Here comes the flood! Stress effects of continuous and interrupted waterlogging periods during the growing season on Scots pine saplings

Timo Domisch[1)]*, Ji Qian [2)], Izabela Sondej[3)], Françoise Martz[4)], Tarja Lehto[5)], Sirpa Piirainen[1)], Leena Finér[1)], Raimo Silvennoinen[6)] and Tapani Repo[1)]

[1)] Natural Resources Institute Finland (Luke), Joensuu, Finland

[2)] College of Horticulture, Hebei Agricultural University, Baoding, China

[3)] Department of Natural Forests, Forest Research Institute, Białowieża, Poland

[4)] Natural Resources Institute Finland (Luke), Rovaniemi, Finland

[5)] School of Forest Sciences, University of Eastern Finland, Joensuu, Finland

[6)] Simitec Ltd., Joensuu, Finland

*Corresponding author, timo.domisch@luke.fi

Keywords: anoxia, flooding, hypoxia, oxidative stress, pinitol, shoot growth, soil, root growth

Running head: Waterlogging stress on Scots pine saplings

Abstract

Future climate scenarios for the boreal zone project increasing temperatures and precipitation, as well as extreme weather events such as heavy rain during the growing season. This can result in more frequent short-term waterlogging (WL) leading to unfavourable conditions for tree roots. In addition, it is decisive whether short-term WL periods during the growing season occur continuously or periodically.

We assessed the effects of short-termed WL on 4-year-old Scots pine (*Pinus sylvestris* L.) saplings after shoot elongation started. Waterlogging (WL) lasted either continuously for 2.5 weeks (ContWL) or non-continuously for five weeks, consisting of three repeated one-week-WL periods (IntWL). Both treatments resulted in the same total duration of soil anoxia. We studied soil gases, root and shoot growth and physiology, and root survival probability and longevity during the experiment. In the final harvest we determined shoot and root biomass, and hydraulic conductance and electric impedance spectra of the root systems.

Soil CO_2 and CH_4 concentrations increased immediately after WL onset and O_2 decreased until anoxia. Waterlogging decreased fine root survival probability but there was no difference between WL treatments. Shoot growth suffered more from ContWL and root growth more from IntWL. Needle concentrations of pinitol increased in the WL saplings, indicating stress. No WL effects were observed in photosynthesis and chlorophyll fluorescence. Increased starch concentration in needles by WL may be due to damaged roots and thus a missing belowground sink. Electrical impedance indicated suffering of WL saplings, although root hydraulic conductance did not differ between the treatments.

Oxidative stress of short-term and repeated WL can have long-lasting effects on shoot and root growth and the physiology of Scots pine. We conclude that even short-term WL during

42 the growing season is a stress factor that probably increases in the future, and can affect
43 carbon allocation and dynamics in boreal forests.

44

45 **Introduction**

46 Future climate scenarios project increasing temperature and precipitation, particularly so at
47 higher latitudes, and during the winter months (IPCC 2014). Increasing precipitation during
48 the winter can result in increased snowfall, increased soil thaw-freeze events after warm
49 spells, 'rain on snow' events and eventually also a higher risk of flooding, meaning an
50 overflow of water and submerging the soil (e.g. Kreuzwieser and Gessler 2010). In Nordic
51 conditions the main hydrological event causing flooding is snow melt in spring before the
52 growing season (Veijalainen et al. 2010) and tree species are adapted to this. However,
53 climatic changes may increase extreme weather events also during summer, thus increasing
54 the incidences of flooding (Kundzewicz et al. 2006). A significant part of the Finnish forests
55 grow on peatlands that are drained for forestry purposes (Finnish Forest Statistics 2018). As
56 peatlands are hydrologically labile ecosystems (Paavilainen & Päivänen 1995) and thus
57 vulnerable for changes in precipitation, stronger and more frequent rain events during the
58 growing season can increase the risks of severe flooding episodes and water-logging (WL),
59 the latter indicating that the water table is not above the soil surface. However, an increasing
60 risk of WL in the future does not hold to peatlands only, but likewise to fine-textured mineral
61 soils, too.

62 Scots pine is the main tree species in drained peatland forests in Finland, and is considered as
63 a species with low flooding tolerance (Glenz et al. 2006). The timing and duration of WL
64 during the annual cycle of trees are decisive for the impacts in roots and shoots. Earlier
65 studies indicated that WL during dormancy is not as stressful as during the growing season

(e.g. Pelkonen 1975, 1979, Wang et al. 2013). On the other hand, WL in combination with soil freezing for six weeks during dormancy affected negatively shoot and root phenology and growth during the following growing season but was not lethal to Scots pine saplings (Roitto et al. 2019). Waterlogging during the early stages of the growing season was less harmful than later in the growing season (Pelkonen 1975, 1979), which is reasonable as the majority of the root growth in boreal trees takes place after the shoot growth has ceased (Abramoff & Finzi 2014). Waterlogging lasting longer than three weeks during the growing season had adverse effects on Scots pine saplings, and was lethal if lasting longer than five weeks (Repo et al. 2016). A short WL of two to three days did not seem to be harmful for Scots pine (Orlov 1966, Pelkonen 1979). However, we do not know whether a short-term and repeated WL would have similar effects as a longer and continuous WL, both with the same duration of anoxia, on the physiology and growth dynamics of roots and shoots.

An immediate effect of waterlogging (WL) is the decrease in soil oxygen (O_2) concentration due to filling of soil pores with water (e.g. Kozlowski & Pallardy 2007). The diffusion of gases is approx. 10 000 times slower in water than in air, and in addition, the solubility of O_2 in water is much lower than compared to O_2 concentrations in the air. This practically results in hypoxia or even anoxia (e.g. Armstrong 1979), and CO_2 concentrations increase (Greenway et al. 2006). Plants are aerobic organisms and an appropriate soil O_2 concentration (approx. 10% volume) is required for normal growth and function. At anoxic conditions mitochondrial respiration is hampered and turns into less efficient fermentation (Dessaux et al. 2009). Waterlogging and O_2 deficiency in the soil lead to an increase in the activity of anaerobic microorganisms whereupon the production of compounds toxic to roots increases (Parent et al. 2008). For instance, ethanol is accumulated in root tissues and ethylene (C_2H_4), is accumulated in the entire plant (e.g. Jackson 2003), thus affecting root growth negatively (Visser & Perik 2007). Waterlogging imposes reducing conditions in the soil (Pezeshki &

Chambers 1985). Consequently, by-products of fermentation start to accumulate in the rhizosphere (e.g. Parent et al. 2008). These conditions cause not only competition between plants and microorganisms for O_2, but lead also to low availability of essential nutrients and production of phytotoxic compounds (Pezeshki & DeLaune 2012). These all result in stress for roots and affect most fundamental plant processes, like gas exchange, nutrient uptake or biomass production (Kozlowski & Pallardy 2007). At low soil O_2 concentrations, the energy production of roots decreases drastically, as the energy yield from carbohydrates is much lower under hypoxic or anoxic conditions as compared to aerobic conditions (e.g. Pucciariello & Perata 2012). In addition to accumulation of C_2H_4 produced by plants and soil microorganisms (Primrose & Dilworth 1976, Xu & Zhang 2015), reducing conditions during WL lead to a substantial increase in methane (CH_4) production particularly in organic soil, as it is the end product of the anaerobic decomposition of organic matter (Topp & Pattey 1997, Abdalla et al. 2016). Under normal conditions, when the water table is low, only deep peat layers are waterlogged and anaerobic, where CH_4 is produced by methanogens, obligate anaerobic microorganisms. Instead, in the upper aerobic layers methane is consumed by methanotrophs (Topp & Pattey 1997, Whalen 2005). However, when the water table is high and the aerobic layer is shallow or even missing, CH_4 can be produced also in the upper peat layers. High CO_2 and CH_4 concentrations are known to hamper root growth or even contribute to root damage (Gilman & Leone 1982, Gliński and Stępniewski 1985, Crawford 1989).

One of the first plant physiological responses to soil WL and O_2 deficiency is stomatal closure, associated with decreased transpiration and photosynthesis (e.g. Sojka 1992, Parent et al. 2008). These lead to an internal water deficit (Parent et al. 2008), and therefore, WL results paradoxically in similar effects as drought. In line with decreasing photosynthesis, lower chlorophyll fluorescence and chlorophyll contents have been observed after WL, too

(Pezeshki 1994, Repo et al. 2016). In order to maintain the metabolic activity in hypoxic conditions, the plant is forced to use root carbohydrate storages for anaerobic respiration to survive WL (Pezeshski & DeLaune 2012). The stress on roots may affect root hydraulic conductance (Tyree et al. 1995). Higher root hydraulic conductance usually shows increased water uptake ability, but when measured as the reverse-flow conductance (K_r), it was found to increase drastically with increasing root frost damage in Scots pine (*Pinus sylvestris* L.), indicating impaired uptake of water and nutrients due to the increased ion leaking of the roots (Korhonen et al. 2018, Di et al. 2019). However, no immediate effects were observed for Norway spruce (*Picea abies* L. Karst) seedlings after WL during dormancy (Wang et al. 2013), suggesting, that in comparison to frost, WL is not directly detrimental to cell membranes.

The objectives of this study were to compare the effects of repeated short-term WL vs. continuous WL in the beginning of the growing season on physiology, phenology and growth of roots and shoots of Scots pine saplings. We tested the following hypotheses: (1) Scots pine saplings will suffer more when subjected to continuous WL compared to repeated WL even though the total duration of anoxia is the same, (2) the effects of continuous vs. repeated short-term WL can be observed by differences in the dynamics of physiology, phenology and growth shoots and roots.

Materials and Methods

Experimental set-up and plant material

The experiment was carried out in four dasotrons (RTR48, Conviron Ltd, Winnipeg, MB, Canada) with four root containers each (Finér et al. 2001). The cylindrical root containers

(height 0.5m and diameter 0.7m) were filled with 15 cm of fine-textured sand at the bottom, including a glycol coil for controlling soil temperature. Above this 30 cm commercial peat (Forest Seedling Peat B1F, Novarbo Ltd, Eura, Finland) was set as a growth media for the saplings. The peat was compressed by hand and fully watered to get uniform bulk densities between the root containers.

