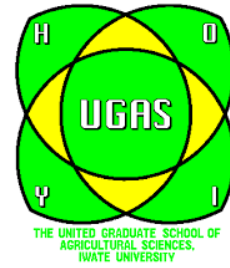


Tree water use strategy in a heavy precipitation region by means of stable isotopes, Japan

Qiqin Zhang



Yamagata University

The United Graduate School of Agricultural Sciences

Japan

December 2019

Main supervisor: M. Larry Lopez C.

Co-supervisor: Kenichi Yoshimura

Chihiro Kato

Contents

1. General Introduction	
1.1. Water and carbon cycles in forest.....	1
1.2. Climate change and the effects to forests.....	2
1.3. Stable isotopes.....	2
1.3.1. <i>Stable isotopes in water</i>	3
1.3.2. <i>Stable isotopes in tree-rings</i>	7
1.4. Objective of this study.....	9
References.....	10
2. Tree xylem water dynamics in three tree species during summer time	
2.1. Introduction.....	16
2.2. Methods.....	18
2.2.1. <i>Study site and meteorological data</i>	18
2.2.2. <i>Sampling design and measurements</i>	20
2.3. Results.....	21
2.3.1. <i>Isotopic variation in monthly precipitation and xylem water</i>	21
2.3.2. <i>Correlation between water $\delta^{18}O$ and δD</i>	23
2.4. Discussion.....	24
2.4.1. <i>Temporal difference of xylem water isotopes</i>	24
2.4.2. <i>Different water uses among tree species</i>	26
2.5. Conclusions.....	27
References.....	27
3. Soil-tree water relationship under extreme climate conditions	
3.1. Introduction.....	32
3.2. Methodology.....	33
3.2.1. <i>Study site and meteorological</i>	33
3.2.2. <i>Sampling design and measurements</i>	34
3.3. Results.....	35
3.3.1. <i>Climatic condition in 2018</i>	35
3.3.2. <i>Isotopic variation in precipitation water, soil water and xylem water</i>	36
3.4. Discussion.....	44
3.5. Conclusion.....	46
References.....	47
4. Monthly variability of cellulose stable isotopes in three tree species	
4.1. Introduction.....	52
4.2. Methodology.....	53
4.2.1. <i>Research sites</i>	53

4.2.2. <i>Sampling design and measurements</i>	53
4.3. Results.....	54
4.3.1. <i>Monthly tree-ring α-cellulose $\delta^{18}O$ and δD</i>	54
4.4. Discussion.....	55
4.5. Conclusions.....	56
References.....	56
5. Final conclusions.....	60
Acknowledgement.....	63

Chapter 1

General Introduction

1.1. Water and carbon cycles in forests

Forests are a very important part of global land, approximately 30% of the global land is covered by forests (Bonan, 2008). Forests are extremely important for the global biochemical cycles (e.g. carbon, oxygen, nitrogen) and supply wood and non-wood products (e.g. berries, mushrooms), in addition, forest also play an important role in ecosystem services, such as climate stabilization, carbon storage, protection of hydrological function, and conservation of habitats for biodiversity (Lindner et al. 2010). But because of the ongoing global climate change, forest distribution may change globally and this will threat forest ecosystem services, one important of these roles is water and carbon cycles. Precipitation will firstly be caught by forest canopy, then return to the atmosphere through evaporation, the rest will reach the forest floor, becoming part of runoff or saturated into soil. through transpiration, Trees take up soil water and ground water and transform this water to leaves, in the end, water will release to the atmosphere as vapor. Transpiration recycles 62000 km³ of water to the atmosphere per year (Jaschko et al., 2013), which is 61% of the total terrestrial evapotranspiration (Schlesinger and Jasechko, 2014). Forests store 45% of terrestrial carbon and contribute 50% of terrestrial net primary production (Bonan, 2008), with 2.41±0.42 pg C year⁻¹ carbon sink from 1990-2007 (Pan et al., 2011). To make sure these cycles working, the stomata of leaves need to be open, so root will take up soil water and ground groundwater and release to the atmosphere by transpiration and CO₂ will absorbed into leaves for photosynthesis to make sugar , and when the environment condition is limiting the available water for trees, stomata will close partially or fully to decrease the water uptake, therefore less CO₂ will absorbed into leaves (McCarroll and Loader, 2004). Because of this, climate change may change either the forest distribution or the species-specific physiological behavior.

1.2. Climate change and the effects to forests

From the year 1880-2012, the global temperature increased 0.85 °C, and it will continue increase 3-4 °C for the next decades (IPCC, 2014), this will result in an increase of aridity in some water limited area (Christensen et al., 2007; Seager et al., 2007), and will cause mortality of small plants (i.e. seedlings and saplings) and mature trees will have less effect of drought stress, but long term drought will also make forest dieback and species migration (Hanson and Weltzin, 2000; Camarero et al., 2015). In Japan, the annual temperature has increased by about 1 °C but the annual precipitation showed no significant trend over the last century (Ichikawa, 2004), in additional, the extreme weather events have increased, from 1901 to 2018 the annual number of extreme dry days have increased, and the extreme wet days also increased (Japan Meteorological Agency, 2017). There is evidence that plant distribution (Chapinet al.,1993; Bokhorstet al., 2007), survival (Van Peeret al.,2004) or net primary productivity and species diversity (Knappet al., 2002) respond to extreme rather than to average conditions (Jentsch & Beierkuhnlein, 2008, Reyer et al., 2013). Thus, the increase of extreme climate events may modify the species composition and dynamic of current forest system.

1.3. Stable isotopes

Isotopes are same chemical elements which have different numbers of neutrons but have same number of protons and electrons. Because the number of neutrons is different, the isotopes have different atomic mass. To write a chemical element isotope, we put a superscript number to the left of the element to represent the total number of protons and neutrons in

the isotope. For example, among the hydrogen isotopes, protium (^1H) has one proton and no neutron, deuterium (^2H) has one proton and one neutron, tritium (^3H) has one proton and two neutrons. Isotopes can be divided into stable isotopes and radioactive isotopes (unstable isotopes). The stable isotopes have nuclei that do not decay to other isotopes on geologic timescales, but may themselves be produced by the decay of radioactive isotopes. Radioactive (unstable) isotopes have nuclei that spontaneously decay over time to form other isotopes. The research of stable isotopes focuses on how natural processes affect the relative abundance of each isotope with respect to the other, which is the ratio of the heavy isotope with respect to the lighter isotope (for example $^{13}\text{C}/^{12}\text{C}$). The most abundant elements in the biosphere are carbon (C), hydrogen (H), oxygen (O) and nitrogen (N), being ^{13}C , ^2H (D), ^{18}O and ^{15}N the stable isotopes of greater interest in plant physiology. The stable isotope composition of a given sample is determined by mass spectrometry, is usually expressed in differential notation:

Eq.1
$$\delta(\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

where δ stands for the isotopic composition, in parts per mil (‰), as referred to a standard (see Table 1). R denotes the ratio of the heavy to light isotope (e.g., $^{13}\text{C}/^{12}\text{C}$), and R_{sample} and R_{standard} are the ratios in the sample and standard, respectively. A higher or positive value means that the isotopic ratio of the sample is higher than that of the standard; a lower or negative value means that the sample is lower than that of the standard.

Element	Isotope	Abundance (%)	Relative mass difference (%)	International standard	Analytical error (‰)	Observed range in plants (‰)
Hydrogen	¹ H	99.985	100	Vienna Standard Mean Ocean Water (VSMOW)	4 to 7	-300 to +20
	² H (also D)	0.0155				
Carbon	¹² C	98.892	8.3	Vienna Pee Dee Belemnite (VPDB)	0.1	-35 to +5
	¹³ C	1.108				
Nitrogen	¹⁴ N	99.635	7.1	Atmospheric Nitrogen (air)	0.2	-10 to +10
	¹⁵ N	0.365				
Oxygen	¹⁶ O	99.759	12.5	VSMOW in water, generally VPDB in CO ₂ or carbonate	0.05 to 0.2	-15 to +35
	¹⁷ O	0.037	(¹⁸ O: ¹⁶ O)			
	¹⁸ O	0.204				

Table.1. Relative abundances of the stable isotopes most common in ecological research in order of increasing mass. Adapted from Sulzman (2008)

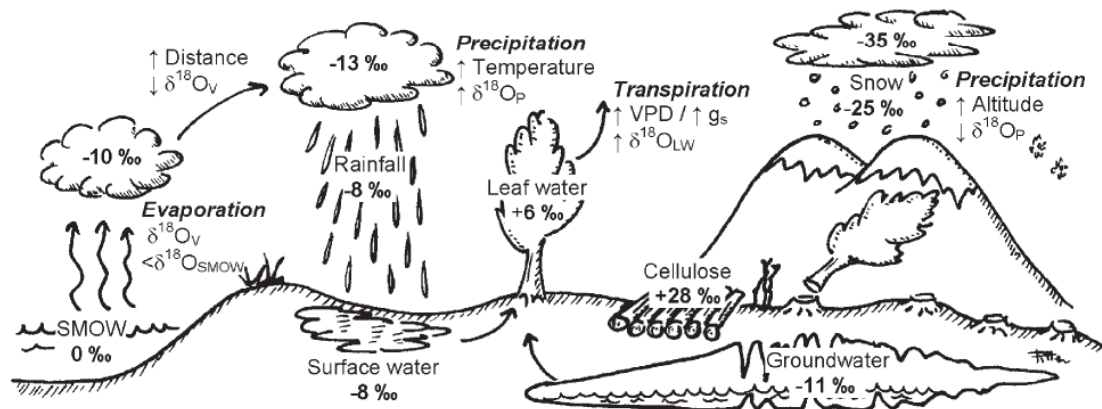
1.3.1. Stable isotopes in water

Water stable isotope composition of xylem water has been applied to determine the source of water for transpiration (Ehleringer and Dawson, 1992; Valentini et al., 1994; Brunel et al., 1995), that is because the water sources available to plants shows different isotopic signatures, which can be traced back from the xylem water stable isotopic composition. For example, the different contribution of seasonal precipitation to soils, streams and groundwater lead to substantial isotopic differences among these water pools (Gat, 1996; Ferrio et al., 2005). In this sense, the relationship between $\delta^{18}\text{O}$ and δD in the world's precipitation follows a predictable linear relationship, referred to as the "Global Meteoric Water Line" (Craig 1961), represent as:

$$\text{Eq.2.} \quad \delta\text{D} = 8 \times \delta^{18}\text{O} + 10$$

that is a direct consequence of the fact that fresh waters of warm regions have more enriched (positive) values of hydrogen and oxygen isotopes, whereas cool regions are isotopically depleted for both elements, this relationship can be shown as Local Meteoric Water Line. Such difference mainly caused by the fractionation occurring during phase changes in the course of the hydrological cycle (fig. 1). light isotopes (¹⁶O and ¹H) evaporate more rapidly than their heavier counterparts, and thus water vapor is isotopically depleted with respect to source

water (e.g. ocean water). the opposite occurs during precipitation, when the heavier isotopes



are selectively precipitated through distillation. The amount of this fractionation varies according to temperature and altitude, resulting in an isotopic composition of precipitation that is positively correlated with temperature, and negatively with altitude (Ferrio et al., 2005).

Fig. 1 Main fractionation steps and typical values of oxygen isotope composition (δ¹⁸O) in a temperate climate. δ¹⁸O SMOW, standard mean δ¹⁸O in ocean water; δ¹⁸O_v, δ¹⁸O_p, δ¹⁸O_{LW}, δ¹⁸O in water vapour, precipitation (either rainfall or snow) and leaf water, respectively; VPD, vapour pressure deficit; g_s, stomatal conductance.

(Ferrio et al. 2005), original data from IAEA/WMO (2001) and Saurer et al. (1997).

When evaporation occurs, light isotopes is easier to evaporate than heavy isotopes, this caused an isotopic enrichment of the liquid phase at the site of evaporation (Craig and Gordon, 1965), and because of this, the isotopic gradience can be observed during soil profile in dry area or during period of drought (Barnes and Allison, 1988). To compare with Global Meteoric Water Line or Local Meteoric Water Line, we can evaluate the evaporation degree of the source water. The Deuterium excess (d-excess) is used for tracing past and present precipitation processes. Represent as:

Eq.3.
$$d - excess(\%) = \delta D - \delta^{18}O$$

d-excess is an index of deviation from the Global meteoric water line, and it is related to air temperature and relative humidity (Merlivat et al., 1979), but recent research showed that d-excess is more related with relative humidity (Pfahl and Sodemann, 2014). Recent research using water stable isotopes has presented a “two water worlds” hypothesis says that the water that trees use for transpiration is different with that for groundwater and streamflow (Brooks et al., 2010). In this theory, there are two types of water: highly mobile water with can infiltrate to groundwater and streamflow quickly; tightly bound water remains in the soil and alter by evaporation processes, this water is used for water transpiration and not mixed with mobile water (Goldsmith et al., 2012; McDonnell, 2014) (fig.2).

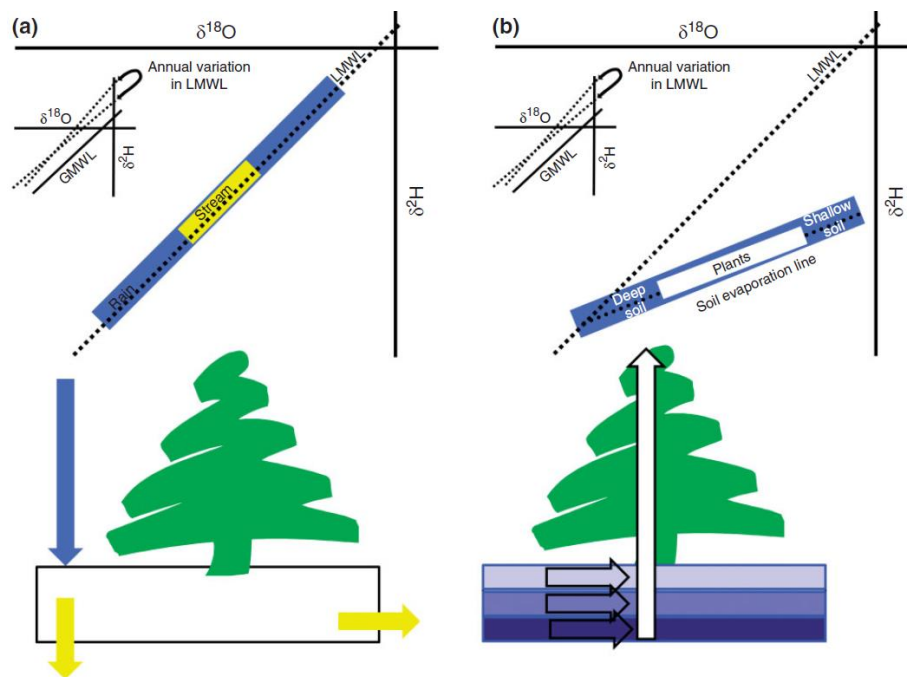


Fig.2. The two water worlds hypothesis in diagrammatic form, showing (a) the mobile water mixing space and schematic representation of mixing and (b) the low mobility water mixing space and schematic representation of mixing. Stream water is lagged and damped relative to the rainfall input signal and therefore represents a narrower distribution of isotope values. Similarly, the plant water values represent a narrower range than the tightly bound soil water, representative of the depth where water is extracted through the soil profile. Shallower soil waters plot farthest away from the meteoric water line (due to evaporation); deepest soil waters plot on the meteoric water line. Adapted from McDonnell, 2014

Water isotopic tracing for plant can be used over seasons (Li et al., 2007), also can be used at inter-specific (Goldsmith et al., 2012; Barbeta et al., 2019) and intra-specific level (Li et al., 2006). There are two important premises that have to be assumed for studying the sources of water for plants (Ehleringer & Dawson, 1992; Voltas et al., 2015): 1) there is no fractionation during water uptake by roots, except for some xerophytic and halophytic plants (Ellsworth & Williams, 2007; Lin et al., 1993), and 2) there is no fractionation during the water transport along the xylem, from the roots to the upper canopy (Ehleringer & Dawson, 1992; Brunel et al., 1995; Walker et al., 2001).

1.3.2. Stable isotopes in tree-rings

Trees uptake CO₂ from air and water from soil water for photosynthesis to make leaf sugar, and finally synthesize the tree-ring for growth. During Photosynthesis, CO₂ go through the stomata into leave during air diffuses, but lighter ¹²C is easier to pass stomata into leave than heavier ¹³C, the CO₂ in leave is depleted in ¹³C compare with the source CO₂ from atmosphere, called fractionation due to diffusion (-4.4‰). The other fractionation occurs when photosynthetic enzyme uses CO₂ to synthesize sugar, called fractionation due to carboxylation. Many environmental factors give stress on trees, which can affect these two processes (fig.3). These two processes resulting that tree-ring carbon stable isotope ratio is isotopically depleted in ¹³C in compare with atmospheric CO₂ (Farquhar et al., 1989), this ratio can be calculated as:

Eq.4.
$$\delta^{13}C = \delta^{13}C_{atm} - a - (b - a) \times (C_i/C_a)$$

Where $\delta^{13}C_{atm}$ is current atmospheric $\delta^{13}C$ value ($\approx -8\text{‰}$), a is fractionation due to diffusion ($\approx -4.4\text{‰}$), b is fractionation due to carboxylation ($\approx -27\text{‰}$), c_i/c_a is the ratio between intercellular and atmospheric CO₂ concentration (Farquhar et al., 1982). According to this equation, $\delta^{13}C$ value is mainly controlled by the ratio c_i/c_a which related to the balance between photosynthesis rate and stomata conductance. High c_i/c_a ratio indicate that stomata

conductance is much higher than photosynthesis rate, which means more CO_2 is available for photosynthesis, more lighter carbon isotope (^{12}C) is used for carboxylation, resulting the low value of $\delta^{13}\text{C}$. low c_i/c_a leads opposite result. The balance between stomata conductance and photosynthesis rate also related with plant intrinsic water use efficiency (WUEi) (Polley et al., 1993; Van de Water et al., 1994). Therefore, carbon stable isotope can also be used for determine plant water use efficiency (Farquhar et al., 1982; Ehleringer et al., 1993).

