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To cite this article: Maximo Larry Lopez Caceres, Sayako Nakano, Juan Pedro Ferrio, Mika Hayashi, Takeshi Nakatsuka, Masaki Sano, Toshiro Yamanaka & Yoshihiro Nobori (2018): Evaluation of the effect of the 2011 Tsunami on coastal forests by means of multiple isotopic analyses of tree-rings, Isotopes in Environmental and Health Studies, DOI: 10.1080/10256016.2018.1495203

To link to this article: https://doi.org/10.1080/10256016.2018.1495203

Published online: 12 Jul 2018.

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Evaluation of the effect of the 2011 Tsunami on coastal forests by means of multiple isotopic analyses of tree-rings

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ABSTRACT
The March 2011 Mega-Tsunami in eastern Japan damaged at different degrees the black pine (Pinus thunbergii) forests along the coast. In order to evaluate the recovery of black pine four years later, tree-ring samples from 9 trees for the period 2002–2014 were analyzed for ring growth and stable isotopes (δ13C, δ15N and δ18O). The results showed that annual tree-ring width decreased approximately 70% from the year 2011 to 2014 compared to the period previous to the tsunami (2002–2010). The multiple isotopic analyses showed that the reduction in growth was caused by soil salinity that prompted stomatal closure and an abrupt increase of tree-ring δ13C. Sea water deposition in the soil did not affect tree-ring δ18O values. Two years after the tsunami, decreasing tree-ring δ13C values caused by apparently photosynthetic recovery did not translate into radial tree-growth, indicating a possible shift in carbon allocation to foliage and mainly roots as a defense mechanism to sodium toxicity. The dual δ13C-δ18O model explains neither the limited growth nor the subsequent recovery in δ13C. Similarly tree-ring δ15N indicated that there was no difference in nitrogen availability before and after the tsunami, suggesting that nutrients were not a limitation but rather soil salinity.

ARTICLE HISTORY
Received 25 November 2017
Accepted 28 May 2018

KEYWORDS
Carbon-13; coastal forests; isotope ecology; nitrogen-15; oxygen-18; soil salinity; tsunami; tree-rings

1. Introduction
Coastal forests in Japan are mainly composed of black pine (Pinus thunbergii Parl.) and have been widely planted to protect inland agriculture and local communities from strong winds and salt-spray coming from the sea [1] and for tsunami mitigation [2]. The Mega-Tsunami that followed a magnitude 9.3 earthquake that struck off the coast of northeastern Japan in March 2011 damaged the coastal forest areas to different degrees [3]. Among the factors that affected the trees, the most significant ones were the physical impact of the incoming and outgoing waves [4], sea water residence in the soil [5,6], deposition of ocean sediments, and their chemical components in the
soil [6–8]. Additionally, negative effects on the rhizosphere were caused by ground subsidence in relation to the deterioration of ground water drainage [6]. Black pine trees are remarkably resilient to changes in environmental conditions such as strong acidity caused by increases in avian nitrogen deposition [9,10] and alkalinity by salt deposition from salt spray [1]. As with most pine species, black pine grows well in sandy to sandy-loamy soils, such as in the coastal soils of Japan [11]. Considering the high water infiltration rate of these soils, salinization largely decreased during the first year after the tsunami because of high precipitation in this region as shown by Ono and Hirai [12]. In contrast, in agricultural areas of Miyagi Prefecture, where fine soils (clay, silt) are common, high salt concentration in the soil was still observed by the summer of 2012 [5].

After the tsunami, the impact of the disturbance on trees was visible by the discoloration of leaves or early defoliation and in some cases irreversible decay in black and red pine (Pinus densiflora) [13]. Until now most studies carried out have focused on the immediate physiological recovery of trees [14]. Annual tree-ring growth and isotope analyses (carbon, oxygen and nitrogen) offer a potential tool to analyze the temporal, physical, chemical and physiological changes in the coastal forest ecosystem following the tsunami. Stable carbon isotope analysis in tree-rings has been widely used to estimate intra-annual as well as inter-annual soil moisture variability in dry environments, given its strong association with changes in stomatal conductance [15–18]. However, in wet environments, like in eastern Japan where drought stress is rare, irradiance and growing season temperature controlled the balance between stomatal conductance and photosynthetic rate and thus the stable carbon isotope ratio [16,19,20]. On the other hand, salinity in the soil generates chemical signals in the roots that prompt stomatal closure [21–23], restricting CO₂ diffusion and supply to ribulose-1.5-bisphosphate carboxylase/oxygenase (RuBisCO) and thereby inhibiting C-fixation. Even for short-time periods high soil salinity is known to cause hyperionic and hyperosmotic stress for plants [24].