Sixteen four-year-old Scots pine saplings (height 88.4 cm ± 5.7 cm) were obtained from a commercial nursery (Meri-Lapin Taimi Ltd, Simo, Finland, 65°41' N, 25°08' E). The saplings had been naturally regenerated next to the nursery in agricultural soil. At the age of three years, they were replanted into pots of 5 litre volume during summer, fertilised once, overwintered at outside conditions in the nursery until the end of February and transported to Joensuu two weeks before the planting into the dasotrons. Before planting they were slowly thawed for one week at a temperature of 3°C and a photon flux density of approx. 100 µmol m^{-2} s^{-1} (6 hours day/18 hours night). The illumination was provided by LED lighting (B100 type, Valoya Oy, Helsinki, Finland). At the time of planting into the dasotrons, the peat soil was watered thoroughly in order to ensure a good contact between the root ball and the surrounding soil. An organic layer (approx. 7 cm depth), taken from a drained pine bog (Onkamo, Tohmajärvi, Finland. 62°16'N, 30°11'E), classified as *Vaccinium vitis-idea* site type II (according to Paavilainen and Päivänen 1995), was placed on the top of the peat layer. A second glycol coil for controlling soil temperature was placed on the top of the organic layer.

The experimental design included three growing seasons (GS) with dormancy periods (D) in between (Table 1). The first growing season (GS1) and dormancy (D1) were used for acclimatization in the dasotron conditions. The WL treatments were carried out in the second growing season (GS2). The second dormancy period (D2) and the third growing season (GS3) were used to monitor the after-effects of the treatments. Each GS consisted of a long-

day and high temperature period followed by three weeks of a short-day period at the end (GS/SD). Irrigation took place once a week with water simulating natural precipitation in Southern Finland (Sallantaus 1992). The volumetric soil water content was maintained approx. at the target values of 0.35 m^3m^{-3} in the surface organic layer and at 0.55 m^3m^{-3} at the bottom of the peat layer.

The WL treatments were initiated 23 days from the beginning of GS2, just when height growth had started, and consisted either of 2.5 weeks of continuous WL (ContWL) or interval WL (IntWL). The IntWL treatment consisted of three one-week-WL periods, interrupted by one-week-periods without WL. During WL, the water table was adjusted up to the organic layer of the containers with water from a nearby lake. The drainage took place by a valve at the bottom of the root containers. The control treatment (CTRL) was irrigated normally for maintaining the target water content.

Measurements of environmental conditions

Soil temperatures (105 T Thermocouple, Campbell Scientific, Shepshed, UK) and volumetric water contents (ThetaProbe ML2x, Delta-T Devices, Cambridge, UK and CS615, Campbell Scientific, Shepshed, UK) were recorded at three depths: one in the surface organic layer and two in the peat layer (10 and 20 cm from the top of the peat layer). Soil O_2 concentrations (Fibox 4 trace and DP-PSt3, PreSens, Regensburg, Germany) were measured at 10 cm from the top of the peat layer, approximately in the middle of the root ball of the saplings.

Sampling and measurements during the experiment

For determining concentrations of soil carbon dioxide (CO_2), and methane (CH_4), silicone tubes (length 1 m) were set at the bottom and on the top of the peat layer. At gas sampling, 40–50 ml of air from the soil tube was sucked into plastic syringes and analysed on the same day, after transferring into 6ml glass vials with a needle (Chromacol®, Sun Sri, Rockwood, TN, USA, caps: BUTYL liner, spring and crimp). Overpressure was avoided by using another needle pushed through the rubber cap on the vial. The gas concentrations were determined using a system of head-space sampler and gas chromatograph (TurboMatrix and Clarus 580 GC, PerkinElmer, Waltham, MA, USA) equipped with a PlotQ capillary column and a two-channel flame ionization detector (FID). Ethylene efflux from the soil was determined ten times during GS2, using a portable C_2H_4 analyser (CI-900, BioSciences, Camas, WA, USA), and analysed as described by Domisch et al. (2019).

Shoot height and trunk diameter were measured weekly during the growing seasons. Shoot height was determined until it did not change anymore, whereas diameter (slightly above the root collar) was measured during the entire growing season.

To assess the maximum and effective photosynthetic quantum efficiency of intact needles, chlorophyll fluorescence was measured from dark-acclimated (20 min) and subsequently light-acclimated green needles (after 3 min at PAR 300 μmol m^{-2} s^{-1}), using a portable fluorometer (Walz PAM-2500, Heinz Walz GmbH, Effeltrich, Germany). Ten green needles were randomly selected and removed from each sapling for the chlorophyll fluorescence measurements, which were replicated three times. The chlorophyll content index (CCI), defined as the ratio of chlorophyll fluorescence at 735 nm and 700–710 nm, was determined from five randomly selected and removed green needles from each sapling (Chlorophyll content meter CCM-300, Opti-Sciences, Hudson, NH, USA). Chlorophyll fluorescence and CCI were measured seven times during GS2 and additionally once during D1 and D2, respectively.

Gas exchange and stomatal conductance were determined with a portable photosynthesis system (LI-6400XT, LI-COR Inc., Lincoln, NE, USA) at light saturation (1700 μmol m^{-2} s^{-1}). Light response curves (measured before starting the beginning of the treatments, data not shown) were used to confirm that photosynthesis was light-saturated at the used measuring irradiance. The number of needles inside the gas exchange cuvette was counted and their length and diameter was measured from sample needles to provide an estimate of the total needle area inside the cuvette.

Carbohydrate analysis was conducted once during D1, five times during GS2 and once during D2. Ten needles developed during GS1, and if feasible, also ten needles developed during GS2, were frozen in liquid nitrogen and stored at -80°C for further processing. The samples were freeze-dried (Christ Alpha 1–4 LD, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany), milled in a ball mill (Fritsch Pulverisette 23, Fritsch GmbH, Idar-Oberstein, Germany) and stored at -20°C until analysis. Soluble carbohydrate and starch were analysed as described previously (Domisch et al 2018). Shortly, soluble sugars were extracted three times from around 15mg DW using hot water (80°C) and analysed by HPLC using a ligand-exchange column (Hi-Plex Ca, 300 × 7.7 mm, Agilent, Santa Clara, CA, USA) and water for elution. Detection was done using an evaporative light scattering detector (Sedex 90LT, Sedere, Olivet, France). Authentic standards (glucose, fructose, sucrose, raffinose, pinitol) were used for quantification. Starch was enzymatically degraded from the pellet after extraction of soluble sugars and glucose was quantified spectrophotometrically (K-GLUC, Megazyme).

Minirhizotron imaging of roots was conducted in three-week-intervals throughout the experiment. The imaging tube (outer diameter 60 mm) was installed prior the start of the experiment horizontally at the lower border of the root plug of the saplings at a depth of 15 cm from the soil surface. At each imaging session, digital images (Bartz BTC-100X Camera

System, Bartz Technology Company, Santa Barbabra, CA, USA) were taken in two directions (up and left) along the tube with a total of 46 frames (13 x 18 mm^2) in both directions. The image analysis was done using the RootView program (Aphalo and Simonic 1999). Root appearance, elongation, death and disappearance were assessed both for short (first order) and long roots (> first order) separately. First order roots without branching were defined as short roots and higher order roots as long roots. In the case the order of individual roots changed from the first order to a higher one, their orders were changed retrospectively for all preceding imaging sessions, as well.

Final harvest

Root hydraulic conductance (K_r, expressed as mg H_2O MPa^{-1} s^{-1}) was measured at the end of the experiment from excised root systems for each sapling using a high pressure flow meter (HPFM, Dynamax, Inc., Houston, TX, USA). The shoot was cut approx. 5 cm above the root collar while the root system remained intact. The bark was peeled off for about 3 cm below the cutting point and the cut surface was attached to the HPFM using a coupling set. The measurement was based on monitoring the flow of water by gradual pressurizing the root from 0 to 0.55 MPa (Tyree et al. 1995), and K_r (termed as reverse-flow K_r) was obtained from the linear part of the relation between applied pressure and water flow (Korhonen et al. 2018, Di et al. 2019).

After finishing the K_r measurements, electrical impedance (EI) spectra of the root systems were determined (Ozier-Lafointaine & Bajazet 2005, Repo et al. 2005), defined as the frequency response of the real and imaginary part at 44 frequencies between 20 Hz and 100 kHz, using an impedance analyser (HP 4284A, Agilent, Palo Alto, CA, USA). A two-electrode measurement set-up was used, where one of the electrodes (stainless steel needle,

diameter 1.5 mm) was inserted into the stem at the root collar and the other electrode into the soil 30 cm apart from the trunk. The measurements were repeated twice with the stem electrodes set perpendicular between the measurements, and the values were averaged. Electrical impedance measurement provides information of the electrolyte balance in cells, and if there are changes in the balance, the proportion of the current along different routes changes. In electrical impedance spectroscopy (EIS), the alternating electric current of different frequencies is driven through the sample. Complex impedance is obtained from the relation between voltage and current (regarding both amplitude and phase) and this is formed of a real part (resistance) and an imaginary part (reactance). A low-frequency current will pass along the routes with the lowest impedance, like along the apoplastic space in plant tissues, whereas a high-frequency current may pass barriers like cell membranes. Impedance of undamaged plant tissue is high at low frequencies but if damaged, the low-frequency impedance decreases (Repo 1988, Repo et al. 1994).

Stems and needles were dried at 60°C until a constant mass and weighed for determining their biomass. Root morphology and biomass were determined for the upper humus layer and three peat layers (each 10 cm thick) by taking sector samples (area 385 cm^2) from each root container. Roots were separated from the soil samples and washed to remove adherent peat soil. Root length, diameter and numbers of root tips were determined by scanning (HP ScanJet 6100, Hewlett Packard, Palo Alto, CA, USA) and by image analysis with the WinRhizo program (v3.1.2, Regent Instruments Inc., Québec, Canada). After measurements, the roots were dried at 60°C and their biomass determined. Finally, the rest of the root system, including the stump, was removed from the containers, cleaned by washing with water and determined for the dry mass as described above.

Calculations and statistical testing

Soil temperature was averaged for the three positions, first for each container, and then also for all treatments, as they did not differ. Similarly, the O_2 data was averaged for the four pots of each treatment. Water content and soil gas data from the bottom position were chosen for the analyses. Pearson correlations were calculated between O_2, CO_2, and CH_4 concentrations, and C_2H_4 efflux of the respective treatments for those dates when the sampling of C_2H_4 coincided with that of CO_2, and CH_4.