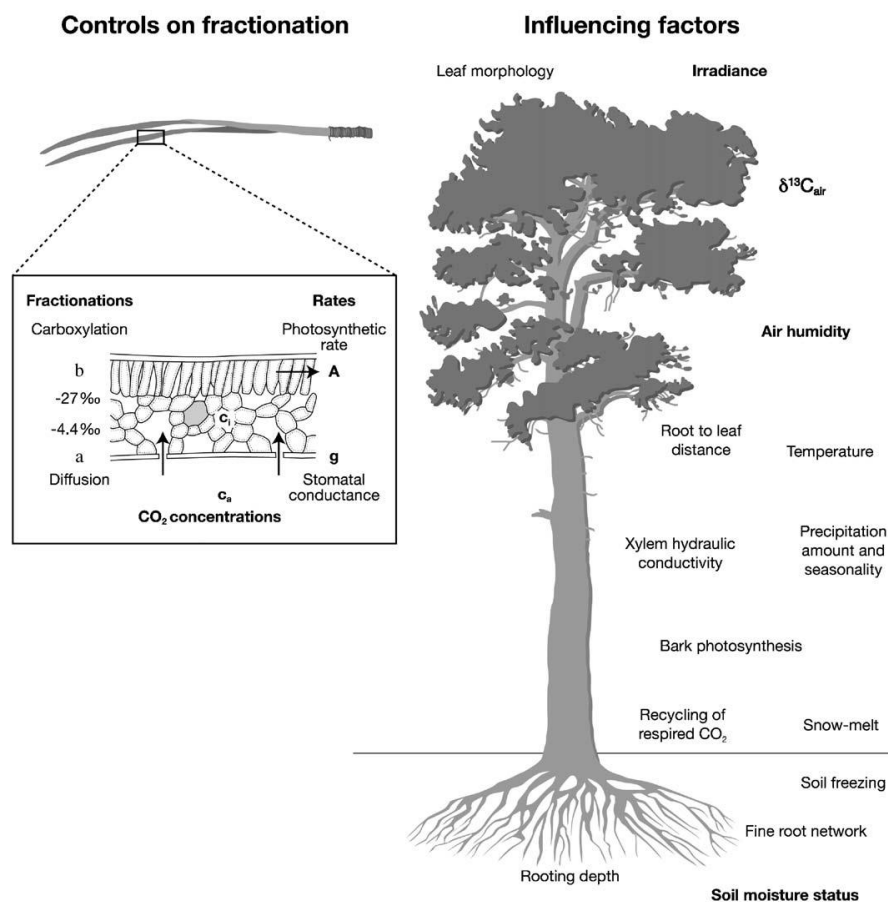


Fig.3. Diagram of a needle-leaf tree showing the main controls on the fractionation of carbon isotopes and the environmental factors that influence them. Adapted from McCarroll and Loader, 2004

Oxygen in tree-rings originally from the soil water uptake by roots, which mainly from precipitation, therefore the oxygen isotope ratio in tree water can represent part of precipitation water oxygen isotope ratio. However, several fractionations occur during the processes to fix the oxygen from water into tree-ring. First occurs in soil water, the upper soil water is isotopically more enriched than deeper soil water because of evaporation enrichment.

Beside seasonal variation in precipitation $\delta^{18}\text{O}$ (Matsubaya and Kawaraya, 2014) can also change the soil water $\delta^{18}\text{O}$. Second occurs in leaf, where transpiration release water into atmosphere, water with lighter isotopes (for example: $^1\text{H}_2^{16}\text{O}$) will loss faster than the one with heavy isotopes (for example: D_2^{18}O), this cause the leaf water enrichment in ^{18}O , can be as much as 20‰ (Saurer et al., 1998). Third occurs in phloem during the sugar transported to trunk for tree-ring cellulose synthesise, the oxygen in sugar from phloem will exchange with water from xylem (Sternberg et al., 1986), and this exchange rate can up to 50% (Roden et al., 2000; Gessler et al., 2014). Thus, tree-ring $\delta^{18}\text{O}$ is not directly represent the source water $\delta^{18}\text{O}$. It depends on several factors, such as: 1) seasonal precipitation $\delta^{18}\text{O}$, which related to air temperature (Dansgaard, 1964). 2) soil water $\delta^{18}\text{O}$ gradient, which is caused by precipitation saturation in cool season and evaporation from soil surface in hot season (Saurer et al., 2016). 3) depth of root, which control from which depth trees are up taking the water. 4) leaf water enrichment during transpiration, which can amplify the oxygen signal contained in the rain water (Cernusak et al., 2016). 5) oxygen exchange rate between sugar from phloem and water from xylem during wood synthesis.

1.4. Objective of this study

The objective of this study is 1) to determine the temporal pattern of temporal variation of stable isotopes in precipitation water, soil water and tree xylem water. 2) to evaluate the impact of extreme climate on trees by using water stable isotopes. 3) to evaluate intrinsic water-use efficiency between different tree species by means of $\delta^{13}\text{C}$. 4) to compare temporal changes in tree-ring $\delta^{18}\text{O}$ with xylem water $\delta^{18}\text{O}$ records as related to the relevance of summer transpiration.

References

- Barnes CJ, Allison GB. (1988). Tracing of water movement in the unsaturated zone using stable isotopes of hydrogen and oxygen. *Journal of Hydrology*. 100. 143-176
- Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE; Fréjaville B, Wohl S, Ogée J. (2019). Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences*, 23. 2129-2146
- Bokhorst S, Huiskes A, Convey P, Aerts R (2007) The effect of environmental change on vascular plant and cryptogam communities from the Falkland Islands and the Maritime Antarctic. *BMC Ecology*, 7. 15.
- Bonan GB. (2008). Forests and Climate Change: Forcings, Feedbacks, and the Climate Benefits of Forests. *Science*, 320. 1444-1450
- Brooks JR, Barnard HR, Coulombe R, McDonnell JJ. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nature geoscience*, 3.100-104
- Brunel JP, Walker GR, Dennett-Smith AK. (1995). Field validation of isotopic procedures for determining sources of water used by plants in a semi-arid environment. *Journal of Hydrology*, 167. 351-368
- Camarero JJ, Gazol A, Sangüesa-Barreda G, Oliva J, Vicente-Serrano V. (2015). To die or not to die: early warnings of tree dieback in response to a severe drought. *Journal of Ecology*, 103. 44-57
- Cernusak AL, Barbour, MM, Arndt SK, Cheesman AW, English NB, Feild, TS, Helliker BR, Holloway-Phillips MM, Holtum JAM, Kahmen A, McInerney FA, Munksgaard NC, Simonin KA, Song X, Stuart-Williams H, West JB, Farquhar GD. (2016). Stable isotopes in leaf water of terrestrial plants. *Plant Cell Environ*, 39. 1087–1102
- Craig H, Gordon L. (1965). Deuterium and oxygen-18 variations in the ocean and the marine atmosphere. In: Proceedings of a conference on stable isotopes in oceanographic studies

- and palaeotemperatures. E. Tongiorgi(ed). Lischi and Figli, Pisa
- Chapin F III, Rincon E, Huante P (1993) Environmental responses of plants and ecosystems as predictors of the impact of global change. *Journal of Biosciences*, 18. 515–524.
- Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, Jones R, Kolli RK, Kwon WT, Laprise R, Magaña Rueda V, Mearns L, Menéndez CG, Räisänen J, Rinke A, Sarr A, Whetton P. (2007) Solomon S et al. (Eds.), Climate Change 2007: The Physical Science Basis. Contributions of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, Cambridge University Press, Cambridge, United Kingdom/New York, NY. CLIMATE CHANGE 2014: Synthesis Report. IPCC
- Dansgaard W. (1964). Stable isotopes in precipitation. *Tellus*, 16:4. 436–468.
- Ehleringer JR, Dawson TE. (1992). Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell and Environment*, 15. 1073-1082
- Ellsworth PZ, Williams DG. (2007). Hydrogen Isotope Fractionation during Water Uptake by Woody Xerophytes. *Plant & Soil*, 291, 93-107.
- Farquhar GD, Ehleringer JR, Hubick KT. (1989). Carbon isotope discrimination and photosynthesis. *Annu Rev Plant Physiol Plant Mol Biol*, 40. 503-537
- Ferrio JP, Resco V, Williams DG, Serrano L, Voltas J. (2005). Stable isotopes in arid and semi-arid forest systems. *Invest Agrar: Sist Recur For*, 14(3). 371-382
- Gat JR. (1996). Oxygen and hydrogen isotopes in the hydrologic cycle. *Annual Review of Earth and Planetary Sciences*, 24. 225-262
- Gessler A, Ferrio JP, Hommel R, Treydte K, Werner RA, Monson RK. (2014). Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. *Tree Physiology*, 34. 796-818
- Goldsmith GR, Muñoz-Villiers LE, Holwerda F, McDonnell JJ, Asbjornen H, Dawson TE. (2012) Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical

- montane cloud forest. *Ecohydrology*, 5. 779-790
- Hanson PJ, Weltzin JF. (2000). Drought disturbance from climate change: response of United States forests. *Science of the Total Environment*, 262. 205-220
- IAEA/WMO, 2001. Global Network of Isotopes in Precipitation. The GNIP Database. Accessible at: <http://isohis.iaea.org>.
- Ichikawa, A., Ed., (2004). Global Warming – The Research Challenges: A Report of Japan's Global Warming Initiative. Springer, 160
- Jentsch A, Beierkuhnlein C (2008) Research frontiers in climate change: effects of extreme meteorological events on ecosystems. *Comptes Rendus Geoscience*, 340. 621–628.
- Knapp A, Fay PA, Blair JM et al. (2002). Rainfall variability, carbon cycling, and plant species diversity in a Mesic grassland. *Science*, 298, 2202–2205
- Li sg, Tsujimura M, Sugimoto A, Sasaki L, Yamanaka T, Davaa G, Oyunbaatar D, Sugita M. (2006). Seasonal variation in oxygen isotope composition of waters for montane larch forest in Mongolia. *Trees*, 20. 122-130
- Li sg, Romero-Saltos H, Tsujimura M, Sugimoto A, Sasaki L, Davaa G, Oyunbaatar. (2007). Plant water sources in the cold semiarid ecosystem of the upper Kherlen River catchment in Mongolia: A stable isotope approach. *Journal of Hydrology*, 333. 109-117
- Lin GH, Sternberg LDL, Hall AE, Farquhar GD. (1993). Hydrogen Isotopic Fractionation by Plant Roots during Water Uptake in Coastal Wetland Plants. In J. R. Ehleringer, A. E. Hall, & G. D. Farquhar (Eds.), *Stable Isotopes and Plant Carbon- Water Relations* (pp. 497-510). New York: Academic Press
- Linder M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Serdl R, delzon S, corona P, kolström M, J. Lexer M, Marchetti M. (2010). Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management*, 259. 698-709

- Matsubaya O, Kawaraya H. (2014). Hydrogen and Oxygen Isotopic Characteristics of Precipitation in Coastal Areas of Japan Determined by Observations of 23 Years at Akita and for 1-2 Years at Other Several Localities. *Geochemical Journal*, 48, 397-408
- Merlivat L, Jouzel J. (1979). Global climatic interpretation of the deuterium-oxygen 18 relationship for precipitation. *Journal of Geophysical Research*, 84. 5029-5033
- McCarroll D, Loader NJ. (2004). Stable isotopes in tree rings. *Quaternary Science Reviews*, 23. 771-801
- McDonnell JJ. (2014). The two water worlds hypothesis: ecohydrological separation of water between streams and trees? *WIREs Water*, 1.323-329
- Jasechko S, Shrp ZD, Gibson JJ, Jean Birks S, Yi Y, Fawcett PJ. (2013). Terrestrial water flux dominated by transpiration. *Nature*, 496. 347-351
- Japan Meteorological Agency, 2017: Climate change Monitoring Report
- Pan Y, Birdsey RA, Fang J, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, David McGuire A, Piao S, Rautiainen A, Sitch S, Hayes D. (2011). A Large and Persistent Carbon Sink in the World's Forests. *Science*, 333. 988-993
- Pfahl S, Sodemann H. (2014). What controls deuterium excess in global precipitation? *Climate of the past*, 10. 771-781
- Polley HW, Johnson HB, Marino BD, Mayeux HS. (1993). Increase in C3 plant water-use efficiency and biomass over Glacial to present CO₂ concentrations. *Nature*, 361. 61-64
- Reyer CPO, Leuzinger S, Ramming A, Wolf A, Bartholomeus RP, Bonfante A, DE Loenzi F, Dury M, Gloning P, Jaoudé RA, Klein T, Kuster TM, Martins M, Niedrist G, Riccardi M, Wohlfahrt G, DE Angelis P, DE Dato G, Francois L, Menzel A, Pereira M. (2013). A plant's perspective of extremes: terrestrial plant responses to changing climatic variability. *Global Change Biology*, 19. 75-89

- Roden JS, Lin GL, Ehleringer JR. (2000). A Mechanistic Model for Interpretation of Hydrogen and Oxygen Isotope Ratios in Tree-Ring Cellulose. *Geochimica et Cosmochimica Acta*, 64. 21-35
- Saurer M, Borella S, Leuenberger M. (1997). ^{18}O of tree rings of beech (*Fagus sylvatica*) as a record of ^{18}O of the growing season precipitation. *Tellus Series B-Chemical and Physical Meteorology*, 49. 80-92
- Saurer M, Robertson I, Siegwolf R, Leuenberger M, (1998). Oxygen isotope analysis of cellulose: an interlaboratory comparison. *Analytical Chemistry*, 70. 2074–2080
- Saurer M, Kirdeyanov AV, Prokushkin AP, Rinne KT, Siegwolf RTW. (2016). The impact of an inverse climate–isotope relationship in soil water on the oxygen-isotope composition of *Larix gmelinii* in Siberia. *New Phytol*, 209. 955–964.
- Schlesinger WH, Jasechko S. (2014). Transpiration in the global water cycle. *Agricultural and Forest meteorology*, 189-190. 115-117
- Sternberg L, Dero M, Savidge R. (1986). Oxygen Isotope Exchange between Metabolites and Water during Biochemical Reactions Leading to Cellulose Synthesis. *Plant Physiology*, 82. 423-427
- Sulzman EW. (2008). Stable Isotope Chemistry and Measurement: A Primer. In: Stable Isotopes in Ecology and Environmental Science: Second Edition. pp 1-21
- Valentini R, Anfodillo T, Ehleringer JR. (1994). Water sources and carbon isotope composition ($\delta^{13}\text{C}$) of selected tree species of the Italian Alps. *Canadian Journal of Forest Research*, 24(8). 1575-1578
- Van de Water PK, Leavitt SW, Betancourt JL. (1994). Trends in stomatal density and $^{13}\text{C}/^{12}\text{C}$ ratios of *Pinus flexilis* needles during alst Glacial-Interglacial cycle. *Science*, 264. 239-243
- Van Peer L, Nijs I, Reheul D, De Cauwer B. (2004). Species richness and susceptibility to heat and drought extremes in synthesized grassland ecosystems: compositional vs physiological

effects. *Functional Ecology*, 18. 769–778

Voltas J, Lucabaugh D, Chambel MR, Feerrio JP. (2015). Intraspecific Variation in the Use of Water Sources by the Circum-Mediterranean Conifer *Pinus halepensis*. *New Phytologist*, 208, 1031-1041

Walker, C. D., Brunel, J. P., Dighton, J., Holland, K., Leaney, F., McEwan, K., Mensforth, L., Thorburn, P., & Walker, C. (2001). The Use of Stable Isotopes of Water for Determining Sources of Water for Plant Transpiration. In M. Unkovich, J. Pate, A. McNeil, & J. D. Gibbs (Eds.), *Stable Isotope Techniques in the Study of Biological Processes and Functioning of Ecosystems* (pp. 57-89). Netherlands: Springer Science & Business Media

Chapter 2

Tree xylem water dynamics in three tree species during summer time

2.1. Introduction

Forests are an important component of the global water cycle as they exchange large amounts of water, originating from different terrestrial sources, with the atmosphere. Stable isotopes (^{18}O and D) have been widely used in the last decades in order to trace such sources of water (rainfall, groundwater, soil water, snowmelt, mist, etc.) available to plants (Ehleringer and Dawson, 1992; Valentini et al., 1994; Brunel et al., 1995; Walker et al., 2001; Asbjornsen et al., 2011; McDonnell., 2014; Martín-Gómez et al., 2017), that is because the isotopic composition of potential water source can be traced back from the values in xylem water. Frequently, the water isotopic composition changes with soil depth, where water from upper soil layers is isotopically enriched in relation to that from deeper layers because of evaporation gradients occurring along the soil profile during dry periods (Barnes et al., 1988). In turn, xylem water isotopes often show strong seasonal variability during wet-dry periods because trees may switch from shallower to deeper soil layers when accessing water under increasing drought stress (Goldsmith et al., 2012; Penna et al., 2013; White and smith, 2015). This shift in the access to different water pools also depends on the type of vegetation and its associated root system. For example, it has been reported (Eggemeier et al., 2008) that *Juniperus virginiana* and *Pinus ponderosa* take up water from different soil depths under the same environmental conditions in the semi-arid sandhills grasslands of Nebraska during the growing season. Similarly, the Mediterranean oak *Quercus ilex* keeps physiological activity for longer periods over acute droughts in the eastern Iberian Peninsula using alternative (deeper) soil water sources, not available for the thermophilic conifer *Pinus halepensis* (Castillo et al., 2016). On the other hand, when water is readily available from precipitation, xylem water isotopes reflect mainly the isotopic composition of precipitation (Li et al., 2006; Li et al., 2007; Barbeta et al., 2015).

