Root Responses of Five Japanese Afforestation Species to Waterlogging

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Received: 13 April 2020; Accepted: 11 May 2020; Published: 14 May 2020

Abstract: The increase of waterlogged environments is of recent concern due to changes in precipitation regimes and the frequent occurrence of extreme rainfall events. Therefore, it is necessary to comprehend the effects and responses of waterlogging for a better understanding of forests and urban afforestation under changing environments. We investigated root responses of five Japanese afforestation species (Pinus thunbergii, Acer mono, Quercus serrata, Alnus hirsuta and Fraxinus mandshurica) to waterlogging. Potted seedlings grown under natural conditions were waterlogged at soil-surface level for 2.5 months during the growing season. The ingrowth core method was used to distinctly measure root growth. As a result, fine root growth during the waterlogging period was significantly decreased for P. thunbergii, A. mono and Q. serrata. Furthermore, root tissue density (RTD) of pre-existing roots was decreased, which suggests root damage such as partial root death and root decay. On the other hand, for A. hirsuta and F. mandshurica, fine root growth was not decreased under waterlogging. For A. hirsuta, although fine root growth continued at the top half, it was decreased at the bottom half. Root damage such as a decrease in RTD was observed for pre-existing roots. For F. mandshurica, root growth continued at the top and bottom half, and root damage of pre-existing roots was not observed at either the top or the bottom. From our results, it was suggested that P. thunbergii was most sensitive, followed by A. mono and Q. serrata. A. hirsuta and F. mandshurica were relatively tolerant; however, the most tolerant was F. mandshurica, as pre-existing roots were not damaged by waterlogging. Overall, root responses could be grouped into three groups: (1) P. thunbergii, A. mono, Q. serrata; (2) A. hirsuta; (3) F. mandshurica. The observed responses may reflect the species’ natural distributions.

Keywords: fine root growth; fine root morphology; root color; ingrowth-core

1. Introduction

Waterlogging is a natural event with varying intensity, frequency, and duration, and can occur in various environments due to multiple factors such as climate and soil conditions (e.g., poor drainage, rich clay soils) [1]. As O2 diffusion in water is 10,000 times slower than in air, roots are exposed to critical hypoxic or anoxic conditions [2] which can lead to mortality and decreases in plant growth [3,4]. Recently, there has been a growing concern that waterlogging environments will increase due to changes in precipitation regimes and extreme rainfall events that are caused by climate change [5].

As the impact of climate change increases, the risk of waterlogging stress is expected to increase in not only forest ecosystems but also urban afforestation sites [6]. At some urban afforestation sites, growth bases are artificially prepared to secure space for root growth. However, in the process of making the growth bases, the surface soil is packed down, due to the heavy machinery used, as also

Forests 2020, 11, 552; doi:10.3390/f11050552 www.mdpi.com/journal/forests
reported at afforestation sites for forestry [7,8]. At compacted soils, drainage and water permeability is decreased, which can lead to waterlogged environments and this is especially of concern at plantation sites along the pacific coast of northern Japan, which are regeneration sites of the coastal black pine forests that were severely damaged by the tsunami caused by the earthquake in 2011 [9]. In addition, one of the concerns of climate change is the rise in sea level, which may result in higher ground water at these coastal forests [10]. However, information on the response to waterlogging is still limited for afforestation species used for plantations and urban afforestation.

Waterlogging tolerance and its injury occurrences have been reported to be highly species dependent [11,12]. For tolerant species, it has been reported that they can survive under waterlogged conditions for several months without injury; on the other hand, for intolerant species, decreases in activities and injuries can occur just after several hours after oxygen deprivation. For tolerant species, anatomical escape and avoidance strategies are performed by forming lenticels [13,14] and adventitious roots [15,16] to increase oxygen input and efficiently transport air to roots to maintain water and nutrient absorption function, and consequently above-ground activity. On the other hand, for non-tolerant species, visible injuries such as leaf wilting and shedding are observed [17,18]. Another common response is the decrease of root biomass [12]. However, in many cases the decrease in root biomass is evaluated by destructive harvest after waterlogging [19,20] and little is known about how waterlogging affects root growth. Furthermore, although it is reported that waterlogging can induce root decay [21], the process and its contribution to the decrease in root biomass are yet to be clarified. As roots have the ability to plasticly change according to the environment [22], morphological and spatial distribution responses under waterlogging may indicate adaptation to waterlogging; however, information on these characteristics is still limited.