For analysing the data obtained by root imaging, the length of all living roots from all frames were aggregated by tube and sampling sessions and for short and long roots separately. The short and long root production in terms of length and number of root tips was determined from the imaging data by pooling the frames at each sampling time for each root container individually. For taking into account the variation between the root containers, the data of each container was normalized using the Euclidian norm:

$$X = \left\{ \frac{x_1}{\sqrt{x_1^2+x_2^2+\cdots+x_n^2}}, \frac{x_2}{\sqrt{x_1^2+x_2^2+\cdots+x_n^2}}, \ldots, \frac{x_n}{\sqrt{x_1^2+x_2^2+\cdots+x_n^2}} \right\}, \quad (1)$$

where X is the normalized root length at the imaging times $t=\{t_1, t_2, \ldots, t_n\}$. By normalisation, small changes in the shape of the curves in a particular period became visible. At each time, production of short and long roots and root tips was calculated as the difference between the subsequent normalised values. The peak in root production during GS3 was almost negligible. A possible reason for this observation could be that the minirhizotron frames got "saturated" of roots and more root growth took place in the lower parts of the containers. The proportions of dead roots were calculated as the percentages of dead roots out of all roots present at the respective imaging sessions, for short and long roots separately.

306 In the final harvest, root characteristics, like biomass, length or number of root tips, were up-
307 scaled for the whole root container by considering the volume of the soil sectors. Roots that
308 were deeper than 30 cm and thus not sampled by the sector sampling, were added to the total
309 root biomass after the final harvest. Specific root length (SRL) was calculated as root length
310 per dry mass for all roots together.

311 Relative shoot height and trunk diameter of the saplings were expressed as values relative to
312 the respective values at the beginning of the WL treatments.

313 Linear mixed models were used for analysing the effects of the WL treatments (CTRL,
314 IntWL or ContWL) on the various plant traits, using sampling time as a repeated factor.
315 Similarly, soil layer was used as a repeated factor when using liner mixed models for
316 analysing WL treatment effects on root length, average diameter, number of root tips, root
317 mass and SRL in the final harvest. Statistically significant effects (P<0.05) of the sampling
318 time and the WL treatments and their interactions were analysed using Bonferroni corrected
319 post-hoc comparisons.

320 Values were ln-transformed when residuals were visibly distributed unevenly. Statistical
321 testing was conducted with the IBM SPSS Statistical package (IBM SPSS Statistics for
322 Windows, Version 25, SPSS Inc., Chicago, IL, USA).

323 One-way ANOVA was used for testing the effects of the treatments at the end of the
324 experiment on biomass and root characteristics, like total root length or mean diameter and
325 root hydraulic conductance.

326 Root survival probability and longevity analyses were conducted for short and long roots
327 separately by generalized Kaplan-Meier statistics, using the 'interval' package in R (version
328 3.5.2). The appearance and death of individual roots was observed at three-week-intervals.
329 Thus the data represented interval as well as right censored data. The survival probability

curves were plotted using the ‘icfit’ function and differences between the treatments were tested with the ‘ictest’ function using an asymptotic logrank k-sample test (permutation form and Sun’s scores, Fay and Shaw 2010). The Kaplan-Meier estimator is typically “undefined" after the last observation if that observation is right-censored (Fay and Shaw 2010), like in our case for those roots that were alive at the end of the experiment. According to Fay and Shaw (2010), this is because the nonparametric maximum likelihood estimation (NPMLE) is not unique in this case, as changes in the survival probability distribution after that last censored observation do not affect the likelihood of the observed data. Root longevities were assessed as the median survival probability estimate, using the ‘Survfit’ function of the ‘survival’ package. Survival probabilities are expressed by the curves as a whole whereas longevity by a point-event when the survival probability curves reach the value of 0.5.

Electrical impedance spectra (resistance and reactance) were examined by Class-Featuring Information Compression (CLAFIC) analysis (Jääskeläinen et al. 1994, Repo et al. 2014). The CLAFIC analysis is based on the principle of artificial intelligence where the training data is compared with the learning data. The measure of how the impedance spectra of different classification groups resemble each other was calculated by comparing the number of IS belonging to each group. In the classification, the unknown spectrum is classified by measuring the length of the projection vector in each subspace k, where k takes into account the fine structure of the impedance spectra.

The experiment consisted of three treatments, distributed over four dasotrons with four root containers. Thus the fourth root container in each dasotron was subjected to one of the three treatments. Before any statistical calculation, the averages of each treatment of each dasotron were calculated, rendering N =4, except for the CLAFIC analysis, where the individual values were used (CTRL, N=5; ContWL, N=5 and IntWL, N=6).

354

355 **Results**

356 *Environmental conditions during the experiment*

357 Waterlogging treatments took place during the first half of GS2 as shown in the graphs for air
358 and soil temperature (Fig. 1A, Table 1). The soil water content reacted immediately to the
359 WL treatments and returned to conditions similar to the CTRL treatment when the WL had
360 ceased (Fig. 1B).

361

362 *Soil gases*

363 Soil O_2 concentrations showed an immediate WL effect. The total duration of hypoxia
364 differed between the two WL treatments (18 days in ContWL and 21 days in IntWL), but
365 both lead to the same total duration of anoxic conditions (16 days). Immediately when WL
366 had ceased, the conditions returned to normoxic, similar to those in the CTRL treatment (Fig.
367 1C, Table 2). Also CO_2 and CH_4 showed very distinct and immediate WL effects (Fig. 2,
368 Table 2). All increased significantly during the ContWL treatment and decreased after WL
369 had ceased, returning to a level similar to that of the CTRL treatment. The IntWL treatment
370 resulted in a zigzag pattern of the soil gas concentrations with the highest peaks during the
371 first WL for CO_2, whereas CH_4 showed the highest peak during the third (and last) WL
372 period (Fig. 2).

373 Ethylene efflux from the soil increased slightly but insignificantly after WL had started, when
374 compared to the level before the WL (Fig. S1, Table 2). The variation in C_2H_4 efflux between
375 the root containers was high, and WL seemed to increase the variation even more. On
376 average, the soils acted as a C_2H_4 source in all treatments and at all measurement occasions.

We observed significant negative correlations between the O_2 and CO_2, and O_2 and CH_4 concentrations, respectively, in both the ContWL and IntWL treatments (N=10, $-0.988<r<-0.818$, $P<0.002$), whereas these relationships were not significant in the CTRL treatment (N=10, $-0.493<r<-0.150$, $P>0.074$). No significant correlations were observed between any gas concentrations and the C_2H_4 efflux (N=8, $-0.616<r<0.19$, $P>0.087$).

Growth and biomass of aboveground parts

The CTRL saplings showed a slight and significantly higher relative height increment than those in the WL treatments during GS2 (Fig. 3A, Table 3). During GS3, these differences became more distinctive, and the CTRL saplings grew relatively faster than those in the ContWL treatment, with the IntWL saplings having intermediate growth (indicated by the significant time*treatment interaction). The relative diameter was not different between treatments during GS2 (Fig. 3B). During GS3, CTRL saplings grew slightly more in diameter than the WL saplings did, but this was not statistically significant (Table 3).

Needle length increment was not significantly affected by WL, but the pattern was very similar to the sapling height increment during GS2 (data not shown). When needle elongation ceased, CTRL needles were slightly, but insignificantly longer than those of the WL saplings (Table S1).

Belowground (roots or stumps or total) and stem biomass at the end of the experiment was not different between the treatments (Table 4, Fig. 4), whereas the IntWL treatment resulted in significantly higher needle biomass compared to that of the ContWL, the CTRL saplings having intermediate values (Table 4, Fig. 4).

400 *Root length production, mortality and survival probability estimates*

401 Waterlogging affected the morphology of the root system significantly (Table 4). Total root
402 length and number of root tips at the end of the experiment were significantly higher
403 (F=7.018, P=0.015 and F=11.8217, P=0.003, respectively) in the IntWL treatment than in the
404 ContWL treatment (830 ± 48 m roots and 142900 ± 14300 root tips per sapling, and 492 ± 56
405 m roots and 68000 ± 8900 root tips per sapling, respectively), the values of the CTRL
406 saplings ranging in between (756 ± 90 m roots and 124500 ± 10100 tips per sapling). When
407 comparing WL treatment effects in the different layers, the saplings in the IntWL treatments
408 had significantly more roots in the 0–10 cm layer than the ContWL saplings (Tables 5 and 6).

409 Root length production was significantly affected by WL (Table 7). Short roots did not react
410 to WL during the first two WL periods in the IntWL treatment, but their production declined
411 drastically after the third WL period, being significantly lower than in the CTRL. The
412 ContWL treatment decreased short root production after WL ceased, although not
413 significantly different from the CTRL (Fig. 5A). Long roots did not react to WL as strongly
414 as short roots did (Fig. 5B). When the WL treatments ended, long root production had
415 decreased significantly in IntWL. Length production of short and long roots recovered
416 towards the end of the growing season, and was on a same level in all treatments. No WL
417 effects were observed during GS3.

418 Waterlogging had very clear and significant effects on the proportion of dead short and long
419 roots (Fig. 5 CD, Table 7). It increased during WL, and became significantly higher in both
420 WL treatments compared to the CTRL when WL ceased.

421 Both short and long root survival probabilities were significantly affected by the WL
422 treatments ($P<0.001$ for both short and long roots), the survival probability being
423 significantly higher in the CTRL treatments (Fig. 6AB). Comparisons between the WL

424 treatments revealed that short and long root survival probability was affected differently:
425 short root survival probability was lowest in the ContWL treatment (P<0.01), whereas for
426 long roots it was lowest in the IntWL treatment (P<0.001).

427 Short root longevity was considerably shorter for WL (326 days for ContWL and 305 days
428 for IntWL) than CTRL treatment (389 days). Long root longevity was the same for CTRL
429 and ContWL (284 days) but lower for IntWL (220 days).

430 Root tip survival probability was significantly affected by WL (P<0.001) (Fig. 6C), but the
431 comparison between the WL treatments revealed that it was not different between the WL
432 treatments (P=0.117). Root tip longevity was 389 days for CTRL but much shorter for
433 ContWL (284 days) and IntWL (220days).

