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博士論文

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）

Division of Earth System Science, Graduate School of Environmental Science, Hokkaido University
北海道大学大学院環境科学院地球圏科学専攻

Rong FAN
樊 荣

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Abstract

With the enhanced global warming in recent years, increasing frequency and severity of the river floods in Arctic regions lead to more waterlogging on plants that are widely distributed in river lowland, finally result in the changes in plant physiology and nutrient condition. In turn, these changes can also affect climate change. Therefore it is necessary to investigate the effects of flooding on plant physiology. For this purpose, we used stable carbon and nitrogen isotope methods. Stable carbon isotopic composition ($\delta^{13}C$) of plants was used to indicate different water regimes in terrestrial ecosystems, and stable nitrogen isotopic composition ($\delta^{15}N$) of plants was used to illustrate nitrogen processes. The willows growing in Indigirka River lowland, Northeast Siberia, was selected for this study.

To investigate the physiology and related foliar $\delta^{13}C$ values of plants in response to different flooding conditions (including waterlogging), we measured the $\delta^{13}C$ values in the leaves of willows species, *Salix boganidensis*, *S. glauca*, and *S. pulchra*. We also monitored changes in plant physiology under several major flooding conditions. The foliar $\delta^{13}C$ values of willows varied, ranging from −31.6‰ to −25.7‰ under the different flooding conditions, which can be explained by the following processes, (i) under normal conditions, the foliar $\delta^{13}C$ values decrease from dry (far from a river) to wet (along a river bank) areas; (ii) the $\delta^{13}C$ values increase in frequently waterlogged areas owing to stomatal closure; and (iii) after prolonged flooding periods, the $\delta^{13}C$ values again decrease, probably owing to the effects of not only the stomatal closure but also the reduction of foliar photosynthetic ability under long period of waterlogging. Based on these results, I conclude or suggest that plant $\delta^{13}C$ values are strongly influenced by plant physiology which responses to the diverse hydrological conditions, particularly the long term of flooding regions, as occurs in the Arctic regions.

To survey the nutrient conditions, foliar $\delta^{15}N$ values and foliar N content of plants in different flooding conditions, we measured the $\delta^{15}N$ values in the same leaves of willows and bulk soil, the concentrations of NO_3^-, NH_4^+, and total dissolved N (TDN) in river water. The foliar $\delta^{15}N$ values of willows varied, ranging from −6.8‰ to 5.3‰

under the different flooding conditions. High foliar δ^{15}N values, which was always found near river, was clearly caused by frequent flooding. Foliar N content ranged from 1.37% to 4.25% and high values were found near river in low water level year 2015 and no significant differences in high water level year 2016 between sites near river or far from river. The high foliar δ^{15}N values caused by flooding can be explained by additional N brought by the river, available soil N in deeper soil because of deeper maximum thaw depth under waterlogging, and also denitrification under reductive conditions. The former two processes which can increase the nitrogen availability may have resulted in the high foliar N content in the frequently waterlogged area near river in 2015, and the last process which may decrease the nitrogen availability and absorption can explain the low foliar N content after prolonged flooding periods in 2016.

Therefore, as one of water-tolerant plants which prefer relatively high water content, willows growing in Indigirka River lowland prefer frequently waterlogging caused by flooding, which can lead to increased carbon fixation and more available nitrogen for the willows. Meanwhile prolonged flooding periods (i.e., continuous waterlogging during whole growing season) may hamper carbon fixation and nitrogen availability for willows. These observations suggest that the flooding level is a key factor determining willows' distributions in Arctic region under warming. The stable isotopic values of plants is a good index to evaluate the plants' feedbacks under different flooding levels including the both physiology and ecology aspect.

Chapter 1 General Introduction

1.1 Global warming and Arctic enhance

Since 1850, the global warming occurred (IPCC, 2007, 2014a) while the global mean surface air temperature showed an average warming of 0.85 °C (from 0.65 to 1.06 °C), and this accelerating warming (Zwiers, 2002) which made past 30-year to be the warmest period of the last 1400 years in the Northern Hemisphere (IPCC, 2014a). At the same time, in Arctic and boreal ecosystems, which cover 22% of the land surface area (Chapin et al., 2000), further Arctic amplification was found, the phenomenon that trends and variability in surface air temperature tend to be larger in the Arctic region than for the Northern Hemisphere or globe as a whole (Serreze & Francis, 2006; Serreze & Barry, 2011). This makes this area one of the most responsive regions to climate change as the hot-spot in biodiversity (Giorgi, 2006).

As a lot of researches have been shown, the Arctic region has a substantial storage on soil organic matter (SOC) (Post et al., 1982; Miller, Kendall & Oechel, 1983; Gilmanov & Oechel, 1995; Michaelson, Ping & Kimble, 1996; Tarnocai, 2000; Tarnocai, Kimble & Broll, 2003; Ping et al., 2008; Tarnocai et al., 2009). And under Arctic warming, microbial breakdown of SOC and the release of the greenhouse gases, usually carbon dioxide and methane, was accelerated (Schuur et al., 2015), which will also lead to further warming (Schuur & Abbott, 2011). This kind of emission of greenhouse gas is considered to be different dynamics between boreal forest and Arctic tundra ecosystems (Apps et al., 1993; Clein & Schimel, 1995).

Under the condition of global warming, plant productive period and its ranges in boreal forest and the Arctic tundra changed. For boreal forest ecosystem, there are evidences showing most tree line shifted northward and to higher elevations sometimes accompanied by a production increase as a whole (Serreze et al., 2000; Gamache & Payette, 2004; Wilmking et al., 2004; Hinzman et al., 2005; Shiyatov, Terent?ev & Fomin, 2005; Pieter S A Beck and Scott J Goetz, 2011; Berner et al., 2013; IPCC, 2014b). For tundra ecosystem, shrub expansion was convinced using time-series

photography (Sturm, Racine & Tape, 2001; TAPE, STURM & RACINE, 2006), satellite images with NDVI analysis (Myneni et al., 1997; Genuo J. Jia and Howard E. Epstein, 2003), plot studies (Joly et al., 2007). The early summer temperature and date of snow melt were found to be the most important factors that define seasonal growth and tree-ring structure (Kirdyanov et al., 2003), as the early snow melt can increase the wood production. Thus the vegetation structure in Taiga-tundra ecosystems is easy to change under fluctuant climatic condition (Troeva et al., 2010). And through changes the greenhouse gas emission, water and energy exchange, vegetation variation under warming may cause feedback effects on global climate (McGuire et al., 2009; Myers-Smith et al., 2011; Huissteden & Dolman, 2012). These all indicate that it is urgent to investigate the feedbacks of vegetation to the changing climate in Siberia region.

1.2 Hydrological changes under Arctic enhance and effects on vegetation

In Northern Eurasia, there is huge magnitude of runoff, and most rivers belongs to the Arctic basin including the top three largest rivers (Shahgedanova, 2002). For Yana-Indigirka-Kolyma lowland, the total annual runoff of these three rivers is 211.5km^3 with the total catchment area of 1.265x10^{6}km^2 (Sokolov, 1964), with the characteristic that flood prevails in spring. The Arctic region is highly sensitive and responsive to climatic changes (e.g., Giorgi, 2006). Thus, increases in atmospheric temperature significantly affect the hydrology in the region, including prevailing floods in spring (Shahgedanova, 2002; Shiklomanov et al., 2007; Tan, Adam & Lettenmaier, 2011). With progress in global warming, Arctic rivers continue to show pronounced changes to their hydrology and ecology (IPCC, 2014b). For example, the average annual discharges of fresh water from the six largest Eurasian rivers to the Arctic Ocean increased by 7% from 1936 to 1999 (Peterson et al., 2002) were found, consistent with the increase from 1977 to 2007 by 9.8% in annual discharges of 19 large rivers which encompassing the entire Arctic region (Overeem & Syvitski, 2010). Across the Russian Arctic, an observed significant shift to earlier spring discharge was also observed (Stone et al., 2002; Tan, Adam & Lettenmaier, 2011; Lesack et al., 2014), which is related to documented changes in

earlier snowmelt and freeze-thaw (Shiklomanov et al., 2007). This kind of earlier timing of spring discharges was most significant in eastern, colder continental climates, because in this area the upward trends in air temperature has the largest impact on spring and summer flows (Tan, Adam & Lettenmaier, 2011). 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 strongly influences riparian plant communities. Spring flood of river is an important restrict factor for plants, for example, the *Pinus* and *Larix* with relatively low water tolerance cannot survive in spring flooding circumstance, and the dwarf shrub with high water tolerance like willow (*Salix* sp.) communities can live constantly undergo spring floods in the large river valleys (Troeva et al., 2010). The willows, belong to *Salix* genus, have around 400 species in total over the world. They are C3 plants and primarily grow on moist soils in cold and temperate regions of the Northern Hemisphere (Mabberley, 1997). There are several productive strategies in willows, including normal seed and floating twigs. They can be carried great distances by the currents. Their species covered both deciduous trees and shrubs (Nelson, 2010), 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). The reason for the high water tolerance in plants usually relate to the development of aerenchyma (Pezeshki, 2001), the mechanisms critical to the ability of plants to cope with anaerobiosis, as it provides a gas transport system which allows atmospheric oxygen to reach underground organs to maintain aerobic respiration and to oxidize various reducing compounds in the rhizosphere (Pezeshki, 2001; Randerson, Moran & Bialowiec, 2011). The increase of aerenchyma in the upper roots (100 mm) was found under flooding, in *Salix viminalis* cuttings under waterlogging for four weeks (Jackson & Attwood, 1996). The rhizosphere oxygenation by radial oxygen loss (ROL) from roots is another important factor great importance for wetland plants to overcome

anaerobic conditions (Pezeshki, 2001). This kind of oxygen supply into the soil environment can be also found in *Salix viminalis* in laboratory experiments (Randerson, Moran & Bialowiec, 2011).

In the wide Indigirka River lowland near Chokurdakh village Russia in Northeastern Siberia, one sixth of a 10 × 10 km^2 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.

1.3 Objective

In past three decades, stable isotope techniques such as $\delta^{13}C$ and $\delta^{15}N$ 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, Ehleringer & Hubick, 1989; Robinson, 2001), which is caused by the priority of light isotope in reactions. The plant $\delta^{13}C$ values, which relate to physiological responses including stomatal conductance and photosynthesis, will definitely change under different soil water conditions. Thus using willow $\delta^{13}C$ values, the flooding effects on willow physiology can be illustrated. And plant $\delta^{15}N$ values, which closely correlate to soil nitrogen dynamics, can be also changed by different soil water conditions. With the help of willow $\delta^{15}N$ 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 in detail in the part of chapter 2, using foliar $\delta^{13}C$ values and other

physiological parameters. And the nitrogen processes in soil under different flooding will be explained in detail in the part of chapter 3, using foliar δ^{15}N 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.

Chapter 2 Effect of floods on willow physiology in Northeastern Siberia using stable Carbon isotope

2.1 Introduction of stable Carbon isotopes in plants

Over the past four decades, stable carbon isotopic composition ($\delta^{13}C$, ‰ relative to Vienna Pee Dee Belemnite, VPDB) of plants has been widely employed as a conventional tool to estimate changes in carbon (C) flux as plant physiological responds to environmental changes. The magnitude of isotopic fractionation is indeed highly dependent on physiological conditions (Farquhar, Ehleringer & Hubick, 1989; Robinson, 2001). For instance, it is well known that carbon isotopic fractionation ($\Delta^{13}C$) in plants is a function of the ratio of leaf intercellular- (C_i) to atmospheric (C_a) CO_2 concentrations (C_i/C_a) (Farquhar & Sharkey, 1982; Farquhar, Ehleringer & Hubick, 1989), as given in equation (2.1):

$$\Delta^{13}C = \delta^{13}C_a - \delta^{13}C_p = a + (b - a) \times \frac{C_i}{C_a} , \quad (2.1)$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ are the $\delta^{13}C$ values of atmospheric CO_2 and photosynthate, respectively; while a and b are the carbon isotopic fractionations associated with CO_2 diffusion and enzymatic carboxylation (carbon fixation) in plant leaves, respectively.

The c_i/c_a ratio is usually determined from the balance between the CO_2 supply controlled by stomatal conductance and CO_2 consumption via the carboxylation related to photosynthetic activity. When the stomata close (e.g., in response to a large water deficit and high evaporation rates due to high ambient temperature, (Meidner & Mansfield, 1968; Willmer & Fricker, 1996)), low CO_2 supply reduces c_i, leading to a decrease in the $\Delta^{13}C$ values and, ultimately, an increase in the $\delta^{13}C_p$ values. On the other hand, when CO_2 consumption decreases as a consequence of reducing photosynthetic activity (e.g., due to the limitations of light and nutrients (Hall & Krishna, 1999)), a large c_i increases the $\Delta^{13}C$ values and, ultimately, decreases the $\delta^{13}C_p$ values. These effects are expressed by equation (2.2),

$$\frac{A}{gc} = C_a - C_i = C_a \times \left(1 - \frac{C_i}{C_a} \right) , \quad (2.2)$$

where A is the photosynthetic rate, gc is the stomatal conductance of CO_2, and gs is stomatal conductance which equals 1.62 times gc (Farquhar, Ehleringer & Hubick, 1989).

Combining equations (2.1) and (2.2), the $\Delta^{13}C$ and $\delta^{13}C_p$ values are given by the standard equation (2.3):

$$\Delta^{13}C = \delta^{13}C_a - \delta^{13}C_p = \mathrm{b} - \frac{\mathrm{b-a}}{C_\mathrm{a}} \times \frac{1.6A}{g_s} \ , \qquad (2.3)$$

Thus, the plant $\delta^{13}C$ values are primarily controlled by both stomatal conductance (*gs*) for CO_2 and photosynthetic activity (*A*) (Farquhar & Richards, 1984). For example, under constant *A*, the $\delta^{13}C_p$ values are controlled mainly by the *gs*. Drought-induced low *gs* decreases the $\Delta^{13}C$ values and increases the $\delta^{13}C_p$ values. In contrast, moisture-induced high *gs* increases the $\Delta^{13}C$ values and decreases the $\delta^{13}C_p$ values (Farquhar & Richards, 1984; Knight, Livingston & Van Kessel, 1994; Korol et al., 1999; Barbour & Farquhar, 2000; Warren, McGrath & Adams, 2001; Huang et al., 2008; Peri et al., 2012). Under constant *gs*, however, the $\delta^{13}C_p$ values are primarily controlled by *A*, which is strongly correlated with light intensity (Yakir & Israeli, 1995) and nutrient availability (Ripullone et al., 2004; Duursma & Marshall, 2006; Kranabetter et al., 2010). Enhanced *A* decreases the $\Delta^{13}C$ values and, ultimately, increases the $\delta^{13}C_p$ values (O'Leary, 1988; Farquhar, Ehleringer & Hubick, 1989).

With respect to the river flooding, there is physiological evidence that stomata can also be closed in response to waterlogging conditions (Gomes & Kozlowski, 1980; Olivella et al., 2000; Copolovici & Niinemets, 2010); although, such of former studies mostly focused on plant physiology but not isotope data, such as the $\Delta^{13}C$ or $\delta^{13}C_p$ values. It is predictable if the CO_2-*gs* term in equation (2.3) decreases due to the low *gs* (stomatal conductance) during waterlogging, low foliar $\Delta^{13}C$ (high foliar $\delta^{13}C_p$) values will appear very similar to the values observed under drought conditions. A few number of studies have reported changes in the $\delta^{13}C_p$ value under both, natural and simulated waterlogging conditions. Anderson et al. (2005) found that tree-ring $\delta^{13}C$ values for the pond cypress (*Taxodium ascendens*) in their natural environments in southeast U.S.A. are positively correlated with the total annual precipitation; similarly, Li and Sugimoto (2017) reported an increase in needle $\delta^{13}C_p$ values for Dahurian larch (*Larix gmelinii*) in waterlogging pot experiments. The latter study attributed an increase in the larch needle $\delta^{13}C_p$ values to low *gs* caused by waterlogging.

Although Anderson et al. (2005) reported a decreased *gs* with an increased *A* under very wet conditions, the most physiological experiments have demonstrated that, not only *gs*, but also *A* is apparently reduced under waterlogging (Gomes & Kozlowski, 1980; Copolovici & Niinemets, 2010; Li & Sugimoto, 2017). Based on these findings,

we hypothesize that the $\Delta^{13}C$ (and $\delta^{13}C_p$) values in plant leaves are not exclusively under the controlling of stomata (*gs*), because the photosynthetic rate (*A*) is also not constant in waterlogging. Moreover, net *A* and chlorophyll contents were observed decreasing without any change in either *gs* or c_i/c_a, in a continuous waterlogging experiment with okra (*Abelmoschus esculentus*) (Ashraf & Arfan, 2005), a waterlogging-tolerant plant. Thus, the possible changes in the foliar $\delta^{13}C$ value under long period (continuous) waterlogging, which are assumed more dependent on changes of photosynthetic rate, are shown in Fig 2.1 under predicted scenarios 1, 2, and 3 (S1, S2, and S3). The increased *A* or further reduced *gs*, or both of them together in long period waterlogging that can lead to S1, with the higher $\delta^{13}C_p$ values. Moreover, there are higher possibilities as S2, reduced *A* with significantly decreased *gs*, or as S3, that under low *gs*, a significant reduction of *A* can lead to lower $\delta^{13}C_p$ values. Thus, $\Delta^{13}C$ (and $\delta\ ^{13}C_p$) values will be potentially changed in terms of frequency, magnitude, and duration of waterlogging.

As mentioned above, because willows in Arctic are exposed to an increase frequency of river floods and have a high chance of being submerged, they are a good candidate species to study the effects of flooding on the $\delta^{13}C_p$ values of leaves in relation to *A* and *gs*. In the chapter 2, I determined the effects of flooding on the $\delta^{13}C$ values in willow leaves under four major hydrological conditions: dry, wet, and short and long period waterlogging (Fig 2.1). I measured the $\delta^{13}C$ values of bulk leaves from willows growing under these flooding regimes in the Indigirka River lowland of Northeastern Siberia.