Therefore, our research objective was to elucidate the effects and responses of waterlogging on afforestation species with a focus on the quantitative and morphological responses of fine roots. The ingrowth-core method was used to distinctively measure root growth during the waterlogging period. In our study, we selected five common Japanese afforestation species—*Pinus thunbergii*, *Acer mono*, *Quercus serrata*, *Alnus hirsuta* and *Fraxinus mandshurica var. japonica*. *P. thunbergii* is the main component of coastal forests and is presently planted at the regeneration sites. *A. mono* and *Q. serrata* are species which are reported to inhabit coastal forests at areas relatively far from the coast line, and are also candidates for plantation at the regeneration sites [23,24]. Those three species represent species which usually do not inhabit waterlogged environments. *A. hirsuta* is known to be native to riparian areas [25], which are waterlogged environments with very slow but regular water movement. On the other hand, *F. mandshurica* are reported to be native to swampy areas with stagnant water [26,27].

We hypothesized that root responses would differ according to their natural distributions. For *P. thunbergii*, *A. mono* and *Q. serrata*, we hypothesized that root biomass would decrease, due to their not being adapted to waterlogged conditions. On the other hand, *A. hirsuta* and *F. mandshurica* would show hypoxic avoidance responses and root biomass would not decrease. However, responses were assumed to differ according to the degree of hypoxia at the natural site; for instance, riparian areas have very slow water movement and swampy areas have stagnant water.

### 2. Materials and Methods

#### 2.1. Plant Material and Waterlogging Treatment

Two-year-old seedlings of five Japanese afforestation species, *Pinus thunbergii*, *Acer mono*, *Quercus serrata*, *Alnus hirsuta* and *Fraxinus mandshurica var. japonica* were purchased from a commercial tree nursery in Hokkaido, Japan. At the end of April 2018, the seedlings were planted in 1/2000 Wagner pots (500 cm², depth 30 cm with a drainage hole) with Akadama soil derived from the loamy B horizon of an Andisol [28]. In total, 120 (24 pots per species) pots were randomly distributed across 12 groups with each group containing 2 pots per species (control and treatment). Each group (2 pots × 5 species) was evenly placed on wooden boards (1 × 2 m). Seedlings were grown under natural conditions.
at the seedling nursery of Tohoku Research Center, Forestry and Forest Products Research Institute. Two weeks after transplanting, a 2000-fold diluted liquid fertilizer was applied (N:P:K = 6%, 10%, 5%, Hyponex Japan, Osaka, Japan) with 1 L of water. The amounts per pot were approximately 30, 50, and 25 mg of N, P, and K, respectively. Depending on the weather, 2–4 L of water was given once or twice a week, until mid-July when the waterlogging treatment began.

In mid-July, after 2.5 months after transplantation, the waterlogging treatment began. Here, 12 pots per species (one pot per species from each group) were subjected to waterlogging. Seedlings subjected to waterlogging were randomly chosen, ensuring that there was no initial size difference between the control and waterlogging group. The waterlogging treatment was carried out by blocking the drainage hole and filling pots with water until the water table reached the soil surface. Water was carefully added at one to three-day intervals to maintain the water table at the soil surface. For the control treatment, the drainage hole was kept open. If it did not rain for over 10 days, the control pots were watered with 2–4 L of water.

The waterlogging treatment began in mid-July and lasted until the end of September (waterlogging period: 2.5 months) when the seedlings were harvested. The total experiment period was from the end of April to the end of September, which was approximately 5 months. The period of waterlogging was set after leaf elongation and height growth had ceased so that effects of waterlogging on above-ground growth would not affect below-ground growth. Furthermore, the treatment was done during the typhoon season when extreme water events are predicted to occur.

### 2.2. Aboveground Measurements

Heights and stem base diameters of all seedlings were measured before and after the waterlogging treatment, and before harvest.