434

435 *Gas exchange*

436 There was a general decrease in net photosynthesis with time, both for GS1- and GS2-
437 needles, but no statistically significant treatment effects were observed (Table 2S, Fig. 7A).
438 Only small non-significant decreases in net photosynthesis of GS1-needles were observed in
439 both WL treatments and a subsequent recovery when WL ceased. No treatment effects were
440 observed in stomatal conductance or transpiration (Table 2S, Fig. 7BC).

441

442 *Chlorophyll fluorescence and chlorophyll content index*

443 Chlorophyll content index was not different between the treatments (Table 3S, Fig. 2S). No
444 treatment effects for chlorophyll fluorescence were observed but the yield in GS2 needles of

445 ContWL was higher than in IntWL and CTRL just after the WL treatment ceased (Table 3S,
446 Fig. 3S).

447

448 *Carbohydrates*

449 There was a general increase of starch concentrations in the GS1-needles at the beginning of
450 GS2 and after a levelling off, a second peak was observed at the end of GS2. These peaks
451 were intensified by the WL treatments (Fig. 8A). For needles developed during GS2,
452 however, no treatment effects were observed (Fig. 8D, Table 8).

453 The overall concentrations of soluble sugars in the needles were not affected by the WL
454 treatments (Fig. 8BE). However, the pinitol concentrations in the GS1-needles were
455 significantly higher in the ContWL saplings during GS2 after WL had ceased, as compared to
456 the CTRL (Fig. 8C). On average, pinitol concentrations in the GS-needles were the lowest in
457 the CTRL (Fig. 9F), but a statistically significant difference between the treatments was seen
458 only at the first sampling occasion after the WL treatments, when pinitol concentrations were
459 higher in the ContWL than in the CTRL treatment.

460

461 *Root hydraulic conductance and electrical impedance*

462 Root hydraulic conductance at the end of the experiment was not affected by treatments,
463 being on average 442 mg H_2O MPa^{-1} s^{-1} and showing rather large variations between the
464 saplings (df=2, F=0.529, P=0.602).

465 The electrical impedance spectra of the roots at the end of the experiment differed between
466 the CTRL and the WL treatments (Fig. 9). In addition, the CLAFIC results suggested a

467 difference between the WL treatments (Fig. 10), since at k-values > 4, all treatments were
468 classified into their own groups.

469

470 **Discussion**

471 Both WL treatments resulted in the same total duration of anoxia, but still differed in their
472 effects on the saplings. We hypothesised that the Scots pine saplings experiencing the same
473 duration of soil anoxia, but subjected to it for a continuous period, will suffer more than
474 saplings subjected to it for interrupted WL. This was true for shoot height increment. The WL
475 treatments started in the phases of the most intensive height growth, and the relative height
476 increment was significantly lower in ContWL than CTRL, while it was not reduced as much
477 in IntWL. The WL effects on shoot height and diameter growth were more pronounced in the
478 following growing season GS3, which can be partly explained by the predetermined growth
479 pattern of Scots pine. This means that the terminal bud, which is formed during the growing
480 season (GS1) before the actual growth year (GS2), has already all the major structures that
481 will appear in the elongated shoot (Lanner 1976, Sutinen et al. 2007). Thus the growing
482 conditions during the current growing season can affect the final shoot height only to some
483 extent (Salminen & Jalkanen 2006).

484 The WL treatments had very clear and distinct effects on the concentrations of soil O_2, CO_2,
485 and CH_4. As soon as O_2 concentrations decreased with the onset of WL, the concentrations of
486 the other gases increased, which has been observed earlier, too (e.g. Greenway et al. 2006,
487 Domisch et al. 2018). The sharp increase of soil CO_2 concentrations is mainly a result of
488 anaerobic catabolism (Greenway et al. 2006), and that of CH_4 is due to anaerobic
489 decomposition of organic matter (Topp & Pattey 1997, Abdalla et al. 2016). Also the
490 extremely low diffusion of gases in water adds to the increases in concentrations. High CO_2

491 and CH_4 concentrations are known to hamper root growth and even result in root damage
492 (Gilman & Leone 1982, Gliński and Stępniewski 1985, Crawford 1989). However, even the
493 highest concentrations in our experiment were probably not detrimental for plant roots
494 (Gilman & Leone 1982, Greenway et al. 2006), although adverse effects cannot be entirely
495 excluded.

496 An increase of C_2H_4 efflux due to WL is also commonly observed (Smith 1976, Kimmerer &
497 Kozlowski 1982), either produced by soil microorganisms or plants. We measured also C_2H_4
498 efflux but its responses to WL were not that clear. There was a tendency for higher efflux due
499 to WL but there were large variations between root containers, and the increase in the WL
500 treatments was certainly not high enough to cause root damage (e.g. Smith & Restall 1971).
501 Domisch et al. (2019) showed C_2H_4 efflux from the soil in spring just after WL and
502 snowmelt, but later the soil acted as a C_2H_4 sink. The fact that the soils acted as C_2H_4 sources
503 in the present experiment, using peat as a growth media compared to mineral soil used by
504 Domisch et al. (2019), is connected to the positive effect of organic matter on C_2H_4
505 production (Goodlass & Smith 1978).

506 Root length production decreased significantly during WL and recovered after WL in both
507 treatments. In contrast to aboveground height growth and biomass, where ContWL had a
508 greater effect than IntWL, the negative effect of IntWL on root production and the proportion
509 of dead roots was more pronounced. One reason could be that the saplings started to recover
510 after WL, but in the case of IntWL, recovery was halted by the returning of the WL, and thus
511 had more severe effects. Repo et al. (2016) suggested a post-treatment shock of re-
512 oxygenation by drainage for Scots pine seedlings after 5 weeks of WL, which was manifested
513 by a further decrease in sap flow in waterlogged seedlings after WL ceased. In the present
514 study, WL periods were much shorter, but the trend for more negative effects of IntWL than
515 ContWL, particularly regarding the proportion of dead roots, fits into this pattern. The

damage seems to be a result of repeated re-oxygenation, as the re-exposure of WL plants to O_2 after soil drainage leads to the formation of reactive oxygen species (ROS) (Armstrong et al. 1994, Ricard et al. 2006). This causes damage within the plant, as cellular membranes can be injured by ROS after re-exposure to O_2 by repeated WL and drainage (e.g. Drew 1992). Particularly the root apical meristem is sensitive to O_2 depletion, as it is the zone of the fastest O_2 consumption (e.g. Drew 1992), and eventually cell deaths lead to a drastic reduction of root growth. Here, this was clearly observed as a significant decrease in survival probabilities of roots and root tips, and as an increase in root mortality due to WL. Also earlier studies have shown that especially root tips are sensitive to WL (Levan & Riha 1986, Grossnickle 1987, Repo et al. 2017). Despite having adverse effects on plant roots when occurring at high concentrations in the soil, there are indications that CH_4 could be produced by plants and have a role in abiotic stress tolerance by counteracting effects of ROS (Li et. al 2019).

Shoot and root biomass at the end of the experiment were affected differently by WL than shoot height, root length growth, and root survival probabilities. At the final harvest, the IntWL saplings had the highest aboveground biomass, which was mainly due to a significantly higher needle biomass. Root biomass at the end of the experiment was not different between the treatments. After a drastic decrease by WL, root length production recovered similarly in both WL treatments, which probably contributed to this lack of difference in root biomass at the end of the experiment. Poot and Lambers (2003) observed a decrease in shoot growth, particularly in leaf area, in woody *Hakea* (an endemic Australian species and tolerant to WL) after recovery from WL, but an increase of root growth at the same time. DeBell et al. (1984) studied the effects of repeated vs. continuous WL in *Pinus taeda*, a moderately flood-tolerant tree species growing in the Southeastern US. They found the highest root biomass in a repeated WL treatment, whereas the lowest was found in a continuous WL. Also Megonigal & Day (1992) found higher C allocation to roots in

repeatedly than continuously flooded bald cypress (*Taxodium distichum*) seedlings. Our results, although a very different species and shorter WL periods, are similar in that we observed a clear decrease of shoot growth but a simultaneous recovery of root growth after WL ceased.

In our study, all saplings survived the WL treatments whereas previously, a five-week WL of Scots pine saplings in the middle of the growing season caused serious damage that appeared in the physiology of aboveground parts and even led to their death (Repo et al. 2016). Although our treatments had no lethal consequences, we still observed significant WL effects, like decreased root production and shoot height growth. Thus our results are in agreement with Repo et al. (2016) who concluded that WL longer than three weeks during the growing season can have severe effects on Scots pine.

We also hypothesised that the WL stress could be detected by differences in the dynamics of physiology, phenology and growth between shoots and roots. However, several physiological characteristics were only slightly and/or not significantly affected by the WL, like gas exchange, chlorophyll fluorescence or chlorophyll index. Similar effects were reported for Norway spruce seedlings (Wang et al. 2013) and Scots pine saplings (Roitto et al. 2019) that were exposed to WL during dormancy. In our experiment, photosynthesis was not significantly affected by WL, despite small declines during the WL treatments. Zaerr (1983) showed clear and immediate decreases of net photosynthesis after WL in Norway spruce and Douglas fir seedlings, but not in Scots pine, which is in accordance with our results. Thus we can conclude that an anoxic period of 16 days was not long enough for significant decreases of photosynthesis, in accordance to Repo et al. (2016), who observed significant WL effects just after three weeks of WL in similar experimental conditions.

564 There was a starch peak in needles in the beginning of GS2, which was intensified by WL.
565 Needle starch content is a result from the balance between carbon assimilation by
566 photosynthesis, phloem export and consumption, i.e. a balance between sources and sinks.
567 Any factor disturbing this balance will result changes in starch content. An increase of starch
568 is commonly observed in spring/early summer when the soil is still too cold for efficient root
569 growth (e.g. Sutinen 1985, Fischer & Höll 1991, Domisch et al. 2002), which would explain
570 the increase in the CTRL seedlings, too. In addition, we observed a second peak in the WL
571 saplings later in the growing season, particularly in needles of older age classes, which is in
572 accordance with the earlier studies (Sudachková et al. 2009, Repo et al. 2016). Our results
573 indicate that photosynthesis is functioning during WL too, producing sugars that are stored as
574 starch in the needles. The accumulation of starch in the needles was probably due to a
575 decreased sink for carbohydrates in other parts of the plant (e.g. Steffens et al. 2005, Repo et
576 al. 2016), particularly in the roots. This decreased sink strength is obviously due to increased
577 root mortality and decreased new root production due to WL. The recovery of root growth
578 after WL indicated, that while root growth is inhibited during WL and starch accumulates in
579 the shoot, the translocation of stored carbohydrates enables fast root growth after WL ceased,
580 being essential for the performance of the whole plant.