2.2 Materials and Methods

2.2.1 Study area

The study site is located in the Indigirka River lowland near Chokurdakh (70°38′N, 147°53′E), Sakha Republic (Yakutia), Russian Federation (Fig 2.2). 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). The Indigirka River lowland, including rivers, lakes, wetlands, hills, and floodplains, is frequently flooded during spring and summer. Soils in the region are loamy or silty-

loamy alluvial soils with black- to grayish-olive color along the riverbanks (Troeva et al., 2010). The average depth of the active layer in soils is approximately 30 cm on land and 1 m near the river in the summer. The local vegetation consists of aquatics, sphagnums mosses, graminoids, shrubs (mainly the willow *Salix* sp. and the dwarf birch: *Betula nana*), alders, larches, and pines. 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). Field experiments were approved by Hokkaido University, and Institute for Biological Problems of Cryolithozone, Siberian Branch of Russian Academy of Science, and North-Eastern Federal University.

2.2.2 Willows in the Indigirka River lowland

The common willow species observed in 2015–2017 were *Salix boganidensis*, *S. pulchra*, *S. glauca*, *S. richardsonii*, *S. viminalis*, *S. alaxensis*, *S. fuscescens*, and *S. hastata*. Most species were ≤1 m tall, except for a few species such as *S. boganidensis*, *S. alaxensis*, and *S. fuscescens*, which were 2–3 m in height. Diameter at breast height (DBH) generally ranged between 1 and 6 cm. Maximum root depth was approximately 1 m at the riverbank (Figure A2.1), but was highly variable and depended on various factors such as the thickness of the active layer which represents the top layer of soil that thaws during the summer and freezes again during the autumn, and moisture levels where the willows grew. Willows were distributed more densely along the riverbanks than on xeric lands.

Observations made with a GardenWatchCam time-lapse camera (Brinno, Inc., Taipei City, Taiwan) showed that the willow leaf buds opened around the first few weeks of June, when the snow had melted and the daily average air temperature had increased to > 0 °C. The leaves and stems grew rapidly, within 10 days after bud opening, and were fully developed by mid-July. Willow leaf biomass peaked by the end of July, an observation consistent with a NDVI study in Alaska (Boelman et al., 2011). Aboveground net primary production (ANPP, newly formed stems and leaves in each year) and the leaf area index (LAI; $m^2\ m^{-2}$) of the willows in 2016 were measured using the direct harvesting method (Jonckheere et al., 2004) in three blocks which were

predominated by willows. Aboveground net primary production (ANPP) was 63, 119 and 117 g m^{-2}·a in each of the three blocks, and the LAI was 0.59, 0.71 and 1.59 in each of the three blocks (Table 2.1).

2.2.3 Plant samples

In the summer of 2015 and 2016, we collected current-year shoots from the locally dominant willows: *Salix boganidensis*, *S. glauca*, and *S. richardsonii* on three sets of 20 m transects (SBoydom, SKA, and SKB) from the river. SBoydom is located between the mainstream Indigirka and the wetland; while, SKA and SKB are situated next to a secondary tributary, Kryvaya (Figure 2.2 and Table 2.2). 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 2.2 and Figure 2.3). PA at SKB and SBoydom were continually waterlogged (i.e. sporadic waterlogging) throughout the growing season in 2015 and continuously waterlogged (i.e. continuous waterlogging) until July in 2016 (Figure 2.3). PB at SKB and PA at SKA were flooded only in 2016 (Figure 2.3).

Four current-year top shoots were collected at each point at the end of the growing season (the end of July) in both 2015 and 2016. 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 2.2 and Table 2.2). In snapshot-sampling, we cannot make the continuous observation, so the water conditions were just recorded as the status when we took samples, such as, in river, flooded, or wetland. At least four current-year shoots were collected at each location to obtain representative data for each site.

The details of sampling sites, locations, species and sampling numbers are shown in Figure 2.2 and Table 2.2. All samples were immediately dried at 60 °C for 48 h after collecting.

2.2.4 Stable carbon isotope analysis

As the previous study mentioned enzymatic activity of young branches often show different metabolic pathway with leaves . like poplar (Wang HM

et al/ Photosynthetica 2007) 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). Isotope ratios explained by the equation (2.4) below:

$$\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000\ (‰), \qquad (2.4)$$

where R_{sample} and $R_{standard}$ represent molar ratios of heavy to light isotopes ($^{13}C/^{12}C$) of the samples and standards, respectively.

The stable carbon isotopic composition was reported in the standard δ notation relative to VPDB (Vienna Pee Dee Belemnite). A laboratory standard was injected after every ten samples to verify that the analytical accuracy was better than 0.1‰. To reduce the effect of sampling heterogeneity in $\delta^{13}C$ within a single site, four samples were measured and the average isotopic composition was reported for each site, with the standard deviation ranging from 0.0‰ to 1.4‰ (average, 0.7‰).

2.2.5 Photosynthetic rate and stomatal conductance analyses

Supporting data on the foliar $\delta^{13}C$ 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. The photosynthetic rate (*A*) of *S. boganidensis*, *S. richardsonii*, and *S. glauca* under different light levels (10 to 955 μmol m^{-2} s^{-1}) was measured to obtain light response curves and thus, to identify the saturation light intensity.

Site SPh near Chokurdakh village, was set up in the summer of 2017 to monitor the conditions in former transects SKA, SKB and SBoydom, since the extremely high flooding caused all these three sites totally submerged for the entire summer of 2017. Under gradient flooding conditions on site SPh (-PA: submerged till July 20; -PB: submerged till July 15; -PC: without submergence during the observation period), temporary changes were measured in the photosynthetic rate (*A*) and stomatal conductance (*gs*) of *S. richardsonii*, *S. glauca,* and *S. boganidensis* in response to a single saturated light exposure at 600 μmol m^{-2} s^{-1} around noon, the rest of chamber conditions were set to match ambient conditions. For each measurement at the points

(PA, PB or PC), a total of 12 leaves from four trees were marked for leaf ADC data recording for more than six times on any leaf of them. Average of all records was calculated for each measurement. Five times of measurement were taken every 2–3 days between July 13, 2017 and July 27, 2017. The leaves were also collected after whole monitoring period to check the foliar δ^{13}C values.

2.2.6 Data processing

Meteorological dataset

Daily temperature records from 1950 to 2016 of Chokurdakh station was obtained by combining two datasets. Those are the Baseline Meteorological Data in Siberia Database (BMDS Version 5.0) from 1950 to 2008 and National Oceanic and Atmospheric Administration (NOAA) website (http://www.ncdc.noaa.gov) from 2009 to 2016. Daily precipitation data was only adopted from BMDS between 1950 to 2008 due to the incompleteness of the NOAA dataset.

Statistical analysis

Linear Mixed Models (LMMs) were used to clarify differences in the foliar δ^{13}C value among willows growing in three transects in 2015 and 2016. Foliar δ^{13}C value was set as the response variable, with flooding conditions was set as the fixed effect, and species (i.e., *S. boganidensis*, *S. richardsonii*, and *S. glauca*) was set as a random effect. Similar analyses by LMMs were also used to figure out any differences in the foliar δ^{13}C value among the willows randomly collected on the Indigirka River lowland in 2015 and 2016. Foliar δ^{13}C value was set as the response variable, the flooding condition was assigned as the fixed effect, and the location (along the mainstream or the tributary), and species (Table 2.2), were set as random effects. Tukey's test was used as a post hoc analysis for multiple comparisons. The lme4 package (Bates, Maechler Martin & Walker, 2015) of R (R Core Team 2015, v. 3.3.2) was used to build the LMMs.

2.3 Results

2.3.1 Foliar δ^{13}C in the transects

Foliar δ^{13}C values differed among SKA, SKB, and SBoydom, and between years (Figure 2.3). Along transect SKA in 2015, the mean foliar δ^{13}C values were −30.3 ± 0.8‰ for PA (close to the river but without submergence), which was much lower than those for PB (−28.0 ± 0.6‰) and PC (−27.5 ± 0.7‰). A similar trend was also observed in 2016, when the water level was high; in this case, the foliar δ^{13}C values for PA (−29.4 ± 0.4‰) was lower than those for PB (−29.2 ± 0.3‰) and PC (−28.6 ± 0.9‰); although, the difference was small. For the inter-annual changes from 2015 to 2016 on transect SKA, an increase in foliar δ^{13}C value was observed for PA (+0.8 ± 0.6‰); whereas, a decrease was recorded for both, PB (−1.2 ± 0.5‰) and PC (−1.0 ± 0.8‰). The flooding conditions on the transects in both years were classified into normal dry, normal wet, continual (i.e., sporadic) waterlogging and long continuous (i.e., long period) waterlogging, abbreviated into “Dry”, “Wet”, “WL” and “LWL”, respectively, from the points far from river to the points near river, depending on the observation during the whole observation period. In detail, normal dry and wet represent the points on land which have not been submerged during the whole observation period, with the soil water content around 20% in dry and 40% in wet. The sporadic waterlogging represent the points sometimes submerged by flooding, and the continuous waterlogging represent the points which were closest to the river and continuous submerged by flooding.

In 2015, the sampling point at PA along both, the SKB and SBoydom transects, were sometimes waterlogged (“WL”, Figure 2.3); whereas, all points PB and PC were not. A similar trend for the foliar δ^{13}C values was found in both, SKB and SBoydom transects, as the foliar δ^{13}C values for PA (−27.4 ± 1.1‰ and −27.3 ± 0.7‰, respectively) were higher than those for PB (−28.5 ± 0.6‰ and −27.4 ± 1.0‰, respectively), and PC (−28.1 ± 0.9‰ and −28.3 ± 0.7‰, respectively) (Figure 2.3). In 2016, although PAs were also but always waterlogged (“LWL”, Figure 2.3), PBs and PCs were not, trends in the foliar δ^{13}C value were apparently different between SKB and SBoydom. In SKB, the foliar δ^{13}C values for PA (−29.0 ± 0.6‰) were slightly higher than those for PB (−29.2 ± 0.5‰) and PC (−29.7 ± 0.9‰); whereas, in SBoydom, values for PA (−29.5 ± 0.7‰) were slightly lower than those for PB (−28.3 ± 0.5‰)

and PC (−28.0 ± 0.8‰) (Figure 2.3). For the inter-annual changes in SKB and SBoydom, decreases in the foliar δ^{13}C value were observed at all points in all transects, except for PC in SBoydom. The differences in the foliar δ^{13}C value between the PB and PC within each transect ranging from −0.5‰ to +0.9‰; however, those for the PA significantly deviated (−2.5‰ to +0.9‰) from the mean value of the PB and PC.

Overall, statistical analysis of the foliar δ^{13}C values from transects (in Figure 2.3) showed a significant difference ($F_{3,42}$ = 42.276, P < 0.01, Figure 2.4) among the four major hydrological conditions (i.e., “Dry”, “Wet”, “WL”and “LWL”, in Figures 2.3 and 2.4). The foliar δ^{13}C values were high in dry and waterlogged continually conditions. Conversely, values under wet and continuous long period waterlogging were consistently low.

2.3.2 Spatial distribution in the foliar δ^{13}C values of willows

Neither significant nor large differences were detected in the foliar δ^{13}C value in any of the willows growing in the 31 randomly selected sampling sites (with different locations) in the basin of Indigirka River lowland during the same sampling periods at the end of the growing seasons of 2015 and 2016 (Figure 2.5). The value of δ^{13}C of willow foliar ranged from −31.1‰ to −25.3‰ in 2015, and from −31.6‰ to −25.7‰ in 2016. Statistical analysis shows that there were no significant differences between the four environmental conditions (flooding, flooded, on land, and wetland) in 2015 or 2016. Moreover, the δ^{13}C values measured for willow leaves collected in a larch forest in 2016 (−27.6 ± 1.2‰) were significantly higher than those sampled anywhere else in the same year ($F_{4,168}$ = 2.58, P = 0.039).

2.3.3 Photosynthetic rate and stomatal conductance

Apparent photosynthetic rate (A) gradually increased asymptotically and reached to about 6 μmol m^{-2} s^{-1} for *S. richardsonii*, about 6–8 μmol m^{-2} s^{-1} for *S. glauca*, and about 8–12 μmol m^{-2} s^{-1} for *S. boganidensis*, under PFD of 400–600 μmol m^{-2} s^{-1} (irradiation of PFD scanning covered the range form from 10 to 955 μmol m^{-2} s^{-1}) (Figure A2.2). Therefore, the saturating light intensity for willow leaves in the Indigirka River lowland was found in the range from 400 to 600 μmol m^{-2} s^{-1}. Maximum

photosynthetic rate *A* recorded in leaves of *S. boganidensis* was the highest among all three species (Figure A2.2).

In the summer of 2017, willows at the SPh-PA were continuously submerged until July 20; willows at the SPh-PB had just come out of the water when monitoring began on July 15; while, willows at the SPh-PC was not submerged during the monitoring period (Figure 2.6a), although all three points (PA, PB and P) at transect SPh were within 20 m from the river. The largest decrease (−1.6±1.4 μmol m^{-2} s^{-1}) in *A* at SPh-PA during the monitoring period, was registered on July 21 when the waterlogging just finished; whereas, after flooding *A* was observed to follow a slow recovery over the last few days (Figure 2.6b). Similar decrease-increase trends in *A* were also detected in the willow leaves at SPh-PB, and also, in this case, the lowest *A* was recorded soon after waterlogging finished on July 18 (−0.7±2.6 μmol m^{-2} s^{-1} lower than first measurement) (Figure 2.6b). However, at SPh-PC, *A* continuously increased, compared to the initial measurement. These values corresponded with the waterlogging gradients in SPh, that *A* was reduced under waterlogging and could recover if waterlogging was over. On the other hand, among points, compared to SPh-PC (0.29±0.12 μmol m^{-2} s^{-1}), the lowest *gs* values were found at SPh-PA (0.16±0.08 μmol m^{-2} s^{-1}); while, intermediate values were recorded in SPh-PB (0.25 ± 0.09 μmol m^{-2} s^{-1})(Figure 2.6c). Thus, *gs*, apparently correlated to the degree of waterlogging among SPh-PA, -PB, and -PC.

2.4 Discussion

2.4.1 River water level and leaf formation

As mentioned before, the willow leaves began opening after the first week in June and finished growing by the end of July. This suggests that the foliar $\delta^{13}C$ values of willow leaves recorded hydrological conditions experienced from early mid-June to the date of collection, which is a longer period than that of the *in-situ* observation and monitoring period for hydrological conditions. However, it is known that the river water level is gradually decreased but by within approximately 1 m during the term of leaf growth (Figure A2.3). Moreover, only small differences were found in the foliar $\delta^{13}C$ values between top and bottom of a single current-year shoot, approximately 0.5±0.1‰ (Figure A2.3), which may have experienced leaf formation over different periods. Thus, in present field observation study, the hydrological conditions observed during July are

assumed to be almost the same or very similar to those for the early part of the growing season.

2.4.2 Foliar δ^{13}C values under different hydrological conditions

In the normal dry-wet SKA transect, in the absence of waterlogging during 2015, *S. boganidensis* grew at PA with more available water than that at PB or PC. The willow leaves at PA were largely depleted in ^{13}C, with the difference of δ^{13}C value by about 2‰, relative to those at PB or PC (Figure 2.3). These results are consistent with the well-known fact that, under dry conditions, low *gs* results in high foliar δ^{13}C values in C3 plants; whereas, under wet conditions, high *gs* leads to low foliar δ^{13}C values (equation 2.3); all of which is consistent with the common findings with respect to *gs* in numerous studies (Chen, Bai & Han, 2002; Peri et al., 2012; Schifman et al., 2012). The decrease in the foliar δ^{13}C value between 2015 and 2016 at PB and PC along SKA and SKB transects, and at PB on the SBoydom transect are thus, also attributable to stomatal regulation of gas exchange between dry and wet conditions.

Willow leaves at the PA of SKB and SBoydom in 2015 have the δ^{13}C values similar to or higher than those in their respective PB, even though PAs and PBs were situated under wet and dry conditions, respectively. The high δ^{13}C values in PAs can be explained by stomatal closure under waterlogging conditions. Decreasing *gs* was demonstrated under extremely wet conditions (Gomes & Kozlowski, 1980; Olivella et al., 2000; Copolovici & Niinemets, 2010; Li & Sugimoto, 2017). We suppose, as has been reported, that flooding reduces root hydraulic conductance and thereby, leaf water potential (Olivella et al., 2000; Islam & Macdonald, 2004), which in turn leads to decreased *gs*. Else et al. (2001) also suggested that stomatal closure upon waterlogging is caused by the production of abscisic acid (ABA), which may be related to the decrease of root hydraulic conductance and leaf water potential. Moreover, in the present study, we observed a rapid recovery in *A* but a slow recovery in *gs* after waterlogging ended (Figure 2.6b, c). Thus, when the waterlogging was short and continual, low *gs* can contribute more to the foliar δ^{13}C values than *A*, resulting in the high foliar δ^{13}C values at SPh-PB, although the lack of statistical significance (Figure 2.6a). The co-occurrence of low *gs* with high foliar δ^{13}C values was also observed under waterlogging in field trials (Ewe & Sternberg, 2003; Anderson et al., 2005) as well as in pot experiments (Li & Sugimoto, 2017). Therefore, we suggest that *gs* contributes

more than *A* to the foliar $\delta^{13}C$ values under sporadic waterlogging caused by medium flooding ("WL"; Figure 2.3). The combined results of this and previous research indicates that increasing of the foliar $\delta^{13}C$ values (+0.8 ± 0.6‰) at SKA-PA between 2015 and 2016 were caused by stomatal closure in response to the waterlogging in 2016 ("WL", Figure 2.3). Very similar increases in the foliar $\delta^{13}C$ value were also observed in the SBoydom-PC, even though it was relatively distant from the river. The wetlands near SBoydom-PC may cause waterlogging similar to that experienced in the riverside sites (Figure 2.3).