### 2.3. Root Measurements

Two ingrowth-cores (diameter, 32 mm; height, 30 cm; 2 mm mesh) were placed in each pot for nine seedlings per treatment for each species—one at seedling transplantation (hereafter, IG1) and the other right before the waterlogging treatment started (hereafter, IG2). IG1 was filled with the same Akadama soil. When placing IG2 in the pot, a steel pipe (diameter, 40 mm) was used to extract soil, and space was made for the new ingrowth-core with minimal soil disturbance and root damage. After installation, the ingrowth-core was filled with new Akadama soil, ensuring that the soil inside the core did not contain roots. Each core was placed on both sides of the seedling, approximately 10 cm away for the seedling stem. Both cores were harvested after 2.5 months of waterlogging. The ingrowth cores were harvested with a sharp steel pipe (diameter, 35 mm) so as to carefully cut the roots along the edge of the ingrowth-core. Harvested cores were placed in plastic bags and stored in a refrigerator at 3 °C until further analysis.

The ingrowth-core was divided into the top and bottom halves (top, 15 cm; bottom, 15 cm) and the obtained roots were analyzed separately. The soil from the ingrowth-cores was thoroughly washed out with water on a very fine sieve (sieve aperture 250 µm). Fine roots were carefully picked from the sieve, carefully washed with a brush, and scanned (EPSON GT-X980). Scans were made without any image correction and under the same light conditions at 800 dpi. After scanning, fine roots were dried at 70 °C for 72 h, and then measured for dry mass. The scanned images were analyzed with WinRHIZO Pro (2012b) (Regent Instruments, Inc., Quebec, Canada) for root length, mean diameter, and volume. Specific root length (SRL) and root tissue density (RTD) were obtained for evaluating fine root morphology and were calculated from the following equations:

\[
SRL \ (m \ g^{-1}) = \frac{\text{Root length (m)}}{\text{Root dry weight (g)}} \tag{1}
\]

\[
RTD \ (g \ cm^{-3}) = \frac{\text{Root weight (g)}}{\text{Root volume (cm}^3\text{)}} \tag{2}
\]
A brightness histogram (0–255) of the scanned images was calculated via area ratios of the total numbers of pixels showing the root regions. Regions showing roots were determined by binarization of images, and brightness was set as the average of colors (R, G, B).

2.4. Statistical Analysis

One-way ANOVA was carried out on each species to determine the effect of the waterlogging treatment on stem base diameter. For root dry weight and morphological characteristics obtained from the ingrowth-core, a non-parametric Mann–Whitney (Wilcoxon) two-sample test was used to determine differences between treatments. The top and bottom of the ingrowth-core were analyzed separately. All calculations were conducted using R (version 3.6.1, The R foundation for Statistical Computing Platform) with a significance level of \( p < 0.05 \).

3. Results

3.1. Aboveground

Visual symptoms, such as needle discoloration, were observed for \( P. \) thunbergii and for \( A. \) mono, change in leaf color and leaf shedding was observed. For \( A. \) mono, leaves started to change color from green to a reddish-brown from the end of July, and all leaves were shed before sampling. For the other three species, effects of waterlogging on leaves were not found. Although limited to personal observation, the timing of lenticel formation was different among species (Figure 1). For \( A. \) hirsuta and \( F. \) mandshurica, lenticles were observed on the stem near the water table after two weeks of waterlogging. For \( A. \) mono and \( Q. \) serrata, lenticle formation was observed after approximately one month, and there were some seedlings that did not show lenticel formation. Furthermore, fine roots and adventitious roots were also observed at the soil surface for \( A. \) hirsuta and \( F. \) mandshurica after 1 month of waterlogging. Concerning stem base diameter growth, after 2.5 months of waterlogging, it was significantly increased under waterlogging for \( P. \) thunbergii, \( A. \) hirsuta and \( F. \) mandshurica (Figure 2).

![Figure 1](image1.png)

**Figure 1.** The stem bases (near water table) of all species under the waterlogging treatment before harvest. In species other than \( P. \) thunbergii, lenticels were observed near the stem bases under waterlogging. Fine roots and adventitious roots were also observed for \( A. \) hirsuta and \( F. \) mandshurica (shown with yellow arrows).
Figure 2. Means (±SE) of stem base diameter (mm) (n = 12, except for Quercus serrata where control was n = 5 and the waterlogging treatment was n = 4). Growth was obtained by subtracting the value before waterlogging treatment from the final value. White bars are results of the control treatment and gray bars are results of the waterlogging treatment. Results of one-way ANOVA are denoted as **, p < 0.01; ***, p < 0.001.