581 We observed higher pinitol concentrations in the needles of the WL saplings after the WL
582 treatments, and significantly so in the ContWL saplings as compared to CTRL saplings.
583 Interestingly, the pinitol concentrations remained higher in the previous-year needles during
584 the whole growing season after the WL exposure. Pinitol is regarded as an antioxidant,
585 protecting the plant (particularly cytoplasm and chloroplasts) against oxidative stress by ROS
586 (Galinski & Truper 1994, Ashraf & Harris 2004). Increased pinitol concentrations are found
587 during drought and cold stress in conifers (Nguyen & Lamant 1988, Lintunen et al. 2016),
588 and it appears to be the case for WL stress, too.

589 We did not observe any visible damage of plant tissue due to WL. However, there were
590 indications that the roots subjected to WL suffered from oxidative stress still after a recovery
591 period with considerable root growth. The impedance spectra of roots of WL saplings
592 indicated some root damage, as their spectra differed from the CTRL saplings. The real parts
593 of impedance at low frequencies representing the apoplastic electrical resistance have been
594 shown to be sensitive to cell membrane damage but also to water content (Repo 1994, Repo
595 et al. 2000). Immediate cell membrane damage in roots has been shown to decrease
596 apoplastic resistance when electrolytes leak into the symplast to the apoplast (Ryyppö et al.
597 1998). The changes in electrical properties of roots support the observations concerning the
598 increased proportion of dead roots and decreased root longevity by WL. However, they are in
599 contrast to the reverse-flow hydraulic conductance of roots, where no difference was found
600 between the treatments, suggesting that the root systems would have recovered from the WL
601 stress during the follow-up growing season.

602 In conclusion, we observed direct and immediate WL effects on the saplings. Waterlogging
603 may occur more frequently in the future as a consequence of increased precipitation and
604 incidences of flooding. Soil CO_2 and CH_4 concentrations very fast increased during WL and
605 immediately decreased when WL ceased. Anoxic soil conditions, as a consequence of WL,
606 did not directly affect photosynthesis functioning, but impaired root growth. Likely as an
607 effect of the damaged root system, WL had also negative consequences on aboveground
608 growth occurring later in the growing season, as well as during the following growing season.
609 Our results indicated also that repeated WL can be more harmful than continuous WL,
610 indicated by increased fine root mortality, even though the total anoxic time was the same.
611 Starch, and particularly pinitol status, as well as electrical impedance spectra were the most
612 sensitive short-term WL stress indicators, in contrast to photosynthesis or chlorophyll
613 fluorescence which were not affected by WL. The results also suggest that even rather short-

term WL can have adverse effects on growth and fitness of Scots pine, even though no lethal effects are observed and root growth seemed to recover after WL ceased.

Supplementary data

Tables S1, S2 and S3

Figures S1, S2 and S3

Conflict of interest

None declared

Funding

This work received funding from the Natural Resources Institute Finland (Luke) and the Academy of Finland (decision no. 311455).

Acknowledgements

We acknowledge Ms Eija Koljonen, Mr Mauri Heikkinen and Mr Matti Savinainen for skilful laboratory and technical work.

Author's contributions

633 Designing the experiment: TD, TL, SP, LF, TR

634 Implementation of the experiment and data collection: TD, JQ, IS, TR

635 Data analysis and interpretation: TD, JQ, IS, FM, RS

636 Manuscript writing: TD, JQ, IS, FM, TL, SP, TR

637

638 **References**

639 Abdalla M, Hastings A, Truu J, Espenberg M, Mander Ü & Smith P (2016) Emissions of
640 methane from northern peatlands: a review of management impacts and implications
641 for future management options. Ecol Evol 13:7080–7102.

642 Abramoff RZ & Finzi AC (2014) Are above- and below-ground phenology in sync? New
643 Phytol 205:1054–1061.

644 Aphalo PJ & Simonic A (1999) RootView — overview and manual. Finnish Forest Research
645 Institute, Joensuu Research Station, Faculty of Forestry, University of Joensuu,
646 http://www.mv.helsinki.fi/aphalo/pdf/manual.pdf.

647 Armstrong W (1979) Aeration in higher plants. In: Woolhouse HW (ed.) Advances in
648 Botanical Research, 7. Academic Press, London, pp. 225–232.

649 Armstrong W, Brändle R & Jackson MB (1994) Mechanisms of flood tolerance in plants.
650 Acta Bot Neerl 43:307–358.

651 Ashraf M & Harris PJC (2004) Potential biochemical indicators of salinity tolerance in
652 plants. Plant Science 166:3–16.

653 Crawford RMM (1989) Studies in plant survival: ecological case histories of plant adaptation
654 to adversity. Blackwell Scientific Publications, Oxford.

655 DeBell DS, Hook DD & McKee WH (1984) Growth and physiology of Loblolly pine roots
656 under various water table level and phosphorus treatments. Forest Sci 30:705–714.

657 Dessaux Y, Hinsinger P & Lemanceau P (2009) Rhizopshere: so many achievements and
658 even more challenges. Plant Soil 321:1–3.

659 Di B, Luoranen J, Lehto T, Himanen K, Silvennoinen M, Silvennoinen R & Repo T (2019)
660 Biophysical changes in the roots of Scots pine seedlings during cold acclimation and
661 after frost damage. For Ecol Manage 431:63–72.

662 Domisch T, Finér L & Lehto T (2002) Growth, carbohydrate and nutrient allocation of Scots
663 pine seedlings after exposure to simulated low soil temperature in spring. Plant Soil
664 246:75–86.

665 Domisch T, Martz F, Repo T & Rautio P (2018) Winter survival of Scots pine seedlings
666 under different snow conditions. Tree Physiol 38:602–616.

667 Domisch T, Martz F, Repo T & Rautio P (2019) Let it snow! Winter conditions affect growth
668 of birch seedlings during the following growing season. Tree Physiol 39:544–555.

669 Drew MC (1992) Soil aeration and plant root metabolism. Soil Sci 154:259–268.

670 Fay MP, Shaw PA (2010) Exact and asymptotic weighted logrank tests for interval censored
671 data: the interval R package. J Stat Softw 36:1–34.

672 Finér L, Aphalo P, Kettunen U, Leinonen I, Mannerkoski H, Öhman J, Repo T & Ryyppö A
673 (2001) The Joensuu dasotrons: A new facility for studying shoot, root, and soil
674 processes. Plant Soil 231:137–149.

675 Fischer C & Höll W (1991) Food reserves of Scots pine (*Pinus sylverstris* L.) I. Seasonal
676 changes in the carbohydrate and fat reserves of pine needles. Trees 5:187–195.

677 Galinski EA & Truper HG (1994) Microbial behavior in salt stressed ecosystems. FEMS
678 Microbiol Rev 15:95–108.

679 Gilman EF & Leone IA (1982) Influence of soil gas contamination on tree root growth. Plant
680 Soil 65:3–10.

681 Glenz C, Schlaepfer R, Iorgulescu I & Kienast F (2006) Flooding tolerance of Central
682 European tree and shrub species. For Ecol Manage 235:1–13.

683 Gliński J & Stępniewski W (1985) Soil aeration and its role for plants. CRC Press, Boca
684 Raton, FL.

685 Goodlass G & Smith KA (1978) Effect of pH, organic matter content and nitrate on the
686 evolution of ethylene from soils. Soil Biol Biochem 10:193–199.

687 Greenway H, Armstrong W & Colmer TD (2006) Conditions leading to high CO_2 (>5 kPa) in
688 waterlogged–flooded soils and possible effects on root growth and metabolism. Ann
689 Bot 98:9–32.

690 Grossnickle SC (1987) Influence of flooding and soil temperature on the water relations and
691 morphological development of cold-stored black spruce and white spruce seedlings.
692 Can J Forest Res 17:821–828.

693 IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of working groups I, II
694 and III to the fifth assessment report of the Intergovernmental Panel on Climate Change
695 (Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)). IPCC, Geneva.

696 Jackson MB (2003) Ethylene and responses of plants to soil waterlogging and submergence.
697 Ann Rev Plant Biol 36:145–174.

698 Jääskeläinen T, Silvennoinen R, Hiltunen J & Parkkinen J (1994) Classification of the
699 reflectance spectra of pine, spruce and birch. Appl Optics 33:2356–2362.

700 Kimmerer TW & Kozlowski TT (1982) Ethylene, ethane, acetaldehyde, and ethanol
701 production by plants under stress. Plant Physiol 69:840–847.

702 Korhonen A, Lehto T, HeinonenJ & Repo T (2018) Whole-plant frost hardiness of
703 mycorrhizal (Hebeloma sp. or Suillus luteus) and non-mycorrhizal Scots pine seedlings.
704 Tree Physiol 39:526–535.

705 Kozlowski TT & Pallardy SG (2007) Physiology of Woody Plants, Academic Press.

706 Kreuzwieser J & Gessler A (2010) Global climate change and tree nutrition: influence of
707 water availability. Tree Physiol 30:1221–1234.

708 Kundzewicz ZW, Radziejewski M & Pinskwar I (2006) Precipitation extremes in the
709 changing climate of Europe. Clim Res 31:51–58.

710 Lanner RM (1976) Patterns of shoot development in Pinus and their relationship to growth
711 potential. In: Cannell MGR & Last FT (eds.) Tree physiology and yield improvement.
712 Academic Press, London, UK, pp 223–243.

713 Levan MA & Riha SJ (1986) Response of root systems of northern conifer transplants to
714 flooding. Canadian Journal of Forest Research 16:42–46.

715 Li L, Wei S & Shen W (2019) The role of methane in plant physiology: a review. Plant Cell
716 Rep. https://doi.org/10.1007/s00299-019-02478-y.