There are two important premises that have to be assumed for studying the sources of water for plants (Ehleringer and Dawson, 1992; Voltas et al., 2015): (1) there is no fractionation during water uptake by roots, except for some xerophytic and halophytic plants (Ellsworth and Williams, 2007; Lin et al., 1993), and (2) there is no fractionation during the water transport along the xylem, from the roots to the upper canopy (Ehleringer and Dawson, 1992; Brunel et al., 1995; Walker et al., 2001). Hence, if no such fractionation occurs at the root level, water is isotopically enriched in the leaves proportionally to the magnitude of transpiration, and this process produces sugars that are isotopically heavier by a mean value of 27‰ in $\delta^{18}\text{O}$ comparison to leaf water (Sternberg et al., 1986).

Until now, most studies on soil water accessibility by plants along the soil profile using isotopic analysis have been conducted in arid and semiarid areas because of evaporation enrichment, but very few have been conducted in wet areas. This may be the case of the humid mixed forests of western Japan, which may be subjected to sporadic episodes of water scarcity in early summer or concentrated extreme rain events with long rainless pauses even within the rainy season period. In the western side of northeastern Japan, the dominant tree species are cedar (*Cryptomeria japonica*), larch (*Larix kaempferi*) and beech (*Fagus crenata* Blume). Cedar is the most commercially important tree species in Japan, with plantations covering an area of approximately 4500 km² (MAFF, 2017), while larch, also native to Japan, is also widely planted for forest industry in northern Japan (4700 km²) (MAFF, 2017) and is distributed as the southern edge of the larch distribution in East Asia. Beech is the most abundant deciduous broad-leaved tree species in Japan, growing in the cool-temperate zone as a late-successional and climax species (Liang et al., 1995). A comparative study on the nitrogen cycle, using nitrogen stable isotopes, of these tree species in the same region revealed differences among them, with beech trees showing higher nitrogen resorption from senescing leaves (Enta et al., 2019; Seidel et al., 2019 a, b), which might be related to their water use patterns. All these

tree species are well adapted to the heavy precipitation conditions of northeastern Japan. However, the species and populations of forest trees inhabiting wet areas may be particularly susceptible to the impact of an even distribution of rain along the growing season in the face of ongoing climate change (Engelbrecht et al., 2007). Thus, the aims of this study were threefold: to determine the temporal pattern of temporal variation of stable isotopes in soil and xylem water of three typical Japanese tree species, the evergreen conifer *Cryptomeria japonica* and the deciduous-but taxonomically contrasting—*Larix kaempferi* and *Fagus crenata*.

2.2. Methods

2.2.1. Study site and meteorological data

The forest stands are located within the premises of the Yamagata University Research Forest (YURF), which is in the Asahi Mountains, 26 km south of Tsuruoka city in Yamagata Prefecture (Fig.1). Three typical Japanese tree species for this region were selected to evaluate their water use patterns: beech (*Fagus crenata*), larch (*Larix kaempferi*) and cedar (*Cryptomeria japonica*). The beech forest stand (N38°33.396', E139°52.641') was located at the highest elevation in the experimental forest (670 m.a.s.l.), and the trees had an average age of 70-80 years. The larch forest stand (N38°33.415', E139°52.566') was located at a slightly lower elevation (650 m.a.s.l.), and the trees had an average age of 45-50 years. Finally, the cedar forest site (N38°32.982', E139°51.796') was located at the lowest elevation (295 m.a.s.l.), and the trees had an average age of 54 years.

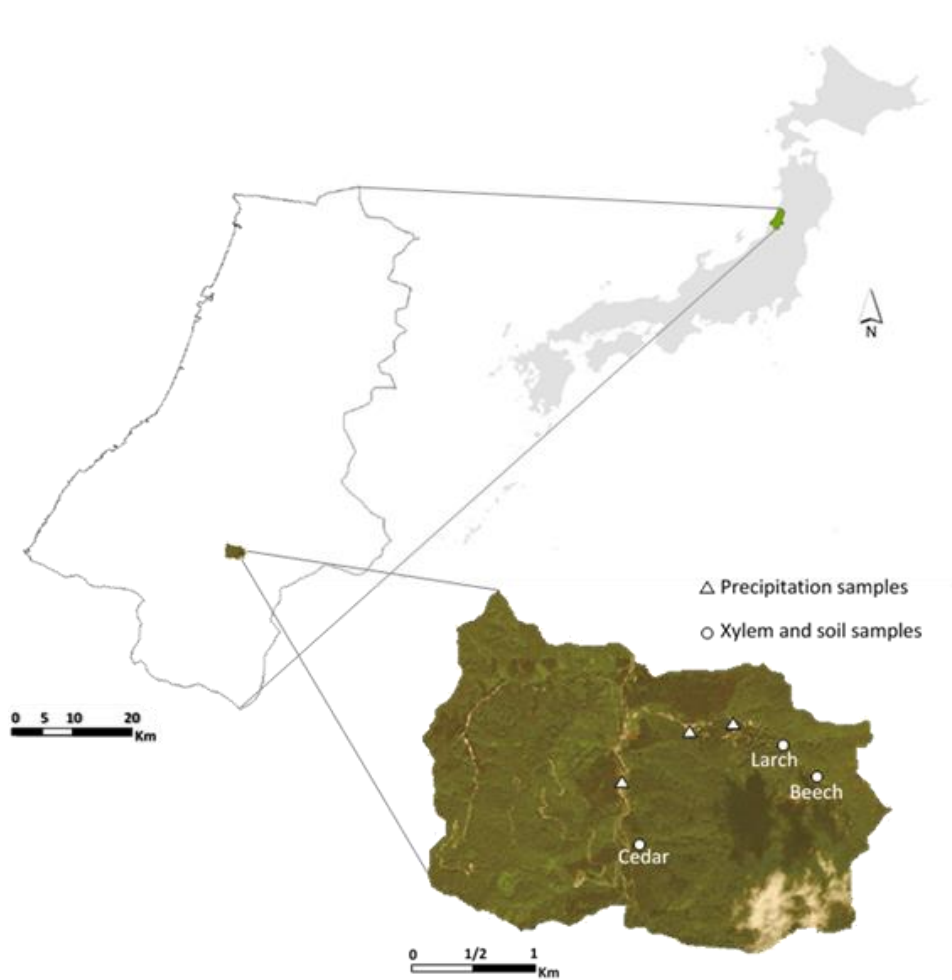


Figure 1. The Yamagata University Research Forest (YURF) is in Shonai Region, Yamagata Prefecture, Japan. The circles represent the xylem and soil sampling sites within the forest, the triangle represent the precipitation sampling spot.

The annual precipitation in this area is 3,090 mm of which 42% falls as snow, the annual mean air temperature is 9.5°C for the period 2012-2017 (Fig. 2). The mean summer precipitation (June, July and August) is 518 mm and mean air temperature is 20.9°C. Monthly precipitation is relatively low from April to September, except for July when the highest values for the growing season are observed as the rainy season sets in for this region. The highest precipitation occurs from October to March, mainly in the form of snow, with the snow melting starting from mid-February and ending by late April or the beginning of May. Average air temperatures are below zero only in January and February, while the highest temperature

corresponds to July and August during summer. Air temperature increases from May through June, and stays approximately stable during July and August. During summer 2017, precipitation was 121 mm (June), 388 mm (July) and 193 mm (August). Most precipitation was concentrated in the first days of July (155 mm), representing with the peak of the rainy season.

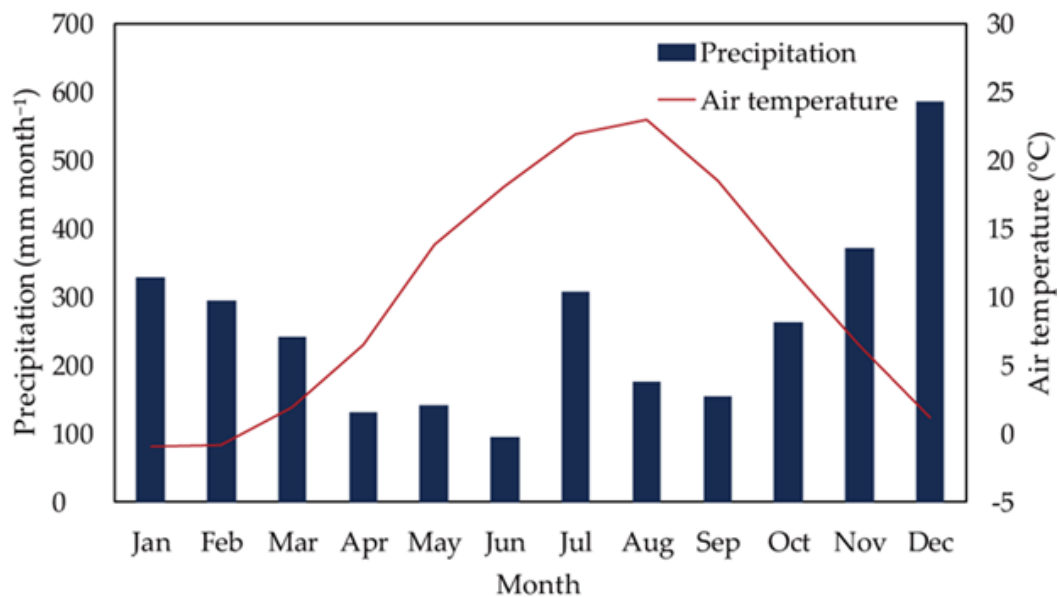


Figure 2. Mean monthly in temperature and precipitation in the study area (2012-2017). (Source: Yamagata University Research Forest Meteorological Station)

2.2.2. Sampling design and measurements

Samples were collected on June 21, July 25 and August 23 (2017). Ten beech, eight larch and ten cedar trees were randomly sampled in each forest for xylem water at each site respectively. The xylem was collected by peeling the bark and phloem from twigs of about 1-cm of diameter taken from the lower part of the crown. In total, we collected 30 samples from cedar and beech trees each and 24 samples from larch trees. The twigs were sampled using telescopic loppers at noon (solar time) approximately. From 07:00 to 09:00 h solar time, soil samples were collected from 0-20 cm, 20-40 cm and 40-60 cm depth using a straight tube probe thoroughly cleaned between consecutive samplings from three soil pits evenly distributed within the area covered by the sam-pled trees at each stand. All twig and soil samples were

collected by screw top test tube (NW-18) and frozen immediately after sampling to prevent evaporation. For the period January-August, precipitation water for isotope analysis was collected immediately after every rain event from 3 plastic bottles, where a 7 cm diameter funnel was inserted, evenly set in open spaces close to the forest sites. To prevent evaporation, the water was stored in airtight flasks and kept frozen until measurements were conducted. The local meteoric water line (LMWL) was estimated as $\delta D = 8 \times \delta^{18}O + 15$ (Matsubaya and Kawaraya, 2014).

Xylem and soil water were extracted using a cryogenic vacuum distillation system (Martín-Gómez et al., 2015) and, together with precipitation water, were analyzed for $\delta^{18}O$ (oxygen isotopic composition) and δD (hydrogen isotopic composition) by isotope ratio infrared spectroscopy (IRIS) using a Picarro L2120-i coupled to an A0211 high precision vaporizer (Picarro Inc., Sunnyvale, CA, USA). The estimated precision, based on the repeated analysis of four reference water samples, was 0.10‰ for $\delta^{18}O$ and 0.40‰ for δD . Residual organic contaminants in the distilled water can interfere with the analysis of plant and soil samples conducted with IRIS (Martín-Gómez et al., 2015). The presence of contaminants was checked using Picarro's CHEMCORRECT post-processing software and corrected (in some few cases) following (Martín-Gómez et al., 2015).

Statistical comparisons among species and months were carried out using a two-way analysis of variance (two-way ANOVA) for stable isotopes of xylem water, soil water and wood α -cellulose. All statistical analysis was conducted with the software JMP (SAS Institute Inc).

2.3. Results

2.3.1. Isotopic variation in monthly precipitation and xylem water

Precipitation water $\delta^{18}O$ and δD values at YURF are shown in Figure 3. They followed

essentially the same trends as the 22-year record of monthly precipitation water $\delta^{18}\text{O}$ and δD found for Akita prefecture (Matsubaya and Kawaraya, 2014), 140 km north of Shonai region. The water isotopic composition of June represented the lowest $\delta^{18}\text{O}$ and δD values observed throughout the year in Shonai.

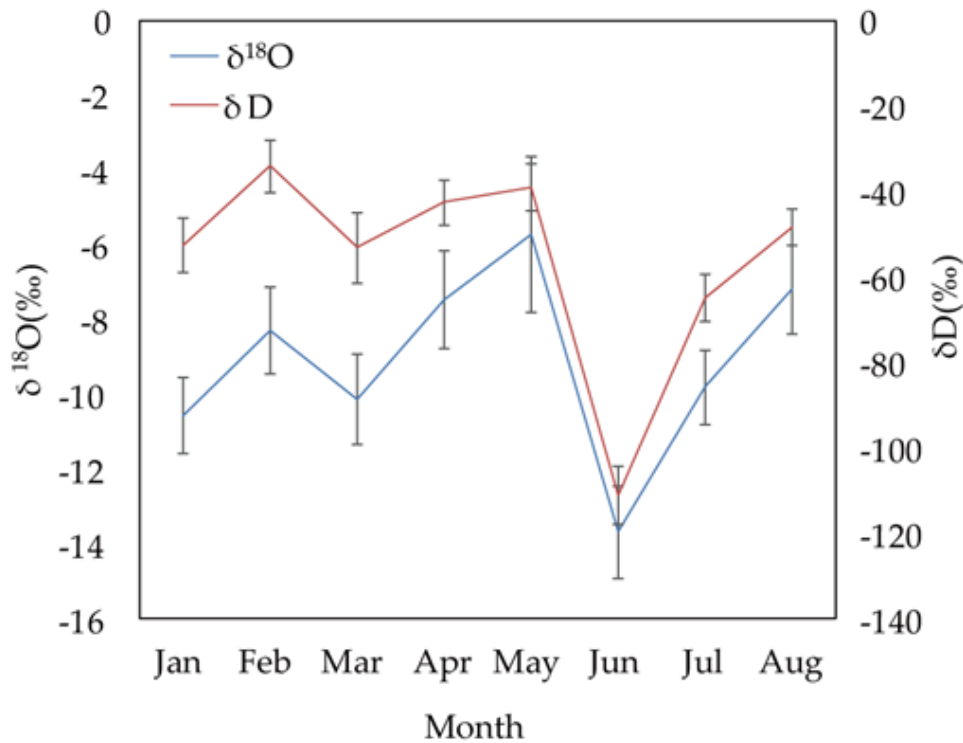


Figure 3. Monthly $\delta^{18}\text{O}$ and δD of monthly precipitation at Yamagata University Research Forest in 2017 (January to August); Values are mean \pm SE (n=3)

Increasing trends in xylem water $\delta^{18}\text{O}$ and δD in all tree species were found in summer (Fig. 4), in agreement with the monthly variation of precipitation water isotopes composition for the same period (Fig. 3, shaded area). For xylem $\delta^{18}\text{O}$, there was no significant difference between species ($p = 0.185$), but for xylem δD there was a significant difference between cedar and beech and larch and beech ($p < 0.001$), with beech being isotopically lighter than the other two tree species during the summer months, especially in August.

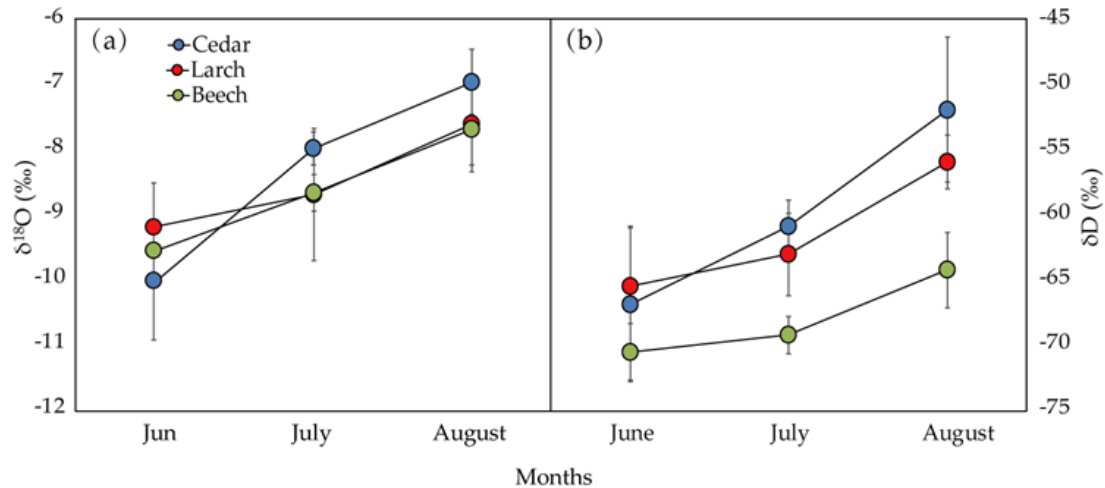


Figure 4. Monthly (a) $\delta^{18}\text{O}$, (b) δD of xylem water from cedar (blue circle), larch (red circle) and beech (green circle); values are mean \pm SE, cedar (n=10), larch (n=8), beech (n=10)

2.3.2. Correlation between $\delta^{18}\text{O}$ and δD

Soil water, xylem water and precipitation isotope records are represented together with the GMWL (Global Meteoric Water Line) and LMWL (Local Meteoric Water Line) in Fig. 5. Soil water $\delta^{18}\text{O}$ and δD at 0-20 cm and 20-40 cm depths (but not soil water at 40-60 cm) were uncoupled from the LMWL in June, which indicated that evaporation was higher in these two layers than in the deeper 40-60 cm soil layer. In contrast, in July and August soil water isotope ratios followed the LMWL, which indicated lower evaporation in these two months. Similarly, the isotopic values of all soil layers showed comparable values in July. Precipitation water isotope ratios increased markedly along the summer months, with values of -13.7 ‰, -9.8 ‰, and -7.2 ‰ for $\delta^{18}\text{O}$, and -111.1 ‰, -64.9 ‰, and -48.5 ‰ for δD in June, July and August, respectively. Xylem water isotope ratios showed relatively similar values as those of soil water isotopes in June (-8‰ to -11‰ for $\delta^{18}\text{O}$, -60‰ to -80‰ for δD); however, xylem water isotopic composition became decoupled from soil water isotopic records in July. Finally, in August, xylem water $\delta^{18}\text{O}$ and δD values for cedar and larch were between the range of values found for soil and precipitation water isotope ratios, but xylem water δD values of beech were always lighter in comparison to the other two tree species δD and closer to those of soil water values

during the study period.