3.2. Belowground

For control treatments, root color was generally close to light or dark brown for *P. thunbergii*, *A. mono*, *Q. serrata* and *A. hirsuta*, and for *F. mandshurica* it was white. Under waterlogging, a visible response was observed: root color darkened (Figure 3a). This change was most significant for *P. thunbergii*, where under waterlogging, the area ratio for darker fine roots (gray scale near < 50) increased (Figure 3b). For *F. mandshurica*, root color was white under both control conditions and waterlogging and only minor effects of waterlogging were observed. For *A. mono*, *Q. serrata* and *A. hirsuta*, the change in root color was not as evident as for *P. thunbergii*. Brightness histogram results of species other than *P. thunbergii* are shown in Appendix A Figure A1a–d.

Figure 3. Scanned image of fine roots (from whole root system) of *Pinus thunbergii* (a). (b) A brightness histogram (set at a gray scale) made by scanned images of *P. thunbergii* obtained from IG1. The blue bars are results of the control treatment and the red bars are results from the waterlogging treatment. Scans were made under the same light conditions at 800 dpi. The brightness histogram of the other four species is shown in Appendix A Figure A1a–d.

The dry weight of fine roots from IG1 was significantly decreased by waterlogging for all species except *F. mandshurica*, and this was more evident at the bottom half for *P. thunbergii*, *A. mono* and *A. hirsuta* (Figure 4a). For *Q. serrata*, although there was a decreasing trend, a statistical difference could not be detected, possibly due to the high variation and limited number of samples.

From IG2, hardly any root growth was observed under waterlogging for *P. thunbergii*, *A. mono* and *Q. serrata*, and the total value of IG2 was significantly decreased. On the other hand, for *A. hirsuta* and *F. mandshurica*, root growth was increased at the top half under waterlogging (Figure 3b). For *A. hirsuta*,
although fine root growth was decreased at the bottom half, the total value of IG2 was not decreased by waterlogging.

Concerning fine root morphology, fine roots obtained from IG1 were calculated by image analysis for fine root diameter, SRL, and RTD. Results of the top and bottom half are shown separately (Table 1) and the analysis was also done separately for the top and bottom half. As a result, except the increase for fine root diameter, SRL, and RTD. Results of the top and bottom half are shown separately (Table 1).

**Figure 4.** Mean (± SE) dry mass of fine roots obtained from (a) ingrowth-core 1 (IG1, throughout the experiment, \( n = 9 \), except for *Quercus serrata* where control was \( n = 5 \) and the waterlogging treatment was \( n = 4 \)), and from (b) ingrowth-core 2 (IG2, only during the waterlogging period, \( n = 9 \), except for *Q. serrata* where control was \( n = 5 \) and the waterlogging treatment was \( n = 4 \)). White bars are results of the control treatment and gray bars are results of the waterlogging treatment. The dashed colored bars are results of the top half and the solid colored bar is of the bottom half. Total refers to the sum of the values of the top and bottom. Results (\( p \) values) of Mann–Whitney (Wilcoxon) two-sample test are shown in the table below each graph.

**Table 1.** Mean (±SE) root diameter (mm), specific root length (SRL; m g\(^{-1}\)), and root tissue density (RTD; g cm\(^{-3}\)).

