博士論文（要約）

Responses of *Pinus thunbergii* seedlings to waterlogging

（クロマツ苗の滞水応答に関する研究）

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1 General introduction

1.1 *Pinus thunbergii* coastal forests in Japan

Pinus thunbergii Parlatore (Japanese black pine) is an evergreen conifer that naturally distributes from the Honshu Island to the Ryukyu Islands (Satake et al., 1989a). *Pinus thunbergii* is known for its high tolerance against harsh coastal environments (infertile soil conditions, drought, salt wind), and from the mid-17th century, they have been widely planted along coastal lines of the Hokkaido island to the Kyusyu island to prevent blowing of sand and mitigate salt wind (Konta, 2013). Another reason that *P. thunbergii* trees are planted at coastal sites because they are known to be a “deep-rooting” species and, therefore, highly resistant to strong winds and tsunamis (Karizumi, 2010). *Pinus thunbergii* coastal forests protect inland areas from sand, salt wind, and tsunamis and provide recreational spaces and beautiful scenery, giving citizens an environment for relaxation and refreshment (Kasetani et al., 2007). As described, coastal forests are an essential component of coastal environments in Japan.

In March 2011, the disastrous tsunami caused by the Great East Japan earthquake heavily damaged about 3660 ha of *P. thunbergii* coastal forests along the Tohoku coastline. The tsunami caused *P. thunbergii* trees to tilt, break, or uproot, and in some cases, whole trees were washed away. At heavily damaged sites where *P. thunbergii* trees were uprooted, it was found from field surveys that the groundwater level was high and *P. thunbergii* trees had shallow root systems (Forest Agency Tohoku Regional Forest Office, 2011; Sakamoto, 2012; Tamura, 2012). On the other hand, at sites where *P. thunbergii* trees developed deep root systems, and the groundwater level was low. At these sites, stem breakage rather than uprooting was observed.

Field reports of Chiba prefecture, unrelated to the tsunami damage, reported the mass withering of *P. thunbergii* at lowland coastal sites (Oda, 2001). At these sites, it was found that vertical root growth was limited by groundwater. These reports and field surveys of the damaged coastal forests, suggested that vertical root development of *P. thunbergii* trees is strongly influenced by groundwater depth.

1.2 Restoration of *Pinus thunbergii* coastal forests and its challenges

Currently, great effort is put into restoring the coastal forests of the Tohoku region. With the knowledge of the effects of groundwater depth on the root system development of *P. thunbergii*, growth banks (2 m from the original ground surface, Fig. 1-1) for planting were constructed by machinery (Murakami, 2015; Takahashi, 2015). These growth banks are aimed to secure enough space for vertical root growth.

At some restoration sites, prolonged waterlogging occurs at the surface soil of the growth banks after rainfall (Ozawa, 2014; Shinomiya et al., 2016; Tohoku Research Center of Forestry and Forest Products Research Institute, 2016); (Fig. 1-2). This is suggested to be poor drainage due to the low saturated permeability coefficient caused by soil compaction from heavy machinery (Shinomiya et al., 2016). A pseudogley horizon was observed at depth 5-30 cm from the waterlogged surface soil at these waterlogged sites. This indicated that due to prolonged waterlogging, the soils of the growth banks were in a reductive state (Fig. 1-2 (d)).

Waterlogging is a condition where the soil is saturated with water. Prolonged periods of soil saturated with water reduce the oxygen availability to roots, and plants are exposed to hypoxic stress. These conditions can limit root respiration and can cause a decline in root function, root death, and rot. Therefore, the occurrence of waterlogging can be a significant problem inhibiting coastal forest restoration.

Several studies have focused on the waterlogging responses of *P. thunbergii* related to the effects of saltwater submergence and tsunami damage (Inoue et al., 2015; Ito and Yoshizaki, 2018; Tateishi et al., 2014). Freshwater waterlogging due to rainfall is the primary concern at the restoration sites, and responses to freshwater waterlogging differ from responses to saltwater waterlogging. Studies such as Oda (2001) have looked into the effect of non-saline waterlogging on *P. thunbergii* seedlings. They reported that *P. thunbergii* seedlings can survive longer under waterlogging when saline concentration is low. Their study observed seedling death after one week of waterlogging with saline water (saline concentration 3.4%). On the other hand, when seedlings were waterlogged with freshwater: saline water = 3:1 (saline concentration 0.8%), seedling death was observed after three weeks. Furthermore, from a field survey, it was reported that the mortality rate of mature *P. thunbergii* trees to freshwater waterlogging due to excessive precipitation from typhoons was less than 1% when the waterlogging duration was three weeks, indicating that they could survive about one month of freshwater waterlogging.

Okida et al. (2015) investigated the influences of saline water submergence (0, 3, 6, 12, and 24 hours) followed by waterlogged/drained conditions (three months, fresh water) on *P. thunbergii* seedlings. Evaluations were made on the survival rate and growth. The mortality of seedlings that experienced 24 hours of saline water submergence was 14% when seedlings were grown under drained conditions. On the other hand, when seedlings were grown under waterlogged conditions, the mortality of seedlings was 30% even when saline water submergence was 0 hours.

Negative effects of waterlogging were also recognized by the author at the restoration sites (Arahama, Sendai City, Miyagi Prefecture), where discoloration of needles was observed (Fig. 1-2 (a), (b), (c)). Needle color was a lighter green and, in some cases, yellowish, and needle length was relatively short. Also, overall, seedlings had poor growth.

Recently, to prevent the occurrence of prolonged waterlogging after rainfall, measures such as plowing the growth banks before planting and the construction of open ditches have been attempted on a trial basis (Murakami, 2015; Ono et al., 2016) (Fig.1-3). These measures are aimed to improving drainage and permeability of the growth bank surface so that heavy rainfall does not result in long-term waterlogging (Noguchi et al., 2021; Ono et al., 2018, 2016).

Although little has yet to be reported on the effect of these measures on the occurrence of waterlogging, both Ono et al. (2016) and Noguchi et al. (2021) reported that plowing has a significant effect on improving soil hardness to about 1.5 m depth. The most extended effect reported so far is 30-months (Noguchi et al., 2021). Hence, plowing before planting may be a beneficial way to improve soil hardness, as well as waterlogging. However, knowledge of the responses of *P. thunbergii* seedlings to various waterlogging conditions, such as the depth and duration of waterlogging, is still lacking. Therefore, responses of *P. thunbergii* seedlings to waterlogging must be elucidated to maintain conditions of the growth banks and efficiently realize coastal forest restoration.

1.3 Research objective

Prolonged waterlogging at the growth bank's soil surface is one of the problems inhibiting and slowing the restoration of Tohoku region's coastal forests. Therefore, this study aims to clarify waterlogging conditions in which *P. thunbergii* seedlings can recover or maintain growth after the release from waterlogging and to contribute to the achievement of the restoration of Tohoku region's *P. thunbergii* coastal forests.

Fine roots (diameter < 2 mm) play a vital role in absorbing nutrients and water from the soil and are critical for the growth and recovery of seedlings after planting. Therefore, this study focused on the influence of waterlogging on the growth and morphological characteristics of fine roots. In chapters 2 through 4, three experiments were conducted. In Chapter 2, waterlogging responses of *P. thunbergii* seedlings and four broadleaved species were elucidated to evaluate waterlogging tolerance relative to other candidate broadleaved species for restoration. In Chapters 3 and 4, waterlogging responses of *P. thunbergii* seedlings were elucidated regarding the depth and duration of waterlogging. Chapter 3 elucidated responses of *P. thunbergii* seedlings to two different waterlogging depths (exposure of only the bottom half of the root system to waterlogging or exposure of the whole root system to waterlogging). Focus was especially made on the vertical distribution of fine root growth and transpiration, a value to estimate the water absorption function of fine roots. Chapter 4 elucidated responses of *P. thunbergii* seedlings to different waterlogging durations (7 days, 17 days, and 32 days), followed by a waterlogging-free recovery period. This chapter focused on responses of fine root water absorption measured as transpiration, especially during and after the release from waterlogging.

In Chapter 5, discussions are made on how results obtained can benefit in solving the problem of waterlogging at the Tohoku region's coastal forest restoration sites. Important suggestions made in this study were 1) the potential use of broadleaved species as alternatives of *P. thunbergii* at long-term waterlogged sites due to rainfall and sites where waterlogging is constant due to high ground water due to geographical factors, 2) if waterlogging is partial (topsoil not waterlogged), *P. thunbergii* can change its fine root growth distribution and maintain aboveground activity, and 3) if the waterlogging duration short, such as several weeks, water absorption function of roots can rapidly recover after the release from waterlogging.

Figures

(a)

(b)

(c)

Figure 1-1

Photos of coastal reforestation sites taken at Noda-hama, Kuji City, Iwate prefecture (July 2018). (a) Growth banks whose heights are approximately 2 m from the original ground. (b) and (c) are photos of where planting of *Pinus thunbergii* has been completed. (b) was taken from the breakwater, and (c) was taken from ground level of the reforestation site.

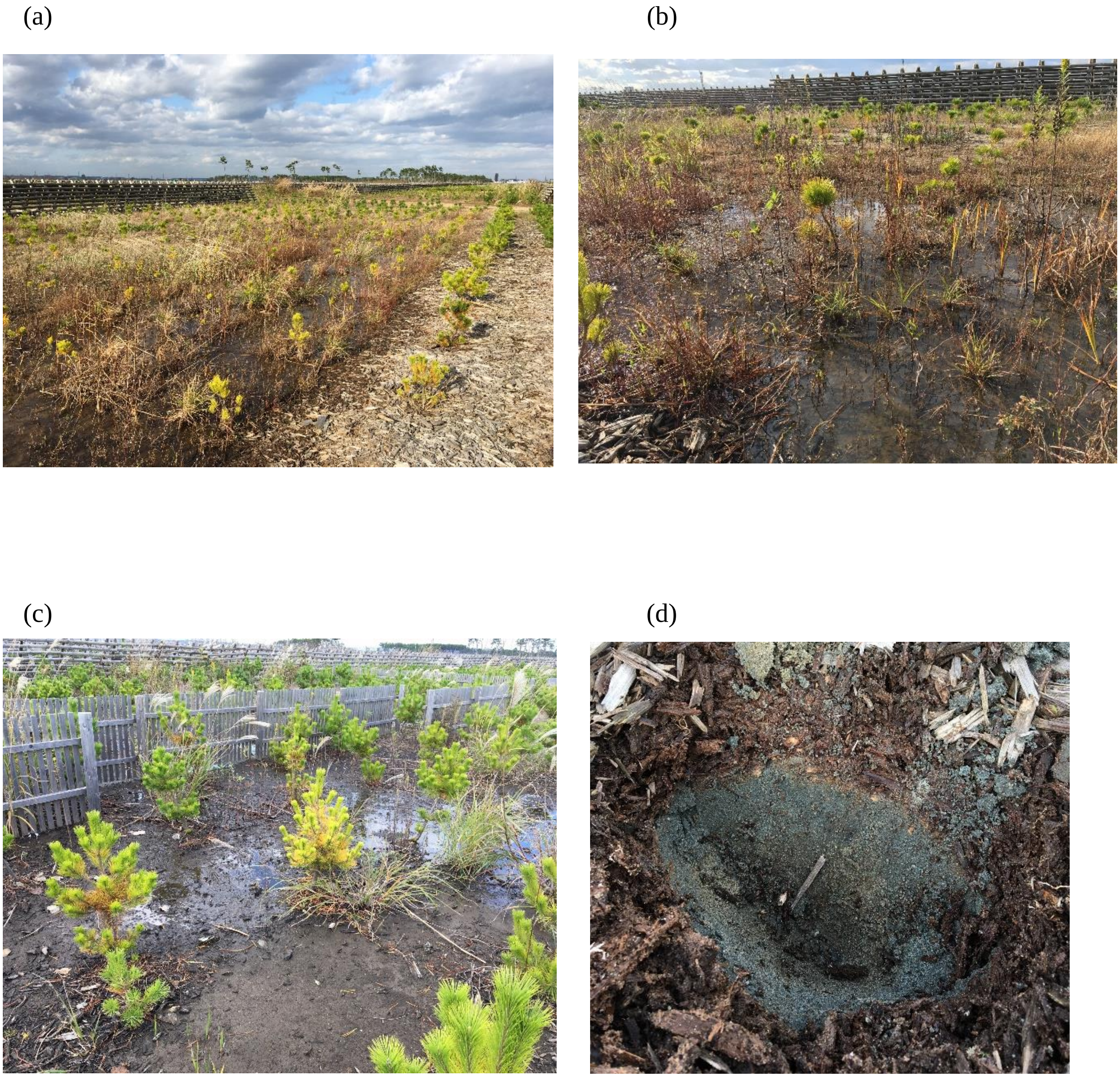

Figure 1-2

Photos of reforestation sites taken at Ara-hama, Sendai City, Miyagi prefecture (October 2017). (a), (b), (c) are photos of waterlogged areas at the growth banks with *Pinus thunbergii* seedlings. (d) is the pseudogley horizon observed near the surface soil of the growth bank.

Figure 1-3

Photos of *Pinus thunbergii* reforestation sites taken at Ara-hama, Sendai City, Miyagi prefecture (October 2017). The red arrow shows the open ditch for drainage.

2 Waterlogging responses of *Pinus thunbergii* and four broadleaved species

2.1 Introduction

This chapter elucidates the waterlogging responses of *Pinus thunbergii* and four broadleaved species to evaluate the waterlogging tolerance of *P. thunbergii* relative to the other candidates for coastal forest restoration. The use of broadleaved species in coastal forests has been recently gaining attention with the aspect of pine wilt disease (Nakamura, 2014) and the growing interest in biodiversity in coastal forests (Forest Agency Tohoku Regional Forest Office, 2011). However, information on species selection and methodology for introducing broadleaved species needs to be further elucidated (Kaneko, 2017; Takahashi and Takahashi, 2017). Therefore, in this chapter, four broadleaved species were chosen with the aspect of waterlogging tolerance. The selected broadleaved species were *Acer mono* Maxim. var. *marmoratum* (G.Nicholson) H.Hara f. *dissectum* (Wesm.) Rehder, *Quercus serrata* Thunb. ex Murray, *Alnus hirsuta* var. *sibrica* (Fischer) C. K. Schn., and *Fraxinus mandshurica* Rupr. var. *japonica* Maxim. In the following paragraphs, representative characteristics are introduced for each chosen broadleaved species.

Acer mono distributes in warm and temperate zones from the Honshu island to the Kyusyu islands (Satake et al., 1989a). *Acer mono* has been planted on a trial basis in the coastal forests of Iwate Prefecture (Hashimoto et al., 2018) and Aomori Prefecture (Nakamura, 2014).

Quercus serrata distributes in warm temperate zones and low altitude areas of temperate zones from the Hokkaido islands to the Kyusyu islands (Satake et al., 1989a). It is a commonly used afforestation species for wood production and is often used as a bed log for shiitake mushroom cultivation. The natural incursion of *Q. serrata* at the Tohoku region's *P. thunbergii* coastal forests has been reported, and its survival after the tsunami has also been observed (Kanno et al., 2014). As *Q. serrata* is one of the dominant species of Satoyama broadleaved forests of the Tohoku region, a stable supply of seeds and seedlings can be achieved (Forest Maintenance Division of Miyagi Prefecture 2012) and is, therefore, a powerful candidate for coastal forest restoration.

Alnus hirsuta distributes from the Hokkaido islands to the Kyusyu islands (Satake et al., 1989a), and is commonly used as a fertilizer tree at sites with infertile soil, such as coastal and sand-break forests (Uemura, 1965). *Fraxinus mandshurica* distributes from the Hokkaido island to the northern part of the Kanto region (Uchiyama, 2016). It has been used for windbreak forests and is a candidate for afforestation in inland areas of coastal forests, especially in the Hokkaido peatlands (Itoh, 1985). *Alnus hirsuta* distributes in mountains and riparian areas (Satake et al., 1989a), and *F. mandshurica* in wetlands in mountainous areas (Satake et al., 1989b). These two species are often used for afforestation in riparian areas (Fujita, 2004; Nakajima, 2014) and were chosen as relatively waterlogging-tolerant species.

In this chapter, the focus was made on the vertical distribution of fine root growth. Fine root growth during the waterlogging period was distinctly measured with the in-growth core method (Li et al., 2013; Makkonen and Helmisaari, 1999; Ostonen et al., 2007). The mini-rhizotron method (Lee et al., 2016; Noguchi et al., 2005) was also used to non-destructively evaluate changes in fine root growth, color, and other visible characteristics (e.g., root hair, mycelial bundles) under waterlogging.

2.2 Materials and Methods

2.2.1 Plant materials and experimental setup

Two-year-old seedlings of *Pinus thunbergii*, *Acer mono*, *Quercus serrata*, *Alnus hirsuta*, and *Fraxinus mandshurica* were purchased from a commercial tree nursery in Hokkaido, Japan. At the end of April 2018, seedlings were transplanted in 1/2000 *a* Wagner pots (500 cm^2, in surface area, 30 cm, in depth with a drainage hole near the bottom) with Akadama soil derived from the loamy B horizon of Andisol. The soil surface was set equal to the pot height. Twenty-four pots per species; in total, 120 pots were prepared. Twenty-four pots of each species were divided into Control and WL (waterlogging treatment), so the mean seedling size did not differ (Table 2-1). Ten pots were placed on a wooden board (1×2 m).

The seedlings were grown under natural conditions at the seedling nursery of Tohoku Research Center, Forestry and Forest Products Research Institute located in Morioka City, Iwate prefecture (Fig. 2-1(a)). Two weeks after transplanting, 1 L of 2000-fold diluted liquid fertilizer (Hyponex 6-10-5, Hyponex Japan, Osaka, Japan) was applied. The amount per pot was approximately 30, 50, and 25 mg for N, P, and K, respectively. Depending on the weather, 2-4 L of water was given once or twice a week until mid-July.

For WL, the waterlogging treatment began on July 13 and ended on September 26 (ten weeks). The waterlogging treatment was conducted from mid-July after needle expansion and elongation had ceased so that effects on fine root growth by the decrease of photosynthetic production through suppressed leaf expansion under waterlogging stress are minor. Furthermore, the waterlogging treatment was conducted under environmental conditions when the seedlings are more likely to experience water stress from waterlogging, such as high temperatures and large vapor pressure deficit.

The waterlogging treatment was done by blocking the drainage hole and filling the pots with water until the water table reached the soil surface (Fig. 2-1 (b)). Water was carefully added at one-to-two-day intervals to maintain the water table at the soil surface. For Control, the drainage hole was kept open, and depending on the weather, 2-4 L of water was given once or twice a week.

On September 26, nine seedlings per group and species were harvested. For *Q. serrata*, four and five seedlings were sampled for Control and WL, respectively. The difference in the number of samplings was due to a lack of leaf expansion and the death of seedlings before the waterlogging treatment was started. The reason for seedling death is unclear, but it may have been due to the initial condition of the seedlings. Fig. 2-2 shows the daily maximum temperature, daily minimum temperature, and daily precipitation during the experiment period (July to September 2018).

2.2.2 Soil property

Soils were sampled at the end of the experiment when the seedlings were harvested. The soil samples were air-dried and measured for pH and EC. Soil pH was measured using a pH meter (F-22, HORIBA, Japan) after suspending 10 g of air-dried fine soil in 25 mL of distilled water. Soil EC was measured using an EC meter (CM-30G, DKK-TOA, Japan) after shaking 10 g of air-dried fine soil in 50 mL of distilled water for one hour.

2.2.3 Survival after over-winter

Three seedlings per treatment and species were over-wintered at the nursery. The seedlings were grown under natural conditions until October 2019. Depending on the weather, 2-4 L of water was given once or twice weekly.