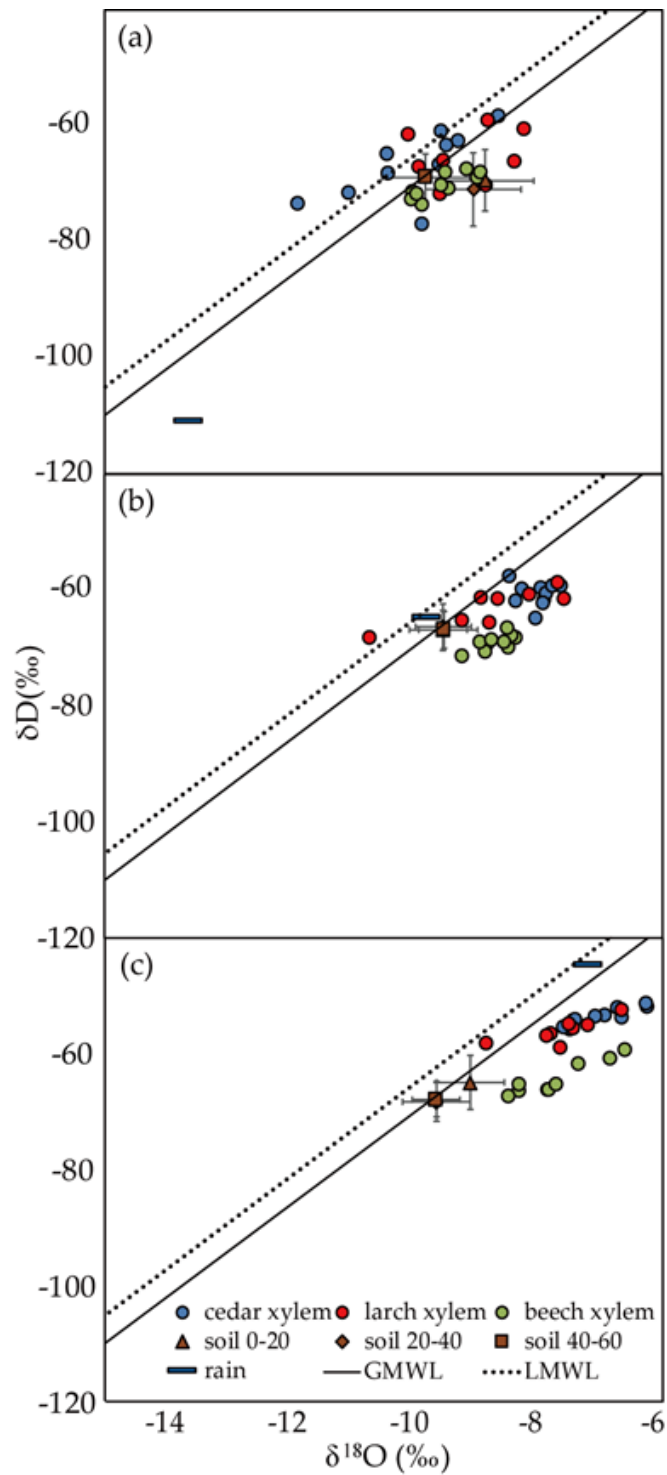


Figure 5. Relationship between δD and $\delta^{18}O$ in precipitation water ($n=3$), xylem water for all tree species (cedar, $n=10$; larch, $n=8$; beech, $n=10$) and the average of soil water isotope ratios at different depths ($n=9$) in (a) June, (b) July and (c) August. The Local Meteoric Water Line (LMWL) and Global Meteoric Water Line (GMWL) are also represented.

2.4. Discussion

2.4.1. Temporal difference of xylem water isotopes

No significant differences in xylem water $\delta^{18}\text{O}$ were found among species, but progressively increasing values were observed from June to August in agreement with the gradually more isotopically enriched precipitation water found along this period. This suggests a tight dependence of xylem water from precipitation water during the peak season when drought stress is absent (Li et al., 2006; Li et al., 2007; Barbeta et al., 2015). In addition, the xylem water isotopic composition of the three species followed closely the different water sources from June to August. However, there were significant differences in xylem water δD between beech and the other two species, as beech δD showed always lighter values, suggesting that the main source of water for this species was soil water – even during months of high precipitation – unlike cedar and larch trees that were likely more prompt to use readily available fresh precipitation water. This was particularly evident in August, when precipitation water was isotopically heavier than the soil water, suggesting that beech trees used soil water as their main water source. Indeed, xylem water δD was in the same range as soil water δD , which was more depleted than precipitation water. According to Goldsmith et al (2012), under dry and wet conditions, different tree species used the same water source as indicated by their xylem isotopic composition. In contrast, cedar and larch trees appeared to use fresh precipitation water when it was available, as shown by xylem water δD values that were closer to those of precipitation water δD . In June (when precipitation was relatively low), cedar and larch trees apparently used water mainly from shallow soil layers where the bulk of their roots are distributed, since xylem water isotopic composition was in the same range as the soil water isotopic values (Eggemeyer et al., 2008; Castillo et al., 2016). Even though beech appeared to use soil water as their main source, during periods of high precipitation, the source of water

is the combination of soil water and fresh precipitation. The same pattern has been reported for *Fagus sylvatica* trees in northern Italy where they use soil and precipitation water during rainy periods but mainly deep soil water and ground water during dry periods (Penna et al., 2013).

In our study, we found an offset between xylem water δD and water sources δD as it has been reported in recent studies (Plavocová et al., 2018; Evaristo et al., 2017; Geris et al., 2017). However, we only detected this offset in August, but not in June and July. In a previous study on seasonal patterns of *Fagus sylvatica* in Czech Republic, the xylem water isotopic composition followed the precipitation isotopic composition (Oerter and Bowen, 2019), which was the case for oxygen isotopes in our study. Coincidentally, *Fagus sylvatica* xylem water δD was reportedly more depleted than the source water, suggesting that fractionation occurred at the soil interface during water uptake (Barbeta et al., 2019). Although we observed a similar phenomenon in August, our data are probably not enough to support the theory that such fractionation occurred in *Fagus crenata*. This remains a topic of future study since it may appear to be a species and temporal-specific phenomenon, as suggested by our results.

2.4.2. Different water uses among tree species

Our results indicated that, among the three tree species, there were two water use patterns during the summer period that were controlled by the precipitation regime and the root distribution of each tree species. In June, when precipitation was relatively low (128.5 mm), the three species took up water from shallow as well as deeper soil layers, as it can be inferred by the similar isotopic composition of xylem and soil water among species. In July, the soil water isotopic composition was strongly controlled by precipitation water. Therefore, it was difficult to discern if trees were preferentially using soil water, fresh precipitation water or both during this month. We assume, though, that trees used mainly (readily available)

precipitation water. In August precipitation water isotopic composition was heavier than soil water isotopic composition but because of lower precipitation in comparison to July, soil water isotopic composition did not reflect precipitation water isotopic composition. Thus, it was possible to identify that cedar and larch used preferentially precipitation water that was readily available, whereas beech used preferentially water from deeper soil layers, which is accessible to this tree species because of its deeper root system distribution in comparison to cedar and beech. Indeed, the cedar and larch root systems are distributed in the surface 30 cm soil layer, while beech fine roots are evenly distributed down to 60 cm deep (Karizumi, 2011).

2.5. Conclusions

Cedar, larch and beech showed different water use patterns. The conifers cedar and larch were more prompt to use readily available precipitation water because of its shallow root system, as revealed by $\delta^{18}\text{O}$ and δD records of xylem, precipitation and soil water. However, these tree species used water stored in different soil layers when precipitation was relatively low. On the other hand, the broadleaf beech used soil water more profusely than the other two species because of its deeper root system, as indicated by xylem δD .

References

- Asbjornsen, H., Goldsmith, G.R., Alvarado-Barrientos, M.S., Rebel, K., van Osch, F.P., Rietkerk, M., Chen, J., Gotsch, S., Tobón, C., Geissert, D.R., Gómez-Tagle, A., Vache, K., Dawson, T.E. (2011). Ecological advances and applications in plant water relations research: a review. *Journal of Plant Ecology*, 4. 3-22.
- Barbeta, A., Mejía-Chang, M., Ogaya, R., Voltas, J., Dawson, T.E., Peñuelas, J. (2015). The

- combined effects of a long-term experimental drought and an extreme drought on the use of plant-water sources in a Mediterranean forest. *Glob. Chang. Biol*, 21. 1213-1225.
- Barbeta, A., Jones, S. P., Clavé, L., Wingate, L., Gimeno, T. El, Fréjaville, B., Wohl, S., Ogée, J. (2019). Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrol. Earth Syst. Sci*, 23. 2129-2146.
- Barnes, C.J., Allison, G.B. (1988). Tracing of water movement in the nsaturated zone using stable isotopes of hydrogen and oxygen. *Hydrol*, 100. 143-176.
- Brunel, J.P., Walker, G.R., Kennett-Smith, A.K. (1995). Field validation of isotopic procedures for determining sources of water used by plants in a semiarid environment. *Journal of Hydrology*, 167. 351-368
- Castillo, J. d., Comas, C., Voltas, J., Ferrio, J. P. (2016). Dynamics of competition over water in a mixed oak pine Mediterranean forest: Spatio-temporal and physiological components. *Forest Ecology and Management*, 382. 214-224.
- Eggemeyer, K. D., Awada, T., Harvey, F. E., Wedin, D. A., Zhou, X. H., Zanner, C. W. (2008). Seasonal changes in depth of water uptake for encroaching trees *Juniperus virginiana* and *Pinus ponderosa* and two dominant C4 grasses in a semiarid grassland. *Tree Physiology*, 29. 157-169.
- Ehleringer, J.R., Dawson, T.E. (1992). Water uptake by plants: perspectives from stable isotope composition. *Plant Cell Environment*, 15. 1073-1082.
- Ellsworth, P. Z and Williams, D. G. (2007). Hydrogen isotope fractionation during water uptake by woody xerophytes. *Plant Soil*, 291. 93-107.
- Engelbrecht, B. M. J., Comita, L. S., Condit, R., Kursar, T. A., Tyree, M. T., Turner, B. L., Hubbell, S. P. (2007). Drought sensitivity shapes species distribution patterns in tropical forest. *Nature*, 447. 80-83.

- Enta, A., Hayashi, M., Lopez, C, M, L., Fujiyoshi, L., Yamanaka, T., Oikawa, A., Seidel, F. (2019). Nitrogen resorption and fractionation during leaf senescence in typical tree species in Japan. *J. For. Res*
- Evaristo, J., McDonnell, J. J., Clemens, J. (2017). Plant source water apportionment using stable iso-topes: A comparison of simple linear, two-compartment mixing model approaches. *Hydrol. Process*, 31. 3750-3758
- Ferrio, J. P., Voltas, J. (2005). Carbon and oxygen isotopes ration in wood constituents of *Pinus halepensis* and indicator of precipitation, temperature and vapour pressure deficit. *Tellus*, 57B. 164-173.
- Geris, J., Tetzlaff, D., McDonnell, J. J., Soulsby, C. (2017). Spatial and temporal patterns of soil water storage and vegetation water use in humid northern catchments. *Sci. Total Environ*, 595. 486-493.
- Goldsmith, G.R., Muñoz-Villers, L.E., Holwerda, F., McDonnell, J.J., Asbjornsen, H., Dawson T.E. (2012) Stable isotopes reveal linkages among ecohydrological processes in a seasonally dry tropical montane cloud forest. *Ecohydrol*, 5. 779-790.
- Karizumi, S. (2011). Tree root illustration, Seibunndo shinkosha: Tokyo, Japan, pp. 62-66, pp. 123-132, pp.296-300, 978-4-416-41005-9.
- Li, S.G., Tsujimura, M., Sugimoto, A., Sasaki, L., Yamanaka, T. Davaa, G., Oyunbaatar, D., Sugita, M. (2006). Seasonal variation in oxygen isotope composition of waters for a montane larch forest in Mongolia. *Trees*, 20. 122-130.
- Li, S.G., Saltos, H.R., Tsujimura, M., Sugimoto, A., Sasaki, L., Davaa, G., Oyunbaatar, D. (2007). Plant water sources in the cold semiarid ecosystem of the upper Kherlen River catchment in Mongolia: A stable isotope approach. *Journal of Hydrology*, 333. 109-117.
- Liang, N.; Nagayama, M.; Nakata, M.; Maruyama, K. (1995). Growth, photosynthesis and nitrogen content in Japanese beech (*Fagus crenata Bl.*) seedlings grown under five

- irradiances. *Photosynthetica*, 31. 257-268.
- Lin, G. H., Sternberg, L.D.L., Hall, A.E., Farquhar, G.D. (1993). Hydrogen isotopic fractionation by plant roots during water uptake in coastal wetland plants. In Stable isotopes and plant carbon-water relations. Ehleringer, J.R., Hall, A.E., Farquhar, G.D. Academic Press, New York, USA pp. 497-510, 978-0-12-233380-4.
- Matsubaya, O., Kawaraya, H. (2007). Genetical relationship of ground water and river water to pre-precipitation suggested from several observation results of their hydrogen and oxygen isotopic ratios at Akita. *地下水学会誌*, 56. 309-320.
- Matsubaya, O., Kawaraya, H. (2014). Hydrogen and oxygen isotopic characteristics of precipitation in coastal areas of Japan determined by observations of 23 years at Akita and for 1-2 years at other several localities. *Geochemical Journal*, 48. 397-408.
- Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S., Dawson, T.E., Feerio, J. P. (2015). Isotope ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources? *New Phytologist*, 207. 914-927.
- Martín-Gómez, P., Díaz, J.P.M., García, J.P. (2017). Water use patterns and physiological interactions in Sub Mediterranean mixed forests: methodological challenges and implications for ecohydrology. PhD thesis, University of Lleida, Pl. de Víctor Siurana, 1 25003 Lleida.
- MAFF. Available online: <http://www.rinya.maff.go.jp/j/keikaku/genkyou/h29/4.html>
- McDonnell, J.J. (2014). The two water worlds hypothesis: ecohydrological separation of water between streams and trees? *WIREs Water*, 1. 323-329.
- Valentini, R., Anfodillo, T., Ehleringer, J.R. (1994). Water sources and carbon isotope composition ($\delta^{13}\text{C}$) of selected tree species of the Italian Alps. *Canadian Journal of Forest Resources*, 24. 1575-1578.
- Oerter, E. J., Bowen, G. J. (2019). Spatio-temporal heterogeneity in soil water stable isotopic composition and its ecohydrologic implications in semiarid ecosystems. *Hydro. Process.* 1-

- Penna, D., Oliviero, O., Assendelft, R., Zuecco, G., van Meerveld, I. (H. J.), Anfodillo, T., Carraro, V., Borga, M., Fontana, G.D. (2013). Tracing the water sources of trees and streams: isotopic analysis in a small prealpine catchment. *Procedia Environmental Sciences*, 19. 106-112.
- Plavcová, L., Hronková, M., Marie Šimkova, M., Květoň, J., Vráblová, M., Kubásek, J., Šantrůček, J. (2018). Seasonal variation of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ in leaf water of *Fagus sylvatica* L. and related water compartments. *Journal of Plant Physiology*, 227. 56-65
- Seidel, F., Lopez, C, M, L., Oikawa, A., Yamanaka, T. (2019a). Seasonal nitrogen partitioning in Japanese cedar (*Cryptomeria japonica*, D. Don) tissues. *Plant and Soil*, 442. 511-529.
- Seidel, F., Lopez, C, M, L., Celi, L., Bonifacio, E., Oikawa, A., Yamanaka, T. (2019b). N Isotope Fractionation in Tree Tissues During N Reabsorption and Remobilization in *Fagus crenata* Blume. *Forests*, 10. 330
- Sternberg, L., Dero, M., Savidge, R. (1986). Oxygen isotope exchange between metabolites and water during biochemical reactions leading to cellulose synthesis. *Plant Physiology*, 82. 423-427.
- Voltas, J., Lucabaugh, D., Chambel, M.R., Ferrero, J.P. (2015). Intraspecific variation in the use of water sources by the circum Mediterranean conifer *Pinus halepensis*. *New Phytologist*, 208. 1031-1041.
- Walker, C.D., Brunel, J.P., Dighton, J., Holland, K., Leaney, F., McEwan, K., Mensforth, L., Thorburn, P., Walker, C. (2001). The use of stable isotopes of water for determining sources of water for plant transpiration. In *Stable isotope techniques in the study of biological processes and functioning of ecosystems*, Unkovich, M., Pate, J., McNeil, A., Gibbs, J.D., Eds., Springer Science & Business Media, Netherlands, pp. 57-89, 978-0-7923-7078-9.
- White, J.C., Smith, W.K. (2015). Seasonal variation in water sources of the riparian tree species *Acer negundo* and *Betula nigra*, southern Appalachian foothills, USA. *Botany*, 93. 519-528.