717 Lintunen A, Paljakka T, Jyske T, Peltoniemi M, Sterck F, von Arx G, Cochard H, Copini P,
718 Caldeira MC, Delzon S, Gebauer R, Grönlund L, Kiorapostolou N, Lechthaler S, Lobo-
719 do-Vale R, Peters RL, Petit G, Prendin AL, Salmon Y, Steppe K, Urban J, Roig Juan S,
720 Robert, EMR & Hölttä T (2016) Osmolality and non-structural carbohydrate
721 composition in the secondary phloem of trees across a latitudinal gradient in Europe.
722 Front Plant Sci 7.

723 Megonigal JP & Day FP (1992) Effects of flooding on root and shoot production of bald
724 cypress in large experimental enclosures. Ecology 73:1182–1193.

725 Nguyen A & Lamant A (1988) Pinitol and myo-inositol accumulation in water-stressed
726 seedlings of maritime pine. Phytochemistry 27:3423–3427.

727 Orlov AJ (1966) Growth and vitality of Scots pine, Norway spruce and downy birch
728 seedlings with flooded root systems. In: Effect of waterlogged soils on forest
729 productivity. Moscow, pp 112–154.

730 Ozier-Lafontaine H & Bajazet T (2005) Analysis of root growth by impedance spectroscopy
731 (EIS). Plant Soil 277:299–2013.

732 Paavilainen E & Päivänen J (1995) Peatland forestry. Ecology and principles. Ecological
733 Studies 111, Springer-Verlag, Berlin-Heidelberg-New York.

734 Parent C, Capelli N, Berger A, Crevecoeur M & Dat JF (2008) An overview of plant
735 responses to soil water logging. Plant Stress 2:20–27.

736 Pelkonen E (1975) Vuoden eri aikoina korkealla olevan pohjaveden vaikutus männyn
737 kasvuun. Effects of Scots pine growth of ground water adjusted to the ground surface
738 for periods of varying length during different seasons of the year. Suo 26:25–32.

739 Pelkonen E (1979) Männyn ja kuusen taimien kyvystä sietää tulvaa vuoden eri aikoina.
740 Seasonal flood tolerance of Scots pine and Norway spruce seedlings. Suo 30:35–42.

741 Pezeski SR (1994) Plant responses to flooding. In: Wilkinson RE (ed.) Plant-Environment
742 Interactions. Marcel Dekker Inc., New York, pp 283–321.

743 Pezeshki SR & Chambers JL (1985) Stomatal and photosynthetic response of sweet gum
744 (Liquidambar styraciflua) to flooding. Can J Forest Res 15:371–37.

745 Pezheski SR & DeLaune RD (2012) Soil oxidation-reduction in wetlands and its impact on
746 plant functioning. Biology 1:196–221.

747 Poot P & Lambers H (2003) Growth responses to waterlogging and drainage of woody *Hakea*
748 (Proteaceae) seedlings, originating from contrasting habitats in south-western Australia.
749 Plant Soil 253:57–70.

750 Primrose SB & Dilworth MJ (1976) Ethylene production by bacteria. J Gen Microbiol
751 93:177–181.

752 Pucciariello C & Perata P (2012) Flooding tolerance in plants. In: Shabala S (ed.) Plant Stress
753 Physiology, CAB Internationa, Wallingford, UK, pp 148–170.

754 Repo T (1988) Physical and physiological aspects of impedance measurements in plants.
755 Silva Fennica 22:181–193.

756 Repo T (1994) Influence of different electrodes and tissues on the impedance spectra of Scots
757 pine shoots. Electro Magnetobiol 13:1–14.

758 Repo T, Zhang M, Ryyppo, A, Vapaavupri E & Sutinen S (1994) Effects of freeze-thaw
759 injury on parameters of distributed electrical circuits of stems and needles of Scots pine
760 seedlings at different stages of acclimation. J Exp Bot 45:823–833.

761 Repo T, Zhang G, Ryyppo A & Rikala R (2000) Electrical impedance spectroscopy of Scots
762 pine (Pinus sylvestris L) shoots in relation to cold acclimation. J Exp Bot 51:2095–
763 2107.

764 Repo T, Leinonen I, Ryyppö A, Finér L (2004) The effect of soil temperature on the bud
765 phenology, chlorophyll fluorescence, carbohydrate content and cold hardiness of Norway
766 spruce seedlings. Physiol Plantarum 121:93–100.
767 Repo T, Laukkanen J & Silvennoinen R (2005) Measurement of the tree root growth using
768 electrical impedance spectroscopy. Silva Fennica 39:159–166.
769 Repo T, Korhonen A, Laukkanen M, Lehto T & Silvennoinen R (2014) Detecting
770 mycorrhizal colonisation in Scots pine roots using electrical impedance spectra.
771 Biosystems Engineering 121:139–149.
772 Repo T, Launiainen S, Lehto T, Sutinen S, Ruhanen H, Heiskanen J, Laurén A, Silvennoinen
773 R, Vapaavuori E & Finér L (2016) The responses of Scots pine seedlings to
774 waterlogging during the growing season. Can J For Res 46:1439–1450.

775 Repo T, Heiskanen J, Sutinen, M-L, Sutinen R & Lehto T (2017) The responses of Scots pine
776 seedlings to waterlogging in a fine-textured till soil. New Forests 48:51–65.

777 Ricard B, Aschi-Smiti A, Gharbi I & Brouquisse R (2006) Cellular and molecular
778 mechanisms or plant tolerance to waterlogging. In: Huang B (ed.) Plant-Environment
779 Interactions. Third edition. CRC Press, Boca Raton, London, New York, pp 177–208.

780 Roitto M, Sutinen S, Wang A, Domisch T, Lehto T & Repo T (2019) Waterlogging and soil
781 freezing during dormancy affected root and shoot phenology and growth of Scots pine
782 saplings. Tree Physiol 39:805–818.

783 Ryyppö A, Repo T & Vapaavuori E (1998) Development of frost hardiness in roots and
784 shoots of Scots pine seedlings at non-freezing temperatures. Can J For Res 28:557–565.

785 Salminen H & Jalkanen R (2006) Modelling variation of needle density of Scots pine at high
786 latitudes. Silva Fennica 40:183–194.

787 Sallantaus T (1992) Leaching in the material balance of peatlands—preliminary results. Suo
788 43:253–258.

789 Schmull M & Thomas F (2000) Morphological and physiological reactions of young
790 deciduous trees (*Quercus robur* L., *Q. petraea* [Matt.] Liebl., *Fagus sylvatica* L.) to
791 waterlogging. Plant Soil 225:227–242.

792 Smith AM (1976) Ethylene production by bacteria in reduced microsites in soil and some
793 implications to agriculture. Soil Biol Biochem 8:293–298.

794 Smith KA & Restall SWF (1971) The occurrence of ethylene in anaerobic soil. Jo Soil Sci
795 22:430–443

796 Sojka RE (1992) Stomatal closure in oxygen-stress plants. Soil Sci 154:269–280.

797 Steffens D, Hütsch BW, Schholz T, Lošák T & Schubert S (2005) Water logging may inhibit
798 plant growth primarily by nutrient deficiency rather than nutrient toxicity. Plant Soil
799 Environ 51:545–552.

800 Sudachkova NE, Milyutina IL & Romanova LI (2009) Adaptive responses of Scots pine to
801 the impact of adverse abiotic factors on the rhizosphere. Russian Journal of Ecology
802 40:387–392.

803 Sutinen ML (1985) Seasonal changes of carbohydrates in Scots pine seedlings. Aquilo Ser
804 Bot 23:37–44.

805 Sutinen S, Aphalo PJ & Lehto T (2007) Does timing of boron application affect needle and
806 bud structure in Scots pine and Norway spruce seedlings? Trees 21:661–670.

807 Topp E & Pattey E (1997) Soil as sources and sinks for atmospheric methane. Can J Soil Sci
808 77:167–178.

809 Tyree MT, Patiño S, Bennink J & Alexander J (1995) Dynamic measurements of roots
810 hydraulic conductance using a high-pressure flowmeter in the laboratory and field. J
811 Exp Bot 46:83–94.

812 Veijalainen N, Lotsari E, Alho P, Vehviläinen B & Käyhkö J (2010) National scale
813 assessment of climate change impacts on flooding in Finland. J Hydrol 391:333–350.

814 Visser EJW & Pierik R (2007) Inhibition of root elongation by ethylene in wetland and non-
815 wetland plant species and the impact of longitudinal ventilation. Plant, Cell and
816 Environment 30:31–38.

817 Wang AF, Roitto M, Lehto T, Zwiazek J, Calvo-Polanco M & Repo R (2013) Waterlogging
818 under simulated late-winter conditions had little impact on the physiology and growth
819 of Norway spruce seedlings, Annals of Forest Science 70:781–790.

820 Whalen SC (2005) Biogeochemistry of methane exchange between natural wetlands and the
821 atmosphere. Environmental Engineering Science 22:73–94.

822 Xu J & Zhang S (2015) Ethylene biosynthesis and regulation in plants. In: Wen CK (Ed.)
823 Ethylene in plants. Springer, Dordrecht, pp 1–25.

824 Zaerr JB (1983) Short-term flooding and net photosynthesis in seedlings of three conifers.
825 For Sci 29:71–78.

826

827 **Table 1.** Environmental conditions in the growth chambers during the experiment as set
828 values. GS1, GS2 and GS3 refer to growing seasons, D1and D2 to dormancy periods and SD
829 to short-day phases at the end of the growing seasons.

830

	Planting and acclimatisation	GS1	GS1/SD	D1	GS2	GS2/SD	D2	GS3
Duration, weeks	2	11	3	10	13	3	10	10
Air temperature (day/night), °C	3/3	20/15	20/15	3/3	20/15	20/15	3/3	20/15
Air RH (day/night), %	90/90	60/85	60/85	90/90	60/85	60/85	90/90	60/85
Soil temperature, °C	3	15	15	2	15	15	2	15
Photoperiod (day/night), hours	6/18	18/6	6/18	6/18	18/6	6/18	6/18	18/6
PAR, µmol m^{-2} s^{-1}	100	400	100	100	400	100	100	400

831

832

833 **Table 2.** Results of linear mixed models, analysing the effects of waterlogging treatment and
834 time on the concentrations of soil oxygen (O_2), carbon dioxide (CO_2), and methane (CH_4)
835 during GS2, and on the efflux of ethylene (C_2H_4), with 'Time' as a repeated factor. Analyses
836 for O_2 concentrations (although measured continuously) were conducted with values
837 measured at the same time when the other gases were sampled. Statistically significant effects
838 (P<0.05) are depicted in bold.