2.2.4 Aboveground measurements

The heights and stem base diameters of all the seedlings were measured before the waterlogging treatment and at the end of the experiment to evaluate waterlogging responses for aboveground growth. The dry weight of aboveground and belowground organs of harvested seedlings were measured. However, leaf dry weight for *A. mono, Q. serrata, A. hirsuta*, and *F. mandshurica* could not be obtained as all leaves could not be collected due to unexpected and sudden leaf shedding before the end of the experiment.

2.2.5 Fine root measurements

The in-growth core method was used to elucidate responses of fine root growth to waterlogging (Li et al., 2013; Makkonen and Helmisaari, 1999; Ostonen et al., 2007). Fine roots that grew during the whole experiment period (end of April to end of September) were measured by in-growth core 1 (IG_1), and fine root growth during the waterlogging period (mid-July to end of September) was distinctively measured by in-growth core 2 (IG_2). The two in-growth cores (diameter, 32 mm; depth, 30 cm; 2 mm mesh) were placed across the seedling, about 10 cm apart from the seedling (Fig. 2-1). IG_1 was placed in the pots with seedlings at the same time as transplanting and was sampled on September 26. Fine roots obtained from IG_1 are fine roots that grew during the whole experimental period (20 weeks) and reflect the effects of waterlogging on fine root color and morphology for seedlings subjected to waterlogging. IG_2 was placed in the pot right before the waterlogging treatment was started. When placing IG_2, a sharpened steel pipe (diameter, 40 mm) was used to remove soil and make space for IG_2 with minimum soil disturbance and root damage. After placement, IG_2 was filled with new Akadama soil up to the same level as the soil surface (30 cm from the bottom of the pot). Fine roots obtained from IG_2 represent fine roots that grew during the waterlogging period (July 13 - September 26, ten weeks).

Sampling of the in-growth cores was carried out with a sharpened steel pipe to carefully cut the roots along the edge of the in-growth core. Harvested in-growth cores were placed in plastic bags and stored in a refrigerator at 4°C until further analysis. The sampled in-growth cores were divided into the top and bottom parts (top, 0-15 cm in depth; bottom, 15-30 cm in depth). The growth and morphology of fine roots were analyzed according to the position, top part or bottom part. The soils from the in-growth cores were thoroughly washed out with tap water on a very fine sieve (sieve aperture 250 μm), and fine roots remaining on the sieve were carefully washed with a brush and scanned with a flatbed scanner (GT-X980, EPSON) at 800 dpi. Scans were made separately for the fine roots obtained from the top and bottom parts. After scanning, the fine roots were dried at 70 °C for 72 hours and then measured for dry weight.

After sampling the in-growth cores, the whole root system was sampled for nine seedlings per species and group at the end of the experiment on September 26. They were carefully washed with tap water and were divided into fine roots (diameter < 2mm) and coarse roots (diameter > 2mm). Roots were dried at 70 °C for 72 hours and then measured for dry weight.

WinRHIZO Pro (2012b) (Regent Instruments, Inc., Quebec, Canada) was used to measure root length (mm), root projected area (cm^2) and root volume (cm^3). Mean root diameter (D_r), specific root length (SRL), and root tissue density (RTD) were calculated from the following equations,

$$\boldsymbol{D_r\ (mm) = \frac{A_{pr}}{L_r} \times 1000}$$

$$\boldsymbol{SRL\ (m\ g^{-1}) = \frac{L_r}{W_{dr}}}$$

$$\boldsymbol{RTD\ (g\ cm^{-3}) = \frac{W_{dr}}{V_r}}$$

where, A_{pr} is root projected area (m^2), L_r is root length (m), W_{dr} is root dry weight (g), and V_r is root volume (cm^3).

Root color often changes with age, where white roots are new and change to brown with time and are classified as dead when they become black (McCormack et al., 2015). In this study, the root color was evaluated by measuring fine root brightness to determine if the roots were dead. Scan images were analyzed using Image J (ver. 1.51). Regions showing roots were determined by binarization of images, and brightness was set as the average of colors (red, green, blue). Fine roots were skeletonized, and the brightness of the pixel located in the middle of fine roots was obtained to avoid the inclusion of the high-brightness pixels due to reflection of light. As the brightness distribution was not a normal distribution, the mode value was selected as the representative value. For each IG_1 and IG_2, the mode (peak of brightness) was calculated as the representative brightness value for the top and bottom parts, respectively.

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2.2.6 Statistical analysis

The Wilcoxon-Mann-Whitney test was used to determine differences between the groups. For analysis of fine roots obtained from the in-growth cores, statistical differences between the groups were evaluated for the top and bottom parts, respectively. All tests were conducted using R (version 3.65.1, The R Foundation for Statistical Computing Platform) with a significance level of $p < 0.05$.

2.3 Results

2.3.1 Visible symptoms of waterlogging on aboveground organs

Visible symptoms were observed in the waterlogging group (WL) for *P. thunbergii* and *A. mono*. For *P. thunbergii,* needle discoloration was observed after about six weeks of waterlogging, and the discoloration became more evident with time. At the end of the experiment, the needle color was a lighter green at WL (Fig. 2-4 (a)). For *A. mono*, leaves started to change color from green to reddish-brown after two weeks of waterlogging, and all leaves were shed before the waterlogging treatment ended (Fig. 2-4 (b), (c)). For the other three species, visible symptoms on leaves were not observed.

For *A. mono*, *Q. serrata*, *A. hirsuta*, and *F. mandshurica,* hypertrophied lenticels were observed on the stem near the water table (Fig. 2-5 (b), (c), (d), (e)). For *A. hirsuta* and *F. mandshurica*, the hypertrophied lenticels were observed for all the seedlings of WL. For some seedlings, adventitious root development was also observed at the soil surface after four weeks of waterlogging (Fig. 2-5 (d), (e)). However, for *A. mono* and *Q. serrata*, hypertrophied lenticel formation was observed after about four weeks of waterlogging, but only on two out of twelve seedlings.

2.3.2 Soil property

Table 2-2 shows the results of soil pH and soil EC. pH did not differ among groups and was approximately pH=6 for all species. Soil EC was larger at WL for all species. For *Q. serrata*, data is not shown due to accidental loss of soil samples.

2.3.3 Seedling growth

Stem base diameter was increased at WL compared to Control for *P. thunbergii*, *A. hirsuta*, and *F. mandshurica* (Fig. 2-6 (a)). The increase in stem base diameter was mainly attributed to the increase in bark tissues for *P. thunbergii* (Fig. 2-7 (a)) and the increase in the xylem for *A. hirsuta* and *F. mandshurica* (Fig. 2-7 (b), (c)). Height growth did not differ between Control and WL for all five species (Fig. 2-6 (b)). For *P. thunbergii*, *A. mono*, and *Q. serrata*, height growth for both Control and was approximately 2 cm for both Control and WL.

2.3.4 Dry weight of seedlings

Leaf dry weight could not be obtained due to unexpected defoliation during the waterlogging treatment for *A. mono*, *Q. serrata*, *A. hirsuta*, and *F. mandshurica*. The stem dry weight of *P. thunbergii* and *F. mandshurica* were larger at WL compared to Control, which resulted in a large stem + branch dry weight (Fig. 2-8). Fig. 2-9 is the result of root dry weight. The total root (coarse + fine root) dry weight of all species except *F. mandshurica* was decreased at WL. Representative images of the whole root system for each species and group are shown in Fig. 2-10.

The root/shoot ratio was calculated from the total root dry weight (coarse + fine root) and the total aboveground dry weight (stem + branch) (Fig. 2-11 (a)). All five species' root/shoot ratios were larger at WL compared to Control (Fig.2-11 (b)).

2.3.5 Seedling survival the following year

Three seedlings per group and species were left to over-winter under non-waterlogged conditions. As a result, all three seedlings of WL died the following year for *P. thunbergii* (Fig. 2-12). For *A. mono*, *A. hirsuta*, and *F. mandshurica,* all seedlings of both groups were alive and developed new leaves the following spring. For *Q. serrata*, one to two seedlings of Control and WL died. The death of seedlings may have been the effects of snow damage during the winter season as seedlings were grown at the nursery.

2.3.6 Fine root growth and morphology (In-growth core method)

Fig. 2-13-1 and 2-13-2 is fine root growth obtained by in-growth core 1 and 2, and Fig 2-13-3 is the net amount calculated from in-growth core 1 and 2. Fine root growth during the waterlogging treatment differed according to species (Fig. 2-13-2). For *P. thunbergii* and *A. mono*, fine root dry weight obtained from IG_2 for WL was decreased compared to Control, indicating that fine root growth was severely inhibited under waterlogging. For *Q. serrata*, a statistical difference between the groups was not detected. For *A. hirsuta* and *F. mandshurica*, the proportion of fine root dry weight of IG_2 at the top and the bottom parts for Control and WL differed. For *A. hirsuta*, although total fine root dry weight did not differ between the groups, the fine root dry weight of WL was large at the top part and small at the bottom part compared to Control. For *F. mandshurica*, although total fine root dry weight did not differ between the groups, the fine root dry weight of WL was larger at the top part compared to Control.

Changes in root morphology under waterlogging were evaluated by comparing mean root diameter, specific root length (SRL), and root tissue density (RTD) of fine roots obtained from IG_1 of Control and WL. Little effect of waterlogging was observed on mean root diameter (Table 2-3). Mean root diameter did not differ between Control and WL for all species and positions, except for *P. thunbergii*, where the mean root diameter was larger at the bottom part than Control. For the other four species, mean root diameter did not differ between the groups at the top and the bottom parts. SRL was also hardly affected by waterlogging, and did not differ between Control and WL except for *A. hirsuta*, where the SRL was larger at the top part of WL than Control (Table 2-4). RTD showed a tendency to decrease under waterlogging, especially at the bottom part (Table 2-5). For *A. mono* and *A. hirsuta*, RTD of WL was smaller than Control at the top and the bottom parts. For *P. thunbergii*, RTD of WL was smaller at the bottom part than Control. Fine root brightness showed a tendency of decreasing under waterlogging for *P. thunbergii*, *A. mono*, and *A. hirsuta*, and values were smaller for WL than Control, and it was observed that fine root color was changed from brown/dark brown to black (Fig. 2-14 (a), (b)).

From fine roots obtained from IG_2, the difference in morphology and color of fine roots that grew during the waterlogging period between Control and WL was compared. As fine roots could not be sampled from some in-growth cores, the number of samples of WL is less than nine for some species of WL.

Mean root diameter did not differ between groups for all species and positions (Table 2-6). SRL responded to waterlogging only for *F. mandshurica*, where SRL was larger at the bottom part for WL than Control (Table 2-7). For RTD, values for WL were smaller compared to Control, which showed a similar trend as IG_1 (Table 2-8). For *A. hirsuta*, RTD of WL was smaller at the top and the bottom parts compared to Control. For *F. mandshurica*, RTD of WL was smaller at the bottom part compared to Control. The value of fine root brightness of WL at the bottom part was small compared to Control for *P. thunbergii* (Fig. 2-15 (b)). In contrast, for *A. hirsuta* and *F. mandshurica*, fine root brightness was increased for WL compared to Control, and this was mainly observed at the top part (Fig. 2-15 (a)).

2.3.7 Fine root growth and morphology (Mini-rhizotron method)

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2.4 Discussion

Fine root growth under waterlogging

The in-growth method and mini-rhizotron method exclusively evaluated fine root growth under waterlogging. For *P. thunbergii*, *A. mono*, and *Q. serrata*, fine root growth was hardly observed under waterlogging (Fig.2-13-2). For these three species, it is suggested that fine root growth was limited due to an energy crisis caused by the lack of energy production from decreased aerobic respiration under hypoxic conditions (Drew, 2002; Sauter, 2013). The decrease in fine root growth may also have been caused by root damage due to reductive conditions and toxic substances (e.g., sulfides, soluble

Fe, and Mn) (Morard and Sylvestre, 1996) resulting from waterlogging.

For *A. hirsuta* and *F. mandshurica*, fine root growth was continued under waterlogging, especially at the top part of the root system (Fig. 2-13-2 (a)). Adventitious roots were also observed. For these two species, the continued root growth at the top part may have been enabled by the production of hypertrophic lenticels, which were observed at the stem base near the water table (Fig. 2-5).

Hypertrophic lenticels are reported to function as an entrance of air (Li et al., 2006; Shimamura et al., 2010) and may have worked to supply oxygen and enabled fine roots to grow under waterlogging. Although hypertrophic lenticels were observed for *A. mono* and *Q. serrata*, fine root growth of both species was inhibited by waterlogging. Although the reason for this is unclear, the produced hypertrophic may not have been functioning as an air entrance.

The difference between *A. hirsuta* and *F. mandshurica* was the vertical distribution of fine root growth under waterlogging. For *A. hirsuta*, fine root growth under waterlogging was mainly observed at the top part of the in-growth core (Fig. 2-13-2 (a)). Additionally, from the mini-rhizotron method, fine root elongation under waterlogging was only observed near the soil surface and was not observed below 5.5 cm in depth (Fig. 2-20-7 (a), (b)). Therefore, under waterlogging, *A. hirsuta* was able to adapt to waterlogged conditions by producing hypertrophic lenticels. However, as fine root growth was mainly observed near the top part, it was found that despite these adaptions, fine root growth under waterlogging is limited to growth near the soil surface.

On the other hand, for *F. mandshurica*, fine root growth under waterlogging was not limited to the top part as fine root dry weight at the bottom part of IG_2 did not differ between the Control and WL (Fig. 2-13-2 (a), (b)). Moreover, the mini-rhizotron observation observed fine root elongation at the middle part of the pot (Fig. 2-21-4, 2-21-5, 2-21-6). From these results, it was suggested that *F. mandshurica* can grow fine roots at lower parts of the root system and adapt well to hypoxic conditions compared to *A. hirsuta*.

Fine root morphology

Root tissue density (RTD) of fine roots is the ratio of root dry mass to root volume. It is a fundamental trait being increasingly used as a key indicator of the plant species' strategy in resource use (Birouste et al., 2014). In this study, the decrease in RTD was especially used as an indicator for 1) the production of aerenchyma (increase in root porosity) or 2) root death (death of root tissue). If fine root growth was observed, along with the decrease in RTD, it was considered that it was due to the production of aerenchymas and a sign of waterlogging adaption. On the other hand, if fine root growth was not observed, and the change in root color from brown to black was observed, it was considered that this was root damage and a sign of fine root tissue death.

The decrease in RTD, along with fine root growth, was observed for the top part of *A. hirsuta* and the bottom part of *F. mandshurica*, obtained by IG_2 (Table 2-8). Therefore, the decrease in RTD is considered to be reflecting the development of aerenchymas. The production of aerenchymas is reported to increase the fraction of root volume occupied with air and provides an internal pathway for oxygen (Armstrong, 1979; Jackson and Armstrong, 1999). Therefore, it was suggested that for *A. hirsuta* and *F. mandshurica*, the production of hypertrophied lenticels and the development of aerenchymas in roots were the adaptions to transport oxygen to waterlogged fine roots.

Changes in mean root diameter, SRL, RTD, and fine root brightness under waterlogging were evaluated using fine roots obtained from IG_1. As fine root dry weight of IG_2 of WL was significantly small compared to Control for *P. thunbergii*, *A. mono*, and *Q. serrata*, its contribution to affect the results of fine root morphology is assumed to be negligible. On the other hand, for *A. hirsuta* and *F. mandshurica*, the dry weight of fine roots that grew during the waterlogging treatment was approximately 30-40% of the dry weight of fine roots that grew during the whole experiment period.

A smaller RTD was observed for fine roots obtained from both IG_1 and IG_2 for *A. hirsuta* at WL compared to Control at both the top and the bottom parts. Therefore, the decrease in RTD for fine roots obtained from IG_1 may be not only due to the decrease in RTD of pre-existing roots but also due to the decrease in RTD of fine roots that grew during the waterlogging treatment, reflecting the development of aerenchyma, especially at the top part. For *F. mandshurica*, changes in SRL and RTD were only detected in IG_2, and fine root morphology was hardly affected by waterlogging.

For *P. thunbergii*, mean root diameter of WL was large at the bottom part compared to Control (Table 2-3). From scan images of fine roots in IG_1 and mini-rhizotron observations, it is suggested that the increase in mean root diameter is due to the decrease in branching resulting from root growth inhibition and also the decrease of root tips under waterlogging (Fig. 2-17-1 (a), (b)). As observed in this study, several studies have also reported the high death rate of root tips, as root tips are particularly sensitive to waterlogging (Levan and Riha, 1986; Repo et al., 2016). As most root tips in coniferous trees are ectomycorrhizas, this may reflect the higher sensitivity of ectomycorrhizas to anoxia than plant roots (Read and Armstrong, 1972).

The darkening in root color under waterlogging was observed at both the top and bottom part for

P. thunbergii, suggesting that root color of *P. thunbergii* was most affected (Fig. 2-14 (a), (b)). Furthermore, RTD of WL was small compared to Control at the bottom part of IG_1 (Table 2-5). Brittle, shriveled, or wrinkled black roots are generally classified as dead (Vogt and Persson, 2001). From studies on kiwi vines, it is reported that the physical loss of root tissue of waterlogged roots was attributed to the detachment of the cortex from the central stele through the dissolution of the entire cell (Smith et al., 1990). Therefore, the decrease in RTD and fine root brightness may suggest heavy damage or death of roots where root tissue was partially lost due to decay. This discussion was also supported by the result where seedling survival of the following year was not observed for *P. thunbergii* seedlings exposed to waterlogging (Fig. 2-12), suggesting that roots exposed to waterlogging for ten weeks were heavily damaged and could not recover.

All seedlings of *A. mono*, *Q. serrata*, and *A. hirsuta* opened new leaves the following year, indicating that the roots exposed to waterlogging were not all dead and were able to recover after being released from waterlogging. From this result, the decrease in RTD and fine root brightness may not have been a sufficient indicator of root death and decay for *A. mono*, *Q. serrata*, and *A. hirsuta*. The reason for this may be due to the effects of iron accumulation under waterlogging (Hayashi and Wakisaka, 1956; Levan and Riha, 1986; X. Wang et al., 2015), and the change in root color may not have been directly related to root death. In this experiment, RTD was evaluated as the mean value obtained from all fine roots from the in-growth cores (total root volume /total fine root dry weight), which included both dead and alive roots. Dead roots can still be connected to alive roots; therefore, for future works, if measurements on the determination of fine root death and RTD are made at a smaller scale (such as each small fine root segment), an additional indicator that can separate the cause of root darkening (the accumulation of iron or root death) may also be found. If so, this method may be a key to separating dead roots from alive roots. Furthermore, root brightness was evaluated by the mode value, which does not include a fluctuation range. In future work, choosing a value that includes a fluctuation range may also result in a more adequate evaluation parameter or indicator.

The decay of fine roots was assumed to be negligible for Control, as the disappearance of fine roots was not observed for all five species from the mini-rhizotron method. The net amount of fine roots (fine roots that were present before the waterlogging treatment started) was estimated by subtracting the dry weight of fine roots obtained at IG_1 (fine roots that grew from the start to the end of the experiment) from that of IG_2 (fine roots that grew only during the waterlogging treatment) (Fig. 2-13-3).

For *A. hirsuta*, the estimated net amount of fine roots was small for WL compared to Control. Furthermore, for *P. thunbergii*, *A. mono*, and *Q. serrata*, the estimated net amount of fine roots was less than 50% for WL compared to Control (Fig. 2-13-3). Therefore, not only was fine root growth decreased under waterlogging, but fine roots that existed before the waterlogging treatment were also partially lost due to fine root decay at WL. However, as *A. mono* and *Q. serrata*, which were exposed to waterlogging, were still alive and able to open new leaves the following year, it is suggested that not all roots were lost, and recoverable roots were still present.

