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<td>Author(s)</td>
<td>FAN, Rong</td>
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<tr>
<td>Citation</td>
<td>北海道大学 [博士 環境科学] 甲第 13382号</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2018-12-25</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/72380">http://hdl.handle.net/2115/72380</a></td>
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<td>Type</td>
<td>theses (doctoral - abstract of entire text)</td>
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<td>Note</td>
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<td>File Information</td>
<td>FAN_Rong_summary.pdf</td>
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The study on physiology and ecology of willows under flooding in
Indigirka River lowland, Northeastern Siberia, using stable isotope tools
(同位体比を用いた北東シベリアインディギルカ河川低地氾濫域
のヤナギの生理学的および生態学的研究)

1 Background and objectives

Since 1850, the global warming occurred (IPCC, 2014a, 2007). At the same time,
further Arctic amplification was found in Arctic and boreal ecosystems. Under the
condition of global warming, plant productive period and its ranges in boreal forest and
the Arctic tundra changed, which will affect carbon fluxes, albedo and will in the end
make feedbacks to global climate. These all indicate that it is urgent to investigate the
feedbacks of vegetation to the changing climate in Siberia region.

In Northern Eurasia, there is a huge magnitude of runoffs, and most rivers belongs
to the Arctic basin with the characteristic that flood prevails in spring. The increases in
atmospheric temperature significantly affect the hydrology in the region, including
prevailing floods in spring (Shahgedanova, 2002; Shiklomanov et al., 2007; Tan et al.,
2011). With progress in global warming, Arctic rivers continue to show pronounced
changes to their hydrology and ecology (IPCC, 2014b). The earlier and increasing
spring discharge usually causes a larger scale of spring river discharges.

Since the topography of the Arctic river lowlands is relatively flat, spring flooding
The willows, belong to *Salix* genus, have around 400 species in total over the world, which are pioneer species that can invade newly formed banks along rivers and streams, that can help to control soil erosion and allow other species to take hold on the newly formed ground (Hendrix, 1984; Nelson, 2010). Also they are usually dominant species in riparian forest after disturbance, namely after flooding (Hendrix, 1984; Nelson, 2010). In the wide Indigirka River lowland near Chokurdakh village Russia in Northeastern Siberia, one sixth of a 10 × 10 km² area is just covered by dwarf shrub willow (*Salix* sp.) (Morozumi, personal communication) and particularly being abundant on river banks, and they are important component for carbon sink in the Arctic. Also the shrub removal experiment in Indigirka catchment can tell the importance of dwarf shrub, with the result that the absence of dwarf shrub can induce permafrost collapse and can finally lead to a source of methane (Nauta et al., 2015). Therefore, we can announce that it is very important to clarify how the river changes affect growth of willow.

In past three decades, stable isotope techniques such as δ¹³C and δ¹⁵N have been widely used in plant physiology ecology studies, because it can provide a lot information on plant growth and nutrient cycling process via revealing differences of isotopic fractionation (Farquhar et al., 1989; Robinson, 2001), which is caused by the priority of light isotope in reactions. The plant δ¹³C values, which relate to physiological responses including stomatal conductance and photosynthesis, will definitely change under different soil water conditions. Thus using willow δ¹³C values, the flooding effects on willow physiology can be illustrated. And plant δ¹⁵N values, which closely correlate to soil nitrogen dynamics, can be also changed by different soil water conditions. With the help of willow δ¹⁵N and nitrogen content values, the flooding effects on nitrogen conditions for willows can be indicated.

Thus in this study, using stable isotope techniques, I aim to clarify the response of physiology in willow species and the nitrogen cycling in soil under different spring flooding conditions. The physiology feedbacks of willow under flooding will be explained using foliar δ¹³C values and other physiological parameters. And the nitrogen processes in soil under different flooding will be explained using foliar δ¹⁵N and nitrogen content values. These data can contribute to estimate what was/is/will be happening in the ecology on the Arctic vegetation against the flooding driven by climate change, particularly around willows in the river lowland. This work will also offer the basic information for future tree ring studies using shrubs, such as willows in mesic
area, and makes reconstruction of spring flooding’s conditions, scales and their effects to vegetational ecophysiology to be possible.

2 Material and methods

The study site is located in the Indigirka River lowland near Chokurdakh (70°38’N, 147°53’E), Sakha Republic (Yakutia), Russian Federation (Figure 1a). Mean annual air temperature in the region between 1950 and 2016 was −13.7 °C, ranging from −33.9 °C in January (the coldest month) to 10.1 °C in July (the warmest month). Mean annual precipitation between 1950 and 2008 was 209 mm year\(^{-1}\) (Yabuki et al., 2011). Between 1970 and 2016, the average intra-annual water level cycle of the Indigirka River was 70±83 mm for April and May (late winter, pre-flooding), increasing to 600±93 mm for June to August (spring and summer, flooding season); then, gradually receding to 343±146 mm for September and October (autumn and early winter, post-flooding), and declining further to 56±26 mm in winter (after October).

