fig. 1b).

Seasonal variation in $\delta^{15}N_{coral}$ for 1993–2007 (CORE1)

The $\delta^{15}N_{coral}$ in CORE1 varied between +0.1‰ and +11‰ in monthly resolution for 1993–2007 (Fig. 3a). The average $\delta^{15}N_{coral}$ for these 14 years was +6.4‰±4‰ (3σ). The monthly $\delta^{15}N_{coral}$ in CORE1, calculated after linear interpolation to replace the partially missing monthly values, was compared with the monthly total precipitation (Fig. 3a). However, no relationship was found between the $\delta^{15}N_{coral}$ and precipitation in a direct comparison. To define the seasonal characteristics of $\delta^{15}N_{coral}$ and precipitation, the 14-year averages of each monthly value of $\delta^{15}N_{coral}$ and total precipitation were calculated (Fig. 3b, 3c). The rainy and typhoon seasons for Ishigaki Island were in May-June and August-October, respectively (Fig. 3c). The $\delta^{15}N_{coral}$ (Fig. 3b) gradually increased from May to October and then gradually decreased over the following 5 months. The 5-month running mean of $\delta^{15}N_{coral}$ had no correlation with that of monthly total precipitation; however, with the exception of 1993–1995 and 2000–2001, the 5-month running mean of $\delta^{15}N_{coral}$ was positively correlated with that of precipitation less than 150 mm/day (R=0.45, P<<0.0001). Heavy rain (precipitation more than 150 mm/day), indicated by white bars in Figure 3, corresponded with several positive peaks in $\delta^{15}N_{coral}$ but not all. From 1993 to 1995, $\delta^{15}N_{coral}$ was continuously high (>+6‰) regardless of precipitation, and significantly different from 1996-2007 (analysis of covariance; ANCOVA test (Schwarz 2011): P<<0.001). During 2000–2001, the 5-month running mean of $\delta^{15}N_{coral}$ had a positive correlation with that of precipitation of less than 150 mm/day (R=0.70, P<0.0005), and the average of $\delta^{15}N_{coral}$ was +5.3‰, which was 0.7‰ lower than during 1996–2007 excluding 2000–2001. This difference was statistically significant (ANCOVA test: P<<0.001).

The 52-year variation in $\delta^{15}N_{coral}$ for 1958–2010 (CORE2)

The $\delta^{15}N_{coral}$ values in CORE2 varied between +0.5‰ and +14.8‰. The average $\delta^{15}N_{coral}$ decreased from +5.4‰ in 1958–1980 to +4.4‰ in 1981–2010. The annual averages of $\delta^{15}N_{coral}$ in CORE2 were compared with that in CORE1 during 1994-2006 (Fig.4a). During 1994-2006, the variations of $\delta^{15}N_{coral}$ were 2.7‰, (between +4.9‰ and +7.6‰) for CORE1, and 3.9‰ (between +2.9‰ and +6.8‰) for CORE2. The $\delta^{15}N_{coral}$ in CORE1 and CORE2 were not correlated. The average values of $\delta^{15}N_{coral}$ were +6.2‰ and +5.0‰ for CORE1 and CORE2, respectively.

The annual averages of $\delta^{15}N_{coral}$ in CORE2 were compared with the total precipitation of the rainy season (May–June) (Fig. 4a). The cross-plot of the average annual $\delta^{15}N_{coral}$ and the total precipitation of the rainy season showed a weak linear relationship of $R=0.39$ ($P=0.009$) for 1958-2010. The co-relationships between $\delta^{15}N_{coral}$ and total precipitation of the rainy season were shifted to ~1‰ lower for $\delta^{15}N_{coral}$ and 51 mm higher for total precipitation from 1958-1980 to 1981-2010 (ANCOVA test: $P=0.036$ (<0.05)). The linear relationships between $\delta^{15}N_{coral}$ and the total precipitation of the rainy season showed $R=0.80$ ($p<<0.0001$) for 1958–1980 and $R=0.72$ ($p=0.0005$) for 1981–2010 (Fig. 4b), which was statically significant except for 1966, 1974, 1995, 1999, 2002, and 2006. The slopes in the former (1958–1980) and the latter (1981–2010) periods were 144 mm %$^{-1}$and 166 mm %$^{-1}$respectively. In 1966, 1974, 1995, 1999, 2002, and 2006, the $\delta^{15}N_{coral}$ and precipitation values of the rainy season were plotted outside the standard deviation (2σ) from the linear regression lines in the former and latter periods (Fig. 4b). The $\delta^{15}N_{coral}$ values for 1966 and 1974 were -2.5‰ and -3.8‰, respectively, which were along the minima and smaller than expected based on the linear trend of $\delta^{15}N_{coral}$ and precipitation in 1958-1980. By contrast, the $\delta^{15}N_{coral}$ values for 1995, 1999, 2002, and 2006 were +2.5‰, +3.9‰, +3.2‰ and +2.4‰, respectively, which were along the maxima and larger than expected based on the linear trend of $\delta^{15}N_{coral}$ and precipitation in 1981-2010.