Fine root phenology

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Dry weight of fine roots that grew during the whole experiment period was over more than twice the dry weight of fine roots that grew during the waterlogging treatment period for Control of all species. From this result, it was shown that fine root growth before the waterlogging treatment was greater compared to fine root growth from mid-July to the end of September. However, even for species such as *A. mono*, *A hirsuta*, and *F. mandshurica*, whose fine root growth was small from mini-rhizotron observation, fine roots could be obtained from IG_2. The reason for the discrepancies between methodologies may be due to the limited area of observation by the mini-rhizotron method, and the increase in observation area by the mini-rhizotron method is suggested. Additionally, the parallel use of multiple methodologies may also increase the degree of precision.

Aboveground

For *P. thunbergii, A. hirsuta*, and *F. mandshurica*, stem base diameter growths for WL were large compared to Control (Fig. 2-6 (a)). The increment in stem base growth under waterlogging has been previously reported in various species (Kozlowski, 1997). Yamamoto et al. (1995a) and Yamamoto et al. (1995b) reported that stem base diameter increments under waterlogging of *F. mandshurica* and *Alnus japonica* seedlings were attributed to the increment in xylem. On the other hand, for *Pinus halepensis* (Yamamoto et al., 1987) and *Pinus densiflora* (Yamamoto et al., 1987; Yamamoto and Kozlowski, 1987), stem base diameter increment under waterlogging was attributed to the increase in bark thickness, which was also observed in this experiment (Fig. 2-7). An increment of stem base diameter caused by the swelling of tissues and cells of bark is called stem hypertrophy, which is not accompanied by an increase in cellular division. On the other hand, stem diameter increment accompanied by the increase in cellular division at the cambium layer is called stem hyperplasia (Angeles, 1990). It is suggested that the stem base diameter increment of *P. thunbergii* was a different response compared to *A. hirsuta* and *F. mandshurica*, as the increment was mainly attributed to the increase in bark tissue rather than the xylem. The observed tissue hypertrophy for *P. thunbergii* may work to eliminate the build-up of potentially toxic gases such as ethylene, which can be produced by waterlogging (Blake and Reid, 1981).

The root/shoot ratio is generally reported to decrease under waterlogging as fine root growth is more typically decreased compared to stem growth (Kozlowski, 1997). From Fig. 2-11 (b), it was found that the root/shoot ratio of WL was small compared to Control for all five species. For WL of *P. thunbergii*, although stem dry weight was large compared to Control, the decrease in root dry weight was greater, resulting in the decrease in the root/shoot ratio.

Furthermore, as the waterlogging treatment was started after the expansion of leaves and after height growths of *P. thunbergii*, *A. mono*, and *Q. serrata* (Fig. 2-6 (b)) had almost ceased, it was suggested that the waterlogging treatment had a smaller effect on aboveground organs compared to belowground organs. Hence, for *P. thunbergii, A. mono*, *Q. serrata*, and *A. hirsuta*, the decrease in root/shoot ratio of WL was attributed to the decrease in total root dry weight resulting from the decrease in fine root growth, the increase in root decay and the increase in aboveground dry weight especially for *A. hirsuta*.

On the other hand, for *F. mandshurica,* the decrease in root/shoot ratio of WL was attributed to the increase in aboveground dry weight, especially the increase in stem dry weight. As the aboveground and belowground growth (from IG_2) were enhanced under waterlogging, waterlogging did not have a negative effect on *F. mandshurica*. This matched the results obtained in previous studies such as Nakae and Manabe (1963). In this study, it was reported that *F. mandshurica* seedlings showed increased growth at the wet treatment (soil water content by weight 103.7%) compared to moist (soil water content by weight 82.5%) and dry treatments (soil water content by weight 47.5%), and increment in root dry weight was also significant. As negative effects were not observed for *F. mandshurica*, it was suggested that compared to the other four species, *F. mandshurica* presents the highest tolerance against waterlogging stress.

2.5 Conclusions

This chapter elucidated waterlogging responses and tolerance of *Pinus thunbergii* and four broadleaved species, focusing on changes in growth, morphology, and color of fine roots. From 1) the severe inhibition of fine root growth under waterlogging, 2) the occurrence of root death and decay, and 3) the death of seedlings the following year, it was found that *P. thunbergii* was most negatively affected by the waterlogging conditions of this experiment compared to the other four broadleaved species, especially *Acer mono* and *Quercus serrata*, which are both species that do not naturally inhabit waterlogged environments. In contrast, *Alnus hirsuta* and *Fraxinus mandshurica* were able to continue fine root growth under waterlogging. For *F. mandshurica*, fine root growth continued near the soil surface, and the bottom part of the root system, and fine root color did not change during the waterlogging treatment. Therefore, it was found that *F. mandshurica* was more tolerant to waterlogging than *P. thunbergii* and the other three broadleaved species.

Tables

Table 2-1 Size of seedlings

Size	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Stem base diameter (mm)	Control	10.2 (0.8)	5.6 (0.8)	3.6 (0.7)	6.5 (1.2)	6.8 (0.8)
	WL	10.1 (1.0)	5.6 (0.6)	3.8 (0.8)	6.5 (0.7)	7.1 (1.2)
Height (cm)	Control	39.0 (3.0)	33.4 (4.7)	32.6 (1.3)	40.9 (2.9)	40.9 (3.6)
	WL	39.4 (5.6)	34.4 (4.1)	32.5 (3.2)	42.2 (4.8)	41.7 (5.7)

Stem base diameters and heights at the end of June for Control and waterlogging group (WL) before the waterlogging treatment was started for each species. Values are means and standard deviation (*n*=12).

Table 2-2 Soil property

Species	Group	pH	EC (mS cm^{-1})
Pinus thunbergii	Control	5.9 (0.0)	31.9 (1.3)
	WL	6.0 (0.0)	36.4 (0.5) *
Acer mono	Control	5.9 (0.0)	32.2 (0.9)
	WL	5.9 (0.0)	38.3 (0.9) *
Alnus hirsuta	Control	5.9 (0.0)	32.2 (0.9)
	WL	5.9 (0.0)	38.3 (0.9) *
Fraxinus mandshurica	Control	5.9 (0.1)	27.2 (1.1)
	WL	6.0 (0.0)	30.0 (0.4) *

The pH and EC of pot soil at the end of the experiment. Values are means and standard deviation (*n*=4). For *Quercus serrata*, the soil could not be sampled due to an accident in sampling. Statistical difference was tested between the groups by the Wilcoxon-Mann-Whitney test (*, p<0.05).

Table 2-3 Root diameter of fine roots obtained from in-growth core 1

Position	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	0.47 (0.10)	0.35 (0.03)	0.21 (0.05)	0.32 (0.03)	0.38 (0.07)
	WL	0.53 (0.08)	0.37 (0.03)	0.18 (0.02)	0.36 (0.09)	0.43 (0.13)
p value		0.27	0.11	0.47	0.31	0.18
Bottom	Control	0.53 (0.15)	0.37 (0.03)	0.22 (0.04)	0.32 (0.09)	0.42 (0.13)
	WL	0.65 (0.12)	0.36 (0.06)	0.27 (0.11)	0.36 (0.09)	0.43 (0.13)
p value		**<0.05**	0.92	0.90	0.36	1.00

Mean root diameter (mm) and standard deviation of each group and species (n=7-9). The Wilcoxon-Mann-Whitney test was used to test the difference between groups within species at the top and bottom part, respectively.

Table 2-4 Specific root length of fine roots obtained from in-growth core 1

Position	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	28.7 (6.9)	39.3 (11.5)	69.5 (35.6)	34.9 (14.1)	28.0 (14.4)
	WL	30.0 (11.7)	47.6 (15.0)	207.5 (76.8)	101.4 (35.1)	40.0 (4.6)
p value		0.86	0.19	0.11	**< 0.01**	0.08
Bottom	Control	22.0 (6.1)	33.67 (8.9)	100.9 (34.1)	50.1 (14.6)	29.1 (17.2)
	WL	20.5 (5.1)	50.5 (22.6)	152.4 (111.9)	84.1 (46.9)	34.4 (13.3)
p value		0.60	0.14	0.71	0.09	0.22

Mean specific root length (m g^{-1}) and standard deviation of each group and species (n=7-9). The Wilcoxon-Mann-Whitney test was used to test the difference between groups within species at the top and bottom part, respectively.

Table 2-5 Root tissue density of fine roots obtained from in-growth core 1

Position	group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	0.23 (0.03)	0.29 (0.04)	0.59 (0.25)	0.40 (0.10)	0.46 (0.24)
	WL	0.19 (0.07)	0.21 (0.06)	0.26 (0.05)	0.18 (0.02)	0.36 (0.04)
p value		0.06	**< 0.05**	0.11	**< 0.001**	0.35
Bottom	Control	0.28 (0.06)	0.30 (0.04)	0.56 (0.13)	0.32 (0.07)	0.38 (0.11)
	WL	0.16 (0.02)	0.23 (0.07)	0.24 (0.08)	0.17 (0.03)	0.32 (0.06)
p value		**< 0.001**	**< 0.05**	0.11	**< 0.001**	0.06

Mean root tissue density (g cm^{-3}) and standard deviation of each group and species (n=7-9). The Wilcoxon-Mann-Whitney test was used to test the differences between groups within species at the top and bottom part, respectively.

Table 2-6 Root diameter of fine roots obtained from in-growth core 2

Position	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	0.47 (0.06)	0.36 (0.04)	0.21 (0.04)	0.32 (0.06)	0.34 (0.05)
	WL	0.37 (0.06)	0.38	0.22	0.34 (0.06)	0.30 (0.03)
p value		0.40	0.48	1.00	0.22	0.18
Bottom	Control	0.49 (0.09)	0.39 (0.06)	0.43 (0.37)	0.35 (0.03)	0.35 (0.10)
	WL	0.60 (0.15)	0.29 (0.14)	0.22	0.32 (0.08)	0.29 (0.08)
p value		0.23	0.35	1.00	0.71	0.38

Mean of root diameter (mm) and standard deviation of each group and species (n=3-9). The Wilcoxon-Mann-Whitney test was used to test the differences between groups within species at the top and bottom part, respectively.

Table 2-7 Specific root length of fine roots obtained from in-growth core 2

Position	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	27.3 (5.9)	27.6 (10.3)	76.9 (15.9)	54.6 (17.8)	43.6 (11.9)
	WL	27.1 (11.0)	33.5 (11.6)	81.4 (32.3)	39.2 (11.0)	43.4 (22.7)
p value		1.00	1.00	0.37	0.18	0.89
Bottom	Control	28.9 (10.4)	28.9	268.5	70.9 (21.5)	42.7 (11.6)
	WL	23.5 (0.2)	41.4 (15.1)	124.3	71.3 (30.9)	83.7 (39.0)
p value		0.79	0.50	0.72	0.12	**<0.05**

Mean specific root length (m g^{-1}) and standard deviation of each group and species (n=3-9). The Wilcoxon-Mann-Whitney test was used to test the differences between groups within species at the top and bottom part, respectively.

Table 2-8 Root tissue density of fine roots obtained from in-growth core 2

Position	Group	*Pinus thunbergii*	*Acer mono*	*Quercus serrata*	*Alnus hirsuta*	*Fraxinus mandshurica*
Top	Control	0.22 (0.05)	0.40 (0.11)	0.40 (0.11)	0.27 (0.06)	0.28 (0.02)
	WL	0.38 (0.03)	0.30	0.10	0.17 (0.02)	0.40 (0.19)
p value		0.42	0.36	0.37	**< 0.01**	0.23
Bottom	Control	0.22 (0.03)	0.29 (0.08)	0.33 (0.03)	0.29 (0.07)	0.35 (0.17)
	WL	0.20 (0.09)	0.24 (0.06)	0.23	0.20 (0.05)	0.24 (0.10)
p value		0.42	0.36	0.29	**< 0.05**	**< 0.05**

Mean of root tissue density (g cm^{-3}) and standard deviation of each group and species (n=3-9). The Wilcoxon-Mann-Whitney test was used to test the differences between groups within species at the top and bottom part, respectively.

Figures

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(a)

(b)

Figure 2-1 Experimental setup

Photos of the experimental setup. (a) is of seedlings grown at the nursey of Tohoku Research Center of FFPRI (Morioka City). (b) is of the waterlogging treatment where the water table was set at the soil surface; the photo is of *Pinus thunbergii*.

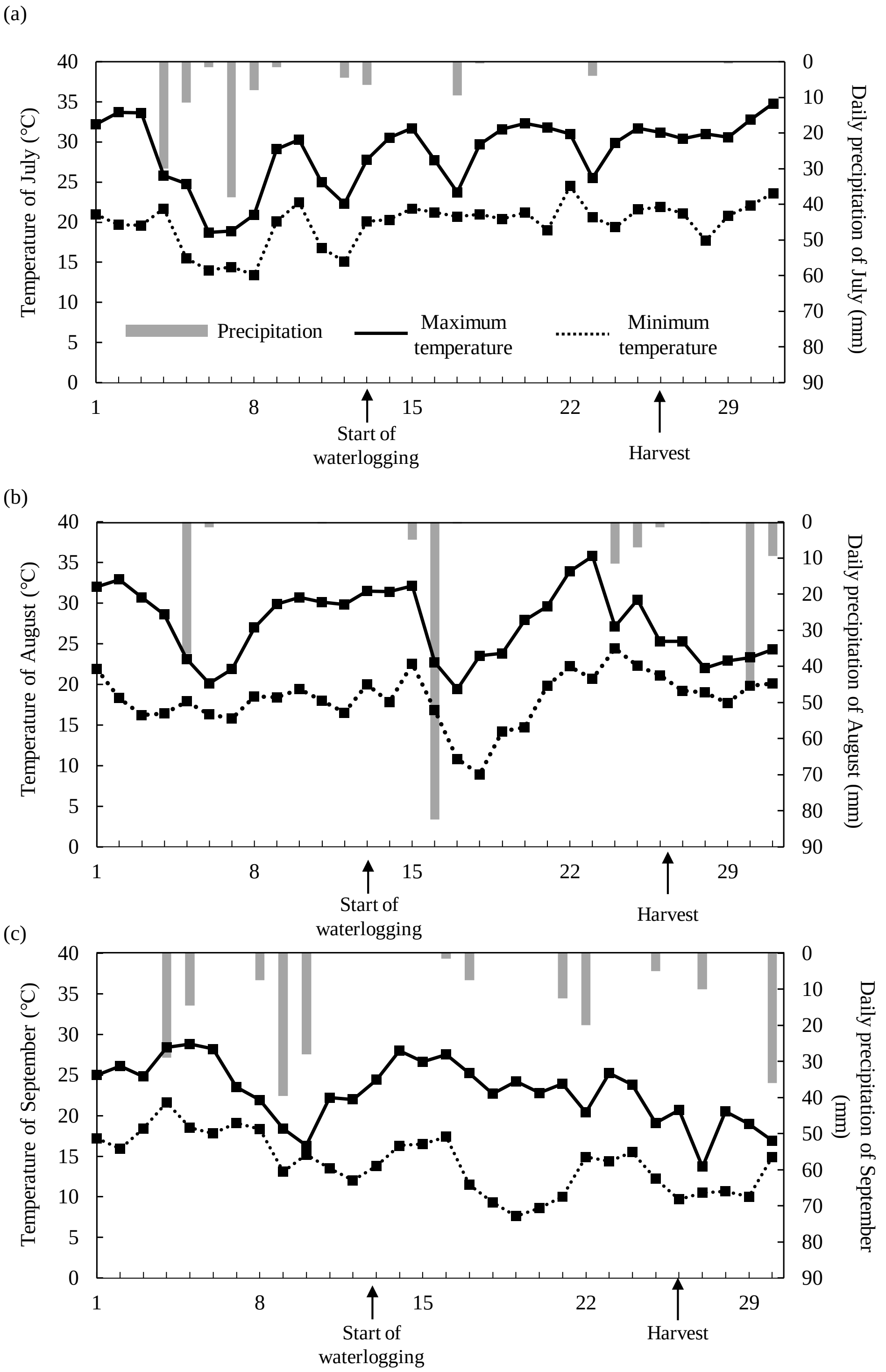


Figure 2-2 Temperature and precipitation during the experimental period

Daily maximum temperature (℃, solid line), daily minimum temperature (℃, dotted line), and daily precipitation (mm, gray bars) for (a) July, (b) August, and (c) September 2018. The horizontal axis is the date of each month. Data was obtained from meteorological observation at NARO Tohoku Agricultural Research (Morioka City, Iwate prefecture).

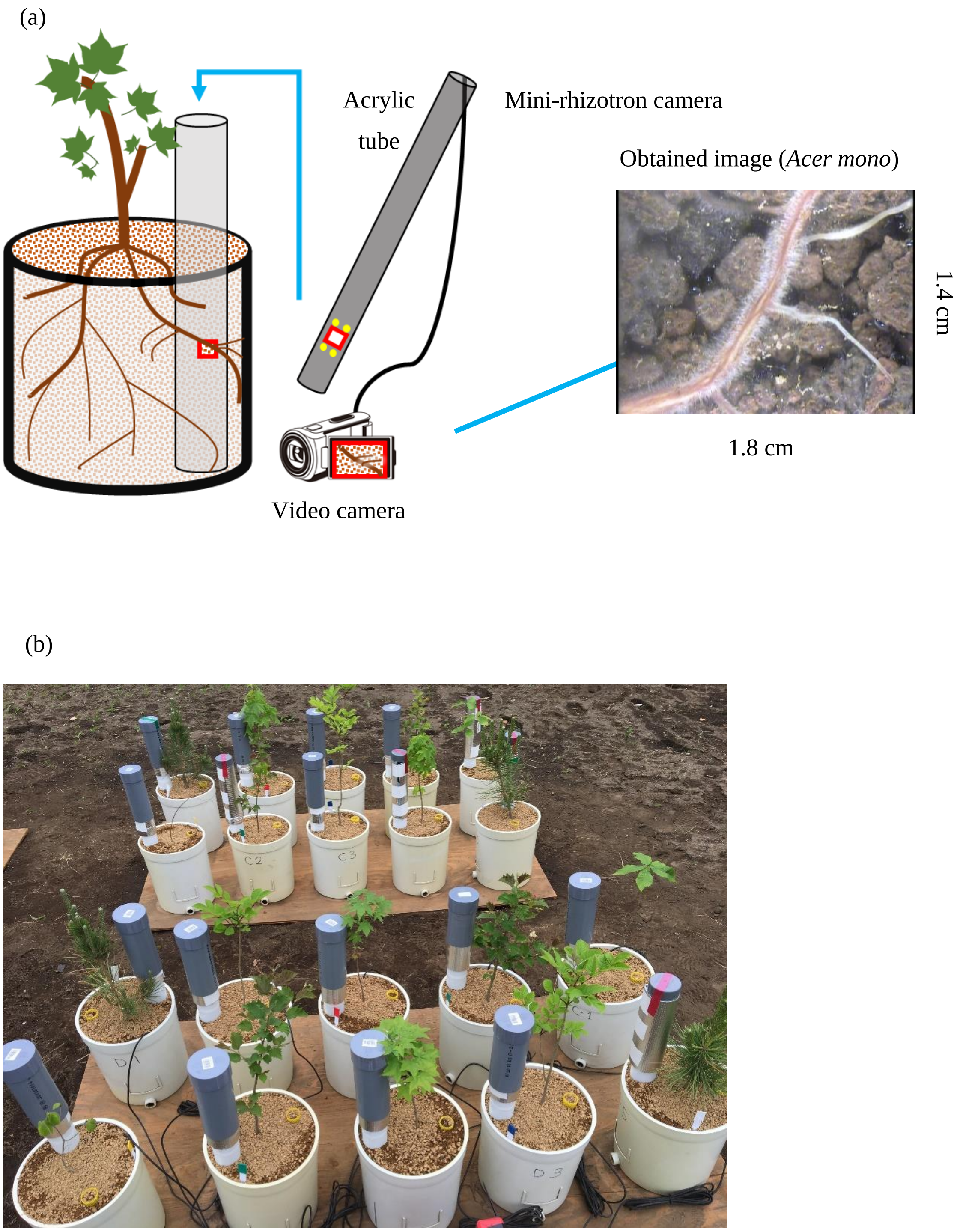
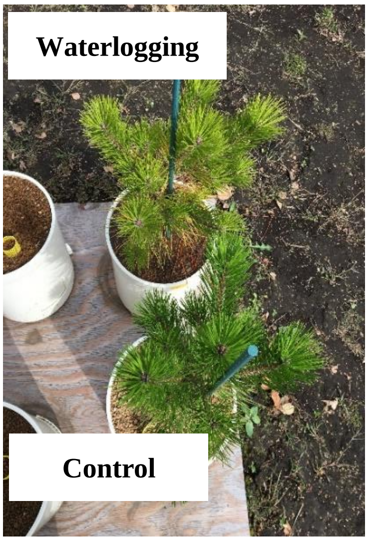


Figure 2-3 Experimental setup for mini-rhizotron method

Photos of the experimental setup. (a) is of the diagram of the mini-rhizotron method and example of obtained image. (b) is of the seedlings used for mini-rhizotron observation (June 8, 2018).