Sporadic waterlogging ("WL", Figure 2.3) related to medium flooding increased the willow foliar $\delta^{13}C$ values. In 2016, however, the foliar $\delta^{13}C$ values at SKB-PA (waterlogging) were only slightly higher (approximately 0.4‰) than those at SKB-PB and SKB-PC. The foliar $\delta^{13}C$ values at SBoydom-PA (waterlogging) were even lower (approximately 1‰) than those at SBoydom-PB. Moreover, between 2015 and 2016 the foliar $\delta^{13}C$ values at PA on SKB and SBoydom decreased by 1.7 ± 0.6‰ and 2.1 ± 0.9‰, respectively, despite waterlogging occurring in both years. To date, very few studies have investigated the reasons for the lack of changes or negative shifts in foliar $\delta^{13}C$ value under waterlogging conditions. We propose that, under the long period waterlogging ("LWL", Figure 2.3) as caused by large flooding observed at PAs on SKB and SBoydom in 2016, the changes in the *A* are another important factor controlling the foliar $\delta^{13}C$ values, besides *gs*. In the present study, low *A* was observed during submergence, before July 20 and more flooded SPh-PA (Figure 2.6c). It has been suggested that waterlogging induces low carboxylation rate by reducing the amount or activity of Rubisco enzyme (Vu & Yelenosky, 1992; Islam & Macdonald, 2004). According to equation (2.3), the foliar $\delta^{13}C$ values should be dependent on both *A* and *gs*; thus, low *A* may have caused the negative shifts in the foliar $\delta^{13}C$ value in LWL compared to WL, as the foliar $\delta^{13}C$ values at SPh-PA were slightly lighter than at SPh-PB, albeit insignificantly so (Figure 2.6a). Therefore, the low foliar $\delta^{13}C$ values observed in the willows at the PAs on SKB and SBoydom in 2016, can be explained by this continuously low photosynthetic activity under long period waterlogging caused by large flooding. The low $\delta^{13}C$ values, *A*, and *gs* under large flooding were previously reported for a pot experiment involving the invasive wetland grass (*Phalaris arundinacea*) (Waring & Maricle, 2012). The aforementioned findings together suggest the hypothesis that long period waterlogging (or large flooding) significantly reduces

the foliar $\delta^{13}C$ values compared to sporadic waterlogging (or small flooding), and that the contribution of *A* and *gs* is highly dependent on the frequency and magnitude of waterlogging events.

Thus, the large differences (Figure 2.4) in hydrological conditions (i.e., "Dry", "Wet", "WL" and "LWL", Figure 2.3) found in transects suggest that the possible foliar $\delta^{13}C$ values can correspond to scenario 3 in Figure 2.1 (i.e. reduced foliar $\delta^{13}C$ values in long period waterlogging), which can be well interpreted by that both *A* and *gs* are affected by hydrological gradients (Figure 2.7).

However, we note that scenario 3 in this study is a very simplified, schematic hypothesis, without quantitative meaning. There are other several potential factors for controlling the foliar $\delta^{13}C$ values, for example mesophyll conductance (*gm*) (Evans et al., 1986). As *gs* and *gm* were found tightly coupled (Vrábl et al., 2009), and both controlled the limitation of CO_2 diffusion. Therefore, in our field study of the determining factors of foliar $\delta^{13}C$ values, we mainly focused on *gs* which can be directly monitored. Detailed changes in the $\delta^{13}C$ value particularly between and within the four hydrological conditions will be illustrated in further studies with determination of these potential factors, including but not limited to *gm* changes under different water regimes, the quantitative meaning of *gs* and *gm* on foliar $\delta^{13}C$ values, and the features of *gs* and *gm* in species with different water tolerance.

2.4.3 Spatial difference in the willow foliar $\delta^{13}C$ value

LMMs analysis of the local scale random sampling indicated that only the willows in the xeric larch forest were slightly but statistically enriched in ^{13}C ($F_{4,168}$ = 2.58, P = 0.039), compared to the other conditions, in 2016. It is accepted that the foliar $\delta^{13}C$ values are higher in dry than in wet conditions, due to stomatal regulation. In contrast, there was no statistical difference in the foliar $\delta^{13}C$ value for either year or among the hydrological conditions tested here (flooding, flooded, on land, and wetland) (Figure 2.5). The first three conditions were situated near the river and at different levels of flooding, whereas the fourth was never affected by flooding, although it was still abundant in water. These results are likely consistent with the lack of difference in the foliar $\delta^{13}C$ value among various hydrological conditions in mesic regions or periods, as reported previously (Garten & Taylor, 1992; Alstad et al., 1999). Nevertheless, relatively minor variations in the $\delta^{13}C$ value in willows growing under these

hydrological conditions cannot be explained by the common dry-wet stomatal regulation theory mentioned above. On the other hand, our transect data in this study can explain why there was a slight variation in the foliar $\delta^{13}C$ value among local scale random sampling sites in response to hydrological gradients as follows.

In 2015, the water level in the river was low. Consequently, the hydrological status of the willows growing nearest the river in the "Flooded" zone, ranged from slight flooding (similar to "Wet" in Figure 2.3 and Figure 2.4; i.e. "small flooding" in Figure 2.7) to sporadic flooding (results in sporadic waterlogging, e.g. "WL" in Figure 2.3 and Figure 2.4; i.e. "medium flooding" in Figure 2.7). Slight flooding near the wet condition zone caused the stomata to open and low foliar $\delta^{13}C$ values. In contrast, medium flooding resulted in stomatal closure and high foliar $\delta^{13}C$ values. Therefore, under the "Flooded" condition, the $\delta^{13}C$ values varied between low and high. This behavior resembles the positive-negative shift in the foliar $\delta^{13}C$ value for the "On land" zone under dry-wet conditions. The same interpretation applies to the foliar $\delta^{13}C$ values measured in the "Wetland" (Figure 2.5).

In contrast, in 2016, the water level in the river was high. Therefore, the hydrological status of the willows growing nearest the river in the "Flooding" zone varied between sporadic flooding (results in sporadic waterlogging, e.g. "WL" in Figure 2.3 and Figure 2.4; i.e. "medium flooding" in Figure 2.7) and continuous flooding (leads to long period waterlogging, e.g. "LWL" in Figure 2.3 and Figure 2.4; i.e. "large flooding" in Figure 2.7). Large flooding reduced both, A and foliar $\delta^{13}C$ values. As was the case for 2015, the conditions in the "Flooded" and "Wetland" zones of 2016 ranged between slight and sporadic waterlogging (similar to "Wet" and "WL", respectively in Figure 2.3 and Figure 2.4), although only waterlogging in "Flooded" zones was caused by floods (i.e. "small flooding" and "medium flooding"; Figure 2.7). Therefore, the foliar $\delta^{13}C$ values ranged between low and high in the "Flooding", "Flooded", and "Wetland" areas. These responses resemble the positive-negative shift in the foliar $\delta^{13}C$ value observed for "On land" under dry-wet conditions.

In previous studies (Garten & Taylor, 1992; Alstad et al., 1999), very small differences in the foliar $\delta^{13}C$ value of plants growing near rivers were detected among the diverse hydrological conditions, where waterlogging frequently occurs. These minor differences can also be explained by the physiological responses of willows to the different hydrological conditions (Figure 2.7). If the $\delta^{13}C$ values in other organs

correlate with those determined by the leaves, then historical records of the wide swings in hydrological conditions could be reconstructed using the $\delta^{13}C$ records, such as those obtained from tree ring cellulose.

2.5 Conclusions

To illustrate the effects of hydrological conditions on the $\delta^{13}C$ values in willow leaves, we measured the foliar $\delta^{13}C$ values of willows at three different points, along three transects near the Indigirka River, under several major hydrological conditions (Figure 2.7). Under normal hydrological conditions, the foliar $\delta^{13}C$ values were lower under wet conditions (along rivers and/or during a wet year) than under xeric conditions (far from the river and/or during a dry year), because the former conditions allowed for stomatal opening. On the other hand, under abnormal hydrological conditions, such as waterlogging, high foliar $\delta^{13}C$ values were found, because medium flooding induced stomatal closure. Moreover, long period waterlogging decreased foliar $\delta^{13}C$ value by reducing photosynthetic activity. Thus, there was a small variation in the foliar $\delta^{13}C$ value (−31.6‰ to −25.7‰) in the Indigirka River lowland, despite large diversity in the hydrological conditions (Figure 2.5). These results demonstrate that the foliar $\delta^{13}C$ values reflect hydrological conditions even in mesic environments (Figure 2.7).

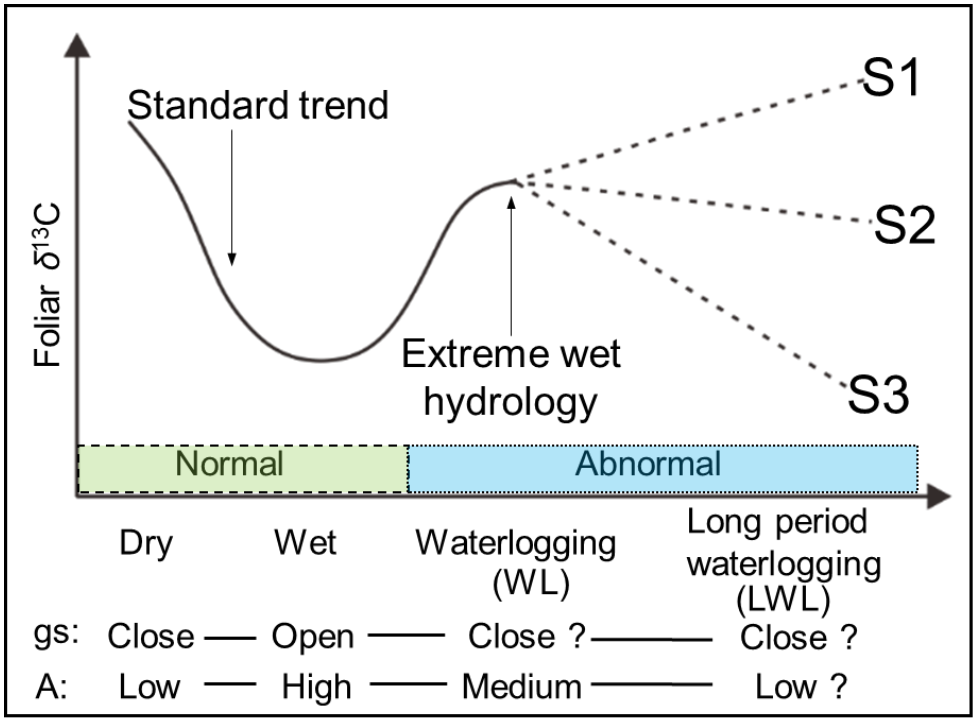

Figure 2.1 Schematic view of the possible foliar $\delta^{13}C$ values under various hydrological conditions. *gs* and *A* are stomatal conductance and apparent photosynthetic activity, respectively. Dry and wet are without waterlogging, and waterlogging and long period waterlogging represent continual and continuous waterlogging, respectively. Possible changes in the foliar $\delta^{13}C$ value are shown for assumed scenarios (S1, S2, and S3).

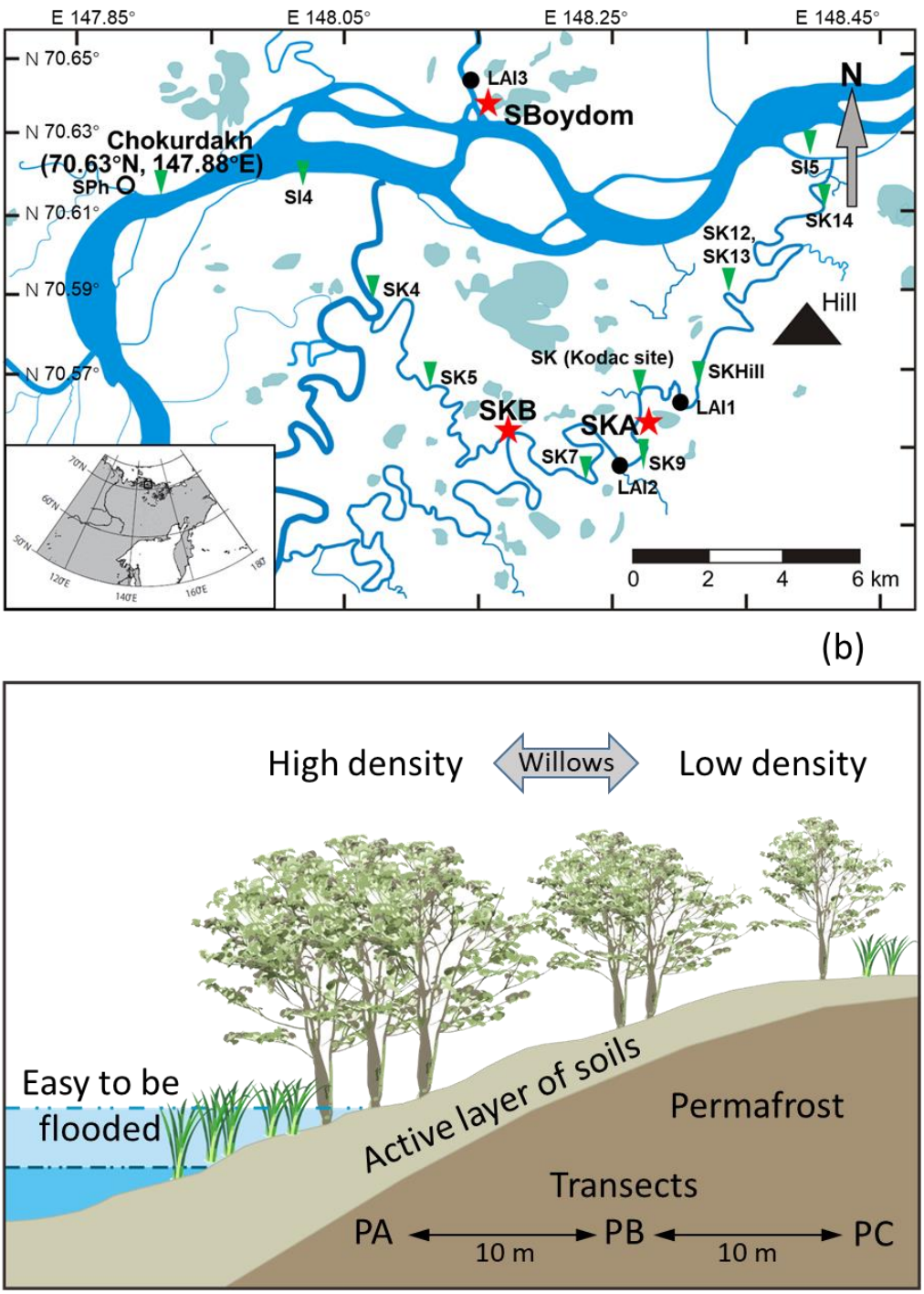

Figure 2.2 (a) Sampling sites near Chokurdakh village in the study region, northeastern Siberia. Thick and thin lines represent the Indigirka River and its tributaries, respectively. Areas filled with light blue represent lakes. Triangles (18), stars (3), filled black circles (3) and empty circle (1) 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.

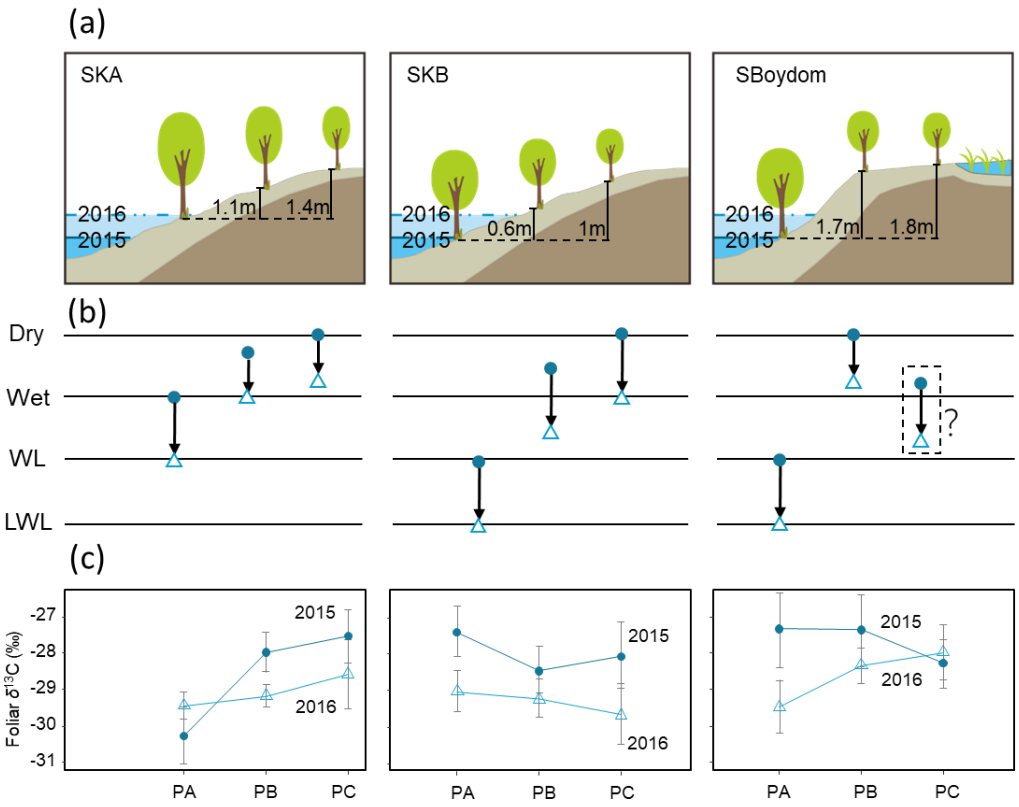

Figure 2.3 (a) Schematic view of each transect with the highest water levels observed in each case in 2015 (blue) and 2016 (light blue) and the height of PB and PC, compared to PA (black line). (b) Possible changes (black arrows) in the hydrological conditions from 2015 (filled circles) to 2016 (open triangles) in each point on transects. Dry and wet are without waterlogging, and WL and LWL represent waterlogging (continual) and long period waterlogging (continuous), respectively. (c) the foliar $\delta^{13}C$ values (‰) found in willows were reported as means ± SD.

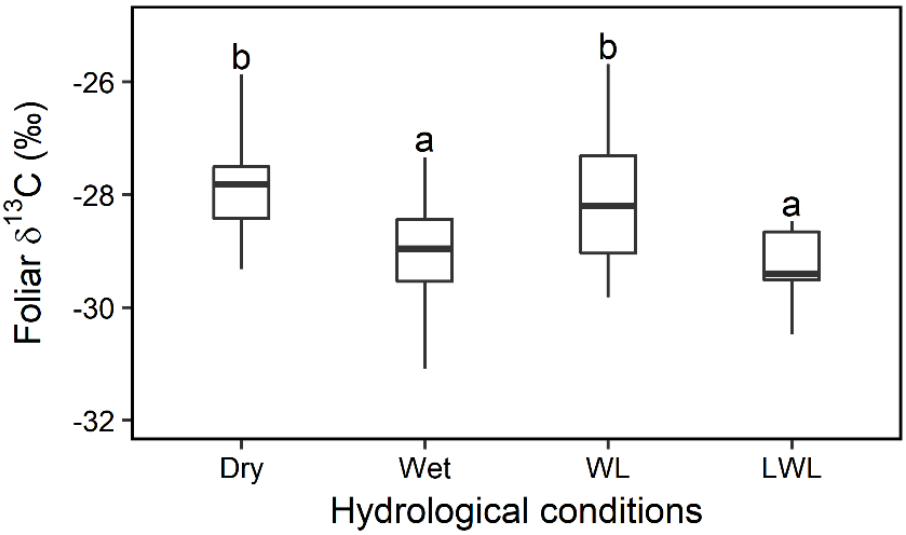

Figure 2.4 Box-and-whisker plot of the statistical analysis for the foliar $\delta^{13}C$ values (‰) under four different hydrological conditions in sampling transects established in year 2015 and 2016. Different letters over the numbers indicate statistically significant differences according to Turkey´s 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.