839

	Numerator df	Denominator df	*F*	*P*
O_2				
Treatment	2	18.368	1319.116	**<0.001**
Time	15	17.718	29914.614	**<0.001**
Treatment*Time	30	17.718	8990.816	**<0.001**
CO_2				
Treatment	2	40.946	94.717	**<0.001**
Time	9	112.855	152.862	**<0.001**
Treatment*Time	18	117.204	33.642	**<0.001**
CH_4				
Treatment	2	48.668	62.446	**<0.001**
Time	9	112.335	101.962	**<0.001**
Treatment*Time	18	110.653	42.727	**<0.001**
C_2H_4				
Treatment	2	28.191	0.368	0.695
Time	9	56.534	0.546	0.835
Treatment*Time	18	56.534	0.918	0.561

840

841

842 **Table 3.** Results of linear mixed models, analysing the effects of waterlogging (WL)
843 treatment and time on relative shoot height and relative diameter (see Fig. 3), with 'Time' as
844 a repeated factor (N=4). Statistically significant effects (P<0.05) are depicted in bold.

845

	Numerator df	Denominator df	*F*	*P*
Relative height				
Treatment	2	5.556	0.680	0.545
Time	33	159.595	6.155	**<0.001**
Time*Treatment	66	159.602	3.558	**<0.001**
Relative diameter				
Treatment	2	9.042	0.785	0.485
Time	43	329.071	39.141	**<0.001**
Time*Treatment	85	272.836	1.346	0.369

846

847

848 **Table 4.** Results of one-way ANOVA, testing the effects of waterlogging on various root
849 characteristics, as well as biomass in the different plant compartments at the end of the
850 experiment (N=4). Values for root length, diameter, root tips and root mass are up-scaled
851 from the sector samples to each root container individually before statistical testing.
852 Statistically significant effects (P<0.05) are depicted in bold.

853

	df	*F*	*P*
Root length	2	7.018	**0.015**
Root diameter	2	3.269	0.086
No of root tips	2	11.827	**0.003**
Root biomass	2	1.514	0.271
Stump biomass	2	1.612	0.252
Total belowground biomass	2	1.128	0.366
Stem biomass	2	2.490	0.138
Needle biomass	2	4.473	**0.039**
Total aboveground biomass	2	4.137	0.053

854

855

856 **Table 5.** Results of linear mixed models, analysing the effects of waterlogging treatment and
857 soil depth on root length, mean root diameter, number of root tips, root dry mass and specific
858 root length (SRL) at the end of the experiment, with 'Depth' as a repeated factor (N=4).
859 Statistically significant effects (P<0.05) are depicted in bold.

860

	Numerator df	Denominator df	*F*	*P*
Root length				
Treatment	2	11.382	4.863	**0.030**
Depth	3	24.570	11.800	**<0.001**
Depth*Treatment	6	24.570	1.066	0.409
Mean root diameter				
Treatment	2	17.207	2.655	0.099
Depth	3	25.859	0.422	0.739
Depth*Treatment	6+	25.859	1.238	0.320
No of root tips				
Treatment	2	12.657	12.808	**0.001**
Depth	3	23.492	18.021	**<0.001**
Depth*Treatment	6	23.492	1.349	0.276
Root biomass				
Treatment	2	7.141	1.386	0.310
Depth	3	18.976	8.518	**0.001**
Depth*Treatment	6	18.976	3.061	**0.029**
SRL				
Treatment	2	12.691	2.216	0.149
Depth	3	24.251	1.072	0.379
Depth*Treatment	6	24.251	1.854	0.130

861

862 **Table 6.** Root length, root diameter, number of root tips, root mass and specific root length
863 (SRL) in the different soil layers in control (CTRL), interrupted (IntWL) and continuous
864 waterlogging (ContWL) at the end of the experiment (± SEM, N=4). Values are up-scaled
865 from the soil sector samples to the whole root containers (excluding the stump). Statistically
866 significant effects (P<0.05) between treatments and within soil layers are indicated by
867 different lowercase letters. Container-wise sums are shown for length, root tips and root
868 mass, whereas the mean for diameter and SRL.

869

Treatment	Layer	Length, m	Diameter, mm	No of root tips	Root mass, g	SRL, m g^{-1}
CTRL	humus	127 ± 40	7.7 ± 0.7	19600 ± 8130	12.2 ± 4.3	12.7 ± 2.7
	0–10 cm	273 ± 43 ab	6.1 ± 0.3	48076 ± 5049 ab	15.2 ± 3.4 a	21.0 ± 5.2
	10–20 cm	196 ± 51	6.8 ± 0.8	31051 ± 5976	9.4 ± 1.9	20.1 ± 1.7
	20–30 cm	140 ± 47	6.9 ± 1.6	21496 ± 7675	6.3 ± 1.8	22.8 ± 4.7
	Sum/Mean	756 ± 90 ab	6.7 ± 0.4	124508 ± 10098 a	44.9 ± 4.0	16.9 ± 1.9
IntWL	humus	118 ± 33	6.4 ± 0.8	19026 ± 5446	5.0 ± 1.1	23.1 ± 1.8
	0–10	354 ± 42 a	9.4 ± 1.4	67645 ± 10258 a	30.0 ± 8.2 b	16.6 ± 3.1
	10–20	201 ± 9	8.7 ± 0.5	33589 ± 2050	11.3 ± 1.3	18.3 ± 2.0
	20–30	188 ± 7	7.5 ± 0.8	28369 ± 3323	9.9 ± 1.2	20.0 ± 2.5
	Sum/Mean	830 ± 48 b	8.0 ± 0.5	142900 ± 14298 a	55.4 ± 10.4	16.1 ± 2.5
ContWL	humus	83 ± 33	7.7 ± 1.7	11401 ± 5328	6.4 ± 2.6	14.1 ± 2.8
	0–10	183 ± 14 b	7.7 ± 0.4	29100 ± 2995 b	13.1 ± 3.5 a	15.7 ± 2.4
	10–20	147 ± 42	8.9 ± 0.7	16661 ± 3959	13.2 ± 2.6	12.0 ± 2.7
	20–30	79 ± 17	8.2 ± 0.8	10854 ± 3481	4.3 ± 0.7	17.9 ± 1.7
	Sum/Mean	492 ± 56 a	8.1 ± 0.4	68071 ± 8902 b	37.4 ± 6.9	13.8 ± 1.2

870

871

872 **Table 7.** Results of linear mixed models, analysing the effects of waterlogging treatments and
873 time on proportions of dead short and long roots (as percentage of all roots present at
874 respective imaging sessions) as well as short and long root production during the experiment,
875 with 'Time' as a repeated factor (N=4). Statistically significant effects (P<0.05) are depicted
876 in bold.

877

	Numerator df	Denominator df	*F*	*P*
Proportion of dead short roots				
Treatment	2	45.159	20.024	**<0.001**
Time	18	133.971	22.424	**<0.001**
Time*Treatment	36	147.468	3.467	**<0.001**
Proportion of dead long roots				
Treatment	2	43.041	21.012	**<0.001**
Time	18	134.191	14.885	**<0.001**
Depth*Treatment	36	134.189	3.045	**<0.001**
Short root production				
Treatment	2	91.230	0.483	0.618
Time	18	141.971	8.286	**<0.001**
Time*Treatment	36	138.335	0.984	0.503
Long root production				
Treatment	2	87.250	0.795	0.455
Time	18	146.776	17.145	**<0.001**
Time*Treatment	36	144.235	1.007	0.468

878

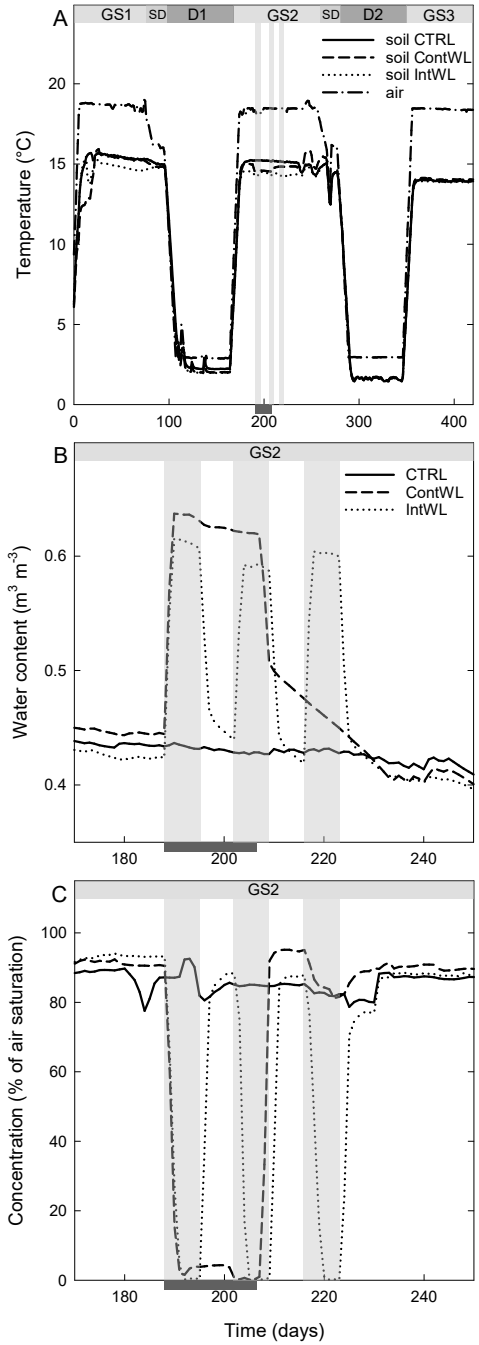
879

880 **Table 8.** Results of linear mixed models, analysing the effects of waterlogging treatments and
881 time on concentrations of starch, soluble sugars and pinitol in needles developed during GS1
882 and GS2, respectively, with 'Time' as a repeated factor. Sampling took place during GS2.
883 Statistically significant effects (P<0.05) are depicted in bold.