(a)



(b)

(c)

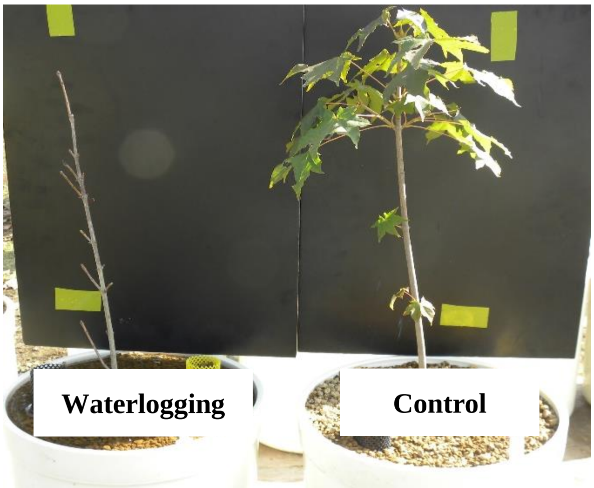


Figure 2-4 Visible symptoms of waterlogging on seedlings

Photos of the visible symptoms observed after waterlogging. (a) is of *Pinus thunbergii* seedlings of Control and waterlogging treatment after ten weeks. (b) is of *Acer mono* seedlings after two weeks of waterlogging. (c) is of *Acer mono* seedlings after eight weeks of waterlogging. For waterlogged seedlings, the shedding of leaves was observed.

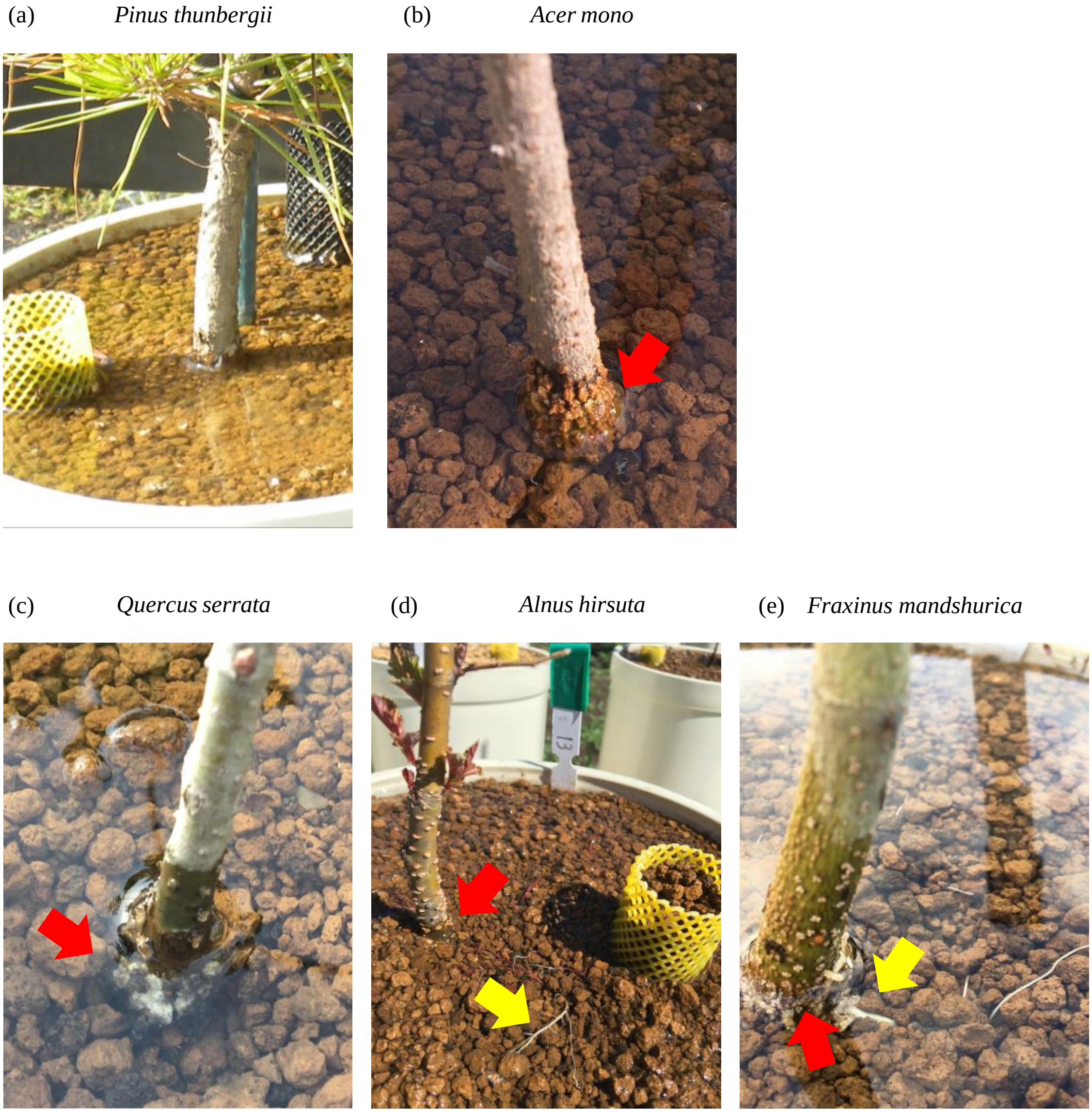
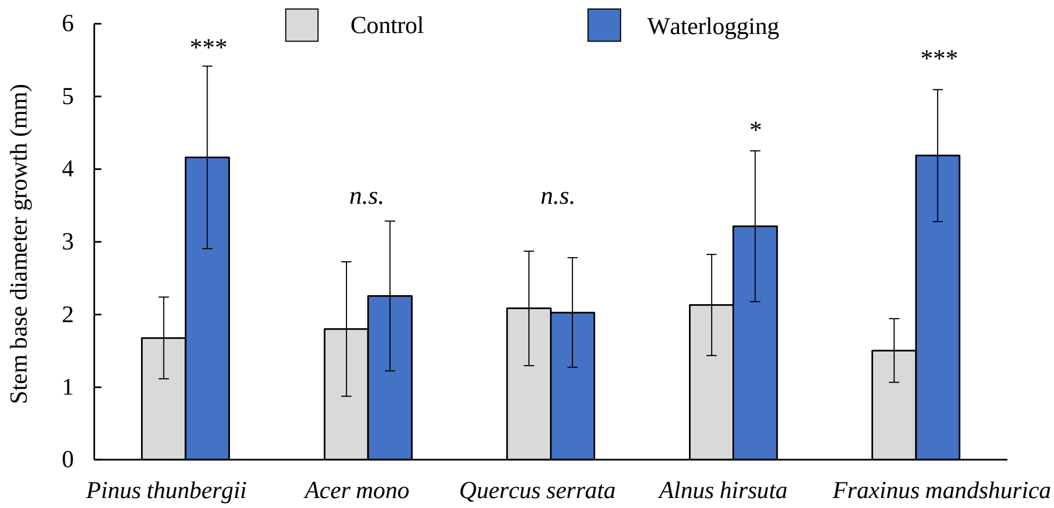


Figure 2-5 Stem base (near water table) of each species of waterlogging

Photos of the stem base of each species under waterlogging. Hypertrophied lenticels were observed on the four broadleaved species near the stem base under waterlogging (shown with red arrows). Roots could also be observed at the soil surface for *Alnus hirsuta* and *Fraxinus mandshurica* (shown with yellow arrows).

(a)



(b)

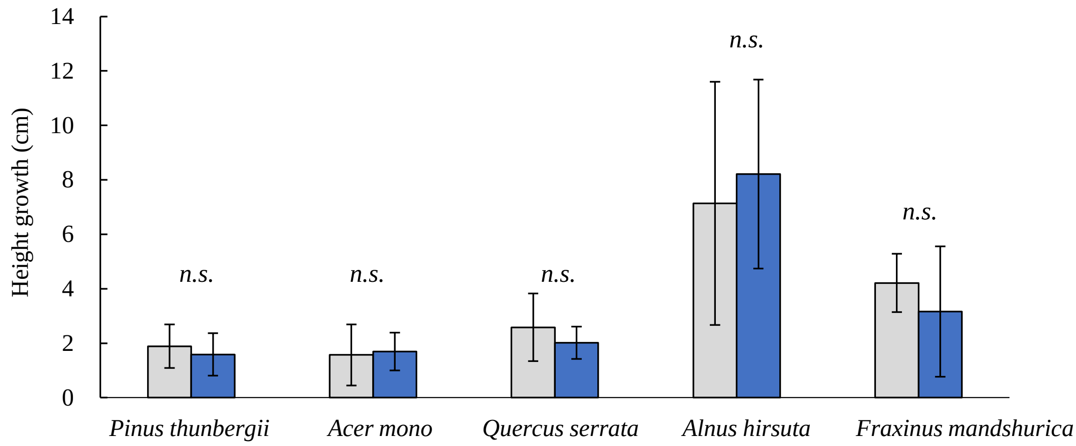


Figure 2-6 Seedling growth

Growth in (a) stem base diameter and (b) height of seedlings for Control and waterlogging group (WL). Growth was obtained by subtracting the value before waterlogging treatment from the final value at the end of the experiment. Results are mean values, and bars indicate standard deviation (n=12, except for *Quercus serrata*, whose Control and WL were n=6 and n=5, respectively). Results of the Wilcoxon-Mann-Whitney test are denoted as: *, $p < 0.05$; ***, $p < 0.001$.

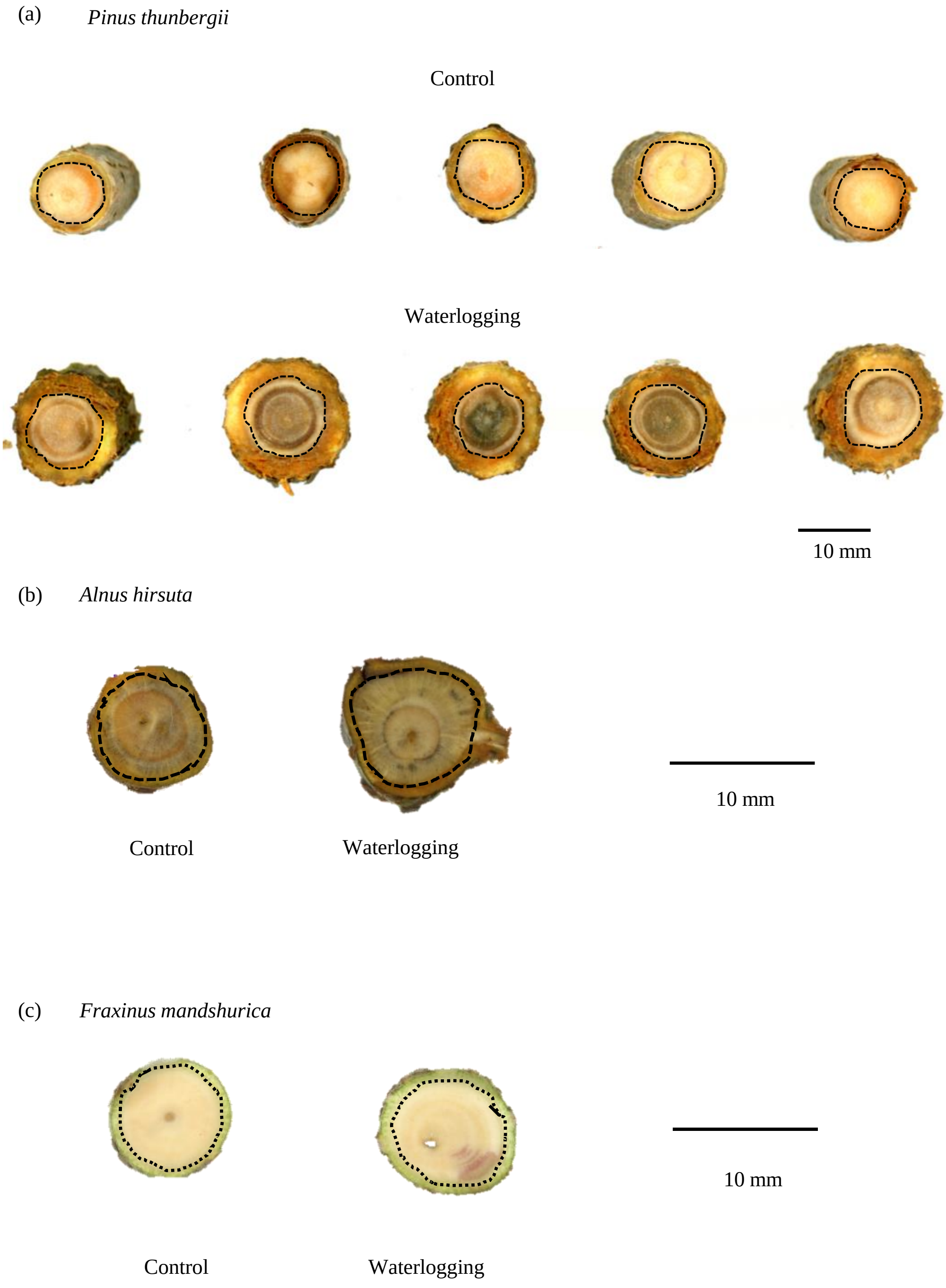


Figure 2-7 Cross section at stem base of seedlings

Scan images of the stem base of seedings for (a) *Pinus thunbergii*, (b) *Alnus hirsuta*, and (c) *Fraxinus mandshurica*. Scan images of the cross sections were made at the stem base. Approximately, inside the hatched lines are the xylem area and outside the hatched lines are the bark tissue.

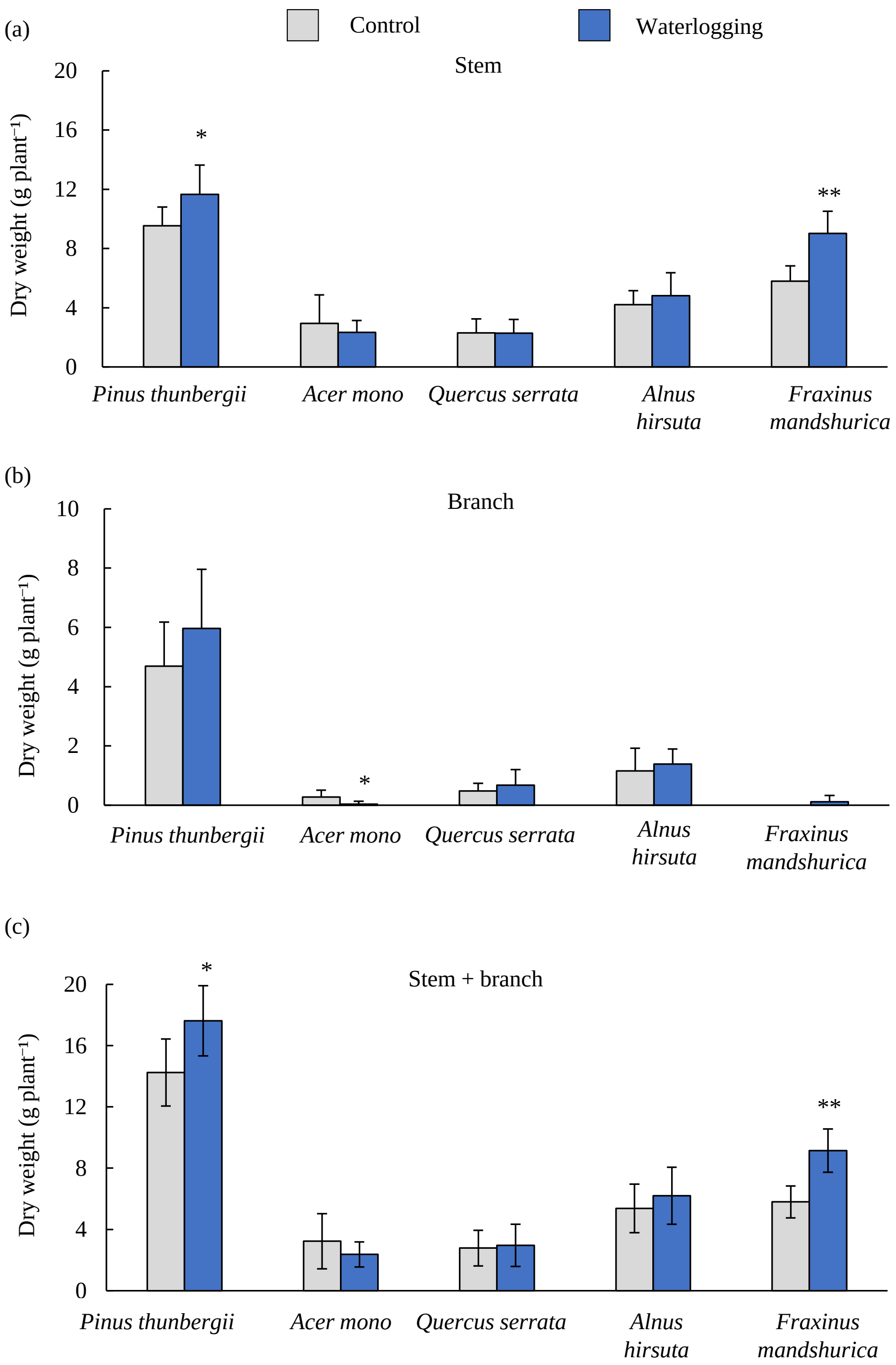


Figure 2-8 Seedling size in dry weight

Dry weight of (a) stem, (b) branch, and (c) stem + branch obtained from seedlings of Control and waterlogging group (WL). Results are mean values, and bars indicate standard deviation (n=9, except for *Quercus serrata*, whose Control and WL were n=5 and n=4, respectively). Results of the Wilcoxon-Mann-Whitney test are denoted as: *, p <0.05, **, $p < 0.01$; ***, $p < 0.001$.