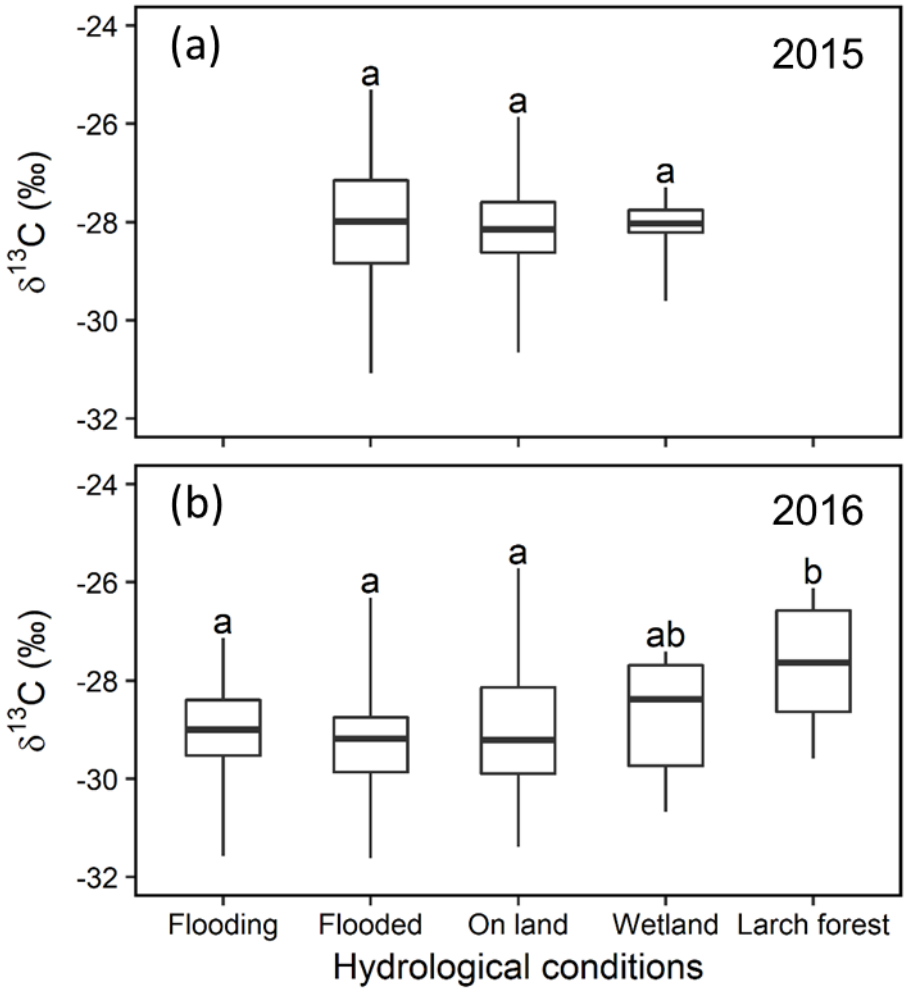

Figure 2.5 Box-and-whisker plot of the statistical analysis for the foliar $\delta^{13}C$ values (‰) under different environments with distinct hydrological conditions at local scale in year 2015 (a) and 2016 (b), different letters over the numbers indicate statistically significant differences according to Turkey´s post hoc test and the Linear Mixed Model.

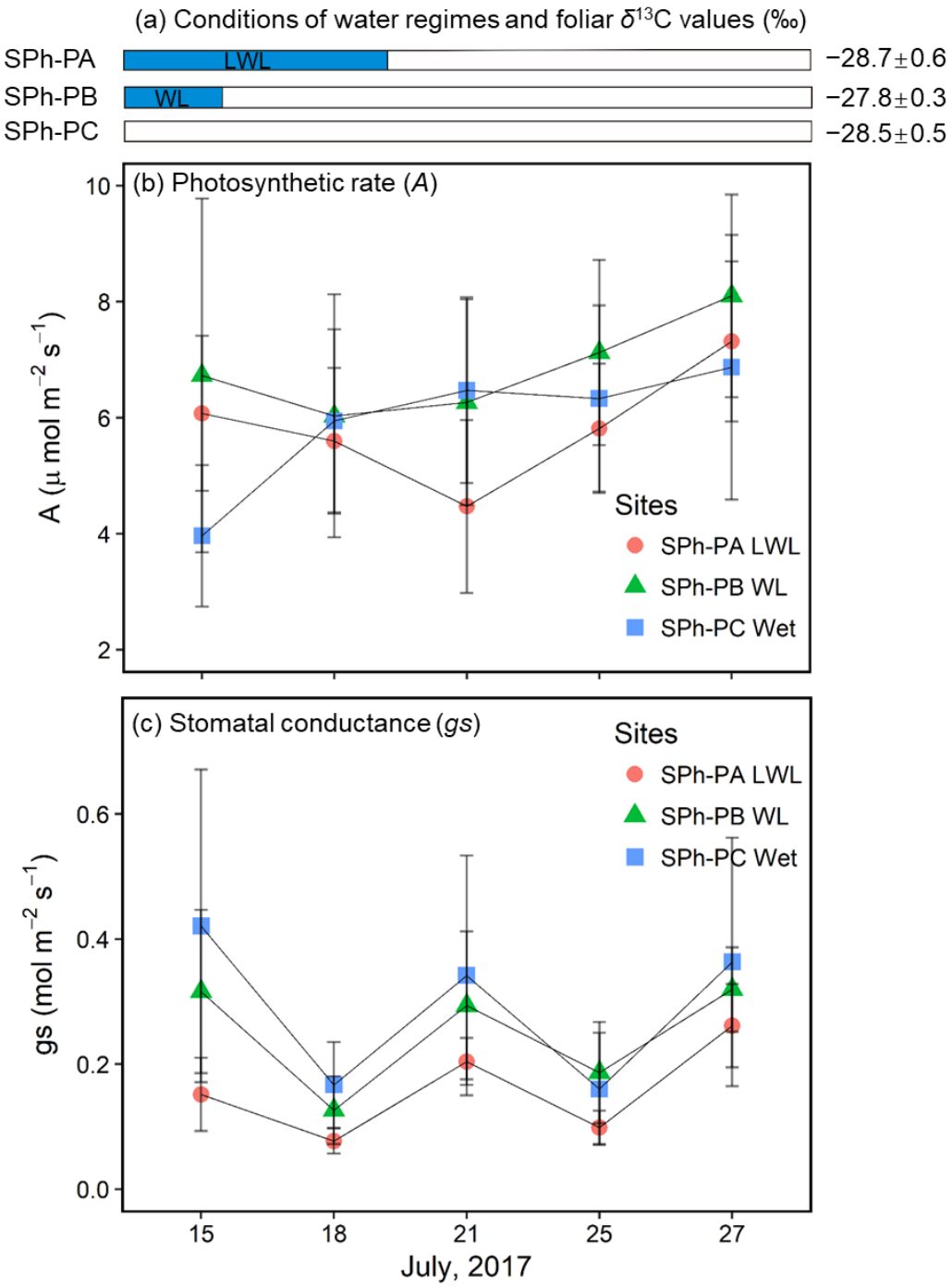

Figure 2.6 (a) Hydrological conditions in SPh-PA (circles), -PB (triangles), and –PC (squares), with the period of submergence is shown in blue shaded bars, with relative foliar $\delta^{13}C$ values (‰), and (b) apparent photosynthetic rate (A, μmol $m^{-2}s^{-1}$) and (c) stomatal conductance (gs, mol $m^{-2}s^{-1}$) with fixed radiation (600 μmol $m^{-2}s^{-1}$), during July 13–27. Means ± SD.

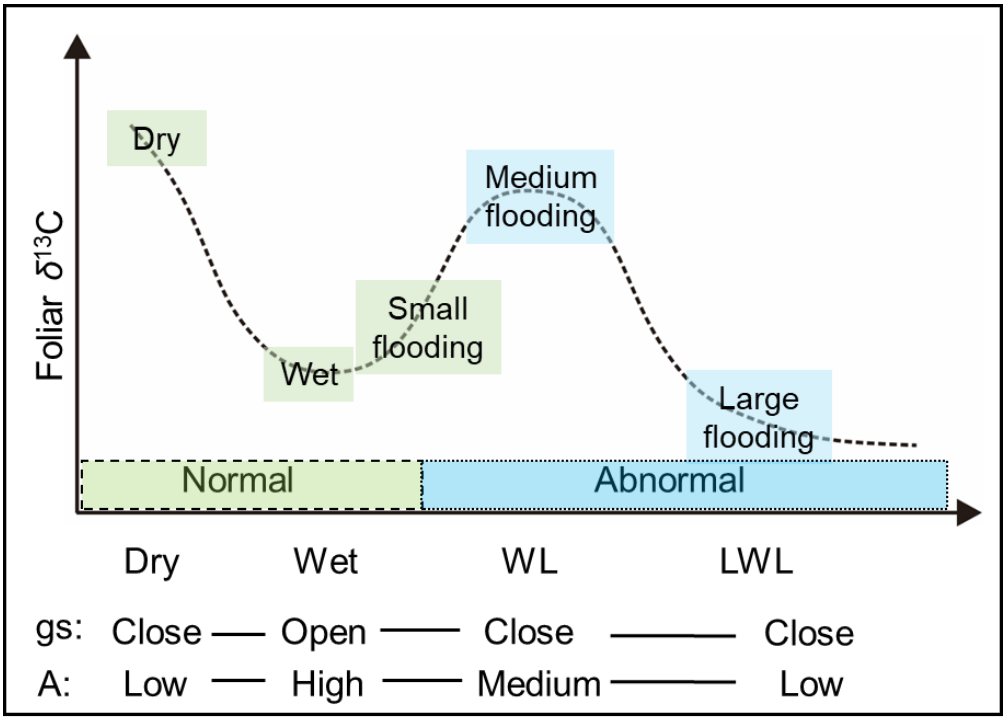

Figure 2.7 The possible foliar $\delta^{13}C$ values with respect to physiological responses to various hydrological conditions. *gs*: stomatal conductance, *A*: photosynthesis activity. Dry and wet are without waterlogging, and WL and LWL represent continual waterlogging and continuous long period waterlogging, respectively.

Table 2.1 Site No., location, distance to river, dominant species, production, ANPP, LAI, δ^{13}C±SD(‰) δ^{15}N±SD(‰) N content ± SD(%) for three 2.5m×2.5m plots in summer of 2016.

Location	Distance to river (m)	Species	Production of leaf(g/m^2·a)	Production of stem(g/m^2·a)	ANPP(g/m^2·a)	LAI	δ^{13}C±SD(‰)	δ^{15}N±SD(‰)	N content±SD(%)
70°33.592'N, 148°17.746'E	> 30 m	*Salix pulchra*	51	11	63	0.59	-27.3±0.2	-3.5±0.1	2.3±0.04
70°32.842'N, 148°15.113'E	3 m	*Salix boganidensis*	85	25	110	1.59	-30.1±0.5	-2.8±0.4	3.1±0.2
70°38.355'N, 148°8.851'E	10 m	*Salix richardsonii*	96	21	117	0.71	-28.4±0.1	-3.8±0.03	2.2±0.1

Table 2.2 Details of sampling site with site No., sampling data, river name, hydrological information, willow species (sampling number) and location of this study. I and K river represent Indigirka and Kryvaya river.

Site No.	Sampling Date	Location	Details	Dominant species composition (sampled number)	**Lat (°)**	**Lon (°)**
15SBoydom	20150725	I river	Transects, position a, b, c: a sometimes flooded(87.5cm), b on land(52.5cm), c on land(52.5cm)	Transects, position a, b, c: a *S. richardsonii (3), S. boganidensis (1)* b *S. richardsonii (4)* c *S. richardsonii (4)*	70.64	148.15
15SI1	20150729	I river	On land	*S. richardsonii (3), S. glauca (2), S. boganidensis*	70.53	147.81
15SI2*	20150729	I river	On land	*S. alaxensis (5)*	70.33	147.49
15SI4	20150731	I river	On land	*S. richardsonii (2), S. boganidensis (2)*	70.62	147.95
15SI5	20150725	I river	Flooding and flooded	*S. richardsonii (2), S. pulchra (3)*	70.62	148.39
15SK	20150725	K river	Flooding and flooded	*S. glauca (1), S. boganidensis (3), S. pulchra*	70.56	148.26
15SK11	20150725	K river	Flooding and flooded	*S. boganidensis (4), S. glauca (2)*	70.56	148.31
15SK12	20150725	K river	Flooding and flooded	*S. boganidensis (2), S. pulchra (3)*	70.59	148.34
15SK13	20150725	K river	Flooding and flooded	*S. boganidensis (2), S. pulchra (2)*	70.59	148.35
15SK14	20150725	K river	Flooding and flooded	*S. richardsonii (2), S. glauca (1), S. pulchra (2)*	70.61	148.41
15SK4	20150731	K river	Flooding and flooded	*S. boganidensis (2), S. alaxensis (2)*	70.59	148.07
15SK5	20150731	K river	Flooding and flooded	*S. boganidensis (4)*	70.57	148.11
15SK9	20150731	K river	Flooding and flooded	*S. glauca, S. boganidensis (4)*	70.55	148.27
15SKA	20150801	K river	Transects, position a, b, c: a nearly flooded(67.5cm), b on land(52.5cm), c on land(31.5cm)	Transects, position a, b, c: a *S. boganidensis (4)* b *S. boganidensis (4), S. glauca (1)* c *S. boganidensis (4), S. glauca (1)*	70.55	148.26
15SKB	20150801	K river	Transects, position a, b, c: a sometimes flooded(70cm), b on land(52.5cm), c on land(50cm)	Transects, position a, b, c: a *S. glauca (4), S. boganidensis (1), S. richardsonii (1)* b *S. glauca (4)* c *S. glauca (4)*	70.55	148.16
15SKHill1,7	20150801	K river	Flooding and flooded	*S. pulchra (1), S. richardsonii (1), S. glauca (2)*	70.56	148.30
15SKHill2,3,4,5,6	20150801	Hill	On hill	*S. reptus, S. richardsonii (2), S. glauca (1)*	70.56	148.31
15SVK*	20150729	Site VK	Wetland, on land and flooded	*S. richardsonii (5), S. glauca (5), S. pulchra (1), S. boganidensis (3)*	70.25	147.47
16SI1*	20160721	I river	On land	*S. boganidensis (8) S. alaxensis*	70.25	147.46
16SI2*	20160721	I river	On land	*S. boganidensis (6), S. alaxensis*	70.33	147.44
16SI3*	20160721	I river	Flooded and on land	*S. boganidensis (8), S. alaxensis*	70.38	147.52
16SI4*	20160721	I river	Flooding and flooded	*S. boganidensis (7), S. alaxensis, S. pulchra (1)*	70.48	147.68
16SI5	20160721	I river	Flooded and on land	*S. boganidensis (8), S. alaxensis*	70.53	147.89
16SI6	20160721	I river	On land	*S. boganidensis (8), S. alaxensis*	70.58	147.88
16SVK-1, 2, 5*	20160721	Site VK	Wetland	*S. richardsonii (8), S. pulchra (1), S. glauca (2), S. boganidensis (1)*	70.25	147.47
16SVK-3, 4, 6, 7, 8*	20160721	Site VK	Larch forest	*S. richardsonii (8), S. glauca (13)*	70.25	147.47
16SK1	20160722	K river	Flooded and on land	*S. pulchra (2), S. boganidensis (6)*	70.55	148.23
16SK2	20160722	K river	Flooding and on land	*S. richardsonii, S. glauca (7)*	70.55	148.27
16SI7	20160723	I river	Flooded and on land	*S. richardsonii (1) S. pulchra (3), S. boganidensis (4)*	70.62	147.95
16SKA	20160723	K river	Transects, position a, b, c: a flooding(70.5cm), b on land(52.7cm), c on land(32.3cm)	Transects, position a, b, c: a *S. boganidensis (4)* b *S. boganidensis (4)* c *S. boganidensis (1), S. glauca (3)*	70.55	148.26
16SKB	20160723	K river	Transects, position a, b, c: a flooding, b flooding(60cm), c flooded(66.2cm)	Transects, position a, b, c: a *S. glauca (4)* b *S. glauca (4)* c *S. glauca (4)*	70.55	148.16
16SBoydom	20160723	I river	Transects, position a, b, c: a flooding, b on land(52.7cm), c on land(42.5cm)	Transects, position a, b, c: a *S. richardsonii (4)* b *S. richardsonii (4)* c *S. richardsonii (4)*	70.64	148.15
16SK	20160723	K river	Flooding and on land	*S. richardsonii (6), S. pulchra (2)*	70.56	148.26
16SK4	20160723	K river	Flooding and on land	*S. pulchra (4), S. boganidensis (4)*	70.59	148.07
16SK5	20160723	K river	Flooded and on land	*S. richardsonii (3), S. pulchra (5)*	70.57	148.11
16SK7	20160723	K river	Flooded and on land	*S. boganidensis (8)*	70.54	148.23
16SAllaiha*	20160724	I river	Flooding and on land	*S. boganidensis (2), S. richardsonii (6), S. pulchra (3)*	70.52	147.30
16SAllaiha-hill*	20160724	Hill	On hill	*S. boganidensis (1), S. glauca (2), S. richardsonii (1)*	70.52	147.28

The Site No. in bold with asterisk is not shown in Figure 2.

Figure A2.1 Root distribution of willows near river. Root length usually reaches to 1 m with the largest abundance in 0~30 cm depth.