The inter-annual variation of oxygen isotopes in tree-rings reflects the different sources of water, thus being able to track changes in the stable isotope composition of precipitation [16], which in turn is associated with temperature, orography and the origin and intensity of precipitation [16,25]. In this regard, the use of alternative water sources with distinct signature, such as the isotopically enriched seawater during marine intrusions [26] can also be reflected in xylem water δ¹⁸O. On the other hand, oxygen isotopes also carry a leaf-derived signal, related to the enrichment of leaf water during transpiration, which imprints a humidity and stomatal response in the sugars that constitute the building blocks for wood cellulose [16,27]. Taking into consideration the stomatal effect on δ¹⁸O, a dual-isotope δ¹³C–δ¹⁸O model can also be used as a complementary stress indicator in the study of tree decline, allowing to discern stomatal and assimilation effects on δ¹³C [28,29].

Nitrogen isotopes in tree-rings have been used in multiple coastal forest sites in Japan to evaluate the long-term effect of avian N deposition on soil fertilization [9,10,30] and nitrogen exchange between black pine and invasive leguminous species [31]. Based on the environmental disturbance caused by the tsunami and the consequent physiological response of trees, as inferred from isotope analysis, we have three hypotheses about the possible effects of the tsunami on pines: (I) Salt deposition in the soil has led to a decrease in tree growth driven by a decrease in stomatal conductance and photosynthetic rate. (II) Trees absorbed a mixture of sea water and fresh water from the soil, which is reflected in the cellulose δ¹⁸O due to the highly enriched signal of sea water. (III) The partial removal of
the AO soil horizon by sea waves and the subsequent deposition of tsunami sediments has changed or decreased the nitrogen availability to trees.

Thus, the objectives of this study are (1) to compare tree growth patterns nine years pre- and four years post-tsunami and (2) to determine by means of stable isotope ratios of carbon, nitrogen and oxygen the factors that have affected coastal forest growth after the tsunami.

2. Methodology

2.1. Study site

The study site is located in the coastal area (38 °24′56″N; 141 °21′02″E) of Ishinomaki in the Miyagi Prefecture, where the coastal forest is composed of black pine (P. thunbergii Parl.), and planted 70 years ago (Figure 1). The mean annual air temperature, precipitation, wind speed and relative humidity for this region is 11.8 °C, 1118 mm, 4.2 m s⁻¹ and 74 %, respectively (Ishinomaki Meteorological station, 38 °25′35″N; 141 °17′36″E, 5.4 km NW of the coastal forest). The distance from the coast line to the mountains is approximately 2 km, which increased the height of the sea water level intrusion. The coastal forest in this area is 30 m wide and 1000 m long. In this area we selected a plot that extended 50 m along the coast in N–S direction and 10 m in E–W direction, with all the area being equally affected by sea water flooding. The meteorological conditions before and after the tsunami were the same in 2002–2010 and 2011–2014 (Figure 2(a)). In 2011, precipitation from May to August was close to the mean value for this period, but in September a typhoon brought 350 mm of rain, which equals 25 % of the annual precipitation for this region. For the period April to October 2011, the cumulative rainfall was 743 mm (Figure 2(b)).

Figure 1. Study site on the coasts of Ishinomaki city (38 °24′56″N, 141 °21′11″E), Miyagi Prefecture, Japan.
2.2. Soil analysis

Soil samples were collected in June 2015 in the selected plot (10 m × 50 m) every 5 m in a grid form at three depths: 0–5, 10–15 and 20–25 cm. In total, 33 samples were collected at each depth. The samples were bagged, brought to the laboratory and air dried. The pH and electrical conductivity (EC) in the soil were measured with a pH-metre (Twin pH B-212; Horiba, Japan) and an EC metre (B-173; Horiba, Japan). EC is used as an indicator of salinity. The soil was mixed with deionized water in a ratio of 1:5 prior to measuring pH and EC.