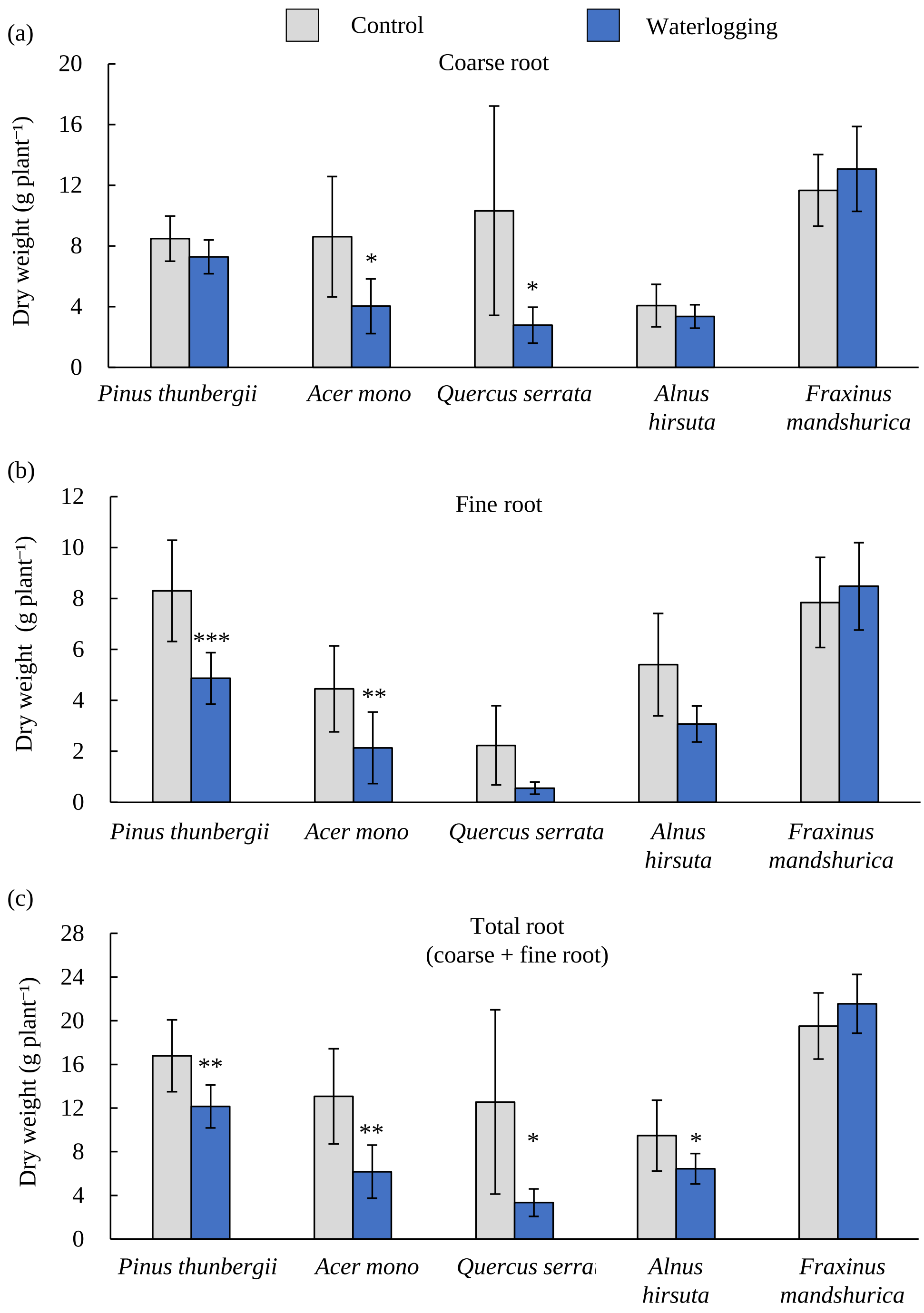


Figure 2-9 Dry weight of roots

Dry weight of the root systems of seedlings of Control and waterlogging group (WL) divided into (a) coarse roots (diameter > 2mm), (b) fine roots (diameter < 2mm), and (c) total root (coarse + fine root). Results are mean values, and bars indicate standard deviation (n=9, except for *Quercus serrata* where Control was n=5 and WL was n=4). Results of the Wilcoxon-Mann-Whitney test are denoted as: *, p <0.05, **, p < 0.01; ***, p < 0.001.

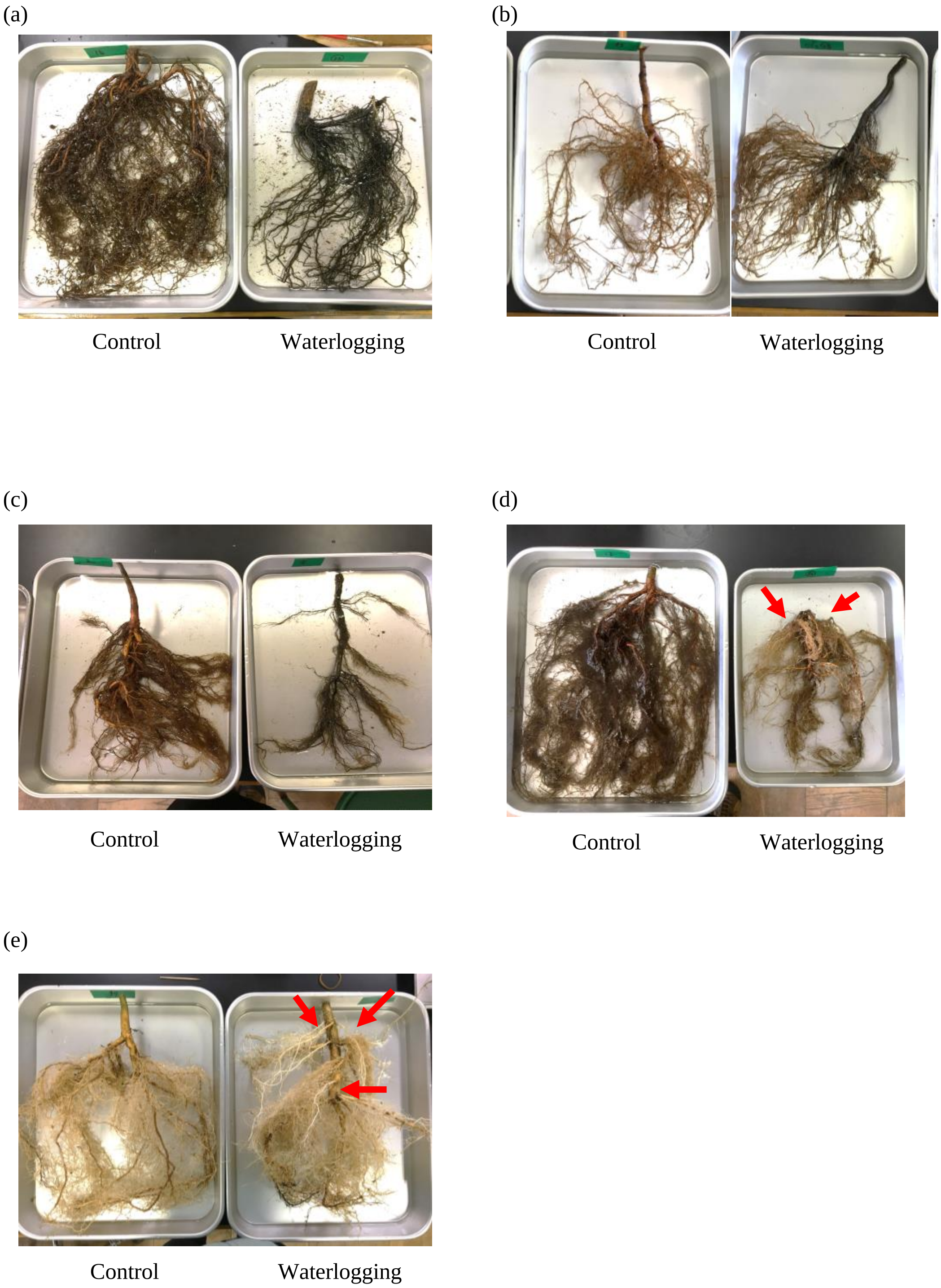


Figure 2-10 Whole root systems of seedlings

A representative image of the whole root system of (a) *Pinus thunbergii*, (b) *Acer mono*, (c) *Quercus serrata*, (d) *Alnus hirsuta*, and (e) *Fraxinus mandshurica* for Control and waterlogging group (WL). Red arrows show roots that were determined to have grown during the waterlogging treatment.

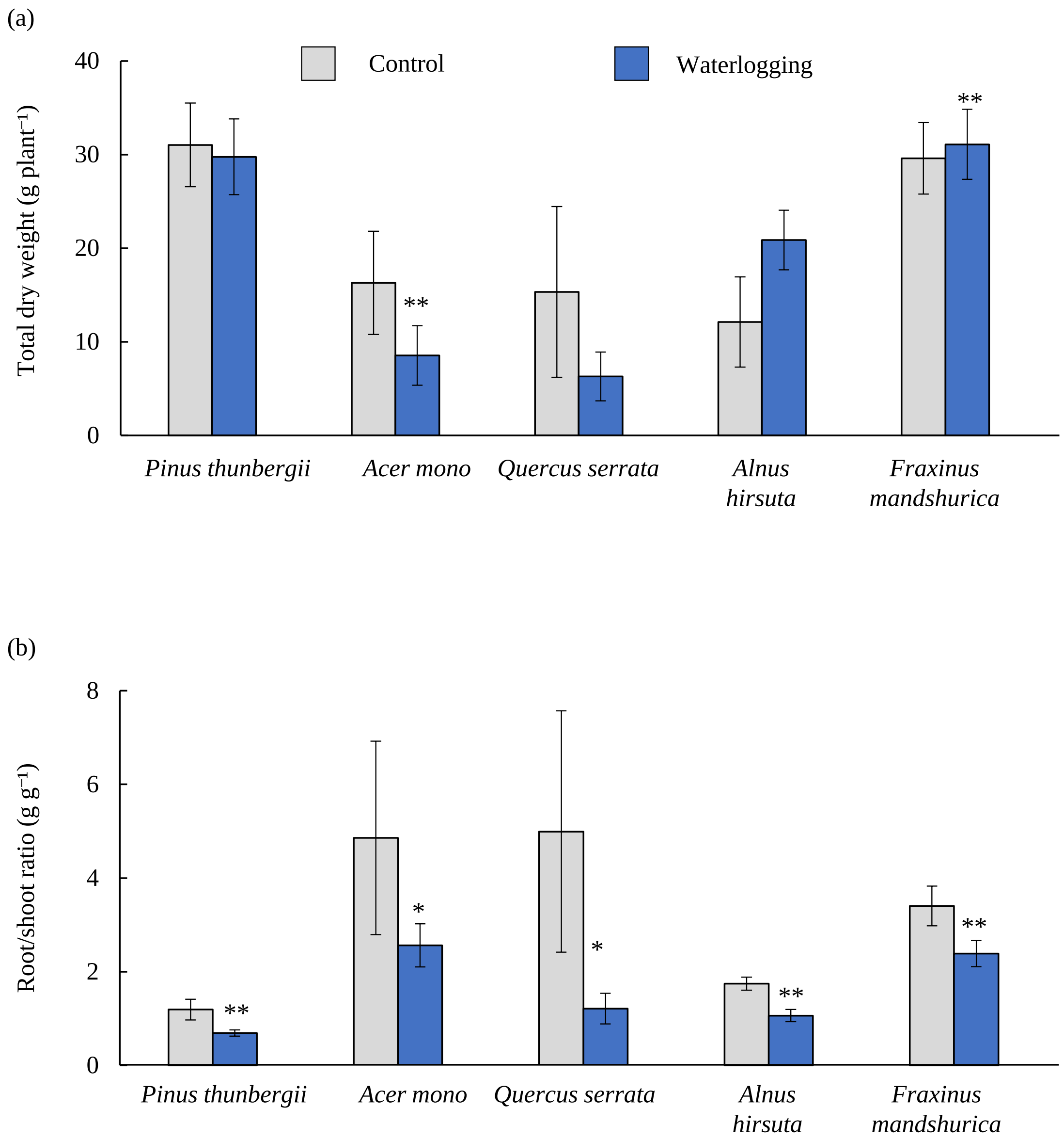


Figure 2-11 Root/shoot ratio

Dry weight of (a) total dry weight (stem + branch + total root) and (b) root/shoot ratio obtained from seedlings of Control and waterlogging group (WL). Results are mean values, and bars indicate standard deviation (n=9, except for *Quercus serrata*, whose Control and WL were n=5 and n=4, respectively). Results of the Wilcoxon-Mann-Whitney test are denoted as: *, p <0.05, **, p < 0.01.

Figure 2-12 *Pinus thunbergii* seedlings of waterlogging group after winter

Pinus thunbergii seedlings from the waterlogging treatment which were left to over winter under natural conditions at the nursery. All three seedlings were determined as dead the following year (photo taken on September 9, 2019).

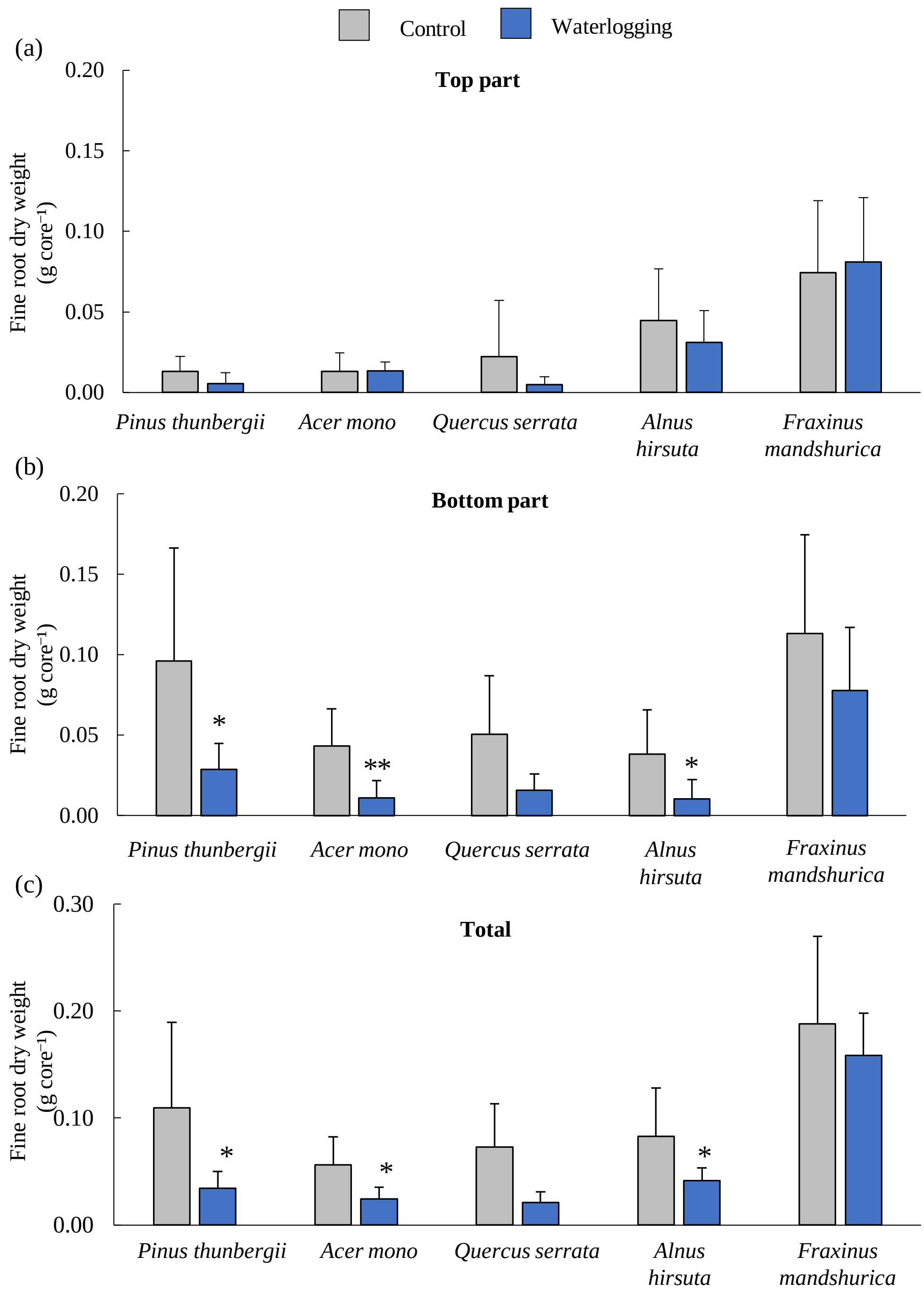


Figure 2-13-1 Dry weight of fine roots obtained from in-growth core 1

Dry weight of fine roots obtained from in-growth core 1 of Control and waterlogging group (WL) for the (a) top part, (b) bottom part, and (c) total (top + bottom). Results are mean values, and bars indicate standard deviation (n=7-9, except for *Q. serrata*, whose Control and waterlogging treatment were n=5 and n=4, respectively. The Wilcoxon-Mann-Whitney test was used to test the statistical differences between Control and WL (*, $p < 0.05$, **, $p < 0.01$).

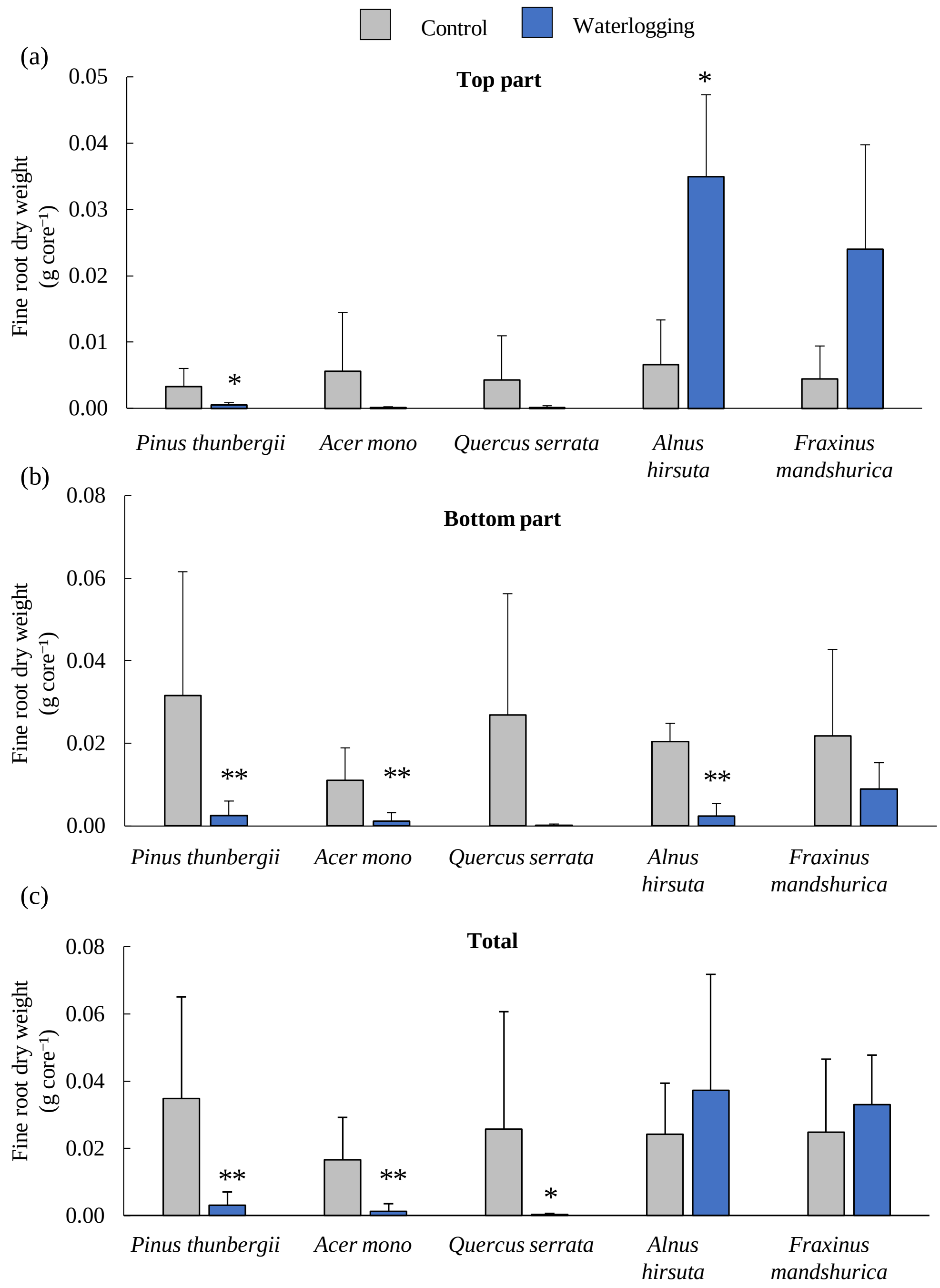


Figure 2-13-2 Dry weight of fine roots obtained from in-growth core 2

Dry weight of fine roots obtained from in-growth core 2 of Control and waterlogging group (WL) for the (a) top part, (b) bottom part, and (c) total (top + bottom). Results are mean values, and bars indicate standard deviation (n=3-9, except for *Q. serrata*, whose Control and waterlogging treatment were n=5 and n=4, respectively). The Wilcoxon-Mann-Whitney test was used to test the statistical differences between Control and WL (*, $p < 0.05$, **, $p < 0.01$).