Discussion

Possible factors controlling $\delta^{15}N_{coral}$

This study examined the assumption that $\delta^{15}N_{coral}$ at the mouth of the Todoroki River records nitrate loading from terrestrial origins, as suggested by Yamazaki et al. (2011b). The $\delta^{15}N_{nitrate}$ that flows from the Todoroki River into the coral reef is +8.5–+9‰ (Fig.3), indicating that it is affected by the land use in the catchment. $\delta^{15}N$ and $\delta^{18}O$ composition of nitrate has been used for identification of denitrification in river watershed. The dual fractionation factor of $\delta^{15}N/\delta^{18}O$ is ~1.3 for denitrification (Fukuda et al. 2003). In Todoroki River water, the fractionation factor of $\delta^{15}N/\delta^{18}O$ was ~3.0 (Fig. 3), which suggests enrichment of ^{15}N in river water is not affected by denitrification and but affected by external nitrogen sources. We measured the $\delta^{15}N$ of total nitrogen in the agricultural water from the dam, the mixture of chemical fertiliser and manure in the sugarcane fields, and the excretion from cattle using same methods of $\delta^{15}N_{coral}$; the results were +2.5‰, +9.1‰, and +26.6‰, respectively. The mixture of each source comprises the nitrate in the Todoroki River water. In this section, we discuss other possible factors controlling $\delta^{15}N_{coral}$.

Terrestrial sediment. Inoue et al. (2014) reported the records of sediment loading in CORE1 (as core L1) and CORE2 (as core L2) using iron (Fe) and manganese (Mn) as proxies. The effect of sediment loading was much smaller at the CORE1 site than at the CORE2 site (Inoue et al. 2014). In CORE2, sediment loading was small until the 1980s and increased remarkably from the 1990s (Inoue et al. 2014). However, the variations in $\delta^{15}N_{coral}$ in CORE1 and CORE2 were not similar to those of the Fe and Mn proxies, suggesting that terrestrial nitrate discharge is not simultaneous with sediment loading.

Light effect. Muscatine and Kaplan (1994) and Heikoop et al. (1998) reported that deeper coral colonies had increased $\delta^{15}N$ in coral tissues due to increasing coral heterotrophy. The light effect on $\delta^{15}N$ in coral tissues was -1~-2‰ between corals living at the surface and those at 30 m depth (Heikoop et al. 1998). $\delta^{15}N$ differences were not observed in live corals at a depth of less than 10 m (Heikoop et al. 1998). In this study, we examined corals living on a shallow reef (less than 2 m depth). The effect of the position of the core top (~50 cm depth differences between the top and side) should be negligible. The seasonal cycle of solar radiation is at a maximum in July-August and a minimum in December-January in Ishigaki Island, as defined from 5-year solar irradiance data (1993-1997) for N25°, E125° (CAYAN solar irradiance database;

http://iridl.ldeo.columbia.edu/SOUCES/.CAYAN/.Si/). The seasonal variation of $\delta^{15}N$ in CORE1 (Fig. 3b) did not correspond with the seasonal cycle of solar radiation. In Reynaud et al. (2009), coral culture experiments also suggested that light intensity does not affect $\delta^{15}N$ in coral tissue. In this study, the variations of $\delta^{15}N_{coral}$ at the mouth of the Todoroki River cannot be explained by light intensity.

Isotope fractionation between DIN and zooxanthellae. Heikoop et al. (1998) suggested that $\delta^{15}N$ in coral tissues may vary due to isotope fractionation between DIN in seawater and zooxanthellae. Isotope fractionation between DIN and dinoflagellates was reported to be less than 3‰ (Needoba et al. 2003). We cannot explain the 14‰ range of variation in coral $\delta^{15}N$ by fractionation of DIN assimilation of zooxanthellae alone. Yamazaki et al. (2011b) observed a correspondence of $\delta^{15}N$ in nitrate and coral skeletons. This result also suggested that the effect of isotope fractionation is negligible at the mouth of the Todoroki River. For CORE1 and CORE2, possible factors controlling $\delta^{15}N_{coral}$ mainly included $\delta^{15}N_{nitrate}$ in ambient seawater.

Coral $\delta^{15}N$ of the nitrate outflow of the Todoroki River

The $\delta^{15}N_{coral}$ in CORE1 was continuously high (>+6‰) in 1993-1995. Blanco et al. (2010) suggested that the controlling factor of NO_3^--N concentration at the Todoroki River was mostly the percentage of sugarcane cover and bedrock type (e.g., limestone) in the watershed. The percentage of sugarcane cover in Todoroki watershed decreased from 68% in 1995 to 52% in 2006-2008 (Fig.5).

Since 1996, The results of CORE1 (Fig.3a) showed that the 5 month running mean of monthly $\delta^{15}N_{coral}$ corresponded with that of precipitation, except during a flood event (precipitation >150 mm/day). The amount of river discharge correlates with precipitation at present (R=0.78, ESM Fig.S3). At the mouth of the Todoroki River, the $\delta^{15}N_{coral}$ could not respond to flood events because the nitrate concentration of the rainwater runoff was 2–10% of the river/groundwater (Fig. 1, Umezawa et al. 2002b). Blanco et al. (2010) reported that the nitrate concentration at the mouth of Todoroki River tended to decrease during flood events in 2006 (NO_3^--N; 1 mg/l of river discharge at ~8 m^3/s) and to increase after the discharge of the Todoroki River had decreased back to baseflow (NO_3^--N; 3 mg/l of river discharge at 0.5~1 m^3/s), which suggested that 1) most of precipitation run off as floods with lower nitrate concentration, and 2) a portion of the rainwater soaked into the soil of the river catchments, converted to groundwater accompanied by nitrate and gathered in the Todoroki River after the flood event. In ESM Fgure S1, the nitrate concentration and $\delta^{15}N_{nitrate}$ at the mouth of Todoroki River were smaller (118 μM and +8.1‰) on 11 June 2011, two week after the flood event (228 mm/day), than in November 2010 (335 μM and +9.1‰) without flood events. This result suggests that floods dilute nitrate concentration and $\delta^{15}N_{nitrate}$ and that precipitation did not correspond with $\delta^{15}N_{nitrate}$ in flood events. The seasonal variation of $\delta^{15}N_{coral}$ in Figure 3b also suggests that rainwater was reserved in the catchment area as groundwater in the rainy seasons and that nitrate gradually seeped into the river with the stored water over the 4-5 months from November to February. The $\delta^{15}N_{coral}$ in CORE1 recorded the loading of nitrate from the land cover soil through the ground to river flow.