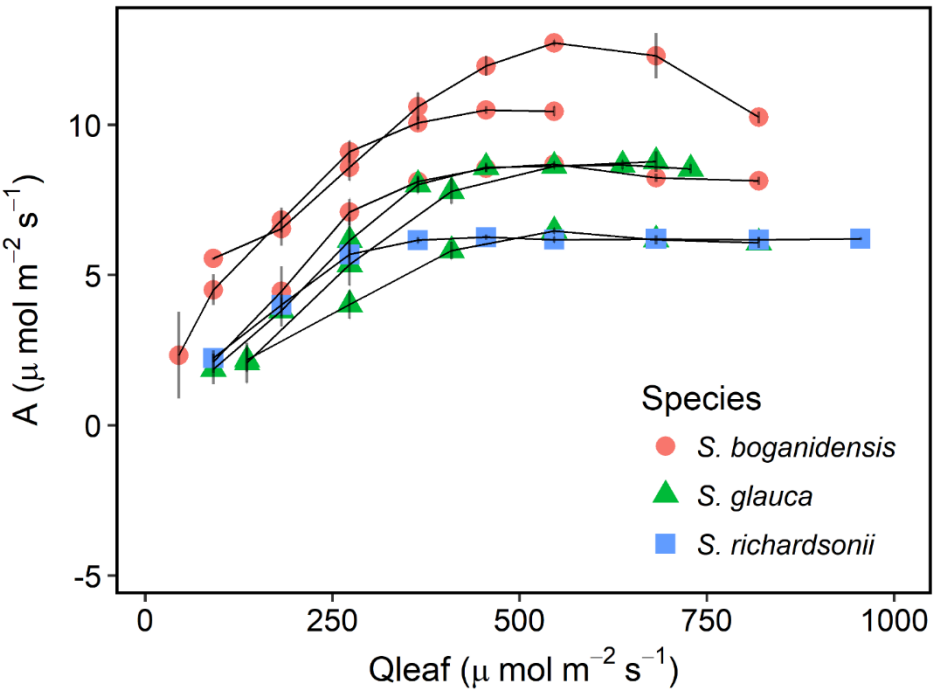

Figure A2.2 Photosynthesis (A) and light (Qleaf) response curve of the willows *S. boganidensis* (red cycles), *S. glauca* (green triangles), and *S. richardsonii* (blue squares). Mean ± SD.

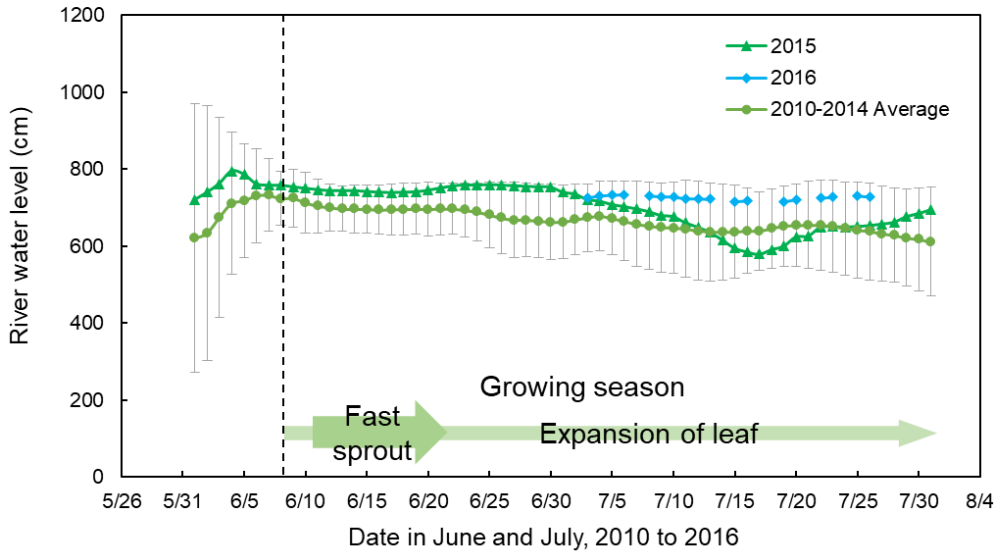

Figure A2.3 The river water level (cm) from 1st June to 31st July, for the average in 2010-2014 (circles), 2015 (triangles) and 2016 (diamonds). Unfortunately there is no complete data in 2016. The arrow lines indicate the observed growing season, with fast growing period in wide arrow line and followed by slow growing period in narrow arrow line.

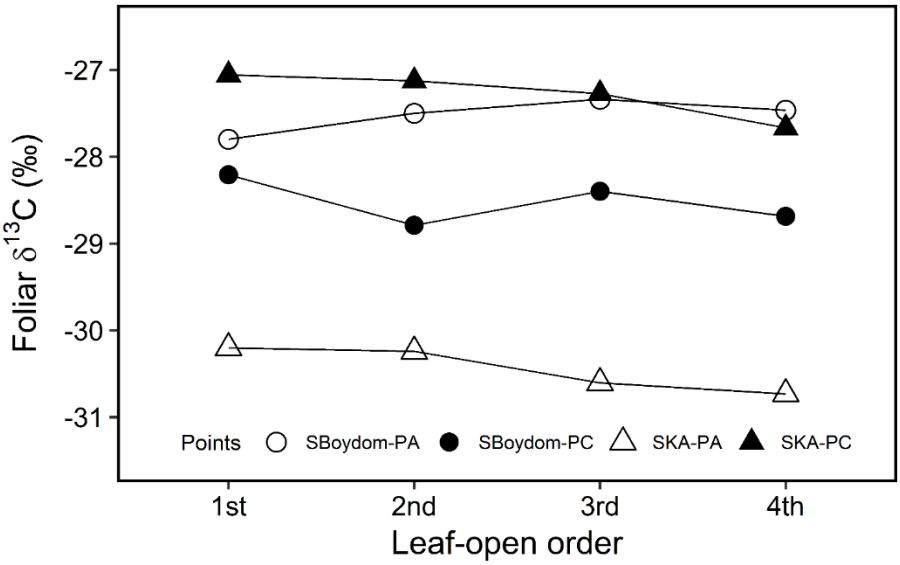

Figure A2.3 The foliar δ^{13}C values (‰) of the leaves according to the leaf-open order, upon single shoot at each point of PA (empty symbols) and PC (filled symbols) in SBoydom (circles) and SKA (triangles). The different points are shown in different symbols.

Chapter 3 Effect of floods on the nitrogen conditions in willows in Northeastern Siberia using stable N isotope

3.1 Introduction of stable N isotopes in plants

The biogeochemistry of the nitrogen cycle, which includes many internal and external transformations and fluxes observed at different timescales, is very complex in natural environment. The faster reactions of lighter isotope (^{14}N) than heavier isotope (^{15}N) in physical and biological (enzymatic) processes, which result in discriminating against the heavier isotope and significant isotopic fractionation, can lead to ^{15}N depletion in products and ^{15}N enrichment in residual substrates (Mariotti et al., 1981; Hogberg, 1997; Robinson, 2001). For example during nitrification processes in soil N cycling, microbial discrimination against ^{15}N causes the ^{15}N-depleted product (i.e., nitrate) and results in the ^{15}N-enriched remaining substrate (i.e., soil organic matter). Also, when the ^{15}N-depleted products leave the system through such as leaching of ^{15}N-depleted nitrate, the remaining soil N pools become enriched in ^{15}N (Mariotti et al., 1981; Hogberg, 1997; Robinson, 2001). There is equation (3.1),

$$\delta^{15}N = (R_{sample}/R_{standard} - 1) \times 1000\ (‰) \quad (3.1),$$

where R_{sample} and $R_{standard}$ represent molar ratios of heavy to light isotopes ($^{15}N/^{14}N$) of the samples and standards, respectively. The important processes which discriminate against ^{15}N in the soil N cycling including mineralization, ammonia volatilization (38‰ to 40‰, (Hogberg, 1997)), microbial nitrification (5‰ to 112‰, (Pérez et al., 2006)) and N immobilization, nitrate leaching, microbial denitrification (11‰ to 75‰, (Barford et al., 1999; Dawson et al., 2002; Sutka et al., 2008)). The discriminations of ^{15}N from soil to plant productions are relatively small (Dawson et al., 2002; Pardo & Nadelhoffer, 2010). Therefore, the $\delta^{15}N$ values of soils and plants are becoming increasingly popular in studies of soil N dynamics and terrestrial N cycling, which are difficult to measure directly at multiple spatial scales (Hogberg, 1997; Robinson, 2001; Evans, 2007; Craine et al., 2009, 2015).

The soil and foliar δ^{15}N values were found high in nutrient-rich ecosystems, because of high N supply, fast biogeochemical N transformation rates and a high potential for isotopically fractionating gaseous N losses relative to pool sizes (Craine et al., 2009). This can be evidenced by correlations between plant δ^{15}N values and net N mineralization and/or nitrification (Miegroet, 1994; Kitayama & Iwamoto, 2001; Pardo et al., 2006; Craine et al., 2009; Watmough, 2010; Cheng et al., 2010), N deposition (Pardo et al., 2006) and foliar N concentration (Pardo et al., 2006; Craine et al., 2009). By contrast, the soil and foliar δ^{15}N values were discovered low in nutrient-poor ecosystems, because the N cycle in nutrient-poor ecosystems is dominated by microbial immobilization and plant uptake, with lower gaseous N losses relative to pool sizes and efficient internal N cycling (Evans, 2007; Pardo & Nadelhoffer, 2010).

There have been many fruitful studies in variation of δ^{15}N values and the identification of the major abiotic and biotic factors across large geographical scales (regional to global) in recent years. In general, the major factors determining large spatial heterogeneity in soil and vegetation δ^{15}N values including temperature, precipitation, mycorrhizal association type, soil fertility and texture, and plant nutrients (Amundson et al., 2003; Aranibar et al., 2004; Hobbie & Högberg, 2012; Craine et al., 2015). At regional to global scales, the δ^{15}N values correlate negatively with mean annual rainfall (Handley et al., 1999; Craine et al., 2009), because of greater ^{15}N-depleted gaseous N losses relative to pool sizes in drier climates (Pardo et al., 2006). Whereas, the correlations between water availability and plant Aδ^{15}N are often reversed at local scales, as wetter areas (e.g., valley bottoms) often show higher δ^{15}N values than nearby drier areas (e.g., hill ridges) in the local landscape (Garten, 1993; Handley et al., 1999; Bai et al., 2009). There are possible explanations, such as higher ammonia volatilization rates and higher microbial activity and thus higher biogeochemical N cycling rates under greater soil moisture (Handley et al., 1999; Pardo & Nadelhoffer, 2010), higher N mineralization and nitrification rates in depressions (Garten, 1993; Bedard-Haughn, van Groenigen & van Kessel, 2003), higher denitrification rates in valley bottoms (Sutherland et al., 1993). However, these explanations can lead to either

adequate or inadequate nitrogen conditions for the plant. Meanwhile, nitrogen is crucial to photosynthesis and ~75% of leaf N can be extracted from chloroplasts (e.g. Evans, 1989). The nitrogen content in C3 plants is tightly correlated to plant photosynthetic capacity (Evans, 1989; Reich et al., 1995; Reich, Ellsworth & Walters, 1998; Tognetti & Peñuelas, 2003; Zhao, Xiao & Liu, 2006). Thus, the nitrogen in plant can be an useful tool to investigate the nutrient conditions and nitrogen cycles in soil. The combination of plant δ^{15}N values and nitrogen contents can be a good indicator to investigate the nitrogen status in plants.

As the willows are easily submerged by flooding growing on the riverbanks, they can be good target to figure out the nutrient availability and variation under frequent river floods in the Arctic. Therefore, in the chapter 3, we tried to determine the effect of floods on the nutrient conditions in willows in Northeastern Siberia using foliar stable N isotope under four major hydrological conditions: dry, wet, and short and long period waterlogging. We measured the nitrogen content and δ^{15}N values of bulk leaves from willows grown sites and soil bulk under these flooding regimes in the Indigirka River lowland of Northeastern Siberia.

3.2 Materials and Methods

3.2.1 Plant and soil samples

Detail information for study area, willows, sampling sites and methods of leaf sampling in willows were shown in part 2, Chapter 2 (Figure 2.2, Table 2.2). Soil samples were also collected at each point on all transects. 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 −20ºC in 50 ml glass vials before I carried them to the lab.3.2.2 Stable nitrogen isotope analysis

Leaf samples were pre-treated with the method described in part 2, Chapter 2. After the soil samples were dried under 105ºC for 48 hours, the soil samples were sieved and

the roots with diameter over 2 mm were removed. The soil samples were milled into fine powder without liquid N_2 and dried again at 60 °C for 48 hours. 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). Bulk nitrogen content was obtained via elemental analyzer (Flash EA 1112, Thermo Fisher Scientific, Bremen, Germany). Isotope ratios explained by the equation (3.1).

The stable nitrogen isotopic composition was reported in the standard δ notation relative to atmospheric N_2. A laboratory standard was injected after every ten samples to verify that the analytical accuracy was better than 0.2‰. To reduce the effect of sampling heterogeneity in $\delta^{15}N$ within a single site, four samples were measured and the average isotopic composition was reported for each site, with the standard deviation ranging from 0.3‰ to 1.5‰ (average, 0.8‰).

3.2.3 River water level measurement and nutrient analysis

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 NO_3^-, NH_4^+, and total dissolved N (TDN) were analyzed using a continuous flow analyzer (Bran and Luebbe, Norderstedt, Germany) in Hokkaido University, the standard deviation of lab standard samples for concentration of NO_3^-, NH_4^+, and TDN are less than 0.15 μmol/L, 0.02 μmol/L and 0.7 μmol/L respectively.

3.2.4 Statistical analysis

Linear Mixed Models (LMMs) were used to clarify differences in the foliar $\delta^{15}N$ values and nitrogen content among willows growing in three transects in 2015 and 2016. Foliar $\delta^{15}N$ values and nitrogen content was set as the response variable, with flooding conditions was set as the fixed effect, and species (i.e., *S. boganidensis*, *S. richardsonii*,

and *S. glauca*) was set as a random effect. Inter-annual variation was checked within a linear model in which fixed effect was set to year. Similar analyses by LMMs were also used to figure out any differences in the foliar δ^{15}N values and nitrogen content among the willows randomly collected on the Indigirka River lowland in 2015 and 2016. Foliar δ^{15}N values and nitrogen content value was set as the response variable, the flooding condition was assigned as the fixed effect, and the location (along the mainstream or the tributary), and species, were set as random effects. Tukey's test was used as a post hoc analysis for multiple comparisons. Linear Mixed Models (LMMs) were also used to test the correlation between the foliar δ^{13}C values and nitrogen content among willows growing in each transects in 2015 and 2016. Also the correlations between maximum thaw depths and foliar δ^{15}N values, between those and nitrogen content on transects, respectively, were checked. The lme4 package (Bates, Maechler Martin & Walker, 2015) of R (R Core Team 2015, v. 3.3.2) was used to build the LMMs.

3.3 Results

3.3.1 Foliar δ^{15}N values and nitrogen contents in the transects

Foliar δ^{15}N values of all transects in 2015 and 2016 showed similar trends (Figure 3.1), that the foliar δ^{15}N values were always higher at PA (near river) and lower at PC (on land). Generally, the transect SKB has the relatively higher foliar δ^{15}N values compared to the other two transects, SKA and SKB. Along transect SKA in 2015, the mean foliar δ^{15}N values were −1.6 ± 0.8‰ for PA (close to the river but without submergence), which was much higher than those for PB (−3.0 ± 0.6‰) and PC (−5.0 ± 0.7‰). In 2015, the sampling point at PA along both, the SKB and SBoydom transects, were sometimes waterlogged; whereas, all points PB and PC were not. The mean foliar δ^{15}N values were 3.0 ± 0.7‰ and 1.2 ± 1.1‰ for PAs in transects SKB and SBoydom, respectively, which were much higher than those for PBs (−2.5 ± 0.6‰ in transect SKB and −4.8 ± 1.0‰ in transect SBoydom) and PCs (−1.9 ± 0.9‰ in transect SKB and −4.5 ± 0.7‰ in transect SBoydom). In 2016, the sampling points, PA along SKA and PB along SKB, were also sometimes waterlogged. In this year, the foliar δ^{15}N values were higher at PAs (0.1 ± 0.4‰ in transect SKA, 3.8 ± 0.6‰ in transect SKB and 1.7

± 0.7‰ in transect SBoydom) than those at PBs (−3.8 ± 0.6‰ in transect SKA, −0.8 ± 0.5‰ in transect SKB and −4.6 ± 0.5‰ in transect SBoydom) and PCs (−4.7 ± 0.9‰ in transect SKA, −1.4 ± 0.9‰ in transect SKB and −3.5 ± 0.8‰ in transect SBoydom) in each transect. The inter-annual variation of foliar δ^{15}N values from 2015 to 2016 was only found significant increases in PA of SKA (increased 1.7±0.6‰, $F_{1,6}$ = 7.889, P = 0.03) and PB of SKB (increased 1.7±0.6‰, $F_{1,6}$ = 30.59, P = 0.001), where the water conditions changed from no submergence to sometimes waterlogged. Overall, statistical analysis of the foliar δ^{15}N values from transects (in Figure 3.2) showed a significant difference ($F_{3,70}$ = 60.184, P < 0.001, Figure 3.2) among the four major hydrological conditions (i.e., “Dry”, “Wet”, “WL”and “LWL”, in Figures 3.1 and 3.2). The foliar δ^{15}N values were high in waterlogging conditions. Conversely, values without waterlogging were consistently low.

In general, foliar nitrogen content in all transects showed higher values in near river points of both 2015 and 2016 although the lack of statistical significance. In 2015, foliar nitrogen content at PAs was 2.8±0.2% in SKA, 2.3±0.5% in SKB and 2.8±0.3% in SBoydom, respectively, a slightly higher than those at PBs (2.2±0.1% in SKA, 2.2±0.2% in SKB and 2.3±0.1% in SBoydom) and PCs (2.4±0.4% in SKA, 2.2±0.2% in SKB and 2.0±0.2% in SBoydom). In 2016, almost all the points showed no significant differences in foliar nitrogen content with the former year, except those at PC in SKA (increased 0.9±0.6%, $F_{1,7}$ = 5.778, P = 0.05) and PA in SBoydom (decreased 0.8±0.3%, $F_{1,7}$ = 13.63, P < 0.01).

There was significant positive correlation between maximal thaw depth (thaw depth in mid-summer in July) and the foliar δ^{15}N values, in both year 2015 (r^2=0.55, p<0.01) and 2016 (r^2=0.61, p<0.01) (Figure 3.3, Table 2.2). Also there was a positive correlation between maximal thaw depth with foliar nitrogen content in year 2015 (r^2=0.17, p=0.007) (Figure 3.3). However, in 2016, the negative correlation was found between these two parameters (r^2=0.26, p=0.006) (Figure 3.3).