Chapter 3

Soil-tree water relationship under extreme climate conditions

3.1. Introduction

The surface temperature has been increased globally and the precipitation patterns also changed since last decade (Schar et al., 2004; Della-Marta et al., 2007; IPCC, 2013). The annual mean surface temperature over Japan increase faster than global annual mean, and significantly high temperature were recorded mostly from 1990s to recent (Ichigawa et al., 2004; Yoshida et al., 2017). The frequency and intensity of extreme climate events has increasing globally, for example the number of cold days is decreasing and the number of warm days is increasing at the global scale (Seneviratne et al., 2012). The increasing of extreme climate events occurs in Japan such as increasing of heavy rain and drought (Ministry of the environment, 2018). The studies about climate change affect ecosystems are more focused on general condition (Bonan et al., 1992; Cao and Woodward, 1998; Porporato et al., 2004; Linder et al., 2010). However, increasing research notices impact of extreme climate on terrestrial ecosystem such as organisms, species and communities (Gutshick and BassiriRad, 2003; Jentsch et al., 2007; Smith, 2011). Forest cover 30% of the land surface and support 80% of the terrestrial biodiversity, and perform essential ecosystem function and service such as carbon sequestration (Boisvenue and Runnings, 2006; Bonan, 2008). It is important to understand the impact from extreme climate event on forest. Evidence showed that extreme drought changes the dominance between species by affecting the recovery phase and initial drought impact, and it may persist if extreme events become more frequent (Cavin et al., 2013). Other studies showed that because of several unusually large amount of precipitation events during 2005-2007 in eastern Siberia, the forests were damaged; needles turned brown, and trees were waterlogged (Iwasaki et al., 2010; Ito et al., 2012; Iijima et al., 2013), and these damage affected the trees in years due to decreased transpiration (Iijima et al., 2013). Water stable isotopes ($\delta^{18}\text{O}$ and δD) are used to trace the water movement for plant during

transpiration (Ehleringer and Dawson, 1992; Allen et al., 2019). The isotope ratio in plant water is highly influenced by the ratio of soil water where plant take up because no fractionation occurs during water uptake by roots (Ehleringer et al., 1992; Lin et al., 1993); also influenced by the water exchange between xylem and phloem tissues (Cernusak et al., 2005), this is caused by evaporative enrichment in leaf due to transpiration (Yakir, 1992). All these processes occur due to transpiration, if the extreme climate influence transpiration, the isotope ratio in plant water may also change. Japanese cedar (*Cryptomeria japonica*) is the most common and popular silvicultural species, distributed in half of the managed forest in Japan approximately (Japan FAO Association, 1997) and covered an area of about 4.5 million hectares (Sasse, 1998). Thus, it is important to understand how extreme climate event effect Japanese cedar. The objective of this research is to determine the affect extreme climate events on Japanese cedar by using water stable isotopes in precipitation water, soil water and xylem water.

3.2. Methodology

3.2.1. Study site and meteorological data

The forest stands are located within the premises of the Yamagata University Research Forest (YURF), which is in the Asahi Mountains, 26 km south of Tsuruoka city in Yamagata Prefecture, The annual precipitation in this area is 3,204 mm of which 42% falls as snow, the annual mean air temperature is 10.14°C for the period 2012-2018 (Fig. 2). The mean summer precipitation (June, July and August) is 651.21 mm and mean air temperature is 20.9°C. Monthly precipitation is relatively low from April to September, except for July when the highest values for the growing season are observed as the rainy season sets in for this region. The highest precipitation occurs from October to March, mainly in the form of snow, with the snow melting starting from mid-February and ending by late April or the beginning of May. Average air

temperatures are below zero only in January and February, while the highest temperature corresponds to July and August during summer. Air temperature increases from May through June, and stays approximately stable during July and August.



Fig.1. Map of YURF. The circles represent the xylem and soil sampling sites within the forest, the triangle represent the precipitation sampling spot.

3.2.2. Sampling design and measurements

Samples were collected once for each one or two weeks from April to October (2018), 14 cedar trees were randomly sampled in forest for xylem water. The xylem was collected by peeling the bark and phloem from twigs of about 1-cm of diameter taken from the lower part of the crown. In total, 250 samples were collected. The twigs were sampled using telescopic loppers at noon (solar time) approximately. From 07:00 to 09:00 h solar time, soil samples were collected from 0-20 cm, 20-40 cm and 40-60 cm depth using a straight tube probe thoroughly cleaned between consecutive samplings from three soil pits evenly distributed within the area covered by the sampled trees at each stand. All twig and soil samples were collected by screw-top test tube (NW-18) and frozen immediately after sampling to prevent evaporation. Finally,

at the end of the growing season tree cores from all trees were collected using a 10-mm diameter increment borer. The corresponding tree rings of 2018 were sliced in monthly sections using an automatic microtome for successive increments based on growth measurements obtained from dendrometers (Manual Dendrometer D1, METER Group, Inc, USA) installed in five trees for each species. For the period April-October, precipitation water for isotope analysis was collected immediately after every rain event from 3 plastic bottles, where a 7 cm diameter funnel was inserted, evenly set in open spaces close to the forest sites. To prevent evaporation, the water was stored in airtight flasks and kept frozen until measurements were conducted.

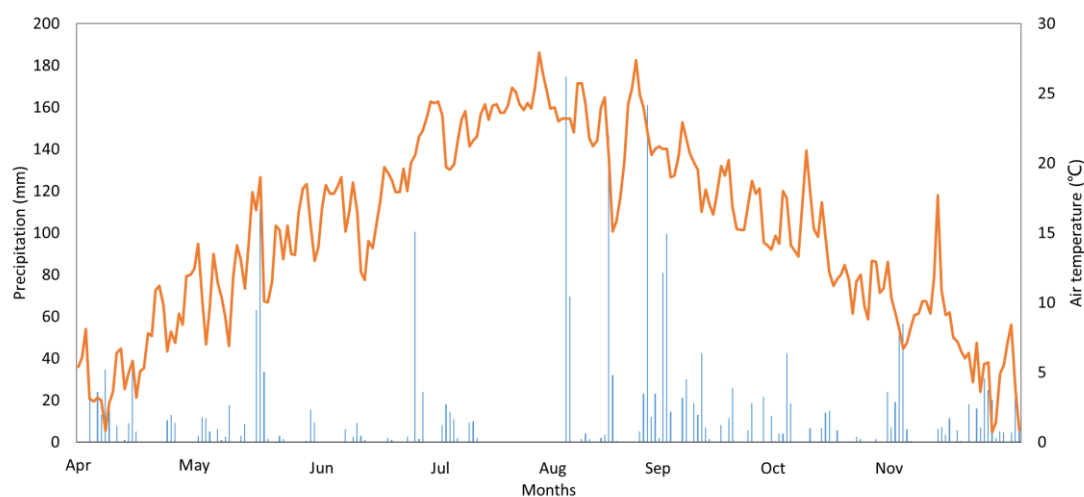
Xylem and soil water were extracted using a cryogenic vacuum distillation system (Martín-Gómez et al., 2015) and, together with precipitation water, were analyzed for $\delta^{18}\text{O}$ (oxygen isotopic composition) and δD (hydrogen isotopic composition) by isotope ratio infrared spectroscopy (IRIS) using a Triple-Liquid Water Isotope Analyzer (912-0050, Los Gatos Research, Inc, Canada) coupled to an A0211 high-precision vaporizer (Picarro Inc., Sunnyvale, CA, USA). The estimated precision, based on the repeated analysis of four reference water samples, was 0.10‰ for $\delta^{18}\text{O}$ and 0.40‰ for δD . Residual organic contaminants in the distilled water can interfere with the analysis of plant and soil samples conducted with IRIS (Martín-Gómez et al., 2015). The presence of contaminants was checked using Picarro's CHEMCORRECT post-processing software and corrected (in some few cases) following Martín-Gómez et al., 2015.

3.3. Results

3.3.1. Climatic condition in 2018

The year 2018 was a very wet year in general, the annual precipitation was 126% of the

average (3090, calculated from 2012-2017), however, during the summer time, the precipitation was 24% of the average in July (308 mm) and 492% of the average in August (177.1 mm), which made July extreme dry and August extreme wet. During summer 2018, there were two extreme dry period: June 2nd to 26th and July 7th to 31th and 4 extreme high precipitation events: June 27th to 29th (126 mm), August 5th-6th (244 mm), 16th-17th (178 mm) and 25th-31th (401 mm).



Figures.2. Daily climate condition in 2018

3.3.2. Isotopic variation in precipitation water, soil water and xylem water

Precipitation water $\delta^{18}\text{O}$ and δD values at YURF from May to October 2018 shown in figure.3. isotopic composition of precipitation was variable in time, the range is from -14.13‰ for $\delta^{18}\text{O}$ and -100.22‰ for δD in beginning of August to. From May to July, the precipitation water stable isotope ratio showed a decreasing trend, the lowest value showed in beginning of August (-14.3‰, -100.22‰), and from July to October the precipitation water stable ratio showed an increasing trend.

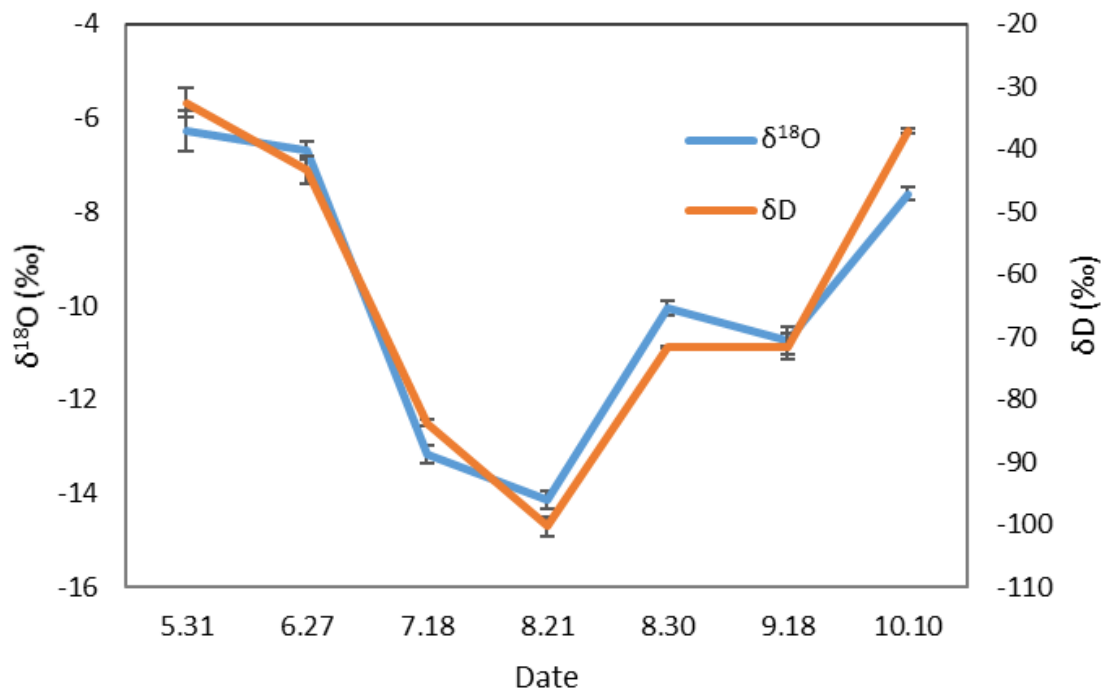


Fig.3. Precipitation $\delta^{18}\text{O}$ ($\pm\text{SE}$) and δD ($\pm\text{SE}$) variation during study period

d-excess ($=\delta\text{D} - 8 \times \delta^{18}\text{O}$) of precipitation showed monthly variation, the range of d-excess is from 8.81‰ to 23.86‰, during end of May to end of June and mid-July to end of August the d-excess decreased, from end of June to mid-July and end of August to beginning of October the d-excess increased.

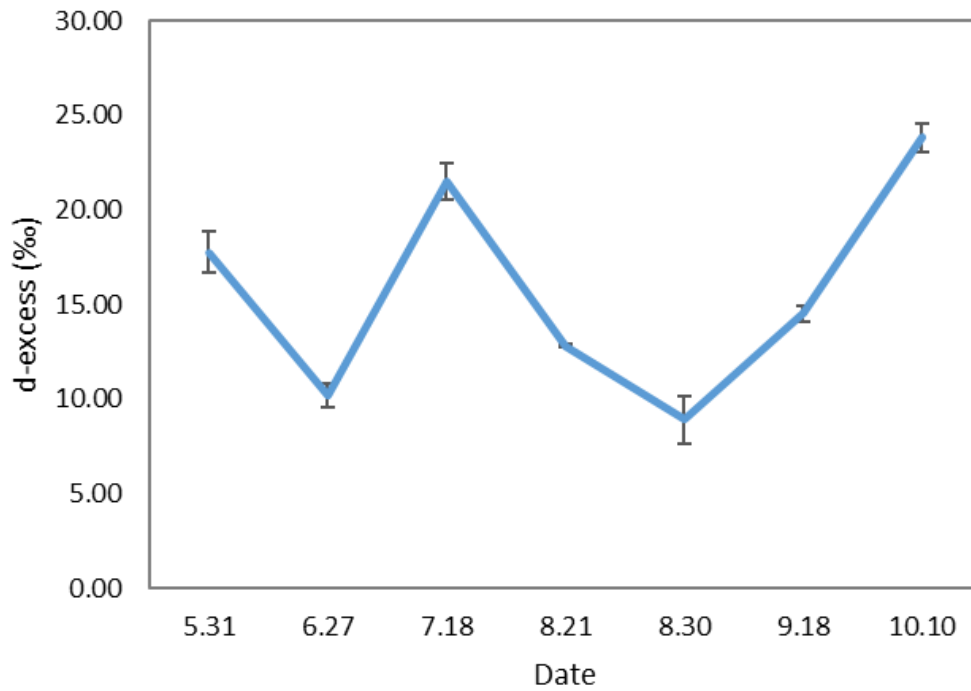


Fig.4. Precipitation d-excess (\pm SE) during study period

Over all the range of soil water isotopic composition in different depth was -5.35‰ to -15.98‰ for $\delta^{18}\text{O}$ and -31.32‰ to -99.84‰ for δD in all depth, and there were no significant different between depth ($P=0.94$), and all depth showed similar trend during the hole study period. From mid-April to the end of May, the soil water isotopic composition showed increase trend, and keep stable from beginning of June to beginning of July, from mid-July to the end of July, the soil water isotopic composition decreased 8‰ for $\delta^{18}\text{O}$ and 50‰ for δD approximately and varied from August to October.

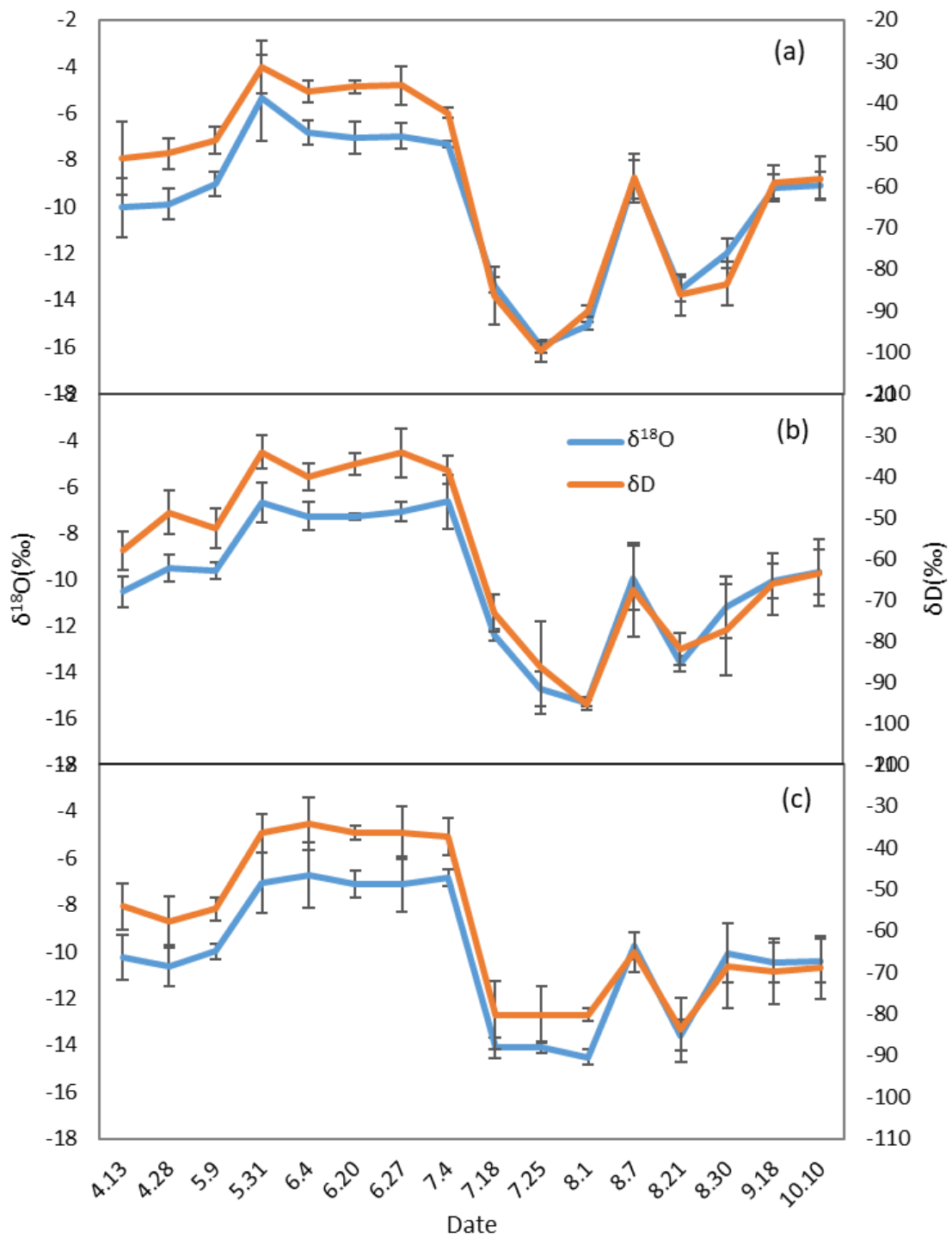


Fig.5. Soil water $\delta^{18}\text{O}$ ($\pm\text{SE}$) and δD ($\pm\text{SE}$) in (a) 0-20 cm, (b) 20-40 cm and (c) 40-60 cm depth