| Species      | Position | Treatment | Root Diameter | SRL     | RTD          |
|--------------|----------|-----------|---------------|---------|--------------|
| *P. thunbergii* | Top      | C \(( n = 9 \)\) | 0.47 ± 0.04 | 27.7 ± 2.6 | 0.23 ± 0.01 |
|              |          | WL \(( n = 7 \)\) | 0.53 ± 0.03 | 29.7 ± 4.8 | 0.19 ± 0.03 *|
|              | Bottom   | C \(( n = 9 \)\) | 0.53 ± 0.05 | 22.0 ± 2.2 | 0.28 ± 0.02 |
|              |          | WL \(( n = 9 \)\) | 0.65 ± 0.04 *** | 20.5 ± 1.8 | 0.16 ± 0.01 ***|
| *A. mono*    | Top      | C \(( n = 8 \)\) | 0.35 ± 0.01 | 39.9 ± 4.3 | 0.29 ± 0.02 |
|              |          | WL \(( n = 8 \)\) | 0.37 ± 0.01 | 47.6 ± 5.7 | 0.21 ± 0.02 **|
|              | Bottom   | C \(( n = 9 \)\) | 0.37 ± 0.01 | 33.7 ± 3.1 | 0.30 ± 0.02 |
|              |          | WL \(( n = 7 \)\) | 0.36 ± 0.02 | 50.5 ± 8.6 | 0.23 ± 0.03 *|
| *Q. serrata* | Top      | C \(( n = 4 \)\) | 0.21 ± 0.03 | 69.5 ± 20.0 | 0.59 ± 0.14 |
|              |          | WL \(( n = 4 \)\) | 0.18 ± 0.02 | 207.5 ± 76.9 | 0.26 ± 0.05 |
|              | Bottom   | C \(( n = 5 \)\) | 0.22 ± 0.04 | 100.9 ± 34.1 | 0.56 ± 0.13 |
|              |          | WL \(( n = 4 \)\) | 0.27 ± 0.07 | 152.37 ± 65.8 | 0.24 ± 0.04 |
Table 1. Cont.

| Species         | Position | Treatment | Root Diameter | SRL       | RTD       |
|-----------------|----------|-----------|---------------|-----------|-----------|
|                 |          |           |               |           |           |
| A. hirsuta      | Top      | C (n = 9) | 0.32 ± 0.01   | 34.9 ± 5.0 | 0.40 ± 0.04 |
|                 |          | WL (n = 9)| 0.36 ± 0.03   | 101.4 ± 35.1| 0.18 ± 0.02*** |
|                 | Bottom   | C (n = 9) | 0.32 ± 0.03   | 50.1 ± 5.2  | 0.32 ± 0.02 |
|                 |          | WL (n = 8)| 0.36 ± 0.03   | 84.1 ± 17.7 | 0.17 ± 0.01*** |
| F. mandshurica  | Top      | C (n = 9) | 0.38 ± 0.02   | 28.0 ± 5.1  | 0.46 ± 0.09 |
|                 |          | WL (n = 9)| 0.35 ± 0.04   | 40.0 ± 4.6  | 0.36 ± 0.04 |
|                 | Bottom   | C (n = 9) | 0.42 ± 0.05   | 29.1 ± 6.5  | 0.38 ± 0.04 |
|                 |          | WL (n = 9)| 0.43 ± 0.05   | 34.4 ± 4.7  | 0.32 ± 0.02 |

White rows (C) are results of the control treatment and gray rows (W) are results of the waterlogging treatment, and the number of samples is also indicated. Values were calculated from roots obtained from ingrowth-core 1 (before waterlogging + during waterlogging). “Top” indicates results from the top half, and “Bottom” indicates results obtained from the bottom half. Results of the Mann–Whitney (Wilcoxon) two-sample test are shown, denoted as: *, p < 0.05; **, p < 0.01; ***, p < 0.001. The analysis was done separately for the top and bottom halves.

4. Discussion

Using the ingrowth-core method, fine root growth under waterlogging was distinctively evaluated. As a result, hardly any root growth was observed under waterlogging for P. thunbergii, A. mono and Q. serrata (Figure 4b). Moreover, a decrease in RTD was observed from IG1, which suggested partial death of pre-existing roots considering the change in root color under waterlogging. Generally, newly produced roots are white and change to brown with time [29,30], and fragile black colored roots indicate dead roots [31]. Under waterlogging, root color significantly changed from dark brown to black (Figure 3a) and the roots were fragile for P. thunbergii, which was also evident from the root brightness histogram (Figure 3b) and a significant decrease in RTD. For A. mono and Q. serrata, the change in root brightness was not as evident as for P. thunbergii, indicating that they may not have been as damaged as P. thunbergii. On the other hand, identification and classification of dead fine roots is still unclear. Although it has been reported that black roots indicate dead roots, in the case of waterlogging, some studies have shown that it may be a result of iron accumulation [32]. As accumulation of iron is suggested to interfere with nutrient uptake [33], black roots nonetheless suggest malfunctioning roots.