The spatial distribution of EC and pH in the study site were plotted by using the software Surfer (Golden software, USA) and combined with the distribution of black pine trees in the study site plotted by the software Forest Window [32].

2.3. Tree-rings

Nine black pine trees, representative of the coastal forest, were selected for tree core sampling. The cores were collected at breast height with an increment borer of 10 mm diameter. For each tree, two opposite radii in the north and west of the tree were collected. The tree cores were polished until the rings were visible, then they were scanned and

Figure 2. Meteorological conditions in Ishinomaki. (a) Rain and air temperature ($T_a$) for the period 2002–2014, (b) Monthly precipitation and cumulative rainfall for the period April–October 2011.
analyzed with the software Windendro (Regents Instruments Inc., Canada). Once the 13-year (2002–2014) tree-ring series were established, the tree-ring indices (TRI) were calculated as the ring width in a given year divided by the average of the ring width for the period 2002–2014 [33].

2.4. Stable isotopes analysis

After cross dating, the individual annual rings starting from 2002 to 2014 were carefully cut (avoiding the surface that was polished by cutting it with a surgical knife) and each ring was put in individual vial bins. Each sample was cut in two small pieces: one portion was used for α-cellulose extraction for δ¹³C and δ¹⁸O analyses and the other for δ¹⁵N analysis. Cellulose purification followed the method of Brendel et al. [34] as modified by Gaudinski et al. [35], and adapted to small-size samples. Briefly, 10–30 mg of wood were soaked with a 10:1 mixture of acetic (80 %) and nitric (69 %) acid at 120 °C for 45 min in 2.0 ml polypropylene tubes, followed by a series of rinses using ethanol, deionized water. The samples were then soaked with 17 % w/v NaOH and repeatedly rinsed with de-ionized water, diluted acetic acid and acetone. Finally, the samples were kept overnight in a desiccator and then placed in an oven for 2 h at 50 °C [36].

The isotopic compositions of carbon (¹³C/¹²C) and oxygen (¹⁸O/¹⁶O) were determined using an elemental analyzer (EA1110, ThermoQuest, Italy) linked to a Delta PLUSXL gas isotope ratio mass spectrometer (ThermoFinnigan, Bremen, Germany). Merck cellulose (Merck KGaA, Darmstadt, Germany) was the standard used for tree-ring ¹³C/¹²C and ¹⁸O/¹⁶O measurements. The stable isotope ratios are expressed using the δ-notation by the equation δ = (R_sample/R_standard − 1)*10³ (‰), where R denotes ¹³C/¹²C and ¹⁸O/¹⁶O of sample and standard, respectively. The analytical precision (σ) is 0.2 %‰ (δ¹⁸O) and 0.1 %‰ (δ¹³C), respectively. The effect of atmospheric δ¹³C decline was corrected using an extrapolation of the trend published by McCarroll et al. [37] and the δ¹³C air during the 1991–2014 periods for the nearest NOAA-GMD network station (Tae-ahn Peninsula, Korea) [38]. Between the two corrections there is no difference in the trends, but a small offset (irrelevant for the purpose of our study), and even for the uncorrected data the observed trends are nearly identical, due to the short period of study (only 12 years). For the NOAA data, we simply subtracted the trend line of δ¹³C air and adjusted to pre-industrial δ¹³C air value (1850: −6.51 %‰; according to McCarroll & Loader [16]).

For the isotopic composition of nitrogen (¹⁵N/¹⁴N analysis, expressed in δ¹⁵N) the portion of each individual ring whole wood was placed in a glass beaker containing excess amounts of a toluene/ethanol mixture (1:1) with intermittent agitation using sonic waves (Branson, Kanagawa, Japan) to facilitate the extraction, and kept overnight at room temperature (15–20 °C). The resultant supernatant solution was decanted off, and the extraction was continued until no more waxes were visible [30]. δ¹⁵N values of dry ring samples were determined using a continuous-flow isotope ratio mass spectrometry (CF-IRMS) on an IsoPrime (GV Instruments, Cheadle Hulme, UK). The in-house standard is acetanilide (−0.89 %‰) and the standard deviation under laboratory conditions is less than 0.2 %‰. Since the amount of wood material for some trees was not enough for δ¹⁵N analysis, only seven trees were used.
2.5. **Statistical analysis**

The tree-ring growth and the isotope ratio were subjected to the analysis of variance (ANOVA) to compare the temporal effect of the tsunami on tree-rings. The difference between means was compared by Tukey’s test at a significance level of 0.05. Standard errors were also calculated.