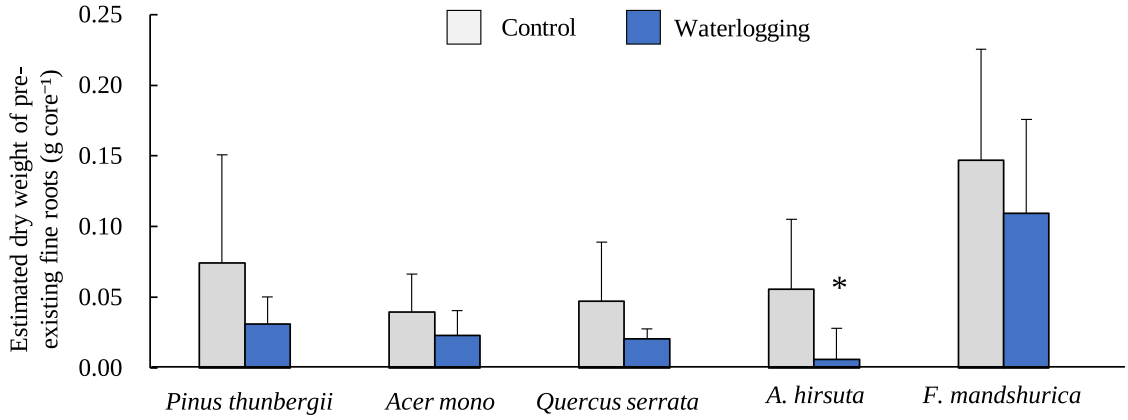


Figure 2-13-3 Net amount of fine roots

The net amount of fine roots (fine roots that were present before the waterlogging treatment started) was estimated by subtracting the dry weight of fine roots obtained at in-growth core 1 (fine roots that grew from the start to the end of the experiment) from that of in-growth core 2 (fine roots that grew only during the waterlogging treatment. Results are mean values, and bars indicate standard deviation (*n*=3-9, except for *Q. serrata*, whose Control and waterlogging treatment were *n*=5 and *n*=4, respectively). The Wilcoxon-Mann-Whitney test was used to test the statistical differences between Control and WL (*, $p < 0.05$).

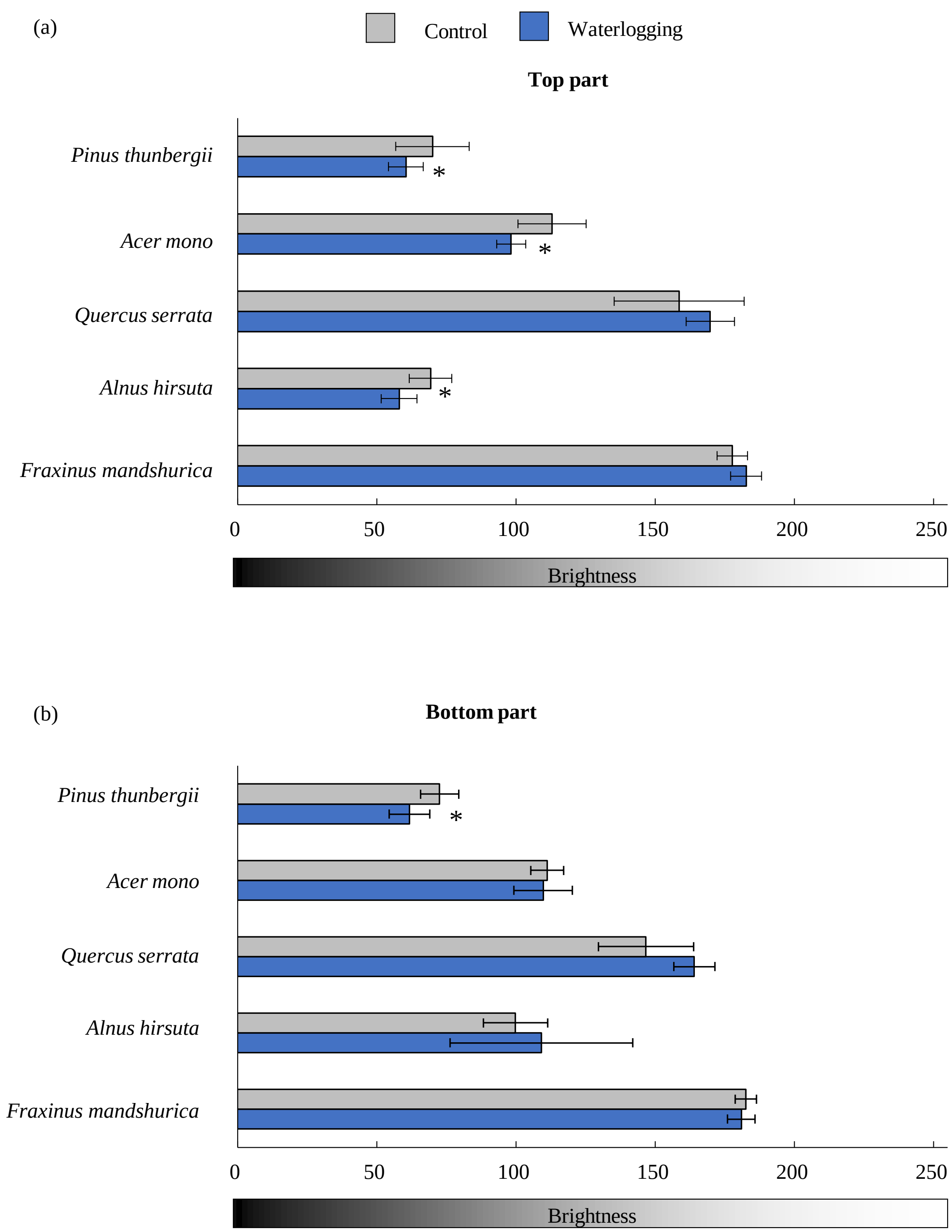


Figure 2-14 Mode brightness of fine roots obtained from in-growth core 1

Mode brightness for the (a) top part and (b) bottom part of Control and waterlogging group. Results are mean values and bars indicate standard deviation (*n*=7-9, except for *Q. serrata*, whose Control and waterlogging treatment were *n*=5 and *n*=4, respectively). *p* values of the Wilcoxon-Mann-Whitney test are denoted as, *, $p < 0.05$.

(a)

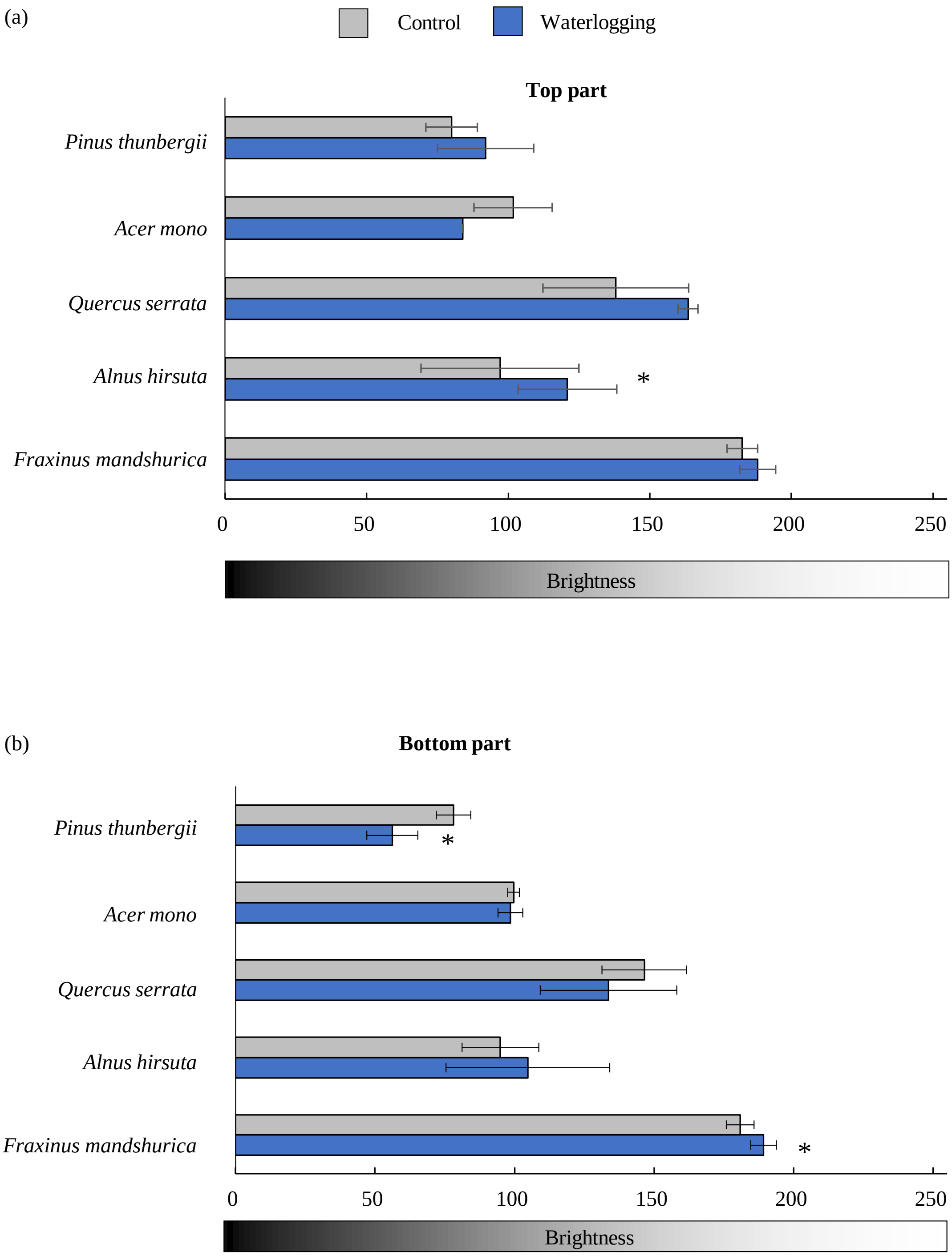


Figure 2-15 Mode brightness of fine roots obtained from in-growth core 2

Mode brightness for the (a) top part and (b) bottom part of Control and waterlogging group. Results are mean values and bars indicate standard deviation (n=3-9, except for *Q. serrata*, whose Control and waterlogging treatment were n=5 and n=4, respectively). p values of the Wilcoxon-Mann-Whitney test are denoted as, *, $p < 0.05$.

3 Responses of *Pinus thunbergii* and *Quercus serrata* seedlings to two waterlogging depths

3.1 Introduction

At coastal forest restoration sites of the Tohoku region, measures such as plowing and the construction of open ditches are being carried out on a trial basis to soften the soil and improve drainage and permeability of the growth bases (Ono et al., 2016). These measures are also expected to improve waterlogging conditions in terms of waterlogging depth and duration. Although these measures are expected to additionally improve the root growth of seedlings through the improvement of soil conditions, evaluations are yet to be made on responses of *P. thunbergii* to different waterlogging depths.

It has been reported that when waterlogging depth is low, the negative effects of waterlogging are smaller compared to when the waterlogging depth is high (Dresbøll et al., 2013; Dreyer, 1994; Jiang and Wang, 2006). When *Quercus robur*, *Quercus rubra*, and *Fagus silvatica* seedlings were submitted to partial waterlogging (water table 6 cm in depth from the soil surface), disorders such as root decay, partial leaf wilting, and the decrease in water potential and photosynthesis rates were mild compared to when the root systems were fully submitted to submergence (water table = soil surface) for four weeks (Dreyer, 1994).

Malik et al. (2001) reported that fine root growth rates of wheat after fourteen days from drainage differed according to the waterlogging depth. Although wheat grown in waterlogged soil at 20 cm below the soil surface (partial waterlogging) fully recovered, those grown in waterlogged soil to the soil surface (full waterlogging) or 10 cm below the soil surface (partial waterlogging) showed limited recovery. Therefore, it is suggested that when only the bottom part of the root system is exposed to waterlogging (partial waterlogging), the negative effects of waterlogging are smaller compared to when the whole root system is exposed to waterlogging.

Concerning root growth under partial waterlogging, it has been reported that root growth rates in waterlogged soil were decreased, while root growth rates grown in soil above the waterlogged zone were increased (Dresbøll et al., 2013). In this study, it was discussed that the increase in root growth rates above the waterlogged zone worked as compensation for the damaged roots exposed to waterlogging. Therefore, it is suggested that fine root growth distribution will change under partial waterlogging.

From field survey of the damaged coastal sites, the relationship between groundwater depth and the root system development of mature *P. thunbergii* trees has been reported (Forestry Agency Tohoku Regional Forest Office 2011; Sakamoto 2012; Tamura 2012; Hirano et al. 2018). It was reported that when the groundwater depth is high, root system development in the vertical direction is limited, and roots mainly grow in the horizontal direction. Furthermore, fine root growth under waterlogging was severely decreased and was small compared to Control (Fig. 2-13-2). Therefore, it is suggested that when only the bottom part of the root system is exposed to waterlogging (partial waterlogging), fine root growth will only continue at the top part of the root system where it is not waterlogged.

This chapter focuses on responses of fine root growth distribution and its relation to the aboveground physiological activity.

3.2 Materials and Methods

3.1.1 Plant materials and experimental setup

Two-year-old *P. thunbergii* and *Q. serrata* seedlings were purchased from a commercial tree nursery. At the end of March 2019, 45 seedlings per species were transplanted in 1/2000 *a* Wagner pots (500 cm², in surface area, 30 cm, in-depth with a drainage hole near the bottom) with Akadama soil deriving from the loamy B horizon of Andisol. The soil surface was set at 26 cm in height from the bottom of the pot. After transplanting, the seedlings were grown under natural conditions at the experimental nursery of Tohoku Research Center, Forestry and Forest Products Research Institute (FFPRI) located in Morioka City, Iwate prefecture (Fig 3-1 (a)). The results of Chapter 2 found that both *P. thunbergii* and *Q. serrata* were relatively sensitive to waterlogging. Furthermore, *Q. serrata* was chosen with *P. thunbergii* as it was found from mini-rhizotron observations that both species continued fine root growth until the beginning of September (Fig. 2-16-1, 2-16-2).

Two weeks after transplanting, 1 L of 2000-fold diluted liquid fertilizer (Hyponex 6-10-5, Hyponex Japan, Osaka, Japan) was applied. The amount of nutrients given per pot was approximately 30, 50, and 25 mg of N, P, and K, respectively. The pots were placed on wooden boards and were watered regularly (once a week, 2-4 L) until the waterlogging treatment began. The seedlings were distributed to three groups (each group consisted of fifteen seedlings) before the waterlogging treatment so that the seedling size did not differ among the groups (Table 3-1). Fig. 3-

2 shows the daily maximum temperature, daily minimum temperature, and daily precipitation from August to September 2019.

In this experiment, two different waterlogging depths were set:

1) partial waterlogging group (Partial-WL)

The waterlogging depth was set at 15 cm from the bottom of the pot. A plastic tube was attached to the drainage hole, and the end of the tube was set at 15 cm in the height of the pot so that the water would overflow from the tube mouth at the set depth (Fig. 3-1 (b)). For this treatment, 0-11 cm in depth from the soil surface was free from waterlogging.

2) full waterlogging group (Full-WL)

The drainage hole near the bottom was blocked with a cork, and water was added until the water table reached the soil surface (waterlogging depth= 26 cm).

The waterlogging depth was maintained at one to two-day intervals by slowly adding water from the soil surface. For Control, the drainage hole was kept open and was watered regularly throughout the experiment period according to the weather (at least once a week, 2-4 L). For *P. thunbergii*, the waterlogging treatments started on August 8 and lasted until October 4, 2019 (eight weeks of waterlogging). For *Q. serrata*, the waterlogging treatments started on August 5 and lasted until September 20, 2019 (six weeks of waterlogging).

At the end of the experiment, ten seedlings out of fifteen of each treatment were harvested (*P. thunbergii*: October 4, *Q. serrata*: September 20). The aboveground organs and roots were dried at 70°C for 72 hours and then measured for dry weight. The roots were divided into coarse roots (diameter > 2 mm) and fine roots (diameter < 2 mm) by root diameter before drying. The other five seedlings per treatment were released from waterlogging and grown under natural conditions until the following spring.

3.1.2 Soil oxidation-reduction potential (Experiment in 2019)

The soil oxidation-reduction potential (Eh, mV) was measured by a platinum electrode, a reference electrode, and a logger (FV-702, Fujiwara Scientific Co., Ltd., Tokyo, Japan). The two probes were set in pots at about 10 cm depth from the soil surface (Fig 3-1 (c)). Probes were set for Control and Full-WL (n=2). The Eh values were recorded at two-to-five-day intervals. On measurement days when water was added, the Eh values were recorded before water addition.

3.1.3 Fine root measurements (Experiment in 2019)

In total, three in-growth cores (diameter, 32 mm; depth, 30 cm; 2 mm mesh) were used to distinctively measure fine root growth. All in-growth cores were placed in the pot at the time of transplanting. In each pot, two in-growth cores were placed on each side of the seedling, about 10 cm apart from the seedling (Fig. 3-1 (c)). These two IG_1s were used to measure fine root growth during the whole experimental period. The third in-growth core (IG_2) was also placed about 10 cm from the seedling, perpendicular to the other two IG_1s. This in-growth core was used to measure fine root growth during the waterlogging treatment. At transplantation, IG_2 was covered with a thin plastic sheet to prevent root penetration before the waterlogging treatment. The plastic sheet was carefully taken away right before the waterlogging treatment was started, enabling fine roots to penetrate IG_2 only during the waterlogging treatment period.

A fourth in-growth core was planned for measuring fine root growth after the release from waterlogging. However, as the waterlogging treatment was started later than the initial experimental plan, a waterlogging-free period after the waterlogging treatment could not be secured, and only three in-growth cores were used in this experiment.

All in-growth cores were harvested on September 20 for *Q. serrata* and October 4 for *P. thunbergii*. In-growth cores were stored at 4°C until further analysis. For the analysis, the in-growth cores were separated into two sections, the "top part (0-11 cm in depth)" and the "bottom part (11-26 cm in depth)", and the fine roots obtained from each part were evaluated separately. The soil from the in-growth cores was thoroughly washed out with water on a very fine sieve (sieve aperture, 250 μm). Fine roots (diameter < 2 mm) were carefully picked from the sieve and washed with a brush. After washing, fine roots were scanned with a flatbed scanner (GT-X980, EPSON) at 800 dpi. After scanning, fine roots were dried at 70 °C for 72 hours and then measured for dry weight.