From the significant decrease in fine root growth and indication of root damage from the decrease in RTD under waterlogging, along with the partial change in color, it was clarified that the generally observed decrease in root biomass is caused by not only the decrease in fine root growth but also due to partial root death [21,34]. In addition to partial root death, the observed decrease in RTD and change in root color may be indicating the decay and loss of roots which had grown before the waterlogging treatment.

Here, we attempted to evaluate the degree of root decay of pre-existing roots. Assuming that the amount of root decay for the control treatment is negligible, the amount of pre-existing roots before the waterlogging treatment can be estimated by subtracting the fine root dry weight of IG2 from that of IG1. From Figure 4a,b, the estimated amounts of pre-existing fine roots for the control treatment can be calculated as 0.07, 0.04, and 0.05 g for P. thunbergii, A. mono and Q. serrata, respectively, and for the waterlogging treatment, those amounts are 0.03, 0.02, and 0.02 g. As a result, the estimated amount of pre-existing roots for the waterlogging treatment was less than 50% of the control treatment. This suggests that for these three species, not only was root growth decreased under waterlogging, but pre-existing roots were also lost due to root decay.

For Acer and Quercus genera, root growth has been reported to decrease, which followed our results of A. mono and Q. serrata (Table 1 of [12]). For Yamamoto et al. [35], the total root mass showed smaller values compared to the control, suggesting that some proportions of roots were lost due to root decay. Root decay of pre-existing roots with senescence of white tips, necrosis of tap root, and waterlogged lateral roots, was also observed in oak species [21], which also supported our results.
For *P. thunbergii*, the decrease in root growth and damage of pre-existing roots were most evident. In previous studies, *Pinus* species such as *P. halepensis*, *P. banksiana* and *P. resinosa* have been reported to be affected by waterlogging negatively, wherein leaf formation, shoot dry weight, and leaf dry weight decreased [36,37]. Therefore, the observed above-ground effects may have been due to the high sensitivity of roots to waterlogging. From field surveys of coastal *P. thunbergii* forests, it has been found that root depth tends to be shallower at sites where the ground water level is high [38,39]. Our results support the field observations wherein root growth was inhibited by waterlogging. Therefore, the change in root distribution of *P. thunbergii* at coastal sites due to the rise of the sea level may be of concern under climate change.

For *A. hirsuta*, root growth continued under waterlogging and this was mostly only observed at the top half (Figures 1 and 3b). At the bottom half, root growth was significantly decreased. It is reported that plants generally invest in roots that are located where the greatest return can be derived, and on the other hand, remove roots located from where the returns are less [40,41]. In the case of this experiment, the degree of waterlogging stress may have been lesser at the upper half, it being closer to the soil surface where oxygen could be supplied. Additionally, lenticels were observed near the stem base and soil surface (Figure 1). In previous studies, it has been reported that lenticels produced under waterlogged conditions are an entrance of air [14,16]; therefore, continuous root growth may have been enabled by air which was supplied from lenticels [42,43].

Although *A. hirsuta* showed continuous fine root growth under waterlogging, the dry weight of IG1 was significantly decreased by waterlogging. This may have been due to the partial death of pre-existing roots, indicated by the decrease in RTD and partial darkening in root brightness. Additionally, the estimated value of pre-existing roots for the control treatment was 0.05 g, which was a similar value to IG1 (whole growth period) and IG2 (only during waterlogging period) of the waterlogging treatment (Figure 4a,b). Although limited to personal observation, the root color of *A. hirsuta* was relatively white at the top and darker at the bottom half (Figure 5a), suggesting that although a substantial proportion of pre-existing roots was lost by root decay at the bottom half; roots were replaced by new root growth during the waterlogging period, especially at the top. Therefore, under waterlogging, new roots may have been produced at the top half to compensate for the damaged and lost roots at the bottom half [41,44].

**Figure 5.** The whole root systems of (a) *Alnus hirsuta* and (b) *Franxius mandshurica* under control and waterlogging. Under waterlogging, root growth was significant near the soil and water surface for *A. hirsuta*. For *F. mandshurica*, adventitious roots were observed near the soil surface and the middle part of the root system, as shown with red arrows.