3.3.2 Spatial distribution in the foliar δ^{15}N values and nitrogen contents of willows

For spatial differences of foliar δ^{15}N values and nitrogen content in randomly local scale sampling along Indigirka River and its tributary Kryvaya (Figure 2.2), it showed the willow foliar δ^{15}N values ranged from −5.6‰ to 5.3‰ and nitrogen content ranged from 1.5% to 3.9% in 2015. In the summer of 2016 for similar sampling strategy, the foliar δ^{15}N values were found between −6.8‰ and 4.9‰ and the foliar nitrogen content were found falling into 1.4% and 4.3%. In general, different foliar δ^{15}N values were found under distinct flooding conditions.

In the analyses of LMMs, even though the locations (i.e., willows growing along mainstream or tributary) were set as the fixed factors, there were still differences found in foliar δ^{15}N values between tall species (e.g., *S. boganidensis*) compared to shorter species (e.g., *S. richardsonii*, *S. glauca*, etc) (Figure 3.4). Thus, effects of locations and species' differences were both need to be removed. In 2015, the willows observed growing under different flooding conditions including flooded (i.e., between slight and sporadic waterlogging), on land (no floods) and in wetland. In 2016, the willows were observed under different flooding conditions including continuous flooding (i.e., long period waterlogging), flooded (i.e., between slight and sporadic waterlogging), on land (no floods), in wetland, and in larch forest. The significant differences in foliar nitrogen content and foliar δ^{15}N values were found in both years. Specific results from Turkey post hoc test from Figure 3.5. In in 2015, it shows that willows growing near river (i.e., continuous flooding and flooded) had higher foliar δ^{15}N and higher foliar nitrogen content values than willows growing on land, however, there was no differences in either the foliar δ^{15}N or foliar nitrogen content between on land and wetland conditions. In 2016, the same results were also found in foliar δ^{15}N values, but there was no significant difference in foliar nitrogen content among flooding conditions including continuous flooding, flooded, on land and wetland. In 2016, willow growing in larch forests showed highest foliar nitrogen content compared to other flooding conditions (also the highest foliar δ^{13}C values can be found in larch forests, from part 2.3.2 in

Chapter 2), however their foliar $\delta^{15}N$ values were same as willows grown on land or in wetland.

The foliar $\delta^{15}N$ values of three LAI measurement block were −3.5±0.1‰, −2.8±0.4‰, −3.8±0.03‰, respectively, at the same time nitrogen content of them three are 2.3±0.04%, 3.1±0.2% and 2.2±0.1% (Table 2.1). The willow growing in the block that locates nearest to river had the highest foliar $\delta^{15}N$ values and nitrogen content.

3.3.3 Soil $\delta^{15}N$ values in the transects

The $\delta^{15}N$ values of bulk soil in the transects varied between different flooding conditions and at different soil depths in 2015 (Figure 3.6). Between different flooding conditions, the enrichment in the $\delta^{15}N$ values of bulk soil under flooding conditions was found. The $\delta^{15}N$ values of bulk soil were higher at PAs which were near river and easily to be flooded, compared to PBs and PCs which were far from river with lower possibilities to be flooded. Additionally, the $\delta^{15}N$ values of surface soil was lighter than deeper soil, indicates that soil $\delta^{15}N$ values increased when soil deepened.

3.3.4 Relative river water level and water nutrient

Relative river water level in July, 2016 was higher than 2015 by around 80 cm in average. The trend in 2015 decreased in start and followed an increase. However, there was only a slightly decreasing in 2016 compared to 2015, which means in 2016 the flooding was severer than 2015 and also last longer.

Average concentrations (SD) of NO_3^-, NH_4^+, and total dissolved N (TDN) of river water in 2015 were 1.76 ± 1.80 μmol/L, 0.39 ± 0.1μmol/L, 19.45 ± 2.61 μmol/L. And they are 2.91 ± 0.89 μmol/L, 0.58 ± 0.26 μmol/L, 21.78 ± 4.54 μmol/L in 2016, respectively. Though there was little sample quantity in 2015, we can still find that there is higher nutrient concentration in 2016 when higher water level appears. Considered the plant mainly using NO_3^- and NH_4^+ while later one was almost not existing in both year 2015 and 2016, there is only concentration of NO_3^- shown in Figure 3.7.

The average concentrations (SD) of NO_3^-, NH_4^+, and total dissolved N (TDN) in

precipitation in July of 2017 were 6.0 ± 4.2 μmol/L, 12.0 ± 9.3 μmol/L, 18.2 ± 13.4 μmol/L, with the maximum 14.1 μmol/L, 26.3 μmol/L, 41.7 μmol/L in individual rain.

3.4 Discussion

3.4.1 Effects on foliar δ^{15}N values from different hydrological conditions

The foliar δ^{15}N values of willows were found low and lack of changes from 2015 to 2016 at points kept with no submergence in both year (i.e., PBs in SKA and SBoydom, and PCs in all transects.) (Figure 3.1). This suggested that the willows growing at PBs and PCs, which were not affected by flooding, reached to the N sources with similar δ^{15}N values, and the N sources were relatively stable if the hydrological conditions kept stable without submergence by flooding.

Meanwhile, the higher foliar δ^{15}N values were always found at PAs than PBs or PCs, as the bulk soil δ^{15}N values were also found higher at PAs which were more closed to river than PBs or PCs (Figure 3.6). Also the local scale randomly sampling showed the similar result that high foliar δ^{15}N values can be found in sites which were closed to the river and easy to be waterlogged under floods. This suggested that waterlogging under flooding conditions increased foliar δ^{15}N values not only in particular sites but also in a large area along river. These indicated that the willows which were growing at PAs near river can reach to N sources with more enriched ^{15}N than the willow which were growing at PBs and PCs far from river. From the field measurement, the maximal thaw depth was found deeper at PAs compared to PCs in all transects in both 2015 and 2016, which suggested the maximal thaw depth can indicate the distance to river (i.e., possibility to be flooded). Thus this also indicated that the flooding increased the foliar δ^{15}N values the in both year 2015 and 2016.

In details, for inter-annual changes of foliar δ^{15}N values from 2015 to 2016, the significant increases were only found at PA of SKA (increased 1.7±0.6‰, $F_{1,6}$ = 7.889, P = 0.03) and PB of SKB (increased 1.7±0.6‰, $F_{1,6}$ = 30.59, P = 0.001). These two points have never been submerged by floods in the period of observation in 2015, but both have been sometimes waterlogged by floods in the period of observation in 2016.

This suggested that the inter-annual changes in foliar δ^{15}N values at PA in SKA and PB in SKB were resulted by changes of hydrological conditions (i.e., from no submergence in 2015 to sometimes waterlogged in 2016). Moreover, the PAs of SKB and SBoydom were sometimes waterlogged with sporadic flooding in 2015, and changed to long period waterlogging with continuous flooding in 2016. However, there were no significant inter-annual changes found in the foliar δ^{15}N values at PAs in SKB and SBoydom from 2015 to 2016. This illustrated that the changes in hydrological conditions from no submergence to waterlogging is the reason for inter-annual changes in the foliar δ^{15}N values.

3.4.2 Effects on foliar nitrogen contents from different hydrological conditions

Generally, the foliar nitrogen contents in all transects showed higher values in near river points in 2015 although the lack of statistical significance (Figure 3.1). Also the high foliar nitrogen contents were found in flooded conditions in local scale randomly sampling in 2015 (Figure 3.5). Meanwhile, the positive correlation between maximal thaw depth with foliar nitrogen content was also found in year 2015 (r^2=0.17, p=0.007) (Figure 3.3).These all suggested that the foliar nitrogen contents might be increased because of flooding conditions in 2015 (i.e., sometimes waterlogged under sporadic flooding).

However for inter-annual changes in foliar nitrogen contents, significant decrease was only found at PA in SBoydom (decreased 0.8±0.3%, $F_{1,7}$ = 13.63, P < 0.01). The hydrological changes at PA in SBoydom were from sometimes waterlogged with sporadic flooding in 2015 changed to long period waterlogging with continuous flooding in 2016. Thus, the decrease in foliar nitrogen contents at PA in SBoydom might be resulted by flooding conditions (i.e., long period waterlogging under sporadic flooding). Also the negative correlation was found between maximal thaw depth with foliar nitrogen content in 2016 (r^2=0.26, p=0.006) (Figure 3.3). These all indicated that the flooding decreased the values the foliar nitrogen content in 2016. Meanwhile, it might also be the reason for the lack of differences in foliar nitrogen contents among

different hydrological conditions in local scale randomly sampling in 2016 under high water level with more sites under long period waterlogging with continuous flooding (Figure 3.5).

3.4.3 Effects on N cycling from flooding

The higher foliar nitrogen contents found in 2015 near river on transects and local scale randomly sampling both suggested that willows grow near river have better nitrogen conditions than willows grow far away from river in this year (Figure 3.1 and Figure 3.5). Between summer of 2015 and 2016, we found higher nitrate concentration under high water level (Figure 3.7), which means river has potential to bring extra nitrate to willow growing near river. Moreover, in the labeling experiment in Arizona (Schade et al., 2005), there were findings that willow grown near a river can utilize nutrient brought by river. Thus, the higher foliar δ^{15}N values found in the willows which were grown near river might be because of the extra N sources from river. Another possible reason is the nutrient release from accelerated thawing permafrost under flooding. From the thaw depth investigation, the points with more frequently flooding had deeper thaw depth in general (Table 2.2), and also the maximal thaw depth increased in newly flooded points. These both suggested waterlogging under flooding conditions, increased thaw depth. Although little is known about plant-available N concentrations in permafrost soils, increased uptake of nitrogen by plants in Alaskan thermokarst areas (Schuur et al., 2007), and increased organic nitrogen concentrations in Arctic rivers as a result of permafrost degradation (Frey & Mcclelland, 2009) suggest that plant-available nitrogen release from thawing permafrost is occurring in some areas. Also, N measurements in Yedoma soils of Northeast Siberia suggest a biologically relevant N release upon thaw (Mack et al., 2010). Soil δ^{15}N values was usually increased with deeper soil in forest mentioned in many studies (Nadelhoffer & Fry, 1988, 1994; Koba et al., 1998; Cheng et al., 2010; Fang et al., 2013). Meanwhile we also found the slightly increase of δ^{15}N values in soil (Figure 3.6), although the real increase might be covered by other processes in the soil near river (e.g., denitrification

and leaching processes). The root distribution of willows was checked and it shows the willow root can reach to the maximal thaw depth. So besides the extra nitrogen sources from river with possible heavier $\delta^{15}N$ values, it was also possible that the enrichment of foliar $\delta^{15}N$ values in willow growing near river was because they can reach to deeper and more nutrient sources with heavier $\delta^{15}N$ values on deeper newly melted permafrost. Thus the positive relationships between maximal thaw depth with both foliar nitrogen contents and $\delta^{15}N$ values in 2015 can be explained by not only extra nutrient brought by river, but also by the deeper nutrient uptake because of deepened thaw depth by flooding as well. 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 & Grogan, 2010). Snow insulates against air temperature effects, at least from a minimum depth of 30-40 cm (Cline, 1997) up to 100 cm in depth (Grogen & Jonasson, 2006) stabilizing soil temperatures and increasing winter soil temperature minima (Walker et al., 1999; Schimel, Bilbrough & Welker, 2004; Nobrega & Grogan, 2007; Buckeridge & Grogan, 2008, 2010). As a result, soil temperatures appear to be sufficient to promote slow winter soil microbial activity that is cumulatively important due to the length of arctic winters. This caused higher soil temperature and higher rate of soil biochemical processes, such as mineralization rate and nitrification. The deepened snow also brought extra nitrogen, as the atmospheric decomposition also have high $\delta^{15}N$ values (Russell et al., 1998; Houlton & Bai, 2009). Many studies suggested the large amount of nitrogen brought by snow melt (YAMAGUCHI & NOGUCHI, 2015; Izuta, 2017). Popova (Popova et al., 2013) observed the inorganic nitrogen decomposition both in summer and winter. I also found the ammonia decomposition in the summer as the average 12.0 ± 9.3 μmol/L. These all suggest the snow accumulated around willows near river also has potential to

bring merit to willow growth and can result in the high nitrogen contents.

This increase of the foliar $\delta^{15}N$ values after sporadic waterlogging can be caused by river bought extra nutrient, more N uptake from potentially deepened thawing permafrost and deepened snow. However, the foliar nitrogen contents were found decreased 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. Because of frequently waterlogged condition and relatively flat topography, the mycorrhizal activities were restrained, so this is not the main point which we are focused on. Soil nitrogen pools and processes were controlled by soil water content and water soluble organic carbon availability, which depend on sedimentation and inundation dynamics (Shrestha et al., 2012), so the observed high water content and reductive condition of oxidation-reduction potential in soil under waterlogging (Shigubara, personal communication) shaded light on denitrification opinion. The previous studies also discovered the maximum rates in valley bottoms tend to flooding (Sutherland et al., 1993) and the lowest rates on arid hill ridges (Groffman & Tiedje, 1989; Van Kessel, Pennock & Farrell, 1993). Moreover, the high DEA (denitrification enzyme activity) was found in the surface soil in denitrification processes in the previous study in riparian buffer zones (Dhondt et al., 2004). Thus, denitrification processes under waterlogging conditions can be another answer to the high foliar $\delta^{15}N$ values in soils at PAs (i.e., the study points which were easily waterlogged). The denitrification processes bring unavailable form nitrogen, lowered availability of nitrogen and may lead to suppress of willow growth. This kind of suppression of growth can be proved by foliar nitrogen content decrease at PA in SBoydom under long period waterlogging in 2016, the negative correlation between foliar nitrogen contents and maximal thaw depth in transects, and also the lack of differences in foliar nitrogen content under different flooding conditions in local scale randomly sampling when more sites were in long period waterlogging under continuous

flooding in high water level year 2016. Thus, in 2016, the larger and longer flooding might be harmful to willow nutrient condition and uptake, rather than beneficial.

The correlations between foliar nitrogen content and $\delta^{15}N$ values in transects were positive in 2015 but negative in 2016. This suggested that the growth willow in low river water level in the year of 2015 were depending on nutrient conditions, and depending on physiological conditions under waterlogging in high river water level in 2016.

3.5 Conclusions

The significant difference of foliar $\delta^{15}N$ values that higher foliar $\delta^{15}N$ values near river caused by flooding has been found in both year 2015 and 2016. Besides the general possible reasons about that waterlogging can cause higher mineralization and nitrification rates thus increase $\delta^{15}N$ values. In specific Indigirka River lowland area, this maybe further related to the extra nitrogen uptakes in 2015 and the denitrification occurred under reductive conditions especially in 2016. The river brought extra nitrate and flooding resulted thaw depth increase are benefits for willow growth in 2015, which was also indicated by higher biomass of willow near river. Meanwhile, the kind of nitrogen loss through denitrification is not good for willow nutrient uptake was proved by decrease of foliar nitrogen content in 2016 under large and continuous flooding. So generally, medium flooding with suitable scale of waterlogging, adequate available water with extra nutrient and deeper thaw depth makes willow growing better, however the large scale flooding injured willow growth with limited nitrogen uptake.

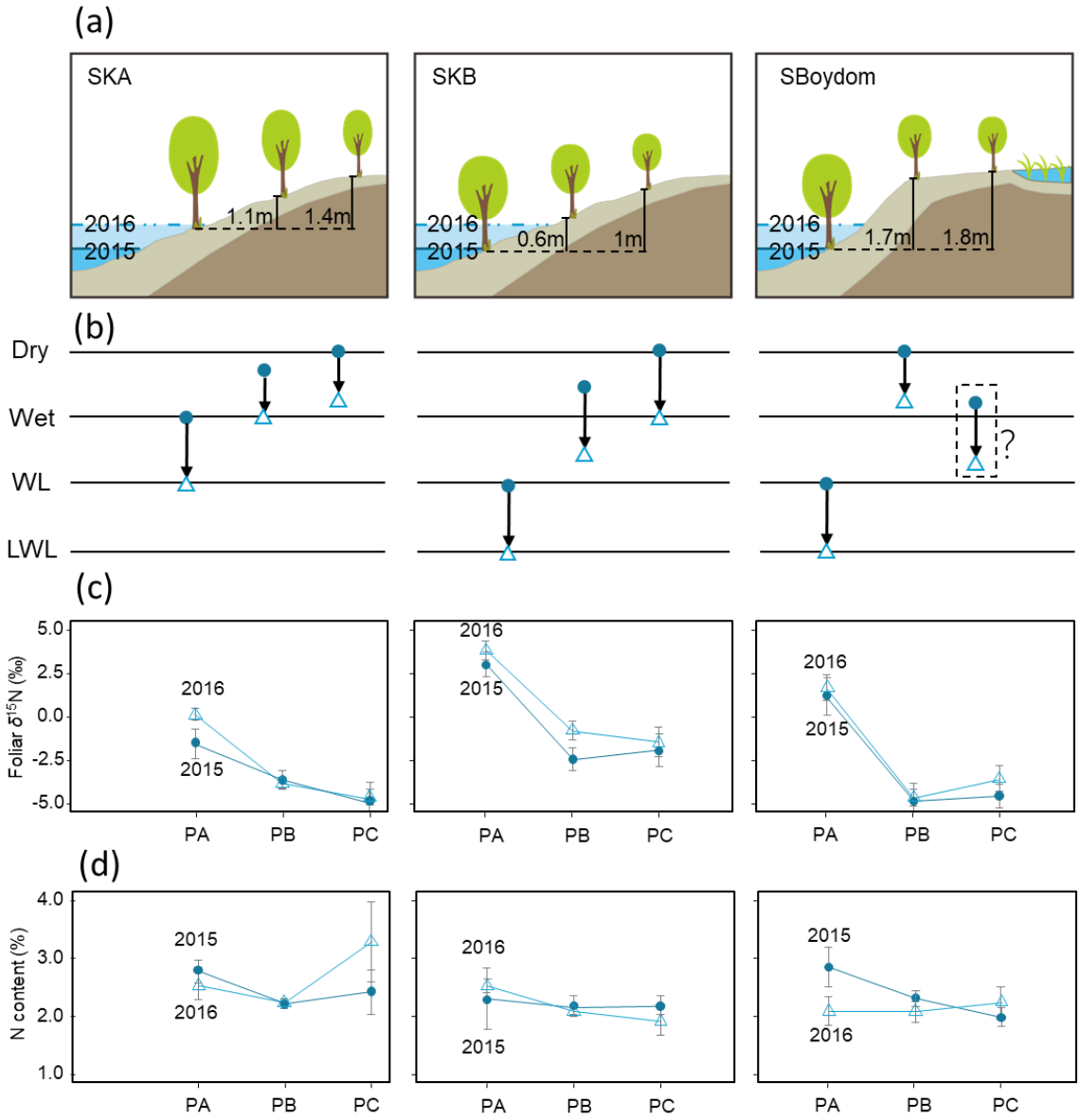

Figure 3.1 (a) Schematic view of each transect with the highest water levels observed in each case in 2015 (blue) and 2016 (light blue) and the height of PB and PC, compared to PA (black line). (b) Possible changes (black arrows) in the hydrological conditions from 2015 (filled circles) to 2016 (open triangles) in each point on transects. Dry and wet are without waterlogging, and WL and LWL represent waterlogging (continual) and long period waterlogging (continuous), respectively. (c) The foliar δ^{15}N values (‰), and (d) the foliar nitrogen content in willows were reported as means ± SD.