3. **Results**

3.1. **Soil chemical characteristics**

Low values of soil EC were found four years after the tsunami in the study site. The range of these values fluctuated between 0.05 and 0.20 dS m\(^{-1}\) in the 0–5 cm soil layer, depending on the area within the plot. The same spatial concentration pattern was observed in the 10–15 cm soil layer with values ranging between 0.07 and 0.23 dS m\(^{-1}\) (Figure 3(a)). The mean values of EC per layer were 0.11 ± 0.04 dS m\(^{-1}\) (0–5 cm layer), 0.12 ± 0.04 dS m\(^{-1}\) (10–15 cm layer) and 0.08 ± 0.02 dS m\(^{-1}\) (20–25 cm layer). Soil pH also showed alkaline values in the 0–5 cm layer (7.3–7.6), while deeper layers showed lower values (5.8–7.0) at 10–15 cm and 5.8 to 6.3 at 20–25 cm (Figure 3(b)). Of the 55 trees in the plot, 16 trees withered within the plot after the tsunami.

3.2. **Changes in tree growth**

For the period 2002–2010, the tree-ring index was in average 1.3 ± 0.3 with lower values found in 2003 (1.01) and 2006 (0.91), which were significantly lower than most of the tree-rings formed in 2002–2010 but significantly higher in comparison to the tree-rings formed after the tsunami (0.4 ± 0.1). The decrease in 2011 was followed by a further decrease in 2012 and 2013 and a slight increase in 2014; however, all of these changes were not significantly different (p < 0.05) (Figure 4).

3.3. **Tree-ring isotope ratios**

δ\(^{13}\)C values were on average −25.2 ± 0.1 ‰ for the period 2002–2010 and −24.0 ± 0.2 ‰ for the period 2011–2014, and they were significantly different (p < 0.05) with the last two years showing a decreasing trend, although not significant in comparison to 2011 and 2012. The annual variation of tree-ring δ\(^{13}\)C for the period 2002–2010 was on average 0.2 ‰ while the difference between 2010 and 2011 was 1.3 ‰ (Figure 5(a)).

The δ\(^{18}\)O values exhibited a high annual fluctuation for the 13-year period. The mean δ\(^{18}\)O values for the period prior to the tsunami (28.0 ± 0.3 ‰) were similar to those following it (27.7 ± 0.5 ‰), except for 2012 when the lowest value (27.0 ‰) was recorded. However, the values observed for 2013 and 2014 were similar to those observed from 2002 to 2010 (Figure 5(b)).

The tree-ring δ\(^{15}\)N did not show any significant variation between the pre-tsunami (−3.8 ± 0.6 ‰) and post-tsunami (−3.7 ± 0.4 ‰) periods (Figure 5(c)). The δ\(^{15}\)N values were in agreement with those found in tree-rings of black pine trees in other undisturbed coastal forests in Japan [9,10,30,31].
4. Discussion

4.1. Soil conditions before and after the tsunami

Salt concentration in the plot four years after the tsunami was relatively low and in agreement with values found in undisturbed coastal forests of Miyagi [6]. According to a

Figure 3. The trees in the plot were in average 70 years old, and trees in light colour were the trees sampled for this study. (a) Soil electrical conductivity (dS m$^{-1}$) at three soil layers (0–5, 10–15 and 20–25 cm) in the study plot, (b) Soil pH at three soil layers (0–5, 10–15 and 20–25 cm) in the study plot.
previous study, soil salinity or ocean water residence time in the soil was short [12] due to high precipitation in May and September 2011 (Japan Meteorological Agency). However, the spatial difference in EC suggests the presence of depressions within the study site where salt accumulation was longer. Accordingly, these areas coincided with a cluster of 16 dead trees found in the study site, which suggests that the surviving trees were exposed to a lower level of salinity or a shorter period of salt exposure.