For *F. mandshurica*, root growth was increased at the top under waterlogging, and root growth was not substantially decreased, which was a different response from *A. hirsuta* (Figure 4b). From Figure 1, lenticel and adventitious roots were observed near the soil surface, which was in common with *A. hirsuta*. However, from Figure 5b, adventitious roots were not only located near the soil surface, as some
were observed at the middle part of the root system. Furthermore, for *F. mandshurica*, pre-existing roots did not show signs of root damage, even at the bottom half. This may have been due to the presence of adventitious root which were located not only near the soil surface but at the middle part. In some species, only a “partial barrier” to radial oxygen loss is reported for adventitious roots [45]. This suggests that adventitious roots that newly grew under waterlogging were able to supply oxygen through not only the roots but also by leaking out oxygen and supplying it to pre-existing roots placed relatively far from the soil surface. The difference in location of where new roots can grow under waterlogging may determine the ability to maintain root condition. The rooting depths of adventitious roots may be related to species type; e.g., deep rooting type tree (*F. mandshurica*) or shallow rooting type tree (*A. hirsuta*) [46].

As for the above-ground response, stem base diameter growth was significantly increased for *P. thunbergii*, *A. hirsuta* and *F. mandshurica* (Figure 2). Stem base increment under waterlogging has been previously reported in various species [12], including both tolerant and sensitive species. For *F. mandshurica*, Yamamoto et al. [26] reported from an anatomical aspect that stem base diameter increment under waterlogging resulted from the increment in xylem. A similar phenomenon was observed for *Alnus japonica* (Thunb.) Steud. seedlings [20]. On the other hand, it has been reported that for conifer species such as *Pinus halepensis* Mill. [36] and *Pinus densiflora* Siebold and Zucc. [47], stem increment under waterlogging was due to the increase in bark thickness. This suggests that stem increment of *P. thunbergii* is a different response compared to *A. hirsuta* and *F. mandshurica*; for those two species, stem growth was increased under waterlogging.

5. Conclusions

Investigations were made on waterlogging responses of roots with a focus on root growth, and changes in morphology of pre-existing fine roots of five common afforestation species. From the obtained results, it was suggested that *P. thunbergii* was most sensitive to waterlogging, followed by *A. mono* and *Q. serrata*. *A. hirsuta* and *F. mandshurica* were relatively tolerant; however, *F. mandshurica* was most tolerant in this experiment, as damage was not observed for pre-existing roots. Our results suggest that for the three sensitive species, afforestation sites should be carefully considered for waterlogging, since groundwater level may increase with the rise in sea level under the on-going climate change.

The observed root responses were grouped into three groups related to the species natural distribution that can be explained by tolerance levels to waterlogging conditions. The three groups are as follows.

1. Inhabits of non-waterlogged environments: *P. thunbergii*, *A. mono* and *Q. serrata*. Inhibition of root growth + damage and loss of pre-existing roots (top and bottom of root system).
2. Inhabits of waterlogged environments (with very slow water movement): *A. hirsuta*. Continuous root growth (only at top) + damage and loss of pre-existing roots (bottom half).
3. Inhabits of waterlogged environments (stagnant water): *F. mandshurica*. Continuous root growth (top and bottom) + no damage of pre-existing roots.

**Author Contributions:** S.F., K.N., and T.T. conceived and designed the experiments; S.F. and K.N. performed the experiments and analyzed the data; S.F. and K.N. wrote the manuscript; T.T. supervised the research. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was financially supported by a Grant-in-Aid for Scientific Research to S.F. (#18J20542) from the Japan Society for the Promotion of Science. This study was also partly funded by the research program I (grant No. #201701) by the Forestry and Forest Product Research Institute “Establishment of guidelines for afforestation of coastal forests on berms that have the merits of high resistance to tsunami” from 2017 to 2019.
Acknowledgments: We thank M. Masumori, H. Kurokouchi, all student members of the Laboratory of Silviculture of the University of Tokyo, and S. Hashimoto of Forestry and Forest Products Research Institute for concrete and fruitful discussion. We appreciate Tohoku Research Center, Forestry and Forest Products Research Institute for extensive support throughout the course of experiment, such as the use of facilities and the nursery. We also thank T. Yoshida of Hokkaido University, Faculty of Engineering for support with image analysis.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Figure A1. (a–d) Brightness histogram shown as a gray scale (0–255) of the area ratio of scanned roots (IG1) for each species. Image analysis was done using ImageJ.

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