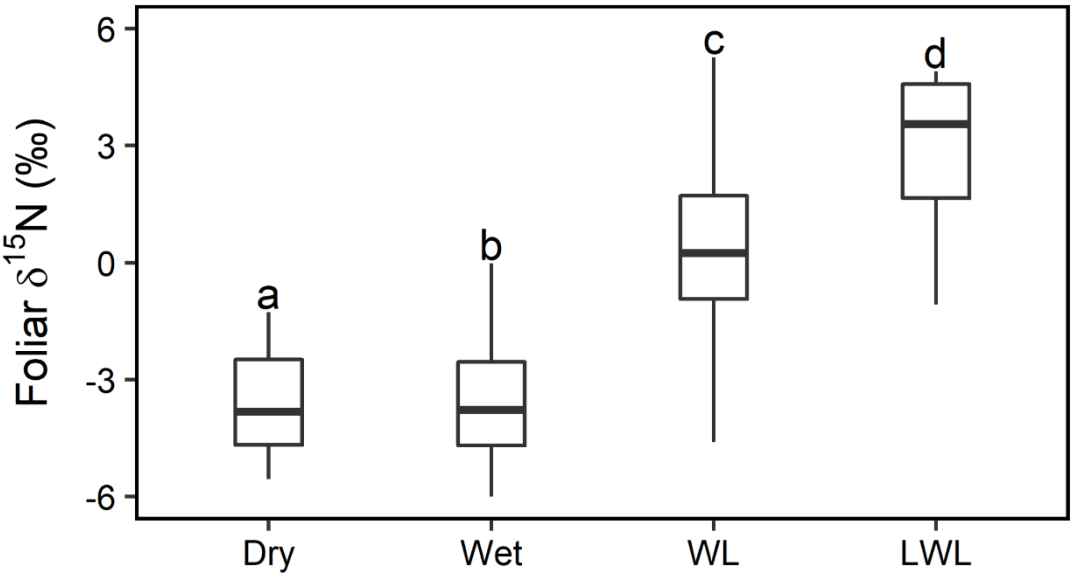

Figure 3.2 Box-and-whisker plot of the statistical analysis for the foliar δ^{15}N values (‰) under four different hydrological conditions in sampling transects established in year 2015 and 2016. Different letters over the numbers indicate statistically significant differences according to Turkey´s post 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.

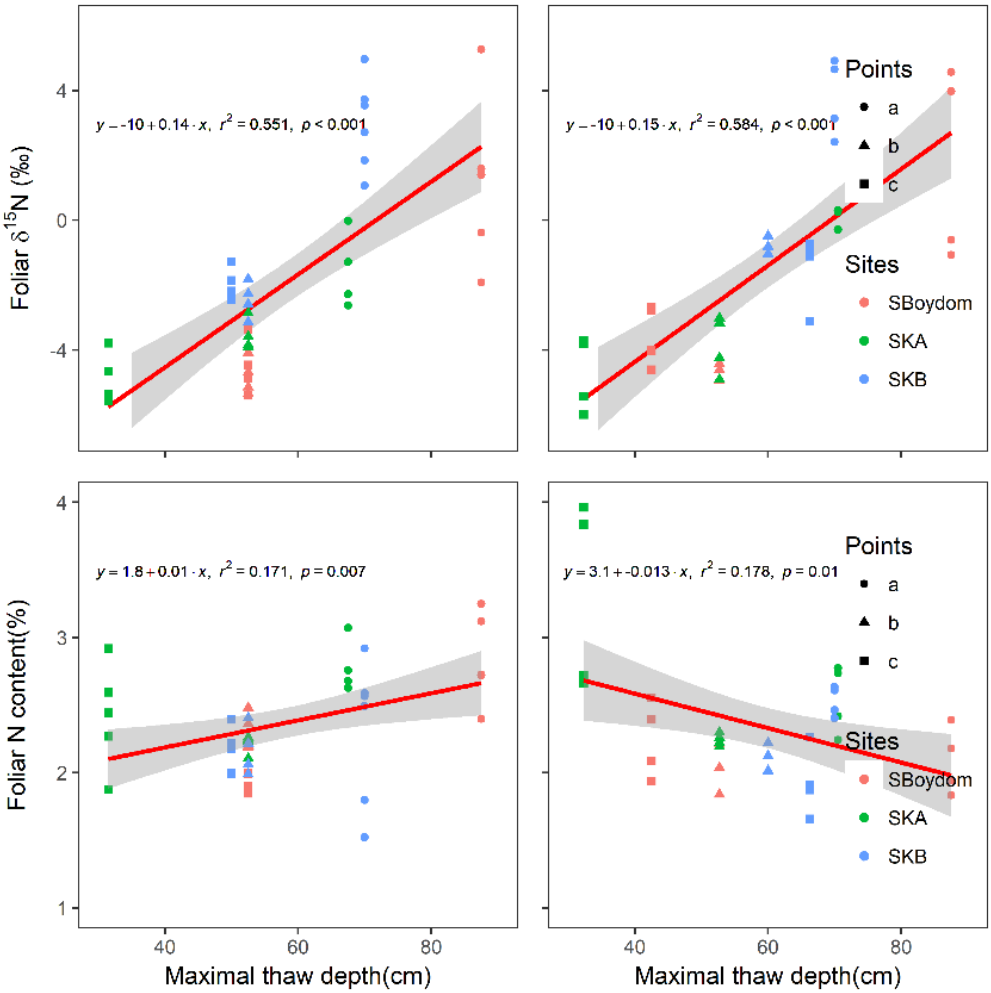

Figure 3.3 Relationships between foliar d15N values (‰) and maximal thaw depth, and between foliar nitrogen content (%) and maximal thaw depth, respectively, in year 2015 and 2016.

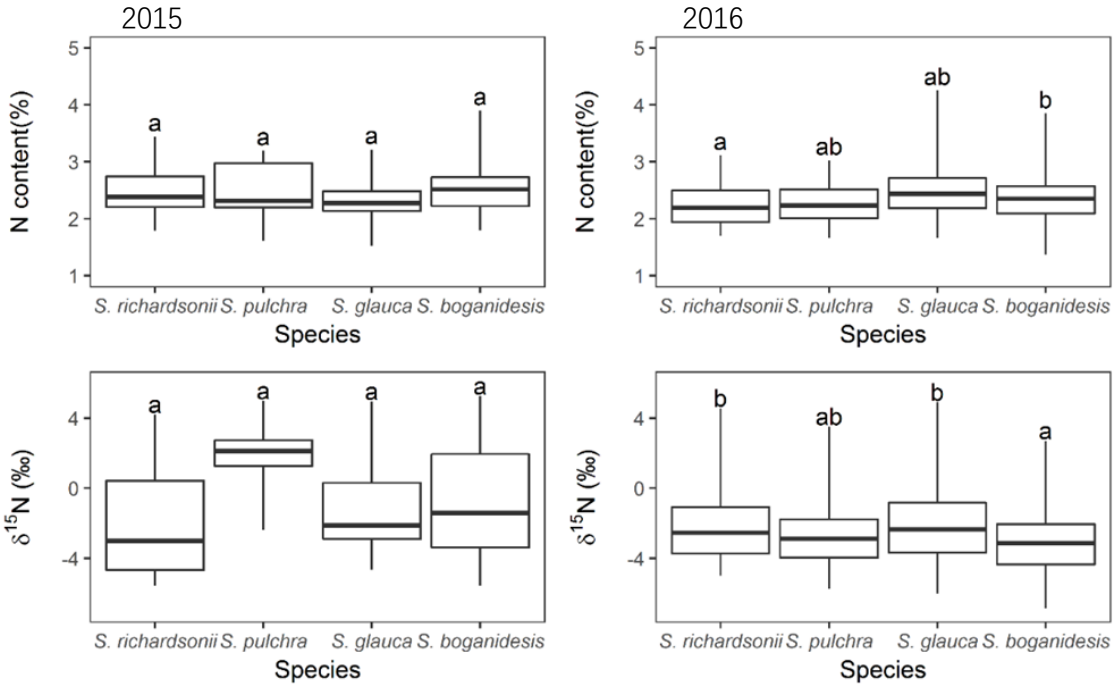

Figure 3.4 Difference analyses in foliar nitrogen content (%) and δ^{15}N values (‰) among different species in year 2015 and 2016 using Turkey post hoc test with linear mixed models.

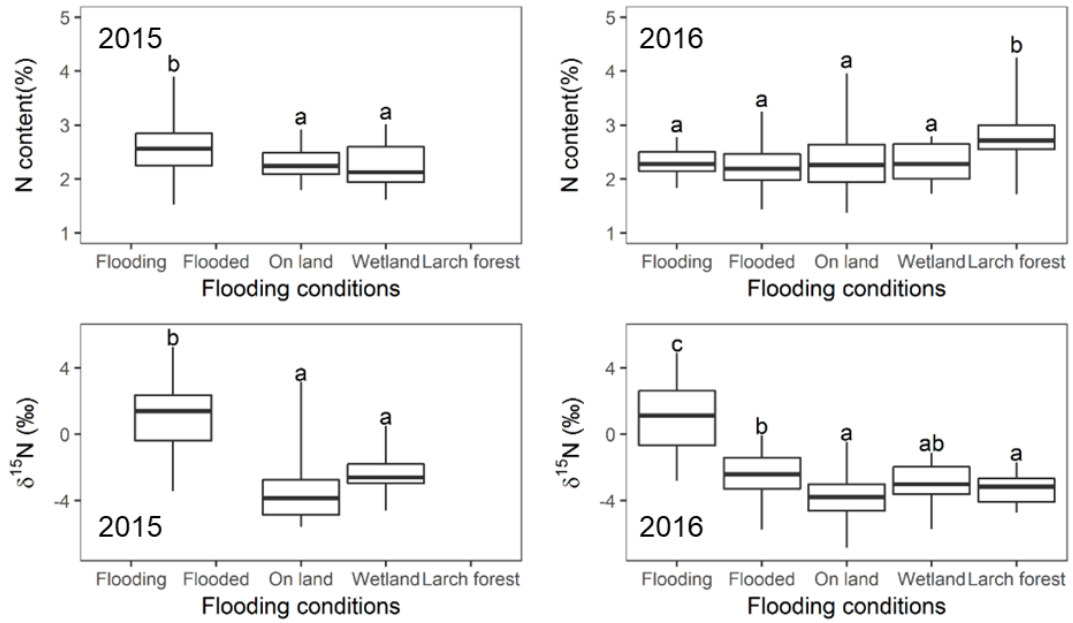

Figure 3.5 Difference analyses in foliar nitrogen content (%) and δ^{15}N(‰) among different flooding conditions in year 2015 and 2016 using Turkey post hoc test with linear mixed models.

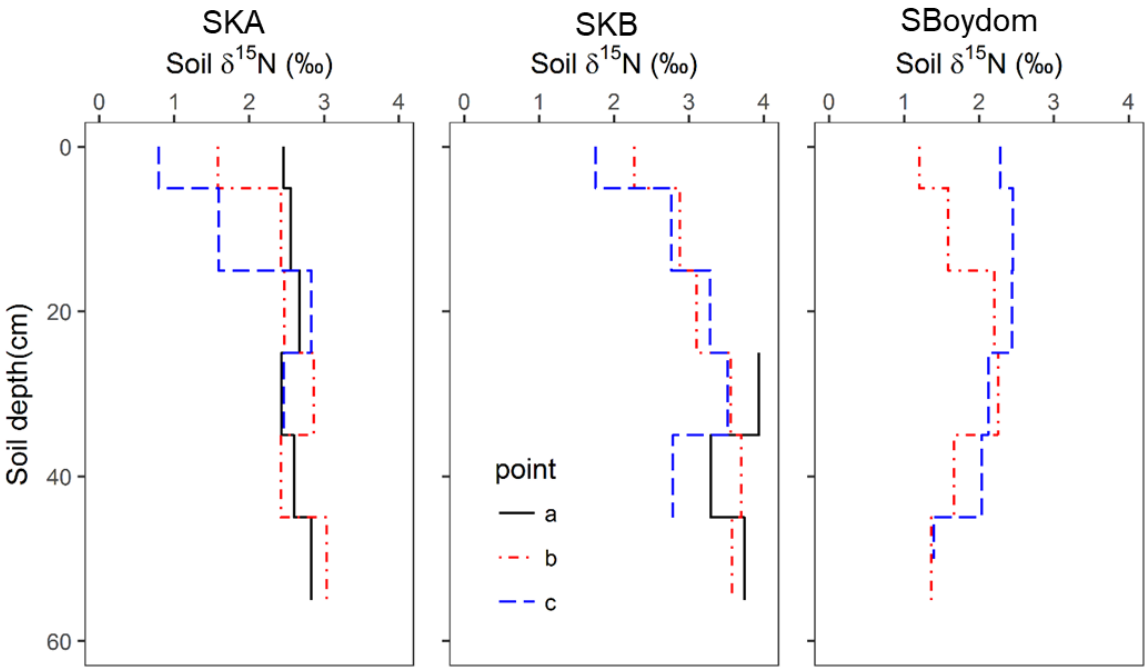

Figure 3.6 Soil δ^{15}N (‰) to maximum thaw depth in 2015 of three transects, SKA, SKB, SBoydom from left to right panel respectively. Black solid line, red dash-dot line, and blue dash line represent PA, PB and PC, respectively.

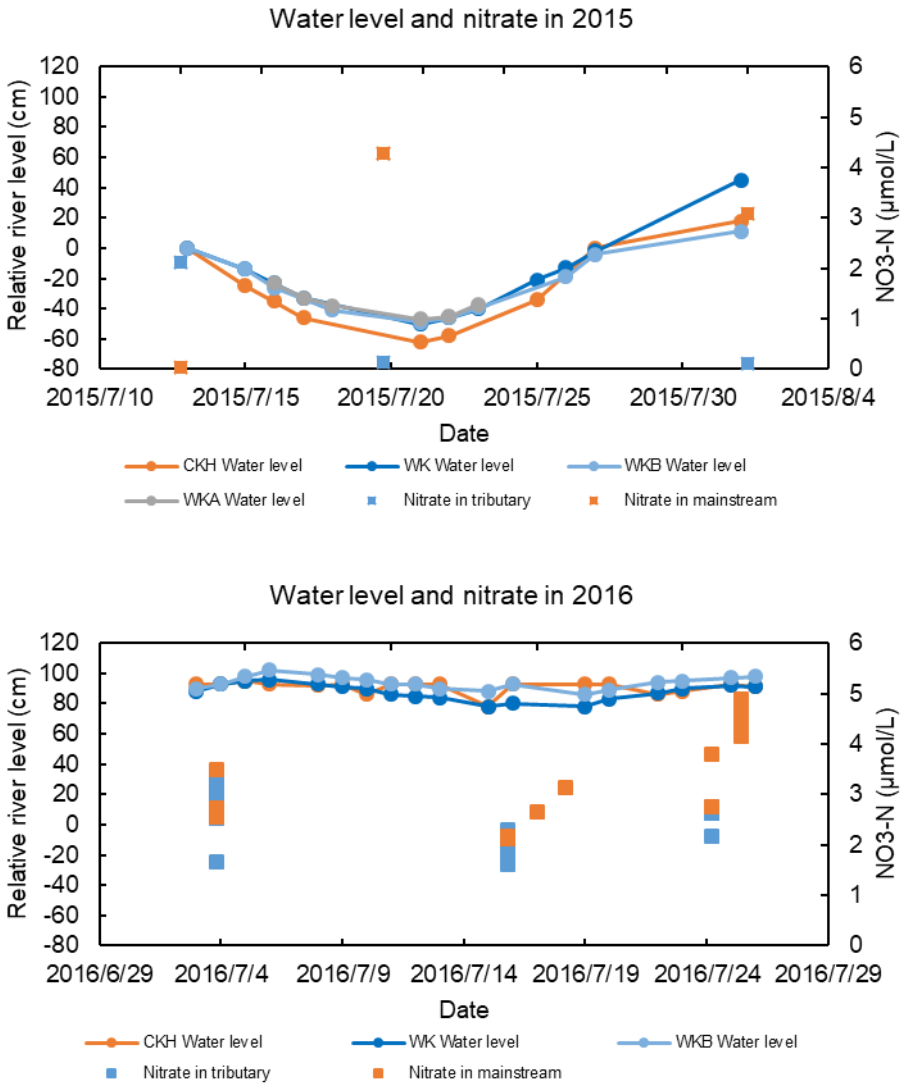

Figure 3.7 Relative water level and nitrate concentration in mainstream Indigirka (CKH) and tributary Kryvaya (WKB and WK).

Chapter 4 General discussions and 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 (chapter 2). The results of foliar $\delta^{15}N$ values and nitrogen contents demonstrated the nitrogen cycling and nutrient conditions for willows under different hydrological conditions (chapter 3). 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 chapter, 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.