The seasonal variation of $\delta^{15}N_{coral}$ suggests that precipitation less than 150 mm/day controlled the nitrate drainage into the Todoroki River through groundwater. However, during 2000-2001, the $\delta^{15}N_{coral}$ was 0.7‰ lower than the previously discussed 14-year average (Fig. 3), the cause of which is unknown. The total monthly precipitation (except daily precipitation over 150 mm/day) in 2000-2001 was consistently higher than the average of total monthly precipitation over the 14 years (126 mm/month), which suggested the river water discharges increased (ESM Fig.S3). The lower $\delta^{15}N_{coral}$ in 2000-2001 might suggest that nitrate was diluted by the rainwater flow that was not converted to groundwater. The $\delta^{15}N_{coral}$ of CORE1 suggested that the nitrate flowed into the coral reef with precipitation and that the land use in the Todoroki River watershed could be the cause of the quantity of discharged nitrate.

During 1994-2006, the average $\delta^{15}N_{coral}$ in CORE1 were 1.2‰ higher than that in CORE2 (Fig.4a). The annual averages of CORE1 were varied around positive peaks of CORE2. These results suggest that CORE1 was more influenced on the nitrate discharge than CORE2 because the colony of CORE1 was located closer to the shoreline than that of CORE2 (Fig.2a). In the Shiraho coral reef, the inner reef distribution of $\delta^{15}N_{nitrate}$ has large gradient from inshore (~+9‰) to offshore

(~+3‰) (Fig. 2a, Yamazaki et al. 2011b). $\delta^{15}N_{coral}$ was sensitively changed by $\delta^{15}N_{nitrate}$ changes with the locations of the coral colonies.

The nitrate sources have shifted with the land-use history in the catchment area

The annual average of $\delta^{15}N_{coral}$ in CORE2 was correlated with the total precipitation in the rainy season (May–June) (Fig. 4b). The 52-year record of the $\delta^{15}N_{coral}$ in CORE2 was divided into two periods: 1958-1980 and 1981-2010 (Fig. 4). Before 1980, the land use of the Todoroki River catchment was primarily paddy fields, which were transformed into sugarcane fields after 1980 (Fig. 5). The reclamation works began in the Todoroki River catchment after the Okinawa reversion was agreed upon by Japan and the U.S. in 1972. The area of the paddy fields decreased after the late 1970s, and sugarcane fields were cultivated using chemical fertilisers beginning in the 1980s. The linear relationships between the annual average of $\delta^{15}N_{coral}$ and the total precipitation in the rainy season were similar between 1958-1980 and 1981-2010; however, the $\delta^{15}N_{coral}$ values decreased approximately +1‰ (Fig. 4b).

We analysed the nitrogen isotopes of the total nitrogen in the soil in sugarcane fields and manure in the Todoroki River catchment using the same methods as in the $\delta^{15}N_{coral}$ analysis. In the present day, the $\delta^{15}N$ of the mixture of chemical and manure fertiliser was +9‰ in the sugarcane fields. The $\delta^{15}N$ of the manure was +26‰, which was approximately three times greater than that of the fertiliser in the sugarcane fields. The slopes of the linear relationships between $\delta^{15}N_{coral}$ and precipitation were similar before and after 1980 (Fig. 4b). Given that the isotopic composition of the nitrogen source term was approximately three times higher prior to 1980, it may be reasonable to assume that the nitrogen loading was only approximately one-third as high to yield similar relationships between $\delta^{15}N_{coral}$ and precipitation before and after 1980. Before the 1980s, the nitrate concentration in the river water was one-third the nitrate concentration at present (1981–2010), which assumes that 1) manure fertiliser was used in the paddy fields before the 1980s, 2) the rate and magnitude of nitrate transport into the coral reef due to the precipitation rate before and after 1980 were the same, and 3) current values of nitrate in the sugarcane soil and manure were applied to the calculation.

Hasegawa (2011) reported that sea grass bed coverage of Shiraho Coral Reef was increased from 1.2% in 1972 to 7.2% in 2004 from the aerial photographs, which also suggested terrestrial nutrient input was increased in these 30 years.