Fine root growth was determined from the fine root dry weight of IG_1 and IG_2. As there were two IG_1s, fine root dry weight, mean root diameter, root tissue density (RTD), and root specific length (SRL) was calculated by adding the values of the two in-growth cores and dividing it by the soil volume of the two in-growth cores. Evaluation methods on mean root diameter, RTD, SRL, and fine root brightness are described in Chapter 2 (refer to section 2.2.3).

3.1.4 Transpiration (Experiment in 2019)

Transpiration rates of *P. thunbergii* were estimated by measuring the evapotranspiration and the evaporation from pots, where the former and the latter were estimated by weighing water loss from pots with seedlings (n=15 per group) and without seedlings (n=3), respectively. Transpiration measurements were made on sunny days without rain. Waterlogging was temporally released for 1.5 days for Partial-WL and Full-WL before measurements. After temporal release of waterlogging, all pots were watered until the water ran out from the drainage hole near the bottom to ensure all pots were well-watered. Weight measurements were done during nighttime (20:00-21:30) to minimize transpiration and evaporation while measuring the pots. The pots were measured for the first weight (before evapotranspiration) and were re-weighed the next night for the second weight (after evapotranspiration). Transpiration per seedling (T, kg pot^{-1}) was estimated from the following equation by using the first weight and the second weight of the pots with seedlings (M_{s1}, M_{s2}) and without seedlings (M_{ws1}, M_{ws2});

$$\boldsymbol{T\ (kg\ pot^{-1}) = \{M_{s1}(kg\ pot^{-1}) - M_{s2}(kg\ pot^{-1})\} - \{M_{ws1}(kg\ pot^{-1}) - M_{ws2}(kg\ pot^{-1})\}}$$

"$M_{S1} – M_{S2}$" and "$M_{WS1} – M_{WS2}$" correspond to evapotranspiration and evaporation, respectively. The average value of the three pots without seedlings was used for evaporation. For six pots of Full-WL, transpiration after four weeks and eight weeks of waterlogging showed negative values, where the averaged evaporation exceeded evapotranspiration (from -0.03 kg to -0.005 kg). For these six pots, transpiration was recorded as zero. After weighing, the pots of Partial-WL and Full-WL were refilled with water. The measurements were done on August 6-7 (before waterlogging), September 3-4 (after four weeks of waterlogging), October 2-3 (after eight weeks of waterlogging, at the end of the waterlogging treatment).

Transpiration rates of *Q. serrata* were measured by a porometer (LI-1600, Li-Cor Inc., Lincoln, NE, USA). The transpiration measurements were done from 10:00 to 14:00 on sunny days without rain. One leaf was chosen for each seedling from the upper part of the seedling crown, and the same leaf was measured for the transpiration rates throughout the experiment. The porometer was set on the leaf for at least one to three minutes to stabilize transpiration rates. At the end of July, the secondary flush of leaves was observed on some seedlings. For these seedlings, one additional leaf was chosen and was measured from September 3 until the end of the experiment. For the five seedlings left to over-winter, the transpiration rate was continuously measured on September 27 (seven days after the release from waterlogging) and October 1 (eleven days after the release from waterlogging).

3.1.5 Leaf traits (Experiment in 2019)

Leaf water content (LWC) and leaf mass per area (LMA) were calculated from leaf area, fresh weight, and dry weight of leaves. For *P. thunbergii*, current-year needles were randomly sampled (20-30 needles, dry weight 0.5-1.0 g) from the top part of the current-year shoot at harvest. For *Q. serrata*, fresh weight and dry weight of leaves were measured for leaves that were measured for transpiration. The sampled leaves were scanned at 800 dpi and dried at 80°C for 72 hours. LWC was calculated as follows using W_f (fresh weight, g) and W_d (dry weight, g),

$$LWC\ (\%) = \frac{W_f - W_d}{W_f} \times 100$$

Leaf area was calculated as leaf projected area (A_{pn}, m²), which was obtained by Image J (ver. 1.51). LMA was calculated as follows,

$$LMA\ (g\ m^{-2}) = \frac{W_d}{A_{pn}}$$

Current-year needles were separated from one-year and older year needles and further divided into green or, brown and red, then dried with other aboveground organs at 80°C for 72 hours. The total aboveground (stem, branches, and needles according to color) was also measured for dry weight.

3.1.6 Additional Experiment in 2020

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3.2 Results

3.2.1 Soil oxidation-reduction potential (Experiment in 2019)

The relationship between the Full-WL treatment and the soil oxidation-reduction potential (Eh) did not differ between both species, and the waterlogging treatment showed a tendency to decrease Eh. However, changes in Eh values for Full-WL treatment differed between #1 and #2, and fluctuations were observed throughout the experiment for both species. The different responses may be influenced by the amount of fine roots located near the probes; where more fine roots are located near the probes, oxygen consumption is suggested to be higher, resulting in a faster decrease and lower Eh values. However, the reasons for the increase in Eh observed under waterlogging (unrelated to release from waterlogging) are unclear. A possible explanation is the effect of oxygen input through rainfall.

For *P. thunbergii*, Eh of Control #1 and Control #2, Eh was about 500-550 mV throughout the experiment. Eh of Full-WL got low compared to Control about five to seven days after the waterlogging treatment was started and was about below 300 mV throughout the experiment, except when it increased due to the temporal release of waterlogging treatment for transpiration measurement (Fig. 3-3 (a)). This result indicated that the full-waterlogging treatment was creating a hypoxic condition. After refilling the pot with water, the Eh of Full-WL #1 became low compared to Control within one week, but the decrease of Eh for Full-WL #2 was moderate compared to Full-WL #1.

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3.2.2 Dry weight of seedlings (Experiment 2019)

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3.2.3 Fine root growth (Experiment in 2019)

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3.2.4 Fine root morphology and color (Experiment in 2019)

The change in mean root diameter, specific root length (SRL), and root tissue density (RTD) under waterlogging was evaluated by comparison of fine roots obtained from IG_I of each group. For *P. thunbergii*, at the bottom part, where it was waterlogged for Partial-WL and Full-WL, mean root diameters were larger than Control, and RTD of Full-WL was smaller than Control (Table 3-3). The value of fine root brightness became low under waterlogging. This matched with the results obtained in Chapter 2, where the darkening of fine roots was observed under waterlogging. In detail, fine root brightness of Full-WL at the top part was low compared to Control, and at the bottom part, fine root brightness of Partial-WL and Full-WL was low compared to Control (Fig. 3-9). On the other hand, fine root brightness of Partial-WL was high at the top part compared to Control and Full-WL, indicating the abundant presence of light-colored newly grown fine roots.

To evaluate changes in fine root morphology and color of fine roots grown under waterlogging, the fine roots sampled from IG_2 were evaluated among the groups (Table 3-4). As a result, significant differences in mean root diameter, RTD, and fine root brightness were detected for *P. thunbergii*. Mean root diameter of Partial-WL at the top part was large compared to Control. RTD of Partial-WL was small at the bottom part compared to Full-WL. Although fine root brightness at the top part did not differ among the groups, fine root brightness of Partial-WL and Full-WL was low compared to Control at the bottom part, where it was waterlogged (Fig. 3-10).

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3.2.5 Transpiration and leaf traits (Experiment in 2019)

Transpiration rates of *P. thunbergii* were measured 1) before waterlogging, 2) after four weeks, and 3) after eight weeks of waterlogging to estimate the response of fine root water absorption function to two waterlogging depths.

Before the waterlogging treatment, transpiration rates did not differ among the groups (Fig. 3-13). After four weeks of waterlogging, transpiration of Partial-WL and Full-WL was low compared to Control, and transpiration of Full-WL was also low compared to Partial-WL. After eight weeks of waterlogging, only transpiration rates of Full-WL were low compared to Control and Partial-WL. There was no significant difference between Control and Partial-WL, and values of Partial-WL had recovered to values that did not differ from Control.

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3.2.6 Results of Experiment in 2020

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3.3 Discussion

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3.4 Conclusions

Under conditions where the root system is exposed to partial waterlogging (vertical direction), *Pinus thunbergii* seedlings were able to recover with time (approximately one month). This recovery in transpiration was enabled by the change in vertical fine root growth distribution, where fine root growth increased at the non-waterlogged top part.

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Tables

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Table 3-3 Root morphology of fine roots obtained from in-growth core 1 of *Pinus thunbergii*

Position	Groups	Mean diameter (mm)	Specific root length (m g^{-1})	Root tissue density (g cm^{-3})
Top part	Control	0.45 (0.08) *a*	27.0 (5.4) *a*	0.25 (0.04) *a*
	Partial-WL	0.52 (0.04) *a*	23.7 (12.0) *a*	0.24 (0.08) *a*
	Full-WL	0.52 (0.05) *a*	22.5 (4.1) *a*	0.22 (0.02) *a*
Bottom part	Control	0.42 (0.04) *a*	26.2 (6.4) *a*	0.29 (0.06) *a*
	Partial-WL	0.49 (0.05) *b*	23.3 (3.2) *a*	0.24 (0.03) *ab*
	Full-WL	0.50 (0.05) *b*	22.3 (5.5) *a*	0.24 (0.02) *b*

Mean root diameter, specific root length and root tissue density of each group at the top and bottom part. Values are means and the standard deviations (n=15). The letters show statistical differences among the groups at the top and bottom part, respectively (Tukey-Kramer or Steel Dwass test, p< 0.05).

Table 3-4 Root morphology of fine roots obtained from ingrowth core 2 of *Pinus thunbergii*

Position	Treatment	Mean diameter (mm)	Specific root length (m g^{-1})	Root tissue density (g cm^{-3})
Top part	Control	0.40 (0.05) *a*	37.3 (9.9) *a*	0.23 (0.03) *a*
	Partial-WL	0.51 (0.05) *b*	28.1 (6.0) *a*	0.23 (0.14) *a*
	Full-WL	0.67 (0.26) *ab*	27.7 (9.1) *a*	0.14 (0.06) *a*
Bottom part	Control	0.41 (0.06) *a*	36.4 (11.8) *a*	0.22 (0.03) *ab*
	Partial-WL	0.53 (0.13) *a*	30.8 (12.0) *a*	0.19 (0.06) *b*
	Full-WL	0.46 (0.10) *a*	24.3 (7.7) *a*	0.27 (0.05) *a*

Mean root diameter, specific root length and root tissue density of each group at the top and bottom part. Values are means and the standard deviations (n=15). The letters show statistical differences among the groups at the top and bottom part, respectively (Tukey-Kramer or Steel Dwass test, p< 0.05).

Figures

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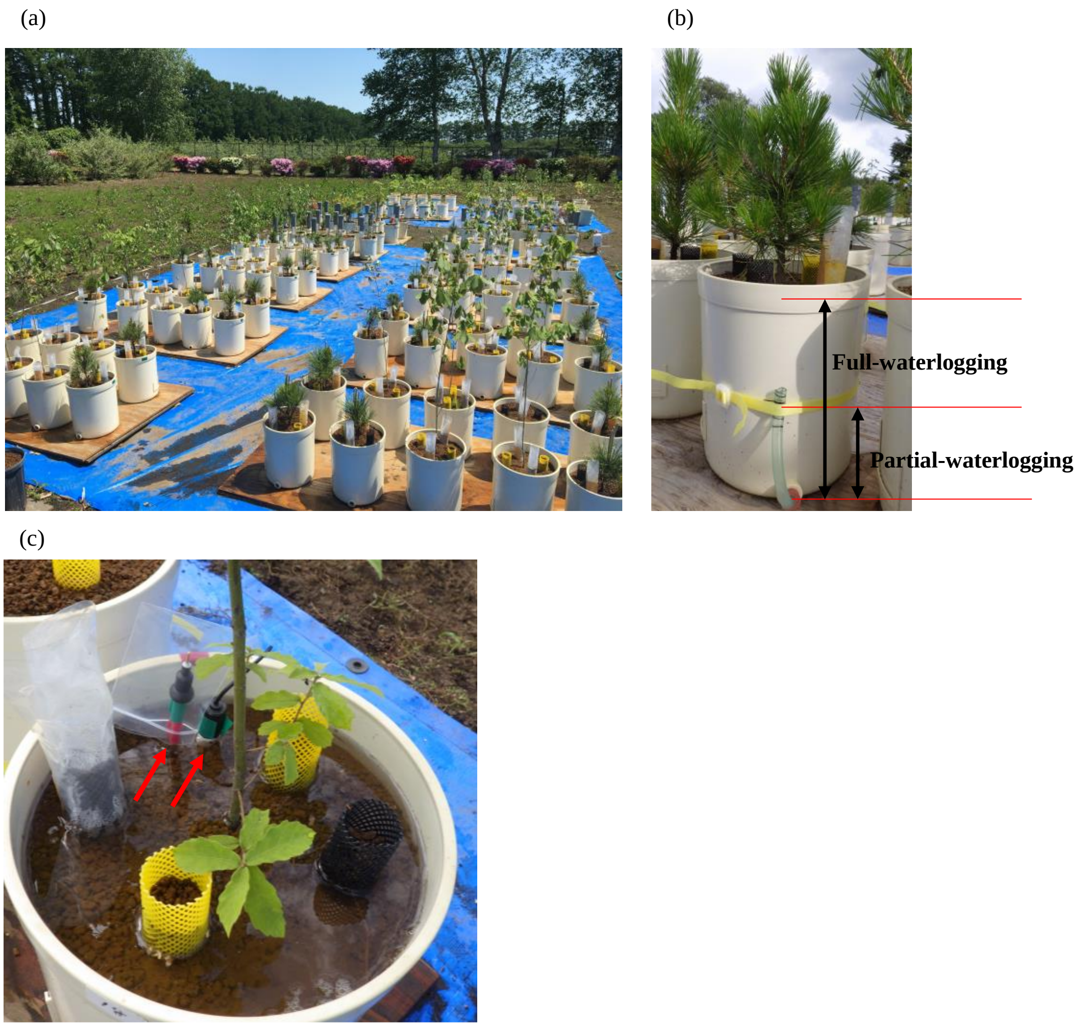

Figure 3-1 Experimental setup

Photos of the experimental setup. (a) is of seedlings grown at the experimental nursery of Tohoku Research Center, FFPRI (Morioka City, Iwate prefecture). (b) is of the waterlogging treatment, where two waterlogging depths were set. For partial-waterlogging treatment, the waterlogging depth was set by using a tube and setting its height 15 cm from the bottom of the pot. (c) is the two electric probes (platinum and comparison probe) used to measure soil oxidation-reduction potential and the ingrowth cores for root measurement. They were all set around the seedling, approximately 10 cm apart. The probes were set at 10 cm depth from the soil surface.

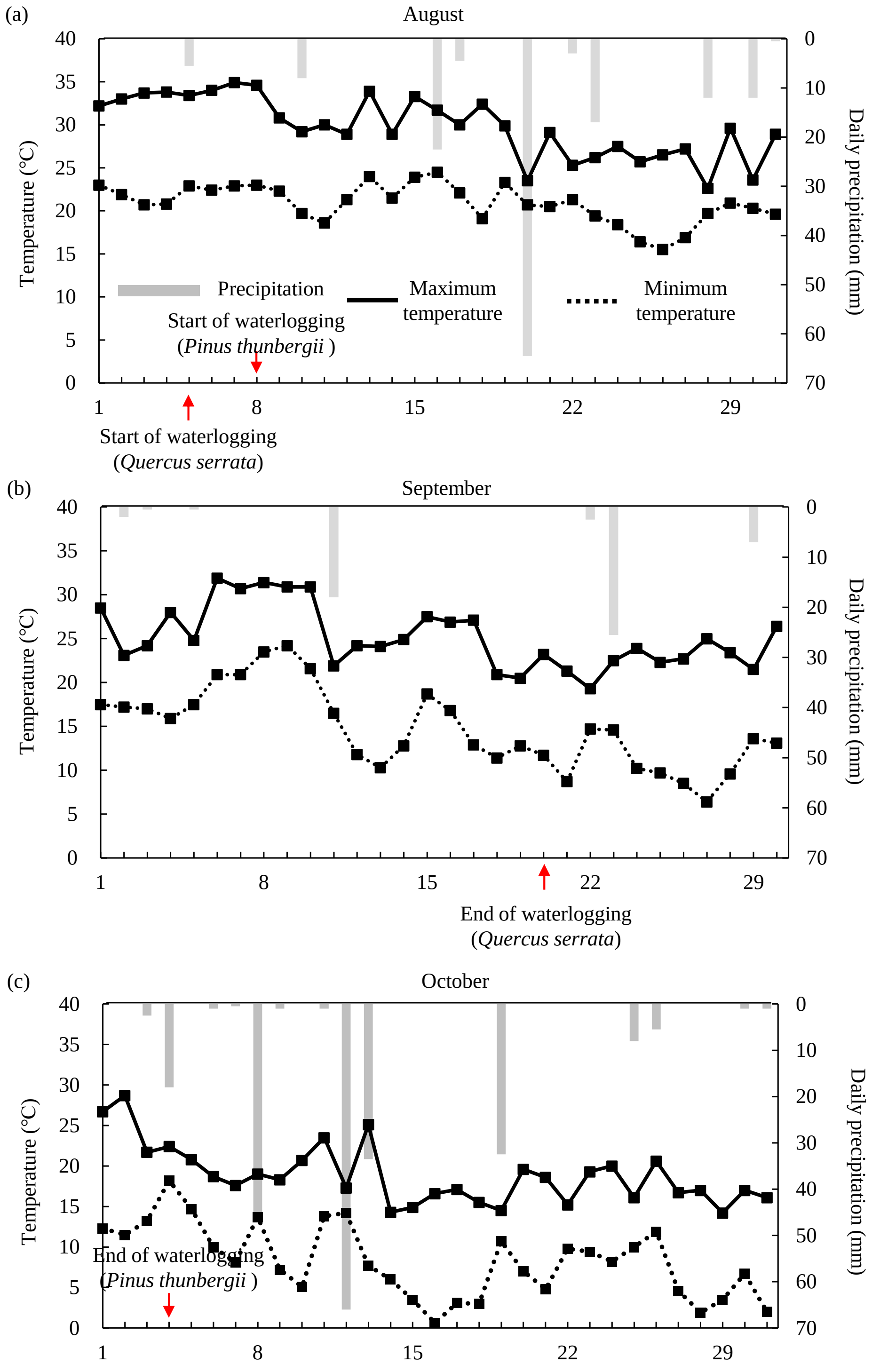

Figure 3-2 Temperature and Precipitation during the experimental period

Daily maximum temperature (°C, solid line), minimum temperature (°C, dotted line) and daily precipitation (mm, gray bars) for (a) August, (b) September, and (c) October, 2019. The horizontal axis is the date of each month. Data was obtained from meteorological observation at NARO Tohoku Agricultural Research near the experimental site (Morioka City, Iwate prefecture).