The waterlogging induced stomatal closure was found in willows, regardless of length and continuity of submergence. Unlike decreases in stomatal conductance under waterlogging no matter what length or continuity of submergence, the decreases in photosynthesis activity were only found during the period of submergence, meanwhile the significant recovery of photosynthesis activity were observed when waterlogging finished. Thus in willows under WL conditions, the significant recovery of photosynthetic activity, during the time interval between two times of waterlogging, reduced the variation of the foliar $\delta^{13}C$ values caused by decreased photosynthetic activity compared to willows under LWL conditions. Therefore, in WL condition, the stomatal closure became the major factor and led to the increased foliar $\delta^{13}C$ values. In LWL conditions, as the waterlogging has never been interrupted, the effects on foliar $\delta^{13}C$ values from decreases of photosynthesis activity cannot be ignored. The continuously decreased photosynthetic activity in LWL conditions resulted in the relatively low foliar $\delta^{13}C$ values compared to WL conditions. These all above can be the reasons for the results that the high foliar $\delta^{13}C$ values were found under medium flooding (resulted in WL conditions) and low foliar $\delta^{13}C$ values were discovered under

large flooding (resulted in LWL conditions) in transects in 2015 and 2016, respectively.

The significant differences of foliar $\delta^{15}N$ values have been found in both year 2015 and 2016 (chapter 3). The high foliar $\delta^{15}N$ values near river were definitely related to flooding. There were studies demonstrated that the nitrogen mineralization and nitrification rates tend to be higher in topographic depressions where runoff converges than on the drier upper slopes (Garten, 1993; Bedard-Haughn, van Groenigen & van Kessel, 2003). Other possible reasons particularly related to Arctic river lowland area included, potential extra nitrogen brought by river flooding, precipitation, snow accumulation, additional nitrogen release from deeper permafrost with global warming (i.e., waterlogging resulted in deeper thaw depth) and denitrification in reductive condition under submergence. The first four explanations increased nutrient pool and the last one decreased the nitrogen composition which can be used by plants (i.e., ammonia and nitrate) in soil. Meanwhile, the foliar nitrogen content in 2015 were higher than 2016. Thus, the nitrogen for willow under medium flooding (resulted in WL conditions) year was more than under large flooding (resulted in LWL conditions). The extra nitrogen caused by river water, precipitation, snow accumulation and flooding resulted thaw depth increase might be the reasons for the high foliar $\delta^{15}N$ values and nitrogen content in 2015. The nitrogen losses from denitrification process under long period waterlogging may lead to the similar high foliar $\delta^{15}N$ values but reversely low nitrogen content in 2016. The medium flooding enhanced the nitrogen availability in willows, but the large flooding weakened it.

Moreover, there were studies suggested that the positive correlation between foliar N concentration and $\delta^{13}C$ implies that nitrogen availability is associated with photosynthetic capacities and decrease of internal leaf CO_2 concentration (Sparks & Ehleringer, 1997; Raeini-Sarjaz et al., 1998). In contrast, a lack of significant relationship between foliar N concentration and $\delta^{13}C$ indicates nitrogen deficiency is not a limiting factor for tree growth (Choi et al., 2005; Zhao, Xiao & Liu, 2006). Combined the data of foliar $\delta^{13}C$ values (data from part 2.3.1, Chapter 2), the relationship between those and nitrogen content in three transects were shown in Figure

4.1. It shows that in 2015 there was a significantly negative correlation between foliar $\delta^{13}C$ values and nitrogen content in SKA (r^2=0.29, p<0.05), and a positive correlation in transects SKB (r^2=0.48, p<0.05) and SBoydom (r^2=0.25, p=0.08). While in 2016, there was only a significant positive correlation between foliar $\delta^{13}C$ values and nitrogen content in SBoydom (r^2=0.33, p<0.05). By checking the results, it indicated that only the willows' growth in transects SKB and SBoydom in 2015 were depending on nitrogen availability in 2015. This also suggested that the willow growth under medium flooding was constrained by nitrogen availability, and the willow growth under large flooding was more related to carbon fixation (i.e., photosynthesis activity).

The possible foliar $\delta^{13}C$, $\delta^{15}N$ and N content values to normal (i.e., dry and wet) and abnormal hydrological conditions (i.e., WL and LWL) were shown in Figure 4.2. There are similar foliar $\delta^{13}C$ values under normal dry and WL conditions, and also similar foliar $\delta^{13}C$ values under normal wet and LWL conditions. Meanwhile, we can find significantly high foliar $\delta^{15}N$ values under frequently flooding conditions (i.e., WL and LWL). Thus we can separate the environments, normal and abnormal with combination application of both foliar $\delta^{13}C$ and $\delta^{15}N$ values. For example, all the raw data from snapshot-sampling were shown in Figure 4.3. If the boundary of foliar $\delta^{15}N$ values between normal and abnormal was set to be 1.5‰. The foliar $\delta^{13}C$ values can be applied to indicate the flooding and waterlogging conditions for plant much easier and clearer. We should note that the precondition to conduct this kind of data analyses is for willows, as the physiological, nitrogenous, isotopic responses for willows under different hydrological conditions have been studied. We should carefully use this theory to other species, such as Larch trees. For example, in the study of Li and Sugimoto (2017), they find only the increased foliar $\delta^{13}C$ values after even LWL waterlogging. This is probably because of the low water tolerance in Larch trees. They were observed dead after waterlogging, which means they cannot live under LWL and cannot further record the significant decreased photosynthetic activity.

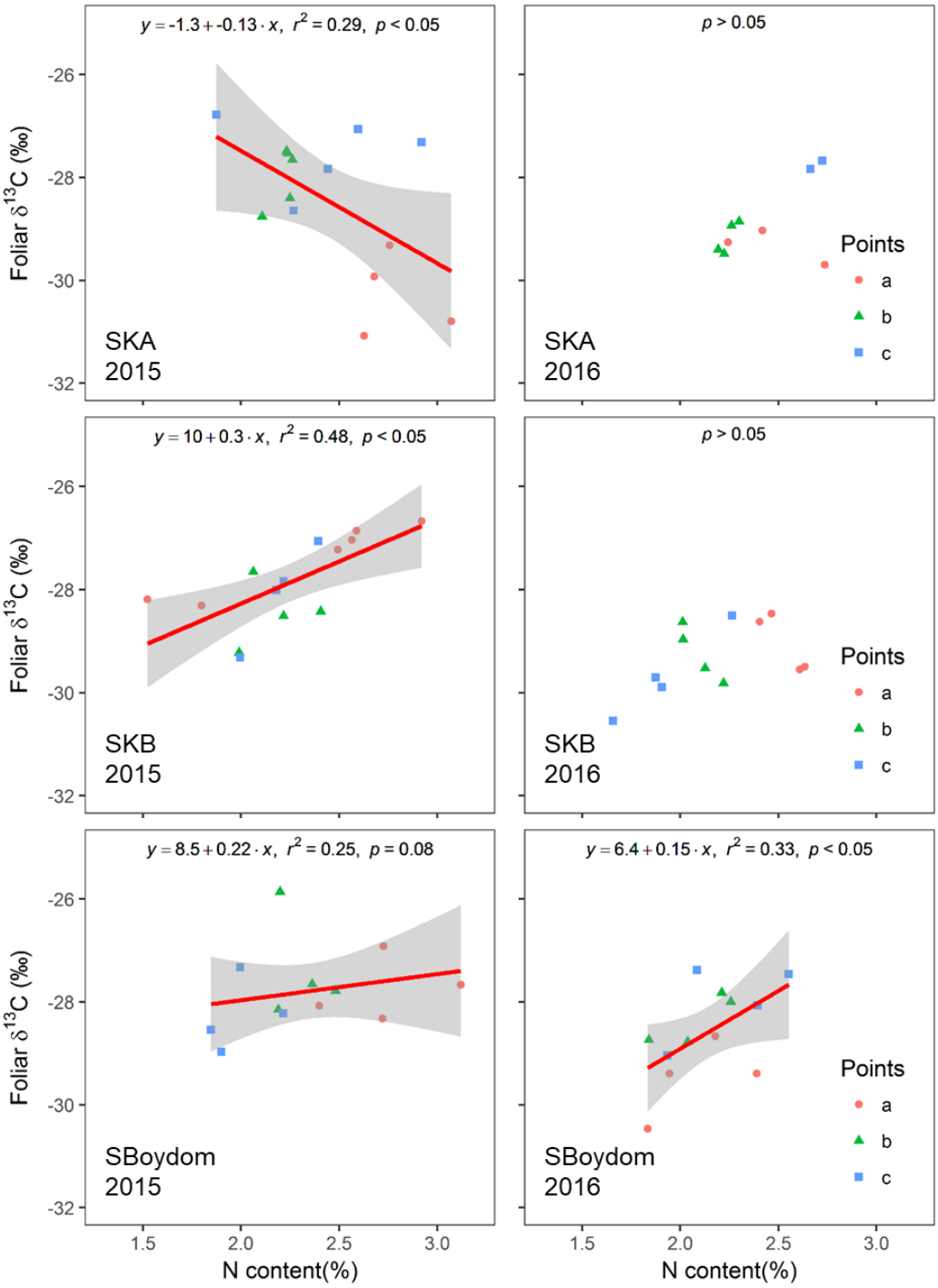

Figure 4.1 Relationship between foliar $\delta^{13}C$ and content (%) of transect SKA, SKB and SBoydom in year 2015 and 2016. For PA (red filled circles), PB (green filled triangles) and PC (filled blue squares).

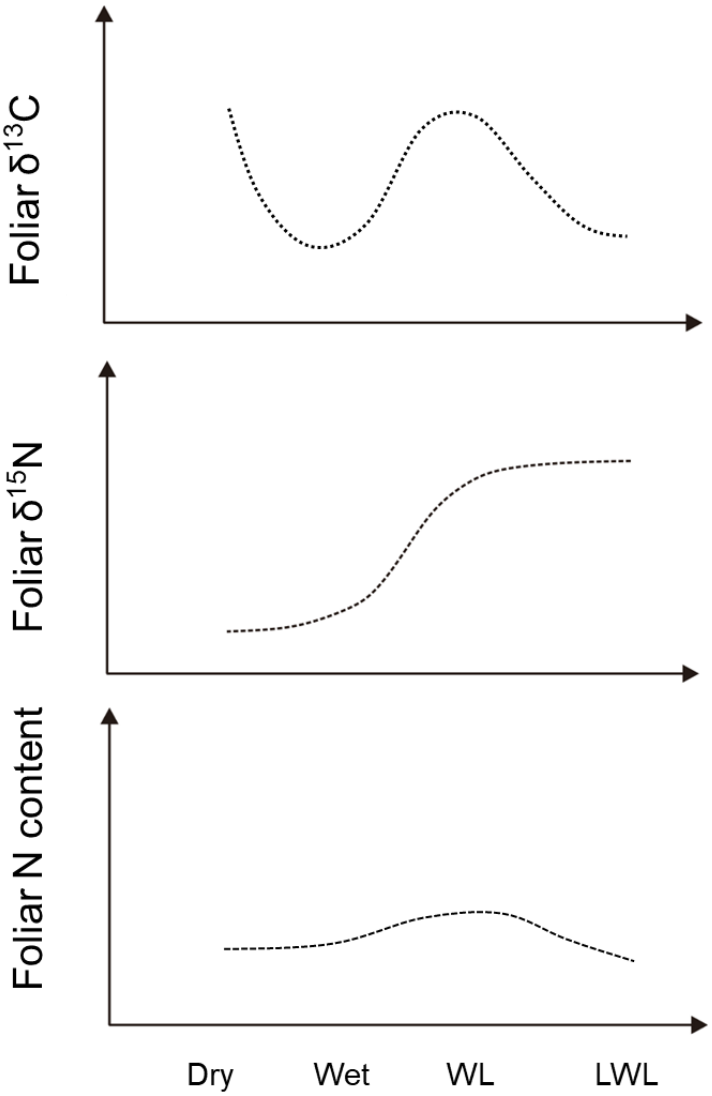

Figure 4.2 The possible foliar $\delta^{13}C$, $\delta^{15}N$ and N content values to normal and abnormal hydrological conditions. WL and LWL represent waterlogging (continual) and long period waterlogging (continuous), respectively.

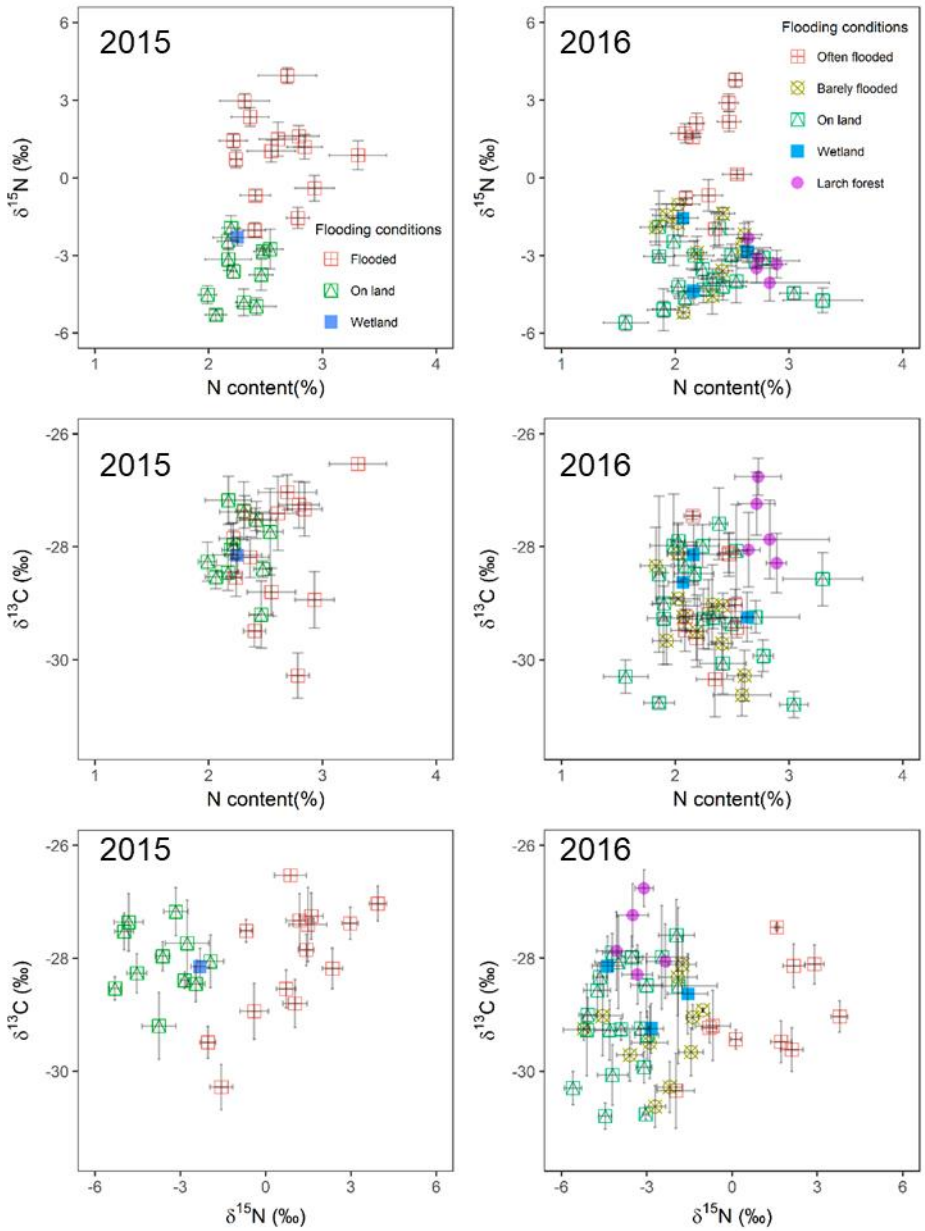

Figure 4.3 All data of foliar $\delta^{15}N$ and $\delta^{13}C$ values (‰) with foliar nitrogen content (%) in year 2015 and 2016. Red square with cross, yellow circle with cross, green square with triangle, blue filled square and purple filled circle indicate different growing conditions, often flooded, barely flooded, on land, wetland and in larch forest. Error bars represent 1 SEM.

Chapter 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 (Figure 5.1). This can be also supported by willows' high water tolerance and distributions. Willows, which were quite preferential and tolerant to high soil moisture, were used to growing in river lowland (Troeva et al., 2010). Through stable isotope tools, the growth of willows was suggested hindered by continuous waterlogging under large flooding (Figure 5.2), 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 (Figure 5.3). 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 (Serreze, Holland & Stroeve, 2007; Stroeve et al., 2007; Comiso et al., 2008; Screen & Simmonds, 2010), which will lead to an increase in fall precipitation and the snow depth (Kohler et al., 2006; Screen & 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 (Table 2.1) and higher foliar nitrogen content (Figure 3.5). 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 (McGuire et al., 2009; Myers-Smith et al., 2011; Huissteden & Dolman, 2012).

If the foliar stable isotope values correlate with those in other organs and tissues (such as tree-ring cellulose), they can be used to reconstruct the hydrological and vegetation changes that have occurred in mesic regions. However, as mentioned before, this study was focused on willows, thus we highly suggest that it is better to carefully check the physiological, nitrogen and stable isotope responses related to changing environment when we want to apply Figure 4.2 into other species, such as larch trees. Thus for future work, we suggest that it is necessary to further clarify of the effects of waterlogging on the foliar or tree ring stable isotopes in many species with both laboratory experiments under controlled conditions and in field, and this will be highly useful for better interpretation of the stable isotopes of plant products and their applications in mesic areas.

There are other important things to note and the further researches are needed. For foliar $\delta^{13}C$ values and physiological changes, as the relationship between $\delta^{13}C$ of plant tissues and water use efficiency is not linear due to multiple factors, not only *A* and *gs*, but also *gm* (mesophyll conductance). It is necessary to pay more attention to the effects of *gm* on the $\delta^{13}C$ values in future studies. Also it is urgent to start the quantitative studies on relationships between plant $\delta^{13}C$ values and water conditions. For foliar $\delta^{15}N$ values and willow nutrient conditions, the measurements of molybdenum concentration

in leaves and nitrogen processes in soil, such as mineralization and nitrification rates, can be better explanations for our hypotheses. In addition, there are findings that the depth of snow covered willows were deeper near river than other plants in other locations. It is better not to separate the effects of deeper snow on nitrogen cycling in future studies.

Figure 5.1 The appearance of willows growing along river bank in 2015 with normal sporadic flooding.

Figure 5.2 The appearance of willows growing along river bank in 2017 with extremely large flooding with continuous waterlogging.

Figure 5.3 The appearance of willows growing along river bank in 2018 after the extremely large flooding occurred in 2017.

Acknowledgments

It is not a fair task to acknowledge all the people who made this Ph.D. thesis possible with a few words. However, I will try to do my best to extend my great appreciation to everyone who helped me scientifically and emotionally throughout this study.

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