4.2. Tree-ring growth and tree-ring δ\textsuperscript{13}C and δ\textsuperscript{18}O

The meteorological conditions in Ishinomaki were similar before and after the tsunami; therefore, the increase of δ\textsuperscript{13}C in tree-ring wood after the tsunami could not be explained by changes in atmospheric conditions. Tree-ring δ\textsuperscript{13}C inter-annual variation from 2002 to 2010 was in the range of 0.1–0.2 ‰, which is in agreement with another study conducted in Japan [21], suggesting that physiological conditions controlling \textsuperscript{13}C/\textsuperscript{12}C do not change drastically from year to year. Thus, the abrupt increase (1.3 ‰) observed from 2010 to 2011 was abnormal, and it appears to be a response to the sudden increase in soil salinity. Salinity in the soil generates chemical signals in the roots that prompt stomatal closure [22–24], restricting CO\textsubscript{2} diffusion and supply to RuBisCO and thereby inhibiting C-fixation. Even for short-time periods high soil salinity is known to cause hyperionic and hyperosmotic stress for plants [39], which could explain the decline in tree-ring growth and the decrease of \textsuperscript{13}C discrimination induced by stomatal closure [40,41] in our study. Since high precipitation in May and especially in September 2011 (400 mm) washed out the salt in the sandy soil [12], the effect of salinity on δ\textsuperscript{13}C should have been negligible in 2012. However, previous studies have reported a high accumulation of sodium, even under short-term salinity exposure in all organs, especially in leaves, leading to a supply restriction of photosynthates to the plant [42–44], which appears to be the case in our study site. Thus, the
effect of salt accumulation in tree compartments on stomatal conductance might have extended longer than the salt residence in the soil itself.

Tree-ring growth in 2011 was relatively higher compared to the following three years because of carbon reserves accumulated in 2010 that could be used for growth in the following year [33], but nevertheless the effect of salinity on the 2011 tree-ring growth was also observed. The further decrease of tree-ring growth from 2012 suggests a combination

Figure 5. (a) Comparison between uncorrected $\delta^{13}$C values (dashed grey line), corrected values using the trend line for $\delta^{13}$C$_{air}$ during the 1991–2014 period for the nearest NOAA-GMD network station in Tae-ahn, Korea [38] (solid grey line), and an extrapolation of the trend published by McCarroll et al. [37] (used in this study). Carbon isotope values ($\delta^{13}$C) from 2002 to 2014: The open circles represent the period 2002–2010 before the tsunami and the shaded circles represent the years after the tsunami of March 2011. Values are mean ± SE ($n = 9$). (b) Oxygen isotope values ($\delta^{18}$O) from 2002 to 2014: The open squares represent the period 2002–2010, before the tsunami and the shaded squares represent the years after the tsunami of March 2011. Values are mean ± SE ($n = 9$). (c) Nitrogen isotope values ($\delta^{15}$N) from 2002 to 2014: The open triangles represent the period 2002–2010, before the tsunami and the shaded triangles represent the years after the tsunami of March 2011. Values are mean ± SE ($n = 7$).
of poor carbon reserves from the previous year [45] and a reduction of photosynthates production as a result of stomatal closure. The decrease of tree-ring $\delta^{13}C$ in 2013 and 2014 in comparison to the previous two years could indicate photosynthetic recovery that was not followed by an increase in tree-ring growth in the same years, apparently due to carbon being allocated to other plant organs (most probably roots), at the expense of stem radial growth. Linder and Rook [46] suggested that carbon allocation shifts from tree growth to fine root structures when they have been exposed to unfavourable conditions, while Cristiano et al. [47] found that more carbon is allocated to the roots as a defense mechanism to sodium toxicity by replacing older sodium-saturated roots with younger roots. It remains to be seen if the carbon allocation pattern will reverse later in favour of stem growth, as it has been observed for cases after severe defoliation for evergreen trees in Spain [48] and deciduous trees in Mongolia [49] caused by insect outbreak. Since pine trees (tree-ring growth) did not exhibit the recovery observed for tree-ring $\delta^{13}C$, the carbon isotopic data recorded here constitute an early signal that can be used to anticipate potential tree recovery.