It was unclear why there were six outliners of $\delta^{15}N_{coral}$ in CORE2. The outliners of $\delta^{15}N_{coral}$ values in the pre-1980s, those for 1966 and 1974 were 2.5 and 3.8‰, respectively, which was lower than expected even though the total precipitation of the rainy season was higher than 700 mm. The years 1966 and 1974 had the second and third most precipitation before 1980, which might indicate that abundant rainwater runoff flowed into the coral reefs through the river and diluted the nitrate concentrations at the river mouth, as was recorded in 2000-2001 for $\delta^{15}N_{coral}$ in CORE1. By contrast, the four maxima $\delta^{15}N_{coral}$ values in 1995, 1999, 2002, and 2006 were 2.4–3.9‰ higher than expected. In typhoon events, it is possible that the nitrogenous nutrients in the fertiliser were directly loaded with sediments into the coral reefs through the Todoroki River. In Ishigaki Island, the sediment loading from the paddy fields into the river is smaller than usual in flood event because the paddy fields work as sediment reservoir (Ikeda et al. 2009). However, instead of the paddy fields, the sugarcane fields prospered in the Todoroki River catchments since 1980, especially after the late 1980s (Fig. 5). The fertiliser inflow with sedimentation might have influenced the four maxima of the $\delta^{15}N_{coral}$. We compared the $\delta^{15}N_{coral}$ values for 1995, 1999, 2002, and 2006 with the total precipitation in the typhoon season (August to October) (triangle points in Fig. 4b). The modified cross-plots in 1995, 1999, and 2006 were plotted within the standard deviation (2σ) from the linear regression lines. However, the typhoon precipitation in 2002 resulted in much higher $\delta^{15}N_{coral}$ values, the reason for which remains unknown and needs future works.

The $\delta^{15}N_{coral}$ at the mouth of the Todoroki River recorded the nitrate discharge caused by the precipitation mainly in the rainy season, and the nitrate sources changed with the changing land use in the Todoroki River catchment. Although future work is needed to give the spatial and temporal inconsistencies of the proxy relationship, the $\delta^{15}N_{coral}$ can be used as a proxy for the source and discharge pattern of terrestrial nitrate in coral reefs.

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Figure Legends

Fig. 1. Land-use and nitrate isotope compositions of the Todoroki River catchment. (a) Land use of the Todoroki river catchment in October 2010. The white (○) and black (●) circles show the nitrate concentrations with the $\delta^{15}N_{nitrate}$ in river water (RW) and ground water (GW), respectively. The RW samples were taken in March and August 2009. The GW, and rainwater were also sampled in August 2009. (b) Cross-plot of the $\delta^{15}N_{nitrate}$ and $\delta^{18}O_{nitrate}$ in RW, GW and rainwater from August 2009. Isotope composition of RW1 was affected by rainwater. Except for RW1, the correlation coefficient was R=0.91 (p=0.01).

Fig. 2. (a) The relationships between the distance of the sampling sites from the shoreline and $\delta^{15}N$ in *Porites* coral skeletons, seawater nitrate, and macro algae (DIN consumers), compiled from Yamazaki et al. (2011a) and Umezawa et al. (2002). $\delta^{15}N$ values decreased from +9‰ inshore to +2~+3‰ offshore. (b)The relationship between the nitrate concentration and the $\delta^{15}N_{nitrate}$ in the seawater of the Shiraho coral reef. The $\delta^{15}N_{nitrate}$ from +2 to +9‰ positively correlates with the natural logarithm of the nitrate concentration (R=0.85, p<0.004).

Fig. 3. (a) The time series of the $\delta^{15}N_{coral}$ (green line graph with dots) in CORE1 from 1993 to 2007 compared with the monthly total precipitation for Ishigaki Island. The blue bar graph shows the monthly total precipitation without the heavy precipitation (>150 mm/day, white bar). The black lines indicate five–month running averages. The 5-month running mean of $\delta^{15}N_{coral}$ was positively correlated with that of precipitation of less than 150 mm/day (R=0.48, P<0.0001), with the exception of 1993–1995 and 2000–2001. From 1993 to 1995, $\delta^{15}N_{coral}$ was continuously high (>+6‰), regardless of precipitation. During 2000–2001, the 5-month running mean of $\delta^{15}N_{coral}$ was positively correlated with that of precipitation of less than 150 mm/day (R=0.62, P<0.0005), and the average of $\delta^{15}N_{coral}$ was +5.3‰, which was 0.7‰ lower than during 1996–2007 excluding 2000–2001 (b) The bar graph of 14-year averages of 12 monthly $\delta^{15}N_{coral}$ in CORE1. (c) The bar graph of 14-year averages of 12 monthly total precipitations.

Fig. 4. (a) The time series of the $\delta^{15}N_{coral}$ in CORE2 from 1958 to 2010 compared with the bar graphs of the total precipitation in the rainy season (May–June). The dotted line shows the analysed $\delta^{15}N_{coral}$ in sub-annual to annual intervals. The green bold line shows the annual averages with the plots of the symbols corresponding with (b). The red solid line shows annual average of $\delta^{15}N_{coral}$ from CORE1. (b) The cross plot between the annual average of $\delta^{15}N_{coral}$ and the total precipitation in the rainy season (May–June); black circles (●) indicate 1958–1981, and white circles (○) indicate 1981–2010. The outliners in 1966 and 1974 are shown as grey circles (●). The outliners of $\delta^{15}N_{coral}$ in 1995, 1999, 2002, and 2006 are showed as white circles (○) compared with precipitation in rainy season (May-June), and white circles connected with dashed line to triangles are compared with precipitation in typhoon season (September-October). The correlation coefficients (R) are 0.80 (P<<0.0001) and 0.72 (P=0.0005) in the former (1958–1980) and latter (1981–2010) periods, respectively. The ranges of standard deviation (2σ) are indicated as green and yellow shades for the former and latter periods. The slopes are 166 mm ‰$^{-1}$ and 144 mm ‰$^{-1}$, respectively. The cross-plots of average $\delta^{15}N_{coral}$ and average total precipitation in May-June are described as a black square (■: +5.4‰, 430mm) and a white square (□: +5.4‰, 430mm) for the former and latter periods.