For our main studies using stable isotope tools, we collected current-year shoots in the summer of 2015 and 2016 along three 20 m transects, SBoydom, SKA, and SKB, from the river. Three points, named PA, PB, and PC, were marked on each transect based on their distance to the river. The maximum thaw depth was always found at PA. This layout was designed based on the differences in intra- and inter-annual flooding conditions (Figure 1b). Current-year shoots were also randomly sampled from willows on the Indigirka River lowland during the same period. A total of 31 sites with different locations as snapshot-sampling were used in 2015 and 2016 (Figure 1a). Four current-year top shoots were collected at each point in transects or snapshot-sampling sites at the end of the growing season (the end of July) in both 2015 and 2016. All samples were immediately dried at 60 °C for 48 h after collecting. Dried leaves were milled into fine powder with liquid N\(_2\) and dried again at 60 °C for 48 h; each sample was then wrapped in a tin capsule and injected into an elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Bremen, Germany), connected to an IRMS (Delta V, Thermo Fisher Scientific, Bremen, Germany) through a continuous-flow carrier-gas system (Conflo III, Thermo Fisher Scientific, Bremen, Germany). The soil samples reached to the permafrost, deepest to 50 cm, were collected every 10 cm depth by mixing two or three samples in the same depth using a hand auger. Note that there is no soil samples collected at the points which were under waterlogging. All soil samples were kept
frozen under −20°C in 50 ml glass vials before I carried them to the lab. The soil samples were also used for stable isotope analyses.

Aboveground net primary production (ANPP, newly formed stems and leaves in each year) and the leaf area index (LAI; m² m⁻²) of the willows in 2016 were measured using the direct harvesting method (Jonckheere et al., 2004) in three blocks which were predominated by willows. Supporting data on the foliar δ¹³C values, the photosynthetic rate and stomatal conductance of willow leaves were monitored in the field in 2017 using a portable porometer (LCpro+, ADC BioScientific Ltd., Hoddesdon, Herts, U.K.) equipped with a conifer chamber and a lighting system. Relative river water level was measured with folding ruler on installed pipe during spring and summer season in 2015 and 2016. The river water samples for nutrient measurement were taken every week in July of 2015 and 2016. The summer precipitation was collected for nutrient analyses in July, 2017. The water samples for nutrient measurement were collected using syringe with filter and preserved frozen before analysis in 10 ml centrifuge tube. Concentrations of NO₃⁻, NH₄⁺, and total dissolved N (TDN) were analyzed using a continuous flow analyzer (Bran and Luebbe, Norderstedt, Germany) in Hokkaido University.

Linear Mixed Models (LMMs) were used to clarify differences in the foliar isotopic values among willows growing in three transects in 2015 and 2016. Similar analyses by LMMs were also used to figure out any differences in the foliar isotopic values among the willows randomly collected on the Indigirka River lowland in 2015 and 2016. Tukey’s test was used as a post hoc analysis for multiple comparisons. The lme4 package (Bates et al., 2015) of R (R Core Team 2015, v. 3.3.2) was used to build the LMMs.

3 Results and discussion

The results of the foliar δ¹³C, δ¹⁵N values (‰) and nitrogen contents (%) and their statistical analyses was shown in Figure 2. The high values of foliar δ¹³C were found in normal dry and sporadic waterlogging conditions, this is because of stomatal closure in these conditions. The former closure was accordance to the general knowledge. The observed closure of stomata in sporadic waterlogging in our field work supported the latter explanation. The low values of foliar δ¹³C were found in normal wet and long period waterlogging, with the different causes. The low values of foliar δ¹³C found in normal wet condition can be contributed to the openness of stomata in wet conditions.
However, the similar low values of foliar $\delta^{13}$C were supposed to relate with the observed decreasing of photosynthetic activity in long period waterlogging conditions.

In transects the high values of foliar $\delta^{15}$N values were always found near river in easily flooded points. In year 2015, not only the high foliar $\delta^{15}$N values was found, the high foliar nitrogen contents were also found. The possible reasons for this high foliar $\delta^{15}$N values and nitrogen contents include, the extra N sources from river, the nitrogen release from accelerated thawing permafrost under flooding. These all suggested that in 2015, flooding potentially brought benefits to the willows growing near river, possibly for river brought extra nutrient and more nutrient uptake from deeper soil. Besides effects from flooding, the deepened snow accumulated around willows near river may impact soil organic matter during the winter by increasing the mobilization of nutrients from litter and organic matter to more labile pools in the microbial biomass and soil solution (Buckeridge and Grogan, 2010). The deepened snow also increased soil temperature and brought extra nitrogen, as the atmospheric decomposition also have high $\delta^{15}$N values (Houlton and Bai, 2009; Russell et al., 1998). In year 2016, the high foliar $\delta^{15}$N values was found, however with the decreased foliar nitrogen contents, after long period waterlogging, the possible reasons includes, such as, the loss in light nitrogen isotope from the process of denitrification by microbes in the rhizosphere, decreased nitrogen uptake under long period waterlogging and the decreases of mycorrhizal activity under waterlogging conditions, can be possible explanations. Thus, in 2016, the larger and longer flooding might be harmful to willow nutrient condition and uptake, rather than beneficial.