The range of xylem water isotopic composition was -5.52‰ to -11.02‰ for $\delta^{18}\text{O}$ and -3.54‰ to -74.15‰ for δD . From mid-April to mid-May, the xylem water isotopic composition increased about 5 ‰ for $\delta^{18}\text{O}$ and 30‰ for δD approximately, and stay stable from mid-May to mid-August, and decreased from -6.52‰ to -8.82‰ for $\delta^{18}\text{O}$ and -46.37‰ to -64.37‰ for

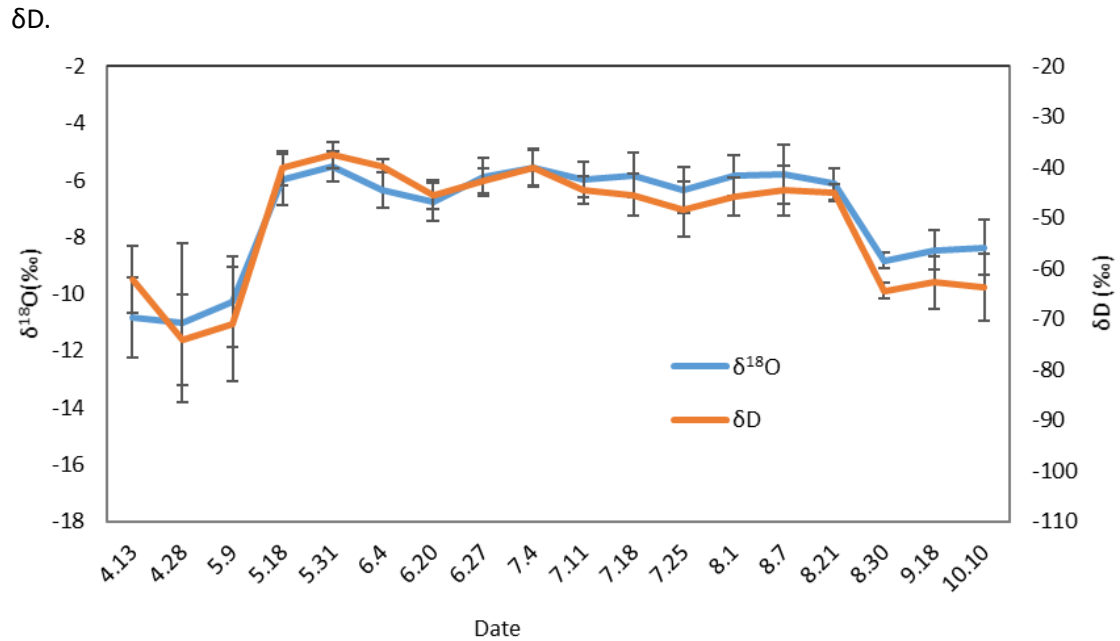


Fig.6. Monthly change of xylem water $\delta^{18}\text{O}$ ($\pm\text{SE}$) and δD ($\pm\text{SE}$) during study period

Soil water, xylem water and precipitation water isotope records are represented together with the LMWL (Local Meteoric Water Line) in Fig. 7, in general, soil water isotope ratio showed no significant difference between depth ($P=0.93$ for $\delta^{18}\text{O}$, $P=0.73$ for δD).

In April, the range of xylem water isotope is 5.57‰ for $\delta^{18}\text{O}$ (-8.47 to -14.04) and 28.12‰ for δD (-53.60‰ to -81.73‰), there was no significant difference in $\delta^{18}\text{O}$ ($p=0.79$) but significant difference in δD ($p<0.01$) between sampling events. The range of soil water isotope ratio is 2.46‰ for $\delta^{18}\text{O}$ (-8.97‰ to -11.43‰) and 17.61‰ for δD (-45.01‰ to -62.62‰), no significant difference was found in both $\delta^{18}\text{O}$ ($p=0.35$) and δD ($p=0.40$) between sampling events.

In May, the range of xylem water was 8.83‰ for $\delta^{18}\text{O}$ (-4.38‰ to -13.21‰) and 58.27‰ for δD (-34.15‰ to -92.42‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between the first sampling events and last 2 sampling events. The range of soil water was 6.16‰ for $\delta^{18}\text{O}$ (-4.06‰ to -10.22‰) and 29.82‰ for δD (-26.88‰ to -56.70‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between sampling events.

In June, the range of xylem water was 3.94‰ for $\delta^{18}\text{O}$ (-4.18‰ to -8.12‰) and 13.52‰ for δD

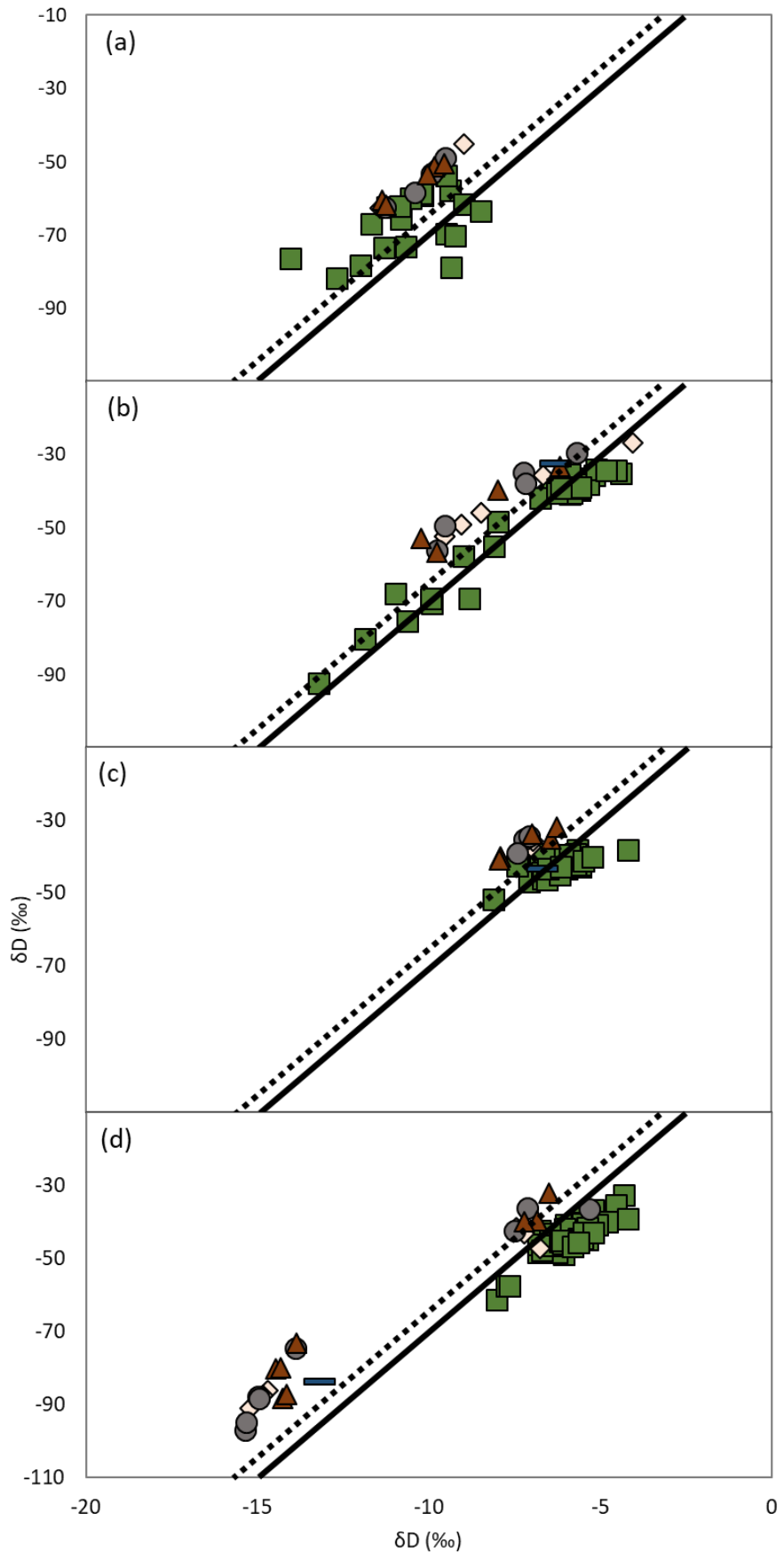
(-38.05‰ to -51.57‰), no significant difference was found in both $\delta^{18}\text{O}$ ($p=0.58$) and δD ($p<0.28$) between the third sampling events and the first 2 sampling events. The range of soil water was 1.67‰ for $\delta^{18}\text{O}$ (-6.27‰ to -7.94‰) and 8.82‰ for δD (-32.02‰ to -40.84‰), no significant difference was found in both $\delta^{18}\text{O}$ ($p=0.23$) and δD ($p=0.72$) between sampling events.

In July, the range of xylem water was 3.83‰ for $\delta^{18}\text{O}$ (-4.20‰ to -8.03‰) and 28.63‰ for δD (-32.86‰ to -61.49‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between the third sampling events and the first 2 sampling events. The range of soil water was 13.26‰ for $\delta^{18}\text{O}$ (-5.33‰ to -18.59‰) and 93.86‰ for δD (-32.11‰ to -125.97‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between first sampling events and last two sampling events.

In August, the range of xylem water was 5.03‰ for $\delta^{18}\text{O}$ (-4.03‰ to -9.06‰) and 29.23‰ for δD (-37.26‰ to -66.49‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between the fourth sampling events and the first three sampling events. The range of soil water was 8.64‰ for $\delta^{18}\text{O}$ (-6.73‰ to -15.37‰) and 55.28‰ for δD (-40.03‰ to -95.31‰), significant difference was found in both $\delta^{18}\text{O}$ ($p<0.01$) and δD ($p<0.01$) between each sampling event.

In September, the range of xylem water was 2.43‰ for $\delta^{18}\text{O}$ (-7.31‰ to -9.74‰) and 18.58‰ for δD (-52.28‰ to -70.86‰). The range of soil water was 2.74‰ for $\delta^{18}\text{O}$ (-8.70‰ to -11.44‰) and 23.53‰ for δD (-54.90‰ to -78.43‰).

In September, the range of xylem water was 3.52‰ for $\delta^{18}\text{O}$ (-6.40‰ to -9.92‰) and 22.52‰ for δD (-50.39‰ to -72.91‰). The range of soil water was 2.82‰ for $\delta^{18}\text{O}$ (-8.42‰ to -11.24‰) and 23.16‰ for δD (-52.71‰ to -75.87‰).



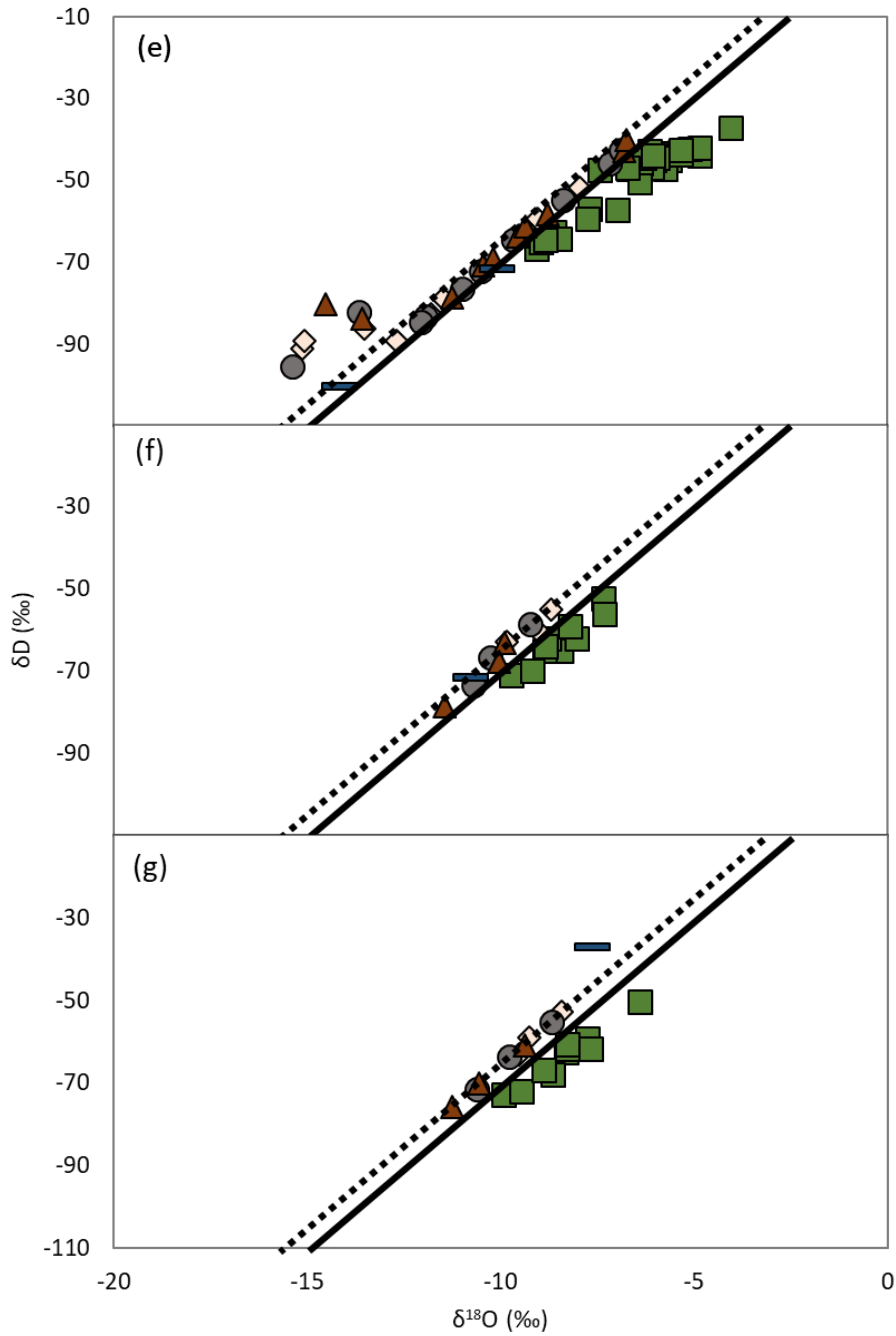


Fig.7. Relationship between δD and $\delta^{18}O$ in precipitation water (blue bar, $n=3$), xylem water (green square, $n=14$) and soil water from 0-20 cm (yellow diamond, $n=9$), 20-40 cm (grey circle, $n=9$) and 40-60 cm (brown triangle, $n=9$) in (a) April, (b) May, (c) June, (d) July, (e) August, (f) September, (g) October. Dashed line represents the Local Meteoric Water Line (LMWL, $\delta D=8 \times \delta^{18}O+15$)

3.4. Discussion

Precipitation water isotopic composition varied in different month, this also showed in d-excess, previous research showed that d-excess of precipitation is considered to be a useful tool for estimating the origin of water vapor in air masses (sea areas / regions where water vapor that causes precipitation evaporates)([Kondoh and Shimada,1997](#); [Lee et al., 2003](#); [Yamanaka et al., 2002](#)). In Japan, the source of precipitation is mainly from 3 regions: the Japan sea, the east China sea and the Pacific Ocean ([Tanoue and Ichiyanagi, 2016](#)). Based the air mass map from NOAA (National Oceanic and Atmospheric Administration, fig.7) we found, the origin of the water that we sampled from different rain event are mainly from these three regions: the precipitation from end of May is from the Pacific Ocean, from June to August were from east China sea and from September to October are from Japan sea. Soil water isotopic composition changed together with precipitation water isotopic composition; this is what we did not find in previous research. For previous research we took soil samples from different depth of soil and extract the water from soil by using cryogenic vacuum distillation system. In this research we set suction cup to take water sample from different depth of soil. Based on the “two water worlds” hypothesis ([Brooks et al., 2010](#); [Goldsmith et al, 2012](#); [McDonnell, 2014](#); [Berry et al., 2017](#)), there are two types of water: tightly bounded water is used by trees and not enter the stream; mobile water related to infiltration, ground water recharge, hillslope runoff and streamflow that possessed a character unrelated to the water taken up by trees. This ecohydrological separation between mobile and nonmobile water is widespread across different biomes ([Evaristo et al., 2015](#)). The previous research we used cryogenic vacuum extraction which extracted both mobile water and nonmobile water, for this research, we used suction cup which extracted mainly mobile water.