Fig. 5. The land-use history in the Todoroki River catchment and pie charts of the area ratios.

Fig. S1. The seasonal variation of nitrogen isotopes and nitrate concentrations at the mouth of the Todoroki River. The nitrogen isotopes in the samples of nitrate concentrations less than 0.5μM are unknown due to the detection limits. The allows indicate the direction of prevailing currents based on Tamura et al. (2007)

Fig.S2. X-radiographs and analytical lines for CORE1 and CORE2.

Fig.S3. Relationship between monthly river discharge and monthly precipitation in the Todoroki River catchment. The data was collected from November 2006 to November 2007 by the government of Okinawa prefecture.

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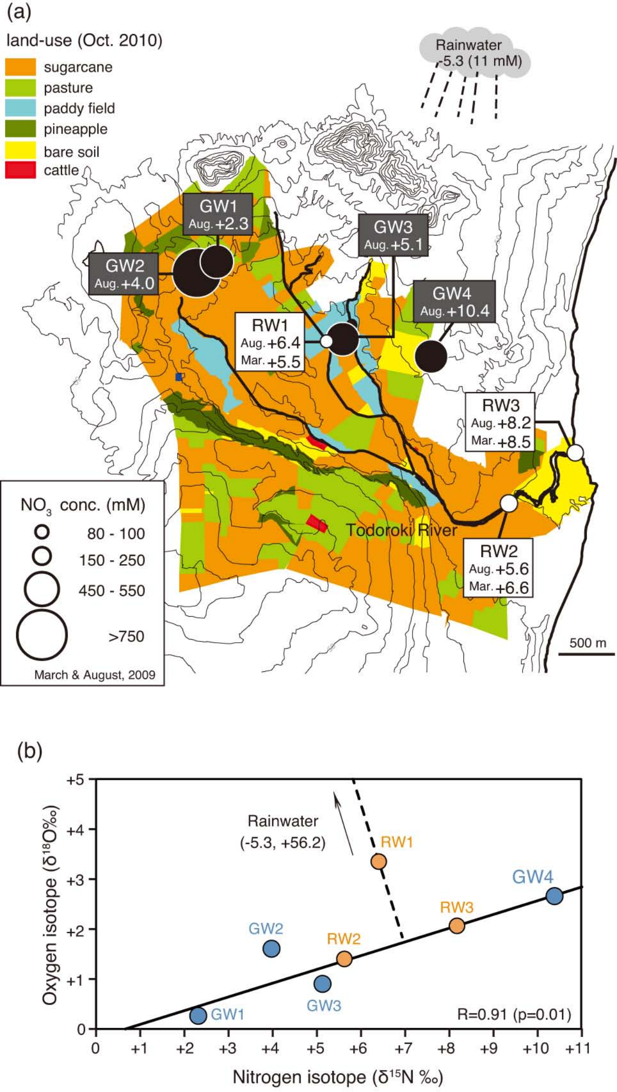
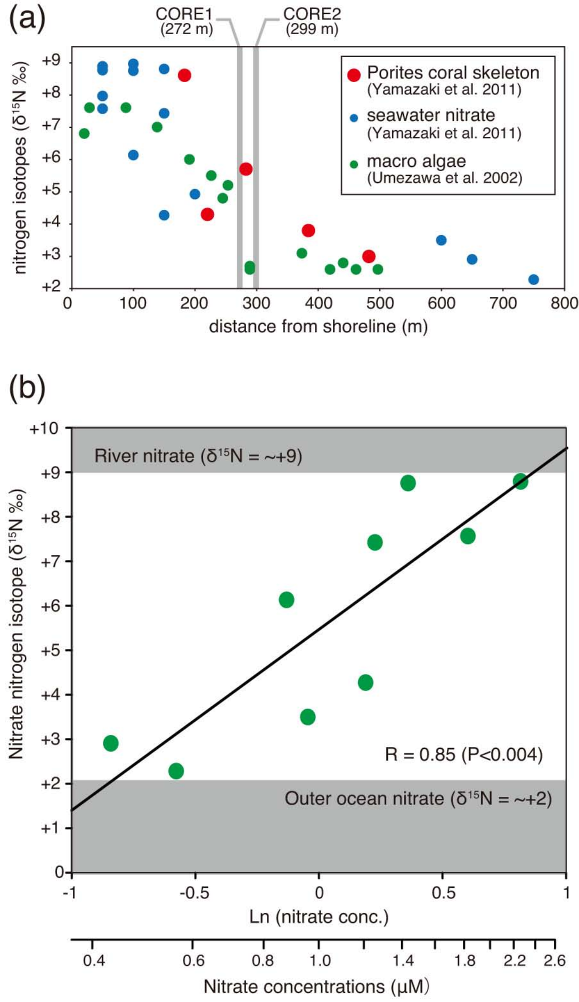
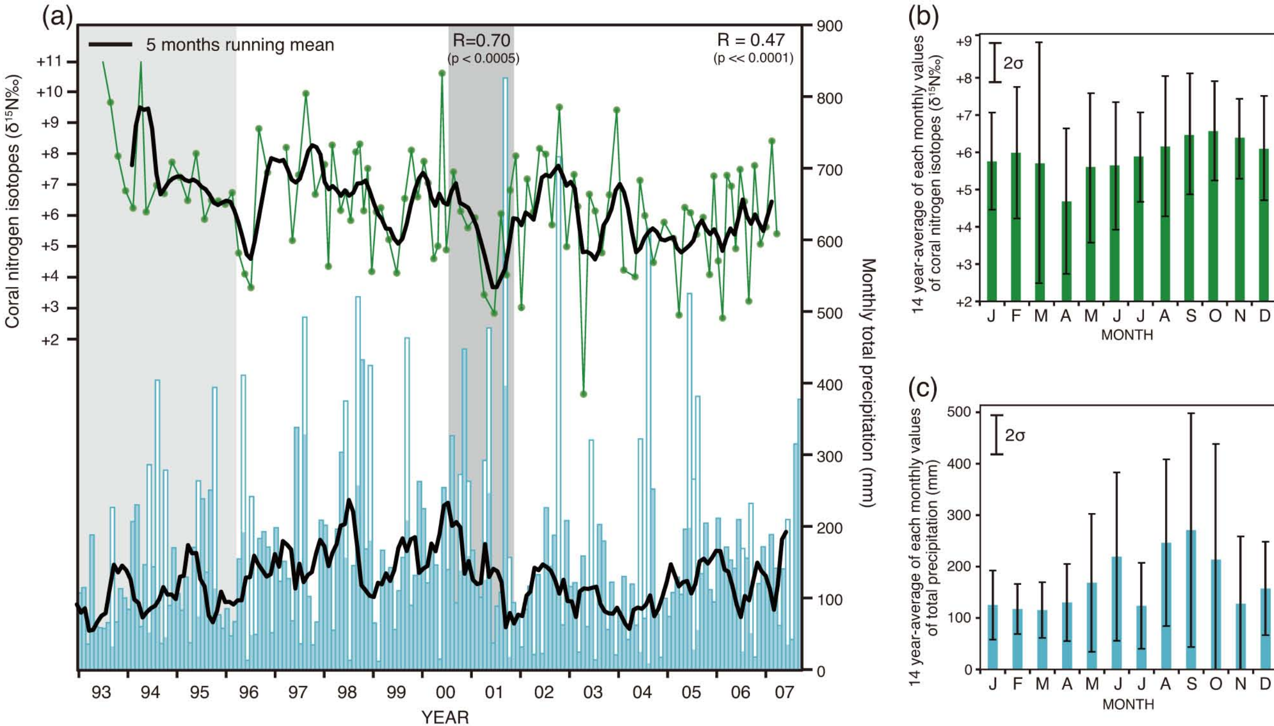
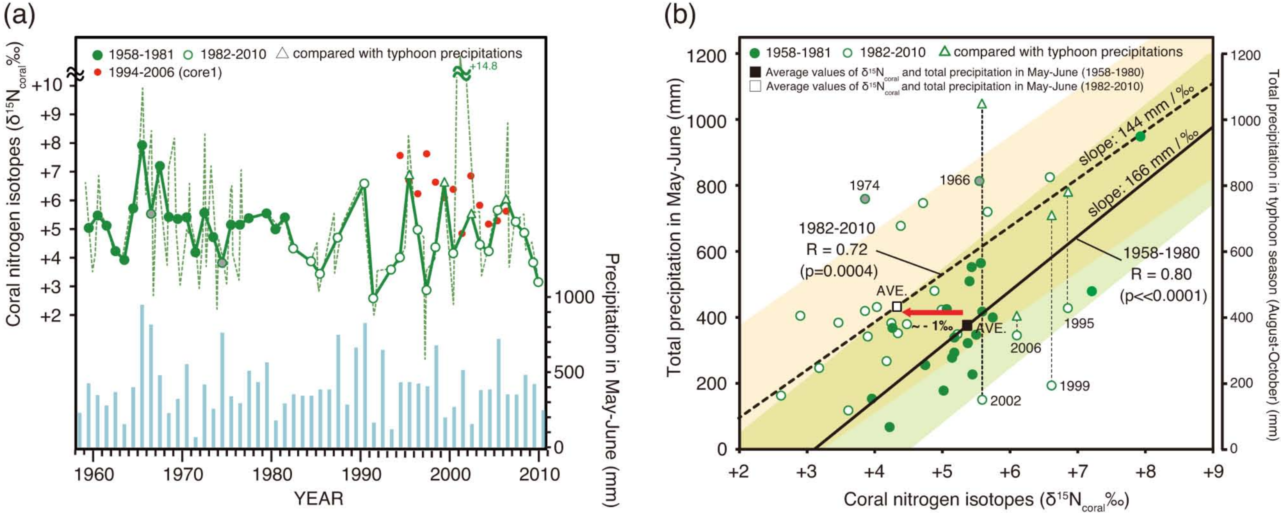