4 Conclusions

The results of foliar $\delta^{13}$C values indicated the variation of foliar $\delta^{13}$C values and the causes in physiological aspects under different hydrological conditions in small scale of landscape. The results of foliar $\delta^{15}$N values and nitrogen contents demonstrated the nitrogen cycling and nutrient conditions for willows under different hydrological conditions. Thus the objectives for this study were focused on how to apply the stable carbon and nitrogen isotopes to indicate both physiological responses and nitrogen conditions in plant in mesic areas, particularly the willows growing under frequently flooded environments in Arctic river lowland. In this study we mainly discussed on stable isotopic variation and correspondent willows’ feedbacks to different levels of
flooding (i.e., abnormal hydrological conditions), including WL (i.e., continual/sporadic waterlogging) and LWL (i.e., continuous long period waterlogging) conditions.

5 Implications

Flooding altered physiology and nutrient for plants in Indigirka river lowland, finally, changed the ecology of willows. Under medium flooding with suitable scale of waterlogging (i.e., WL), the adequate available water with additional nutrient uptake made willow growing better. However, the large scale flooding with long period waterlogging (i.e., LWL) injured willow growth through not only decreased photosynthesis activity, but also reduced nitrogen availability. In the end, the above data can contribute to estimate what was/is/will be happening in the ecology on the Arctic vegetation against the flooding driven by climate change, particularly around willows in the river lowland.

Apparently, using foliar stable isotope tools, the enhanced growth of willows were found under normal medium flooding, unlike larch and pine trees, which will increase carbon fixation. Through stable isotope tools, the growth of willows was suggested hindered by continuous waterlogging under large flooding, which resulted in the decrease of carbon fixation, however, the large flooding with continuous long period waterlogging were more fatal for their competition plants (e.g., larch trees). After the extremely large flooding, the competition plants were almost extinct, at the mean time the willows can be still alive. Also as the extremely flooding event made large currents and left thick nutrient-enriched sediment, which are good pathways and material for willows with multiple seedling strategies. It can be predictable that the willow, as a kind of pioneer species, can distribute to larger area in Arctic river lowland after extreme events.

Under the enhanced warming, the increase of reduction in sea ice can be found (Comiso et al., 2008; Screen and Simmonds, 2010; Serreze et al., 2007; Stroeve et al., 2007), which will lead to an increase in fall precipitation and the snow depth (Kohler et al., 2006; Screen and Simmonds, 2010). Finally resulted in more frequently extreme event in spring and summer in Arctic region, such as Yana-Indigirka-Kolyma lowland (Shiklomanov et al., 2007). Under the more frequently event, in general, historical floods were more advantageous to willow growth (i.e., more carbon fixation) and
nutrient conditions (i.e., accelerated mineralization and nitrification rates), which can be proved by willow growing near river had more available water, higher production and higher foliar nitrogen content. In this conditions, willows may distribute to larger area and take over the areas occupied by larch trees (Troeva et al., 2010). The albedo, carbon fixation and emissions of greenhouse gases can be changed because of vegetation succession and can result in further feedbacks to climate changes in the future (Huissteden and Dolman, 2012; McGuire et al., 2009; Myers-Smith et al., 2011). If the foliar stable isotope values correlate with those in other organs and tissues (such as tree-ring cellulose), they can also be used to reconstruct the hydrological and vegetation changes that have occurred in mesic regions.
Figure 1 (a) Sampling sites near Chokurdakh village in the study region, northeastern Siberia. Thick and thin lines represent the Indigirka River and its tributaries, respectively. Areas filled with light blue represent lakes. Triangles (18), stars (3), filled black circles (3) and empty circle (1) indicate the sampling sites, three transects (SKA, SKB and SBoydom), three sites for production measurement (LAI1~3) and one site for photosynthesis monitoring (SPh). More sampling sites see appendix Table 2.2 (b) A schematic illustration of a transect.
Figure 2 Box-and-whisker plot of the statistical analysis for the foliar $\delta^{13}$C, $\delta^{15}$N values (‰) and nitrogen contents (%) under four different hydrological conditions in sampling transects established in year 2015 and 2016. Different letters over the numbers indicate statistically significant differences according to Turkey’s post hoc test and Linear Mixed Model. Dry and wet are without waterlogging, and WL and LWL represent continual waterlogging and continuous long period waterlogging, respectively.
Figure 3 All data of foliar $\delta^{15}$N and $\delta^{13}$C values (‰) with foliar nitrogen content (%) in year 2015 and 2016. Red square with cross, yellow circle with cross, green square with triangle, blue filled square and purple filled circle indicate different growing conditions, often flooded, barely flooded, on land, wetland and in larch forest. Error bars represent 1 SEM.