Tree-ring $\delta^{18}O$ showed a strong annual variability before and after the tsunami, indicating that the factors controlling $^{18}O/^{16}O$ change from year to year as previously found by Nakatsuka et al. [21]. During 2011, trees could have absorbed a mixture of sea and rain water; however, the tree-ring $\delta^{18}O$ did not show a drastic increase for 2011 or for the following years, agreeing with the observation of trees growing under long-term salt exposure [26]. The values of tree-ring $\delta^{18}O$ after the tsunami were in the same range as the values found in the tree-rings during the period 2002–2010. The lack of an abrupt change in the cellulose $\delta^{18}O$ after the tsunami could indicate strong salt discrimination during root water uptake or the $\delta^{18}O$ of the source soil water and the ocean water is similar and thus no difference was found in the tree-rings before and after the tsunami. In a pot experiment with black pine seedlings exposed to salinity, it was observed that the salt content was twice as high in the roots as in the aboveground parts of the tree [43].

Therefore, assuming that there is a $\delta^{18}O$ difference between the soil water and the ocean water we propose the following scenario: a mixture of sea water and fresh water moved from roots to leaves through the xylem, but since stomatal closure reduced photosynthates production, a limiting sucrose supply induced rapid cellulose formation, which accounted for the minimum exchange of oxygen in the sucrose in the phloem [50] with the xylem water [26] or post-photosynthetic exchange of carbonyl oxygens with non-$^{18}O$ enriched xylem water [14].

Tree-ring $\delta^{13}C$ annual variation is small in comparison to the annual variation of $\delta^{18}O$, where short decreasing and increasing trends were more pronounced even under similar annual conditions before the tsunami (years 2002–2010). After the tsunami, this annual variation in $\delta^{18}O$ continued despite the abrupt increase of $\delta^{13}C$ in 2011 and the subsequent decrease (although not significant) in 2013 and 2014. Considering that in these years soil salinity had already considerably decreased to concentrations similar to those before 2011 [6], the variation in tree-ring $\delta^{18}O$ was not related to soil salinity and it did not appear to be in synchrony with changes in $\delta^{13}C$ before and after the tsunami.

The dual $\delta^{13}C$–$\delta^{18}O$ model [28] was used to elucidate the physiological mechanisms related to the isotopic variability in organic matter, caused by the impact of the tsunami. This method makes it possible to evaluate the interaction between stomatal
conductance and photosynthesis in a plant. The observed increase in δ\(^{13}\)C, without changes in δ\(^{18}\)O, would be interpreted as an increase in assimilation rates which was supported neither by the limited tree growth nor by the subsequent recovery in δ\(^{13}\)C. This highlights the limitations of this model which is more fit to organic matter synthesized at the leaf level and cannot be applied to wood [51] nor does it take into account the spatial variability of δ\(^{18}\)O in source water and the dampening of the δ\(^{18}\)O signal in the leaf organic matter by the post-photosynthetic synthesis of wood cellulose [27,52].

4.3. Tree-ring δ\(^{15}\)N

Out of the three stable isotope ratios used in this study, δ\(^{15}\)N is the one that is less affected by annual climatic variations, but it is instead directly affected by changes in the soil N source [10,30,31]. The values observed in the tree-ring δ\(^{15}\)N were in the same range as those observed for black pine tree-rings in undisturbed coastal forests of Japan [9,31] and represented the spatial variability in soil inorganic nitrogen. Thus, tree-ring δ\(^{15}\)N from 2011 to 2014 was not affected by the physical and chemical changes in the soil caused by the tsunami. It appears that in the selected plot, either the tsunami did not remove the upper soil layers (A\(_O\) horizon) [12] where lower \(^{15}\)N concentration occurs or the nitrogen acquisition was mainly from deeper soil layers which were not removed by the tsunami. Furthermore, the microorganisms responsible for nitrogen acquisition [53] were likely not affected by salt water, especially since 

**Acknowledgements**

We deeply thank the help provided by all the students from the Laboratory of Watershed Preservation of Yamagata University for their assistance during the fieldwork. Dr. J.P. Ferrio was supported by a long-term invitation fellowship from the Japan Society for the Promotion of Science (JSPS, L-14560). We also want to thank Dr. G. Hill for the English correction of the paper.
Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

Dr. J.P. Ferrio was supported by a long-term invitation fellowship from the Japan Society for the Promotion of Science (JSPS), [grant number L-14560].

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