(a)
land-use (Oct. 2010)
sugarcane
pasture
paddy field
pineapple
bare soil
cattle
Rainwater
-5.3 (11 mM)
GW1
Aug. +2.3
GW2
Aug. +4.0
GW3
Aug. +5.1
GW4
Aug. +10.4
RW1
Aug. +6.4
Mar. +5.5
RW3
Aug. +8.2
Mar. +8.5
RW2
Aug. +5.6
Mar. +6.6
Todoroki River
NO_3 conc. (mM)
80 - 100
150 - 250
450 - 550
>750
March & August, 2009
500 m
(b)
Rainwater
(-5.3, +56.2)
RW1
GW4
RW3
GW2
RW2
GW3
GW1
R=0.91 (p=0.01)
Oxygen isotope ($\delta^{18}O$‰)
Nitrogen isotope ($\delta^{15}N$ ‰)
0
+1
+2
+3
+4
+5
+6
+7
+8
+9
+10
+11

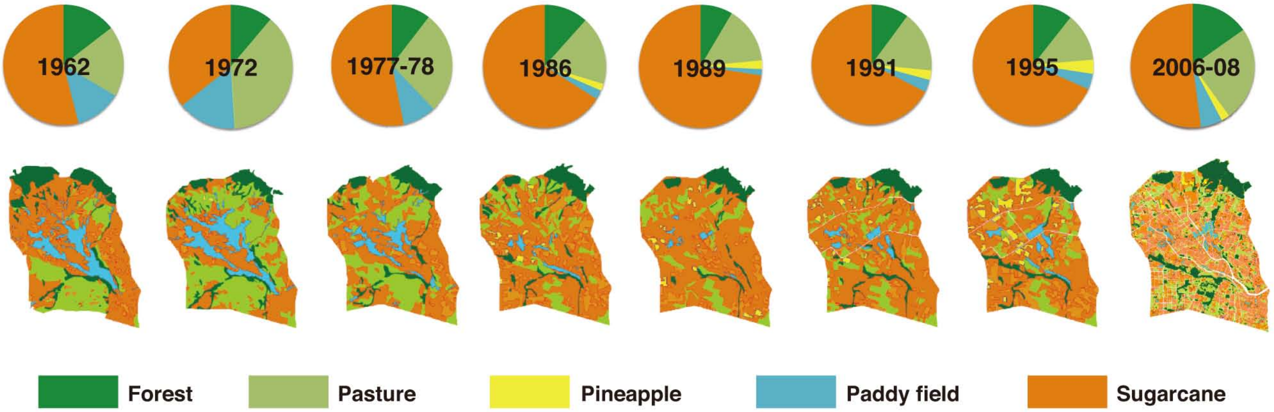


(a)
CORE1 (272 m)
CORE2 (299 m)
Porites coral skeleton (Yamazaki et al. 2011)
seawater nitrate (Yamazaki et al. 2011)
macro algae (Umezawa et al. 2002)
nitrogen isotopes (δ15N ‰)
distance from shoreline (m)
(b)
River nitrate (δ15N = ~+9)
R = 0.85 (P<0.004)
Outer ocean nitrate (δ15N = ~+2)
Nitrate nitrogen isotope (δ15N ‰)
Ln (nitrate conc.)
Nitrate concentrations (μM)

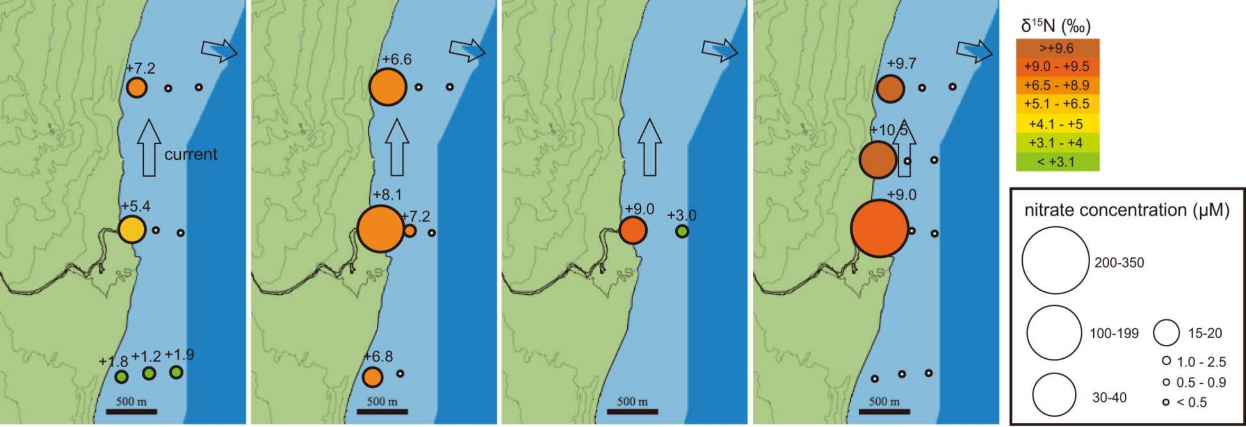

(a)
5 months running mean
R=0.70
(p < 0.0005)
R = 0.47
(p << 0.0001)
Coral nitrogen isotopes (δ15N‰)
+11
+10
+9
+8
+7
+6
+5
+4
+3
+2
Monthly total precipitation (mm)
900
800
700
600
500
400
300
200
100
0
93
94
95
96
97
98
99
00
01
02
03
04
05
06
07
YEAR
(b)
2σ
14 year-average of each monthly values of coral nitrogen isotopes (δ15N‰)
+9
+8
+7
+6
+5
+4
+3
+2
J F M A M J J A S O N D
MONTH
(c)
2σ
14 year-average of each monthly values of total precipitation (mm)
500
400
300
200
100
0
J F M A M J J A S O N D
MONTH