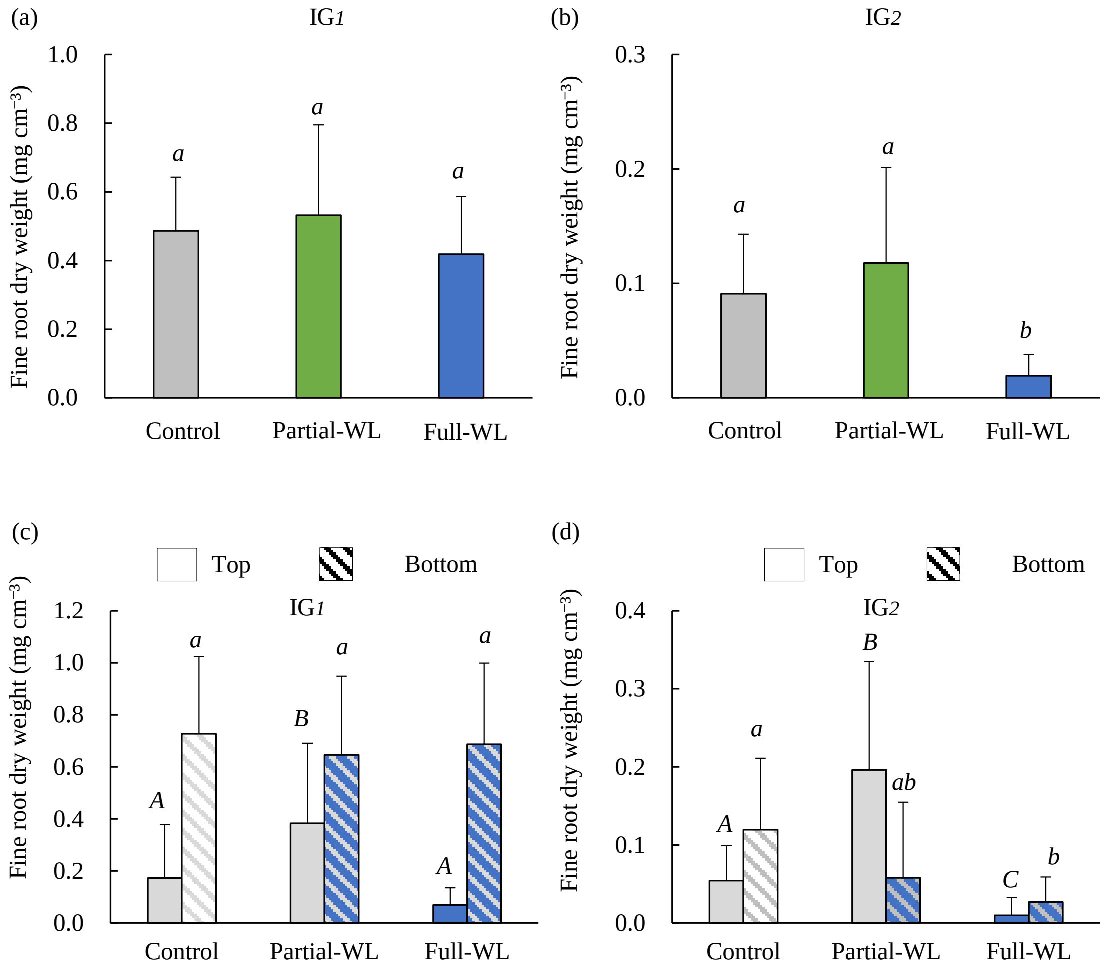

Figure 3-7 Fine root dry weight of *Pinus thunbergii* obtained by in-growth cores

Fine root dry weight of (a) in-growth core 1 (IG_1) and (b) in-growth core 2 (IG_2) of Control, partial waterlogging (Partial-WL) and full waterlogging (Full-WL), respectively. For (c) and (d), solid bars are results from the top part (0-11 cm depth) and hatched bars are the results from the bottom part (11-26 cm depth). Bars which are colored with blue are results obtained from waterlogged conditions. Results are mean values, and bars indicate standard deviation (n=15). The letters indicate statistical differences among the groups (Tukey-Kramer test, p< 0.05). For (c) and (d), capital letters are results of the top part, and lower-case letters are results of the bottom part.

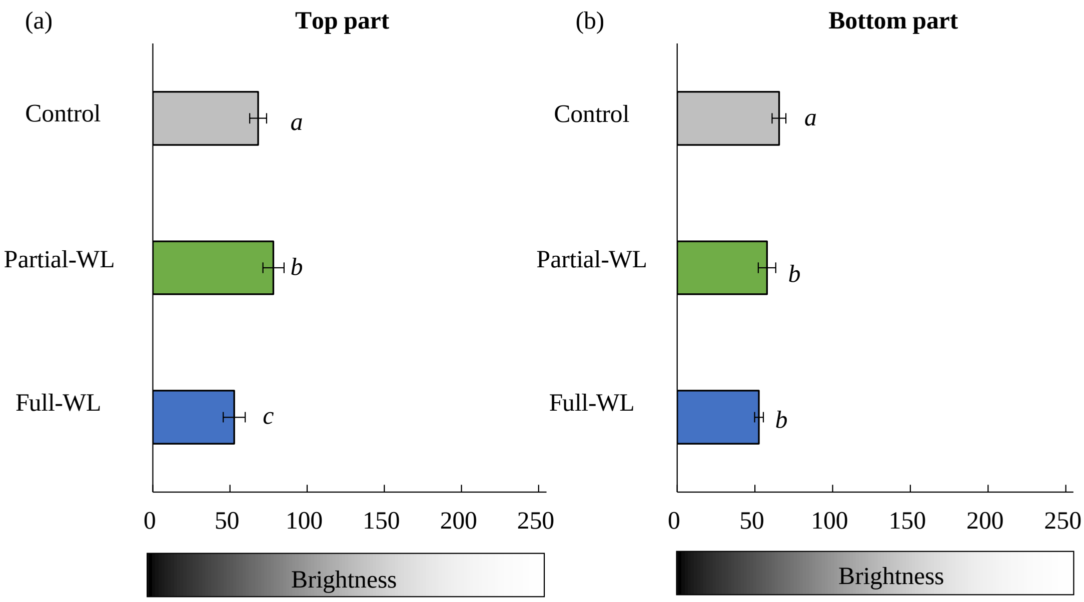

Figure 3-9 Mode brightness of fine roots obtained from in-growth core 1 for *Pinus thunbergii* Mode brightness of fine roots for the (a) top part and (b) bottom part. Results are mean values and bars indicate standard deviation (n=15). The letters show statistical differences between the groups (Tukey-Kramer test, p <0.05).

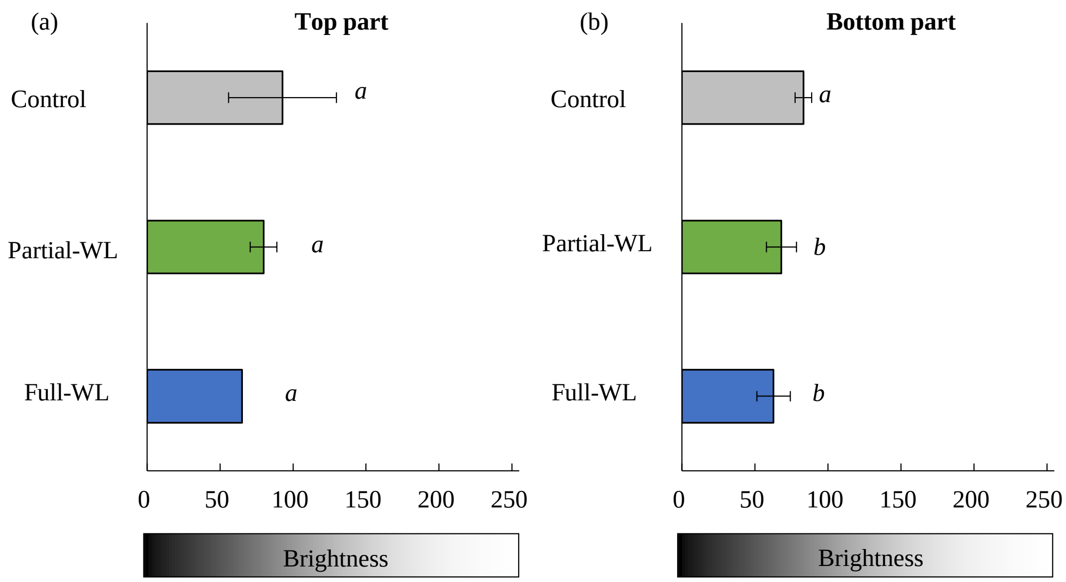

Figure 3-10 Mode brightness of fine roots obtained from ingrowth core 2 for *Pinus thunbergii* Mode brightness for the (a) top part and (b) bottom part. Results are mean values and bars indicate standard deviation (n=15). The letters show statistical differences between the groups (Tukey-Kramer test, p <0.05).

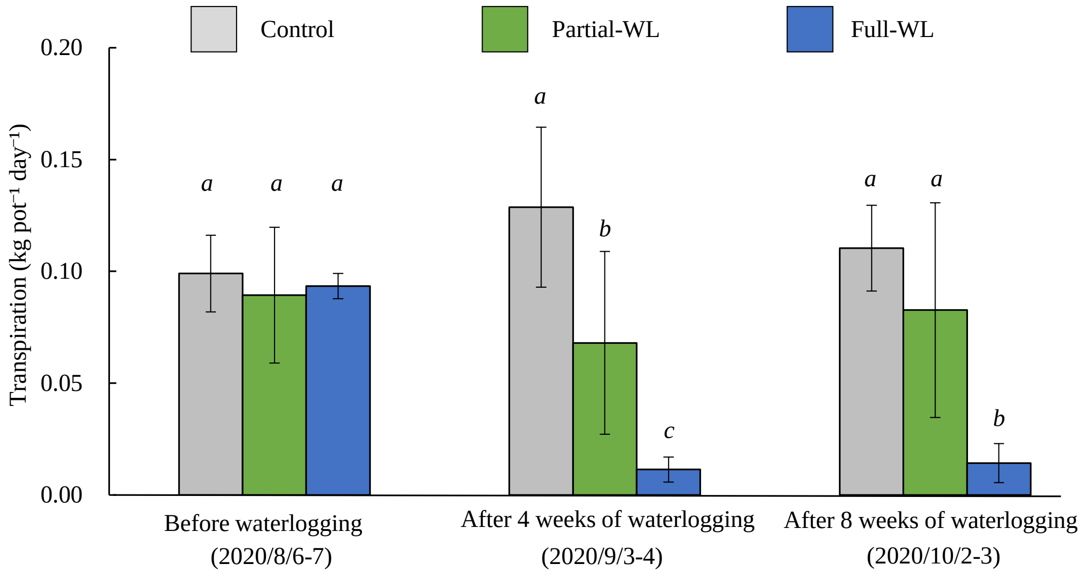

Figure 3-13 Transpiration of *Pinus thunbergii* seedlings

Transpiration of *P. thunbergii* seedlings before waterlogging treatment (August 8-6), after 4 weeks of waterlogging (September 3-4) and after 8 weeks of waterlogging (October 2-3). Gray bars, green bars, blue bars are the result of Control, partial waterlogging (Partial-WL) and full waterlogging (Full-WL), respectively. Results are mean values and bars indicate standard deviation (n=15). The letters indicate statistical differences between the groups at each measurement period (Tukey-Kramer test or Steel Dwass test, p <0.05).

4 Responses of *Pinus thunbergii* seedlings after three different waterlogging durations

今後学術論文に掲載予定のため削除。５年以内に公開予定。

5　General discussion

今後学術論文に掲載予定のため削除。５年以内に公開予定。

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Abstract

Knowledge of the waterlogging responses of *Pinus thunbergii* is of urgent need, especially related to the restoration of the Tohoku region's coastal forests after the disastrous tsunami that occurred in March 2011. With the construction of growth bases, coastal forest restoration is ongoing. Growth bases are being constructed to secure space for vertical root growth, as root depth is reportedly strongly related to tsunami tolerance. However, in some cases, prolonged waterlogging occurs at the surface of the growth bases after rainfall due to poor permeability and drainage caused by heavy machinery compaction.

Discoloration in needles, decreases in shoot growth, and inhibition of vertical root growth have been reported as waterlogging responses of *P. thunbergii* from previous field observations and surveys. Waterlogging is a condition where the soil is saturated with water. Prolonged periods of waterlogging reduce the oxygen availability to roots. These conditions limit root respiration and can cause a decline in root function, root death, and rot. Therefore, waterlogging may be a critical concern in coastal forest restoration.

To prevent the occurrence of waterlogging, measures, such as plowing or construction of open ditches on the top of the growth bases, have recently been attempted on a trial basis. These measures are aimed to improve drainage and permeability of the surface soil of the growth bases so that heavy rainfall does not result in long-term waterlogging. It has been reported that plowing improves soil hardness to about 1.5 m depth, and effects are maintained. Hence, plowing before planting may be a beneficial way to improve soil hardness and waterlogging. However, knowledge of the responses of *P. thunbergii* seedlings to various waterlogging conditions, in terms of depth and duration, is still lacking.

This study focuses on fine roots (diameter < 2 mm) as they play a vital role in absorbing nutrients and water from the soil and are critical for the growth and survival of seedlings after planting. Fine roots can plastically change their growth pattern and morphology according to environmental conditions. The effects on not only mass but elucidations on morphological characteristics and spatial distribution must also be made to understand waterlogging responses of *P. thunbergii* seedlings better.

The research objective of this study is to obtain knowledge on the waterlogging responses of *P. thunbergii* and contribute to achieving restoration of the Tohoku region's *P. thunbergii* coastal forests. Especially responses regarding waterlogging depth and duration were elucidated to reveal

conditions in which *P. thunbergii* seedlings can recover or maintain growth after the release from waterlogging. This dissertation consists of 5 chapters. Chapter 1 describes the general background and objective of this study. Chapter 2 elucidated the waterlogging responses of *P. thunbergii* and four broadleaved species and compared the waterlogging tolerance of *P. thunbergii* relative to the other four broadleaved species. The four broadleaved species were selected from different natural habitats related to waterlogged environments. Chapters 3 and 4 elucidated the responses of *P. thunbergii* to different waterlogging depths and durations. Chapter 5 is the overall discussion and conclusion. In the following paragraphs, Chapters 2 through 4 are briefly summarized.

In Chapter 2, *P. thunbergii*, *Acer mono*, *Quercus serrata*, *Alnus hirsuta*, *Fraxinus mandshurica* seedlings were exposed to ten weeks of waterlogging, where the water table was set at the soil surface. By comparing the waterlogging responses of *P. thunbergii* and four broadleaved species, it was clarified that *P. thunbergii* was most negatively affected by waterlogging, and this was attributed to 1) the death of pre-existing fine roots (grown before waterlogging), 2) the significant inhibition of fine root growth under waterlogging and 3) the death of seedlings the following year. For pre-existing fine roots, root color turned from brown to black under waterlogging, and this change in root color was most evident for *P. thunbergii*. Root tissue density (RTD) was also significantly decreased. These results suggested root tissue death and waterlogging caused root damage.

Moreover, *P. thunbergii* seedlings that were subjected to waterlogging died the following growing season, indicating that roots were dead and did not recover or grow new roots after the waterlogging treatment ended. Fine root growth inhibition was also observed for *A. mono* and *Q. serrata*, species that do not naturally inhabit waterlogged environments. On the other hand, fine root growth continued under waterlogging for *A. hirsuta* and *F. mandshurica*, species that naturally inhibit waterlogged environments.

Chapter 3 suggested that *P. thunbergii* can adapt to partial waterlogging and maintain aboveground activity by changing its fine root growth distribution, especially in the vertical direction. In this chapter, responses of seedlings under 1) no waterlogging (Control), 2) partial waterlogging (Partial-WL, waterlogging depth=15 cm from the bottom), 3) full waterlogging (Full-WL, waterlogging depth= from the bottom to the soil surface, 26 cm) were evaluated. Focus was made on fine root growth distribution, fine root morphology, and transpiration, which was measured to evaluate fine root water absorption. The waterlogging duration was eight weeks during the growing season. Fine roots that grew 1) during the whole experiment period and 2) during the waterlogging period were distinctively measured using the in-growth core method. Fine roots were evaluated on growth and morphological characteristics for the root system's top (11 cm) and the bottom part (15 cm), respectively.

As a result, fine root growth and transpiration were significantly decreased at full-WL. Furthermore, pre-existing fine roots of Full-WL (top and bottom part) and Partial-WL (bottom part) showed symptoms of damage (darkening in root color and decrease in RTD), as observed in Chapter 2. As transpiration was also decreased, it was suggested that fine root water absorption function was also negatively affected. Fine root growth was significantly increased for Partial-WL compared to Control and Full-WL at non-waterlogged top part. Additionally, transpiration of Partial-WL, which had decreased after four weeks of waterlogging, showed no significant difference compared to Control after eight weeks. This recovery in transpiration is likely to be attributed to the increase in fine root growth at the non-waterlogged top part, which compensated for damaged roots at the waterlogged bottom part. From these results, it was shown that although *P. thunbergii* cannot adapt to waterlogging when the whole root system is exposed to waterlogging, it can adapt to partial waterlogging by plastically changing the vertical distribution of fine root growth and increasing fine root growth at the top part.

Chapter 4 elucidated the effects of three different waterlogging durations on *P. thunbergii* seedlings. Focus was especially made on responses of fine root growth and transpiration during and after the release of waterlogging. In this chapter, four treatments were set: 1) no waterlogging (Control), 2) short-term waterlogging (Short-WL, seven days), 3) medium-term waterlogging (Mid-WL, 17 days), 4) long-term waterlogging (Long-WL, 32 days). The waterlogging treatment was carried out by maintaining the water table at the soil surface. Fine roots that grew 1) before and during waterlogging treatment and 2) after being released from the waterlogging treatment were distinctively sampled using the in-growth core method. Fine roots were measured for growth and morphological characteristics. A portable photosynthesis measurement system measured transpiration rates throughout the experimental period to evaluate the effects of waterlogging on fine root water absorption function.

As a result, for Long-WL and Mid-WL, pre-existing fine roots showed a darkening in root color and a decreasing trend in RTD. Transpiration rates were also decreased by waterlogging compared to Control. On the other hand, for Short-WL, although transpiration rates showed a decreasing trend after waterlogging, hardly any changes in fine root color and morphological traits were observed. Transpiration rates after the release from waterlogging recovered to a value that did not differ from Control within a week for Short-WL and Mid-WL, suggesting that the effect of waterlogging on water absorption function was reversible and could recover after the release from waterlogging. Furthermore, fine root growth after the release from waterlogging increased compared to Control.

For Long-WL, responses after the release from waterlogging largely varied within the group. Some seedlings could recover transpiration rates quickly, as observed in Mid-WL. On the other hand, two seedlings could recover transpiration rates to values that did not differ from Control approximately two weeks after release from waterlogging. For these two seedlings, fine root growth was observed after the release from waterlogging, suggesting that these seedlings could slowly recover water absorption function by replacing severely damaged roots with new roots. Hence, when waterlogging is short, the fine root water absorption function damage is reversible. On the other hand, when the waterlogging duration is longer than 17 days, the effect of waterlogging on fine root water absorption function is irreversible, and recovery requires new fine roots.

This study elucidated valuable knowledge on the waterlogging responses of *P. thunbergii*, allowing a better understanding of its adaption strategy to waterlogging. Mainly, this study suggests the following: First, the potential use of broadleaved species such as *Alnus hirsuta* and *Fraxinus mandshurica* instead of *P. thunbergii* at sites where the waterlogging frequently occurs up to the ground surface. Planting species other than *P. thunbergii* is suggested for sites where the groundwater is consistently high due to geographical factors and sites where prolonged waterlogging due to rainfall is difficult to improve with time due to the microtopography of the growth bases, such as hollows. For sites where prolonged waterlogging due to rainfall is expected to improve with the improvement of the growth base's soil physical properties with tree growth, *P. thunbergii* seedlings should be planted after soil permeability and drainage have improved by planting waterlogging tolerant species. However, at places especially near the coastline (the front line of coastal forests), considerations of salt wind stress should also be made other than waterlogging when selecting species other than *P. thunbergii*. Second, if waterlogging is maintained "partial" (topsoil not waterlogged), *P. thunbergii* seedlings can change their fine root growth distribution and maintain aboveground activity. Third, if the waterlogging duration is relatively short, such as several weeks, the water absorption function of roots can rapidly recover after the release from waterlogging.

Although limited to the experiment conditions of this study, knowledge was obtained of conditions in which *P. thunbergii* seedlings can maintain growth and survive, which is expected to benefit the restoration of the Tohoku region's coastal forests.