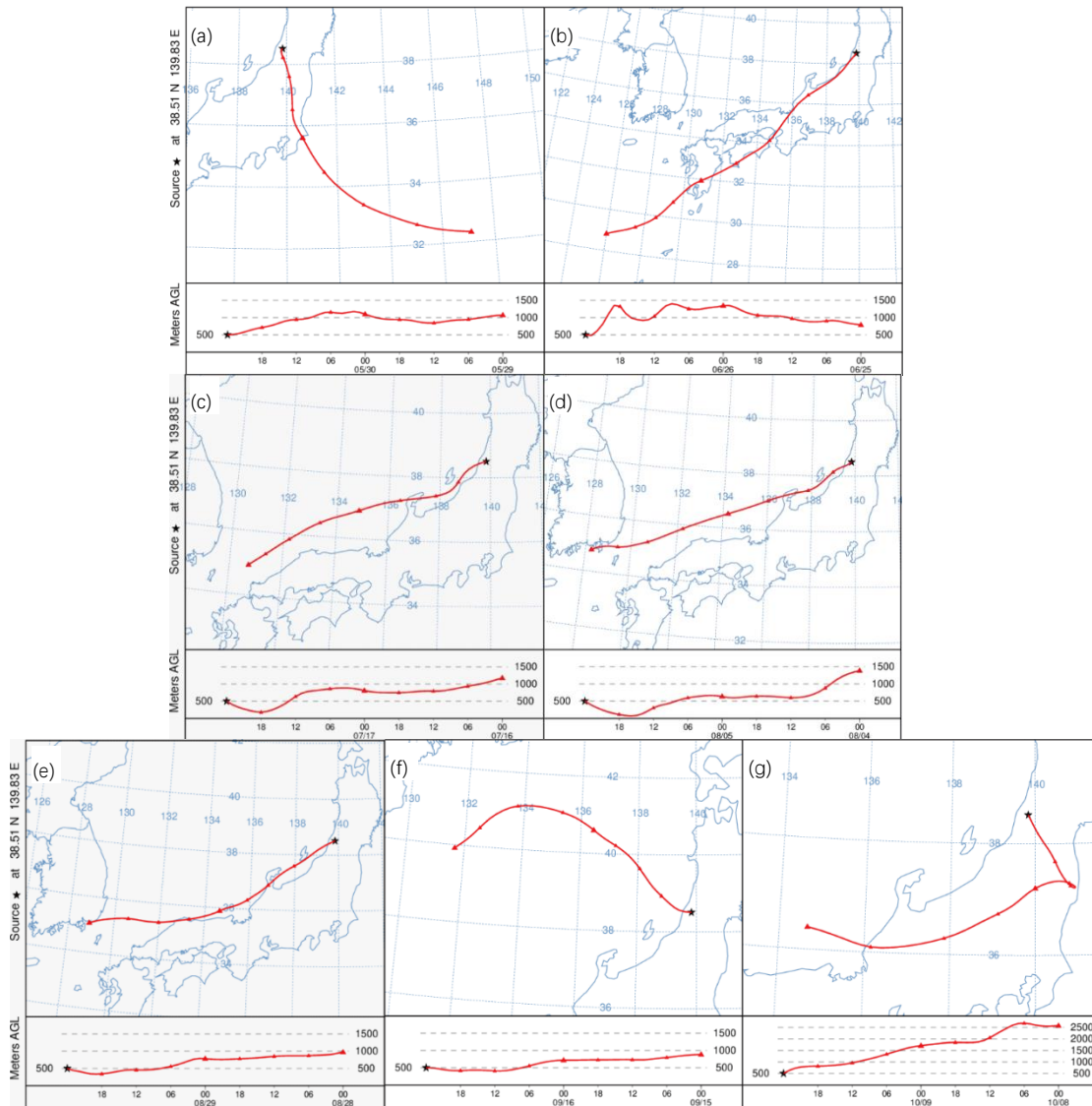


Fig.8. The air mass movement from (a) May 31st, (b) June 27th, (c) July 18th, (d) August 6th, (e) August 30th, (f) September 17th, (g) October 18th

In April and May we have found high variation in xylem water isotope ratio (fig.7. a, b) in different sampling event. Xylem water for April to mid-May showed similar isotopic composition, and more enriched than end of May. In Shonai area, snow start melting from end of March and finish at beginning of May, cedar trees start transpiration from mid-March before snow start melting (Osonoi et al., 2017). From April to mid-May, Cedar may mainly use the snowmelt water for transpiration. In the end of May, the cedar trees are mainly use precipitation water for transpiration. The variation of soil water isotope ratio is also caused by snow melt and precipitation. In June, the xylem water, soil water and precipitation water

isotope ratio were in the same range during the whole month, also closed to end of May, which means during this period, the soil water that cedar trees are using is coming from recent precipitation. In July, the xylem water isotope ratio was still in the same level with May and June, but the soil water isotope ratio showed high variability between sampling events, soil water from beginning of July is isotopically more enriched than from mid and end of July, and precipitation water isotope ratio is similar with the soil water from mid to end July. This may be caused by the water stress during this period. The precipitation in July 2018 was only 24% of the average precipitation in July, so there was a water stress during this period. Trees will decrease transpiration under water stress through closing stomata (Hsiao, 1973; Maruyama and Toyama, 1987). And during this period, the main water source for tree water use is probably storage water in trees. Several studies showed that tree storage water can provide a significant part of the total diurnal even seasonal water use (Čermák et al., 1982, 1984, 2007; Borchert et al., 1994; Kumagai et al., 2009). During July the $\delta^{18}\text{O}$ and δD of storage water is probably different with soil water, the former is closer to the soil water $\delta^{18}\text{O}$ and δD in June. In August the $\delta^{18}\text{O}$ and δD of xylem water is still in the same range with that in July. The precipitation in August 2018 was 842 mm, trees were waterlogged during this period, Iijima et al., 2013 found that waterlog can cause the decreasing of transpiration and reduction of stomatal conductance, and thus resulting a similar change in xylem water $\delta^{18}\text{O}$ and δD with July.

3.5. Conclusion

$\delta^{18}\text{O}$ and δD in xylem water showed similar trend with soil water and precipitation water in most of time, but in July and August was more enriched than soil water and precipitation. This is mainly caused by the extreme climate condition. In the year 2018, cedar showed similar water

use strategy to response the different extreme climate events but for different propose. In July cedar decrease transpiration, reduce stomatal conductance and use more storage water in trunk to against drought. In August, cedar decrease transpiration and reduce stomatal conductance to against waterlog.

References

- Allen ST, Kirchner JW, Braun S, Siegwolf RTW, Goldsmith GR. (2019). Seasonal origins of soil water used by trees. *Hydrology and Earth System Sciences*, 23. 1199-1210
- Berry ZC, Evaristo J, Moore G, Poca M, Steppe K, Verrot L, Asbjornsen H, Borma LS, Bretfeld M, Hervé-Fernández P, Seyfried M, Schwendenmann L, Sinacore K, De Wispelaere L, McDonnell J. (2017). The two water worlds hypothesis: Addressing multiple working hypotheses and proposing a way forward. *ecohydrology*, 11
- Bonan G, Pollard D, Thompson S. (1992). Effects of boreal forest vegetation on global climate. *Nature*, 359. 716–718
- Borchert R. (1994). Soil and stem water storage determine phenology and distribution of tropical dry forest trees. *Ecology*, 75. 1437-1449
- Brooks R, Barnard R, Coulombe R, McDonnell JJ. (2010). Ecohydrologic separation of water between trees and streams in a Mediterranean climate. *Nat Geosci*, 2010, 3, 100–104
- Cao M, Woodward F. (1998). Dynamic responses of terrestrial ecosystem carbon cycling to global climate change. *Nature*, 393. 249–252
- Cavin L, Mountford EP, Peterken GF, Jump AS. (2013). Extreme drought alters competitive dominance within and between tree species in a mixed forest stand. *Functional Ecology*, 27. 1424-1435
- Čermák J, Úlehal J, Kučera J, Penka M. (1982). Sapflow rate and transpiration dynamics in the full-grown oak (*Quercus robur L.*) in flood plain forest exposed to seasonal floods as related

- to potential evapotranspiration and tree dimension. *Biol. Plant*, 24. 446-460
- Čermák J, Jeník J, Kučera J, Žídek V. (1984). Xylem water flow in a crack willow tree (*Salix fragilis* L.) in relation to diurnal changes of environment. *Oecologia*, 64. 145-151
- Čermák J, Kučera J, Bauerle WL, Phillips N, Hinckley TM. (2007). Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth douglas-fir trees. *Tree Physiology*, 27. 181-198
- Cernusak LA, Farquhar GD, Pate JS. (2005). Environmental and physiological controls over oxygen and carbon isotope composition of Tasmanian blue gum, *Eucalyptus globulus*. *Tree Physiol*, 25. 129–146
- Della-Marta PM, Haylock MR, Luterbacher J, Wanner H. (2007). Doubled length of western Europe summer heat waves since 1880. *Journal of Geophysical Research-Atmospheres*, 112.D15103
- Ehleringer JR, Dawson TE. (1992). Water uptake by plants: perspectives from stable isotope composition. *Plant, Cell and Environment*, 15. 1073-1082
- Evaristo J, Jasechko S, McDonnell JJ. (2015). Global separation of plant transpiration from groundwater and streamflow. *Nature*, 525. 91–94.
- Goldsmith, G.R., Muñoz-Villers, L.E., Holwerda, F., McDonnell, J.J., Asbjornsen, H., Dawson T.E. (2012) Stable isotopes reveal linkages among ecohydrological processes in a season-ally dry tropical montane cloud forest. *Ecohydrol*, 5, 779-790.
- Gutschick VP, BassiriRad H. (2003). Extreme events as shaping physiology, ecology, and evolution of plants: toward a unified definition and evaluation of their consequences. *New Phytologist*, 160. 21–42.
- Hsiao T C. (1973). Plant responses to water stress. *Annu. Rev. Plant Physiol*, 24. 519-570.
- Ichikawa A, Ed. (2004). Global Warming – The Research Challenges: A Report of Japan’s Global Warming Initiative. *Springer*, 160 pp.

- Iijima Y, Ohta T, Kotani A, Fedorov AN, Kodama Y, Maximov TC. (2013). Sap flow changes in relation to permafrost degradation under increasing precipitation in an eastern Siberian larch forest. *Ecohydrology*, 7. 177–187
- Ito S, Ohta T, Kotani A, Maximov TC, Kononov AV. (2012). Interannual variation of the carbon dioxide flux in an eastern Siberian larch forest. Proceedings of 1st International Conference on “Global Warming and the Human-Nature Dimension in Siberia: Social Adaptation to the Changes of the Terrestrial Ecosystem, with an Emphasis on Water Environments”, 7-9 March 2012, Research Institute for Humanity and Nature, Kyoto, Japan, 140-143.
- Iwasaki H, Saito H, Kuwao K, Maximov T C, Hasegawa S. (2010). Forest decline caused by high soil water conditions in a permafrost region. *Hydrol. Earth Syst. Sc.*, 14. 301–307
- IPCC, 2013: Climate Change 2013: *The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp
- Japan FAO Association, 1997. Forests and Forestry in Japan, second ed. *Japan FAO Association, Tokyo, Japan*. 64pp
- Jentsch A, Kreyling J, Beierkuhnlein C. (2007). A new generation of climate-change experiments: events, not trends. *Frontiers in Ecology and the Environment*, 5. 365–374
- Kondoh A. and Shimada J. (1997). The origin of precipitation in eastern Asia by deuterium excess. *水文・水資源学会誌*, 10, 6. 627–629
- Kumagai T, Aoki S, Otsuki K, Utsumi Y. (2009). Impact of stem water storage on diurnal estimates of whole-tree transpiration and canopy conductance from sap flow measurements in Japanese cedar and Japanese cypress trees. *Hydrological processes*, 23. 2335-2344

- Lee K, Grundstein AJ., Wenner, DB, Choi M, Woo N and Lee D. (2003). Climatic controls on the stable isotopic composition of precipitation in Northeast Asia. *Climate Research*, 23. 137–148
- Lindner M, Marosched M, Netherer S, Kremer A, Barbati A, Gracia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, Lexer MJ, Marchetti M. (2010). Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management*, 259. 698-709
- Maruyama K, Toyama Y. (1987). Effect of water stress on photosynthesis and transpiration in three tall deciduous trees. *J. Jpn. For. Soc*, 69(5)'87. 165-170
- McDonnell J. (2014). The two water worlds hypothesis: ecohydrological separation of water between streams and trees? *WIREs: Water*, 2014, 323-329
- Osonoi T. (2018). Evaluation of snow melt water used by Japanese cedar at the start of the growing season in Shonai region. Master thesis, Yamagata University.
- Porporato A, Daly E, Rodriguez-Iturbe I. (2004). Soil water balance and ecosystem response to climate change. *Am. Nat*, 164. 625–632
- Sasse J. (1998). The Forests of Japan. *Japan Forest Technical Association, Tokyo*
- Schar C, Vidale PL, Luthi D, Frei C, Haberli C, Liniger MA, Appenzeller C. (2004). The role of increasing temperature variability in european summer heatwaves. *Nature*, 427. 332-336
- Seneviratne SI, Nicholls N, Easterling D, Goodess CM, Kanae S, Kossin J, Luo Y, Marengo J, McInnes K, Rahimi M, Reichstein M, Sorteberg A, Vera C, Zhang X. (2012). Changes in climate extremes and their impacts on the natural physical environment. In: *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation* [Field, C.B., Barros V, Stocker TF, Qin D, Dokken DJ, Ebi KL, Mastrandrea MD, Mach KJ, Plattner G-K, Allen SK, Tignor M, Midgley PM (eds.)]. *A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge University Press, Cambridge,

UK, and New York, NY, USA, pp. 109-230.

Smith MD. (2011). An ecological perspective on extreme climatic events: a synthetic definition and framework to guide future research. *Journal of Ecology*, 99. 656–663.

Tanoue M and Ichiyonagi K. (2016). Deuterium excess in precipitation and water vapor origins over Japan: A review. *日本水文科学会誌*, 46, 2. 101-115

YAKIR D. (1992). Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant, Cell and Environment*, 15. 1005-1020

Yamanaka T, Shimada J and Miyaoka K. (2002). Footprint analysis using event-based isotope data for identifying source area of precipitated water. *Journal of Geophysical Research*, 107(D22). 4624

Yoshida K, Sugi M, Mizuta R, Murakami H, Ishii M. (2017). Future Changes in Tropical Cyclone Activity in High-Resolution Large-Ensemble Simulations. *Geophysical Research Letters*, 44. 9910-9917.

Chapter 4

Monthly variability of cellulose stable isotopes in three tree species

4.1. Introduction

During the formation of cellulose in the tree ring, sugars in the phloem can exchange up to 50% of their oxygen atoms with xylem water, adding an extra source of variability (and uncertainty) to the interpretation of tree-ring oxygen isotopic composition, $\delta^{18}\text{O}$ (Rodén et al., 2000; Gessler et al., 2014). Thus, tree-ring $\delta^{18}\text{O}$ partly represents the isotopic signature of source water, and partly the isotopic enrichment of leaf water, in variable proportions depending on the relevance of each process on the water economy of the tree (Labuhn et al., 2014). Additionally, the carbon isotopic composition ($\delta^{13}\text{C}$) of tree rings can be used to estimate intrinsic water-use efficiency (WUEi), or the ratio of net photosynthesis to stomatal conductance of water (Farquhar et al., 1982; Eleringer and Vogel, 1993; Leavitt et al., 2003), which provides information on the water-carbon balance of the plant at the leaf level. Stable isotopes in tree-ring cellulose changes by the environment condition of the year they formed, thus, it is widely used for climate and environment studies (Lipp et al., 1991; Rodén et al., 2000; Kagawa et al., 2003; McCarroll and Loader, 2004; de Boer et al., 2019). Lopez et al., 2018 using stable isotopes in tree ring to determine the damage of 2011 Mega-Tsunami in eastern Japan, the $\delta^{13}\text{C}$ ratio increased significantly after because of the tsunami in 2011 and 2012, but from 2013, the $\delta^{13}\text{C}$ ratio decreased, suggesting that trees started recover from the tsunami. Some studies focus on the seasonal change of tree ring stable isotopes (Leavitt and Long, 1991; Leavitt and Wright, 2002; Helle et al., 2002; Kagawa et al., 2003; Skomarkova et al., 2006; Tei et al., 2013). Helle et al., 2012, analyzed tree-ring $\delta^{13}\text{C}$ from north Ireland, and they found a significant variation between earlywood and late wood: the highest $\delta^{13}\text{C}$ value showed in summer which is the period to formed earlywood, and the lowest $\delta^{13}\text{C}$ value showed in the end of the late wood and the beginning of the earlywood, this may cause the water stress in the mid of growing season. Kagawa et al., 2003 found a negative correlation between tree-ring

index and tree ring late wood $\delta^{13}\text{C}$ at the dry site mainly dominated by pine trees in eastern Siberia, and this negative correlation is probably can explain by the drought condition. The aim of this study is 1) to evaluate intrinsic water-use efficiency of each tree species by means of $\delta^{13}\text{C}$. And 2) to evaluate the correlation between seasonal climate condition and seasonal cedar tree-ring $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$.

4.2. Methodology

4.2.1. Research sites

See chapter 2 and 3 for study site description.

4.2.2. Sampling design and measurements

Samples were collected in the end of August 2017 and end of September 2018. In 2017, 10 cores from Cedar and beech, 8 cores from larch were collected at each site using a 10-mm diameter increment borer. In 2018, 10 cores from cedar we collected. The corresponding tree rings of 2017 and 2018 were sliced in monthly sections using an automatic microtome for successive increments based on growth measurements obtained from dendrometers (Manual Dendrometer D1, METER Group, Inc, USA) installed in five trees for each species. Wood slices were cut in fine pieces and purified to α -cellulose (Ferrio and Voltas, 2005). For carbon and oxygen isotope analysis, 0.30–0.40 mg of dry α -cellulose was weighed into tin foil capsules and combusted using an elemental analyzer (EA1110, Thermo Quest, Italy) interfaced with a Delta PLUSXL gas isotope ratio mass spectrometer (Thermo Finnigan, Bremen, Germany). Isotope ratios were expressed as per mil deviations using the δ notation relative to Vienna Pee Dee Belemnite (for carbon) and Vienna Standard Mean Ocean Water (for oxygen) standards. The accuracy of the analyses (SD of working standards) was 0.06‰ ($\delta^{13}\text{C}$) and 0.25‰ ($\delta^{18}\text{O}$).