(a)
1958-1981
1982-2010
compared with typhoon precipitations
1994-2006 (core1)
+14.8
Coral nitrogen isotopes ($\delta^{15}N_{coral}$‰)
+10
+9
+8
+7
+6
+5
+4
+3
+2
1000
500
0
Precipitation in May-June (mm)
1960
1970
1980
1990
2000
2010
YEAR
(b)
1958-1981
1982-2010
compared with typhoon precipitations
Average values of $\delta^{15}N_{coral}$ and total precipitation in May-June (1958-1980)
Average values of $\delta^{15}N_{coral}$ and total precipitation in May-June (1982-2010)
Total precipitation in May-June (mm)
Total precipitation in typhoon season (August-October) (mm)
1200
1000
800
600
400
200
0
slope: 144 mm / ‰
slope: 166 mm / ‰
1974
1966
1982-2010
R = 0.72
(p=0.0004)
1958-1980
R = 0.80
(p<<0.0001)
AVE.
~ - 1‰
AVE.
2006
1995
1999
2002
+2
+3
+4
+5
+6
+7
+8
+9
Coral nitrogen isotopes ($\delta^{15}N_{coral}$‰)



Land-use history of Todoroki watershed

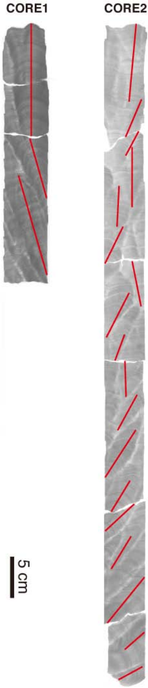


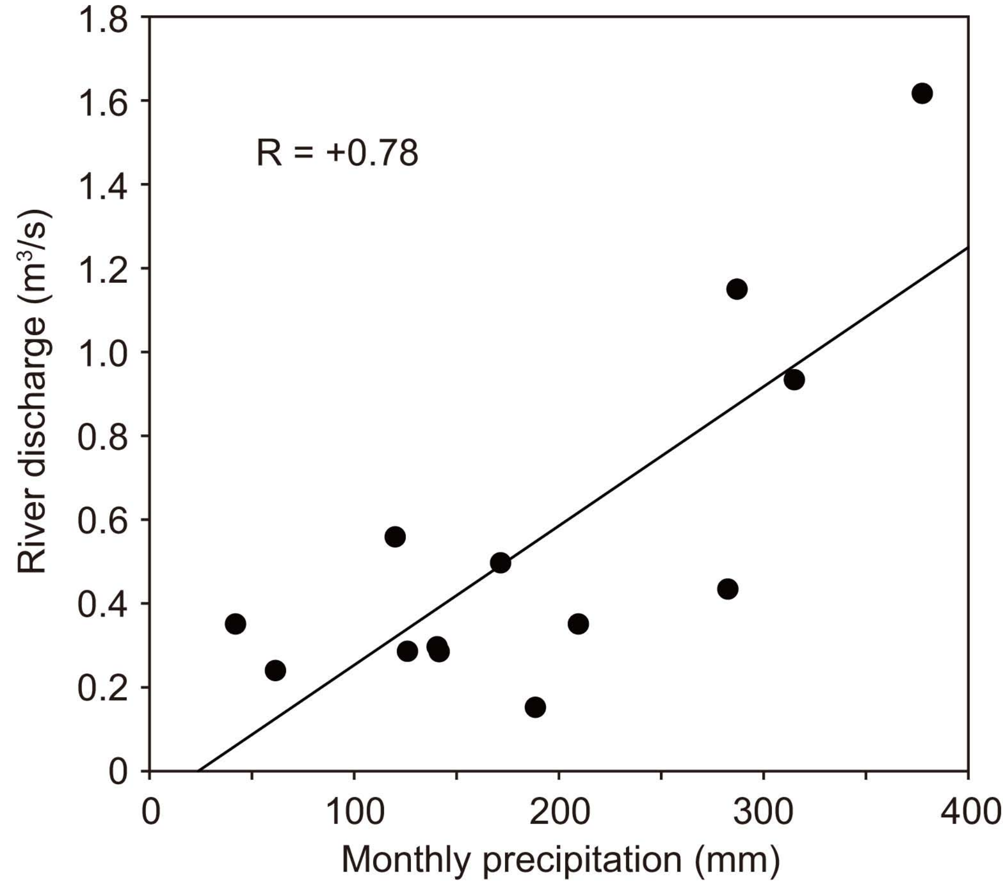
Mar 2011
Precipitation rate: 8.7 mm/day

Rainy season (Jun 2011)
Precipitation rate: 18.5 mm/day
Flood events: 288 mm/day (5/28)

Aug 2009
Precipitation rate: 6.3 mm/day

Nov 2010
Precipitation rate: 12.8 mm/day


CORE1
CORE2
5 cm



1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
River discharge (m3/s)
R = +0.78
0
100
200
300
400
Monthly precipitation (mm)