4.3. Results

4.3.1. Monthly tree-ring α -cellulose $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

Wood α -cellulose $\delta^{18}\text{O}$ (hereafter, wood $\delta^{18}\text{O}$) showed a decreasing trend along the summer months in 2017 for the three species (Fig. 2a). The range of cedar tree ring isotopes was 2.96‰ (27.84‰ to 30.08‰) for $\delta^{18}\text{O}$ and 1.32‰ (-23.87‰ to -25.19‰) for $\delta^{13}\text{C}$. The range of larch tree ring isotopes was 3.17‰ (24.00‰ to 27.17‰) for $\delta^{18}\text{O}$ and 2.67‰ (-24.27‰ to -26.94‰) for $\delta^{13}\text{C}$. The range of beech tree ring isotopes was 2.53‰ (23.84‰ to 26.37‰) for $\delta^{18}\text{O}$ and 2.95‰ (-25.16‰ to -28.11‰) for $\delta^{13}\text{C}$. In general, significant difference was found in both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ between species ($p < 0.01$). The highest $\delta^{18}\text{O}$ values were found in June which were significantly higher than wood $\delta^{18}\text{O}$ in July and August, however this was only found in cedar ($p < 0.01$). On the other hand, no significant temporal variation of wood $\delta^{18}\text{O}$ was found for larch and beech. There were no significant seasonal differences in wood $\delta^{13}\text{C}$ (Fig. 2b). However, species-specific differences were found for both wood $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$, with the highest values recorded in cedar, followed by larch and beech ($p < 0.001$).

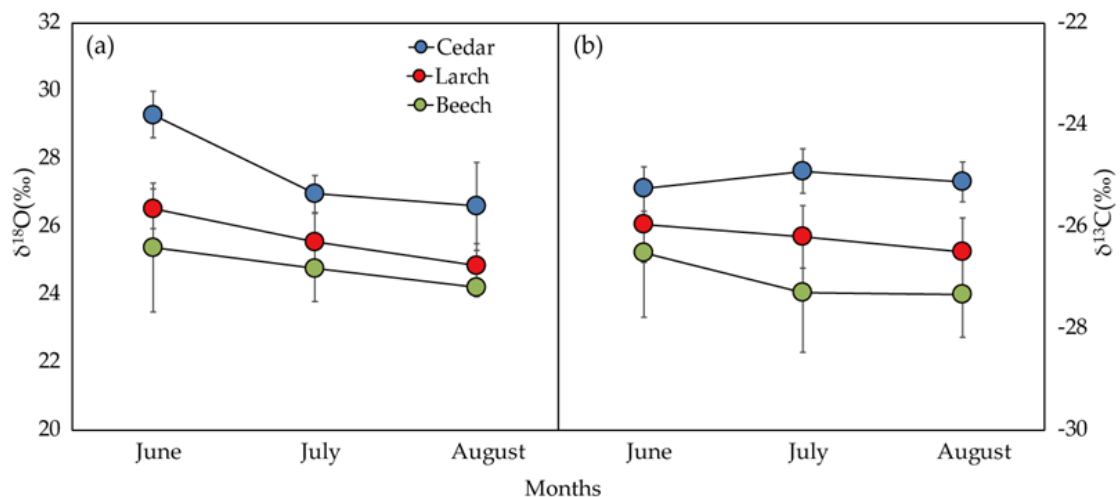


Figure 1. Monthly tree ring (a) $\delta^{18}\text{O}$ and (b) $\delta^{13}\text{C}$ in cedar (blue circle), larch (red circle) and beech (green circle) in 2017; values are mean \pm SE, cedar ($n=10$), larch ($n=8$), beech ($n=10$)

Cedar tree-ring α -cellulose stable isotope ratio for cedar in 2018 showed decreasing trend like

2017, the $\delta^{18}\text{O}$ is isotopically enriched than the same period of 2017. The range of cedar tree ring isotopes was 8.55‰ (25.68‰ to 34.23‰) for $\delta^{18}\text{O}$ and 1.99‰ (-24.71‰ to -26.70‰) for $\delta^{13}\text{C}$. significant difference was found in $\delta^{18}\text{O}$ between September and first three months ($p < 0.01$).

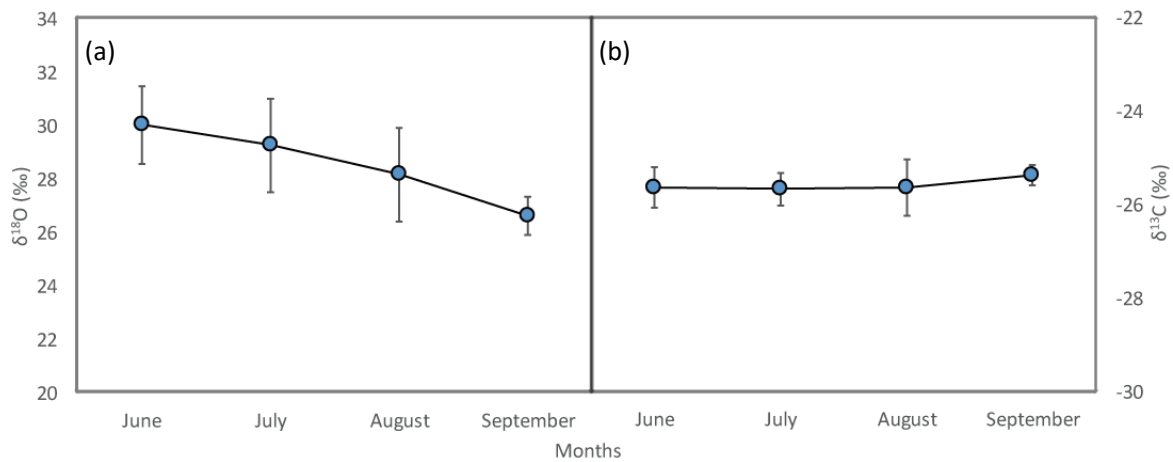


Figure. 2. Monthly cedar tree ring (a) $\delta^{18}\text{O}$ and (b) $\delta^{13}\text{C}$ (values are mean \pm SE, n=10)

4.4. Discussion

The monthly values of wood $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ were in the range of values found for other tree species in northeastern (Lopez et al., 2018) and northern (Nakatsuka et al., 2004) Japan. Wood $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ showed species-specific differences, where the highest values corresponded to cedar, followed by larch and beech. Under similar environmental conditions, species with low $\delta^{13}\text{C}$ values usually maintain a high-water potential, indicating low WUEi, whereas species with high $\delta^{13}\text{C}$ may show lower water potential and high WUEi (Gebrekirstos et al., 2011). Therefore, we assume that the difference found among $\delta^{13}\text{C}$ are related to differences in intrinsic water-use efficiency (WUEi). The higher WUEi of cedar is probably driven by enhanced stomatal regulation of water losses for this species, as suggested by higher $\delta^{18}\text{O}$ values (Scheidegger et al., 2000; Shestakova., 2017). This means that $\delta^{18}\text{O}$ may also be used as an indicator of stomatal regulation in heavy rainfall areas. Monthly wood $\delta^{18}\text{O}$ values showed opposite trends

with respect to xylem water $\delta^{18}\text{O}$ for all three species, which is apparently caused by leaf water enrichment, since strong oxygen exchange between phloem and xylem would have produced similar trends for both wood and xylem $\delta^{18}\text{O}$; in addition, the transport of exported carbohydrates from leaves to developing stems, where cellulose is formed, and the corresponding biochemical fractionation associated with cellulose synthesis could not strongly influence the wood $\delta^{18}\text{O}$ monthly trend (Roden et al., 2000). Dendrometer records showed that the maximum growth occurred in June and decreased sharply in July and August, which suggests that enhanced transpiration (related to higher photosynthetic rates) led to higher leaf water enrichment in June (i.e. higher wood $\delta^{18}\text{O}$) and a decrease in July and August. Thus, monthly wood $\delta^{18}\text{O}$ values likely reflected different degrees of leaf water enrichment caused by differences in monthly transpiration rates.

For the study in 2018, we found stronger negative correlation between tree-ring $\delta^{13}\text{C}$ and air temperature ($r=-0.83$) than precipitation ($r=-0.31$). Leavitt et al., 1991 found stronger negative correlation between tree-ring $\delta^{13}\text{C}$ and precipitation than temperature in north America. In Shonai region there is enough annual precipitation for tree growth, and the air temperature is relatively low during growing season, this condition gives a positive affect to tree growth, this also showed in tree-ring $\delta^{13}\text{C}$.

4.5. Conclusions

The differences in wood $\delta^{13}\text{C}$ pointed to differences in WUEi, with the evergreen cedar showing a consistently higher WUEi than the deciduous larch and beech. For Cedar, the air temperature showed positive affect to tree growth when precipitation is high enough.

References

de Bore HJ, Robertson I, Clisby R, Loader NJ, Gagen M, Young GHF, Wagner-Cremer F, Hipkin

- CR, McCarroll D. (2019). Tree-ring isotopes suggest atmospheric drying limits temperature-growth responses of treeline bristlecone pine. *Tree Physiology*, 39. 983-999
- Ehleringer, J.R., Vogel, J.C. (1993). Historical aspects of stable isotopes. In *Stable Isotopes and Plant Carbon-Water Relations.*, Ehleringer, J.R., Hall, A.E., Farquhar, G.D., Eds., Academic Press, New York, USA, pp. 9-18
- Farquhar, G.D., O'Leary, M.H., Berry, J.A. (1982). On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology*, 9. 121-137, 978-0-12-233380-4.
- Ferrio, J. P., Voltas, J. (2005). Carbon and oxygen isotopes ratio in wood constituents of *Pinus halepensis* and indicator of precipitation, temperature and vapor pressure deficit. *Tellus*, 57B. 164-173.
- Gebrekirstos, A., van Noordwijk, M., Neufeldt, H., Mitlöhner, R. (2011). Relationship of stable carbon isotopes, plant water potential and growth: an approach to assess water use efficiency and growth strategies of dry land agroforestry species. *Trees*, 25. 95-102.
- Gessler, A., Ferrio, J. P., Hommel, R., Treydte, K., Werner, R. A., Monson, R. K. (2014). Stable isotopes in tree rings: towards a mechanistic understanding of isotope fractionation and mixing processes from the leaves to the wood. *Tree Physiology*, 34. 796-818.
- Helle G, Schleser GH. (2003). Seasonal variations of stable carbon isotopes from tree-rings of *Quercus petraea*. In: *Tree Rings in Archaeology, Climatology and Ecology, Volume 1 Proceedings of the DENDROSYMPOSIUM 2002, April 11th – 13th 2002, Bonn/Jülich.* 66-70
- Kagawa A, Naito D, Sugimoto A, Maximov TC. (2003). Effects of spatial and temporal variability in soil moisture on widths and $\delta^{13}\text{C}$ values of eastern Siberian tree rings. *Journal of geophysical research*, 108. No. D16
- Labuhn, I., Daux, V., Pierre, M., Stivenard, M., Girardclo, O., Féron, A., Genty, D., Masson-Delmotte, V., Mestre, O. (2014). Tree age, site and climate controls on tree ring cellulose

- $\delta^{18}\text{O}$: A case study on oak trees from south-western France. *Dendrochronologia*, 32. 78-89.
- Leavitt SW, Long A. (1991). Seasonal stable-carbon isotope variability in tree rings: possible paleoenvironmental signals. *Chemical Geology (Isotope Geoscience Section)*, 87. 59-70
- Leavitt SW, Weight WE. (2002). Spatial expression of ENSO, drought, and summer monsoon inseasonal D^{13}C of ponderosa pine tree rings in southern Arizona and New Mexico. *Journal of Geophysical Research*, 107(D18). 4349
- Leavitt, S.W., Idso, S.B., Kimball, B.A., Burns, J.K., Sinha, A., Stott, L. (2003). The effect of long-term atmospheric CO_2 enrichment on the intrinsic water-use efficiency of sour orange trees. *Chemosphere*, 50. 217-222.
- Lipp J, Trimborn P, Fritz P, Moser H, Becker B, Frenzel B. (1991). Stable isotopes in tree ring cellulose and climatic change. *Tellus*, 43B. 322-330
- Lopez, C. M. L., Nakano, S., Ferrio, J. P., Hayashi, M., Nakatsuka, T., Sano, M., Yamanaka, T., Nobori, Y. (2018). Evaluation of the effect of the 2011 Tsunami on coastal forests by means of multiple isotopic analysis of tree-rings. *Isotopes Environ. Health stud*, 54. 494-507.
- McCarroll D, Loader NJ. (2004). Stable isotopes in tree rings. *Quaternary Science Reviews*, 23. 771-801
- Nakatsuka, T., Ohnishi, K., Hara, T., Sumida, A., Mitsuishi, D., Kurita, N., Uemura, S. (2004). Oxygen and carbon isotopic ratios of tree-ring cellulose in a conifer-hardwood mixed forest in northern Japan. *Geochemical Journal*, 38. 77-88.
- Roden, J. S., Lin, G. L., Ehleringer, J. R. (2000) A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta*, 64. 21-35.
- Scheidegger, Y., Saurer, M., Bahn, M., Siegwolf, R.T.W. (2000). Linking stable oxygen and carbon isotopes with stomatal conductance and photosynthetic capacity: A conceptual model. *Oecologia*, 125. 350-357.

Shestakova, T. A., Voltas, J., Saurer, M., Siegwolf, R. T. W., Kirilyanov, A, V. (2017). Warming Effects on *Pinus sylvestris* in the Cold-Dry Siberian Forest-Steppe: Positive or Negative Balance of Trade? *Forests*, 8. 490.

Skomarkova MV, Vaganov EA, Mund M, Knohl A, Linke P, Boerner A, Schulze ED. (2006). Inter-annual and seasonal variability of radial growth, wood density and carbon isotope ratios in tree rings of beech (*Fagus sylvatica*) growing in Germany and Italy. *Trees*, 20. 571-586

Tei S, Sugimoto A, Yonenobu H, Hoshino Y, Maximov TC. (2013). Reconstruction of summer palmer drought severity index from $\delta^{13}\text{C}$ of larch tree rings in east Siberia. *Quaternary International*, 290-291. 275-281

Chapter 5

Final conclusions

Cedar, larch and beech showed different water use patterns. Cedar and larch were more prompt to use readily available precipitation water because of its shallow root system, as revealed by $\delta^{18}\text{O}$ and δD records of xylem, precipitation and soil water. Beech used soil water more profusely than the other two species because of its deeper root system, as indicated by xylem δD . For all tree species, xylem water and wood α -cellulose $\delta^{18}\text{O}$ showed opposite trends due to differences in leaf water enrichment among tree species caused probably by differences in monthly leaf transpiration. This based on past studies Corroborates the well-known fact that evergreen trees that at the leaf level have a lower stomatal conductance than deciduous trees, which leads lower ^{13}C discrimination. The differences in wood $\delta^{13}\text{C}$ pointed to differences in WUEi, with the evergreen cedar showing a consistently higher WUEi than the deciduous larch and beech. However, at the whole tree level, canopy transpiration is higher in cedar than in larch because of higher leaf area index, this appears to be related to its high demand for nutrient such as N and P, which is higher for beech followed by cedar and larch. $\delta^{18}\text{O}$ and δD in xylem water showed similar trend with soil water and precipitation water in most of time, but in July and August was more enriched than soil water and precipitation. This mainly caused by the extreme climate condition. In the year 2018, cedar showed similar water use strategy to response the different extreme climate events but for different propose. In July cedar decrease transpiration, reduce stomatal conductance and use more storage water in trunk to against drought. In August, cedar decrease transpiration and reduce stomatal conductance to against waterlog.

The results of this study showed clearly that even in high precipitation area like the one in Shonai, where the annual precipitation is 3000mm there are periods of less water availability or long-periods that can extend to one or two months without precipitation. This is indeed more relevant when this rainless period take place in summer.

The root system in the broad leaf beech appears to cope better with this climate pattern and

represents in general the adaption of natural tree species to their environment since beech forest are native of this area. In comparison the plantations represented by cedar and larch appear to be more dependent on the readily available water from frequent precipitation and in the future with more climatic fluctuations, especially in the precipitation regime could become the most affected once. However, even within these two species, there is a difference in how they handle soil water availability, which is proper of conifer evergreen and deciduous species, represented this later by larch.

It is worth mentioning that within these two conifer species, cedar adapts better in lower altitudes while larch is mostly distributed in higher altitudes. From the point of view of water use, the results suggest that larch are better adapted to slow snowmelt patterns because of lower air temperature in higher altitudes that could make water available for longer periods, especially during the “dry” months of April and May, when more resources are needed for leaf formation.

This pattern is also followed by the other two species but beech has a deeper root system that allows the access of deeper water pools and cedar starts transpiring when snow depth is still 2m high ensuring water supply because of higher air temperatures at lower altitudes.

Based on the oxygen isotopic values found in tree-rings, it was not possible to discern how significant snowmelt was for the three tree species studied because apparently the first month of water uptake and consequent carbon assimilation was not invested on growing the tree basal area but was more used for leave or fine root production. This is because of no signal of wood growth during the first months of the growing season in any of the tree species, contrary to what has been reported in previous studies, where stem wood grows during the snowmelt period.

Finally, this study shed lights on the water use strategy of trees in wet region, which are exposed to sever changes in the precipitation regime as it has been observed in recent years.

More research and probably more extensive sampling of other plant tissues will provide information of snowmelt water use by trees, that we could not find in our results, despite the immense amount of snowmelt water that is available in these forests.

Acknowledgement

I would like to thank my advisors, M. Larry Lopez C., Kenyichi Yoshimura, Chihiro Kato for their continuous support during my PhD. Study. I would like also thank all members of the department of crop and forest science (University of Lleida), the department of disaster prevention, meteorology and hydrology (Forestry and Forest Products Research Institute) and in my laboratory for their help in my study in form of sampling and analysis.