Analysis & needle age	Treatment	Numerator df	Denominator df	*F*	*P*
Starch					
GS1	**Treatment**	2	14.709	5.047	**0.021**
	Time	6	16.629	20.247	**<0.001**
	Treatment*Time	12	16.629	2.011	0.093
GS2	Treatment	2	18.938	1.705	0.209
	Time	3	25.562	26.862	**<0.001**
	Treatment*Time	6	25.562	0.850	0.544
Soluble sugars					
GS1	Treatment	2	15.882	2.091	0.156
	Time	6	17.524	4.459	**0.007**
	Treatment*Time	12	17.524	0.680	0.749
GS2	Treatment	2	11.777	2.253	0.148
	Time	3	25.677	42.044	**<0.001**
	Treatment*Time	6	25.677	2.181	0.078
Pinitol					
GS1	**Treatment**	2	29.849	4.811	**0.015**
	Time	6	18.620	16.101	**<0.001**
	Treatment*Time	12	18.620	1.352	0.271
GS2	**Treatment**	2	12.137	5.622	**0.019**
	Time	3	26.345	66.154	**<0.001**
	Treatment*Time	6	26.345	1.532	0.207

884

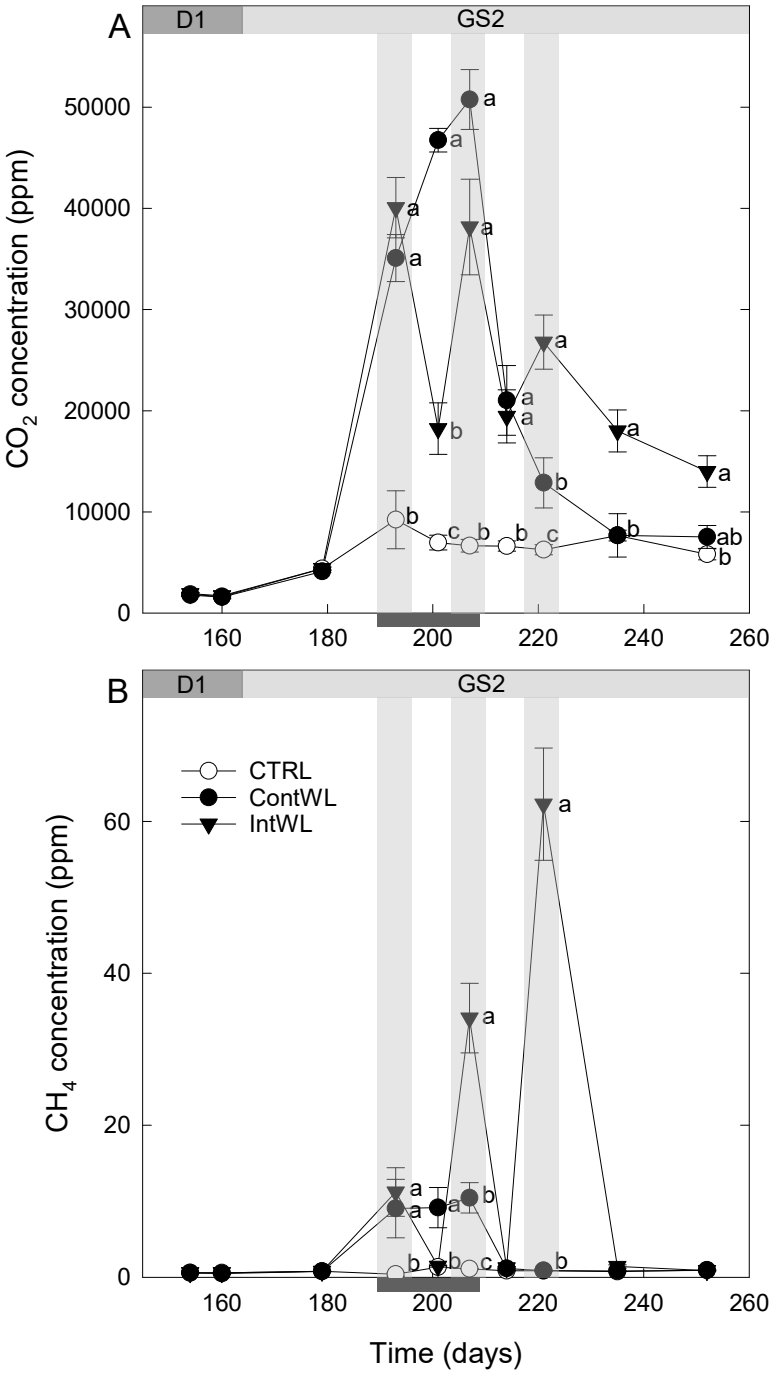
885



886

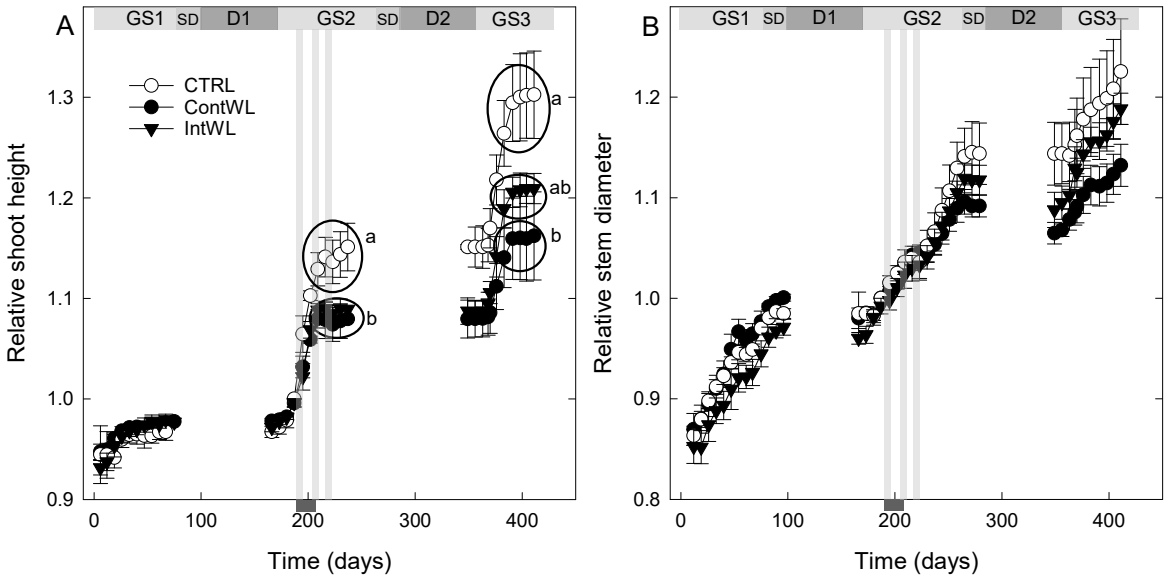
887 **Figure 1.** (A) Air and soil temperatures, (B) soil water content and (C) soil oxygen
888 concentration during the experiment (N=4). Soil temperatures and water contents are
889 averages of three depth positions (see text for more details). Continuous waterlogging
890 (ContWL) is indicated by the dark grey bar below the x-axis and interrupted waterlogging
891 (IntWL) by light grey columns. Time indicates days from the beginning of the experiment.
892 Different phases of the experiment are indicated in the upper part of the figures (see Table1).

893



894

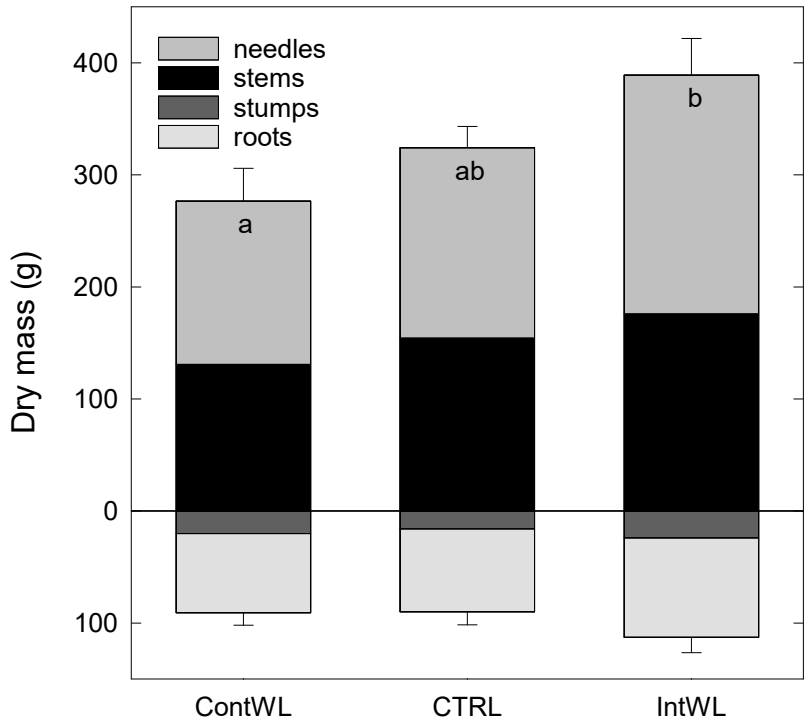
895 **Figure 2.** Concentrations of CO_2 (A) and CH_4 (B) at the bottom of the peat soil layer in the
896 end of the first dormancy period (D1) and during the treatment season (GS2) (N=4, ± SEM).
897 Continuous waterlogging (ContWL) is indicated by the dark grey bar below the x-axis and
898 interrupted waterlogging (IntWL) by light grey columns. Different lowercase letters indicate
899 statistically significant differences between treatments within sampling times (P<0.05). Time
900 indicates days from the beginning of the experiment.



901

902 **Figure 3.** Relative shoot height (A) and stem diameter (B), normalized to the values just
903 before the WL treatments (N=4, ± SEM). Waterlogging periods are depicted by grey shaded
904 bars. Continuous waterlogging (ContWL) is indicated by the dark grey bar below the x-axis
905 and interrupted waterlogging (IntWL) by light grey columns. Groups of statistically
906 significant differences between the treatments are indicated by ovals and different lowercase
907 letters (P<0.05). Different phases of the experiment are indicated in the upper part of the
908 figures (see Table1). Time indicates days from the beginning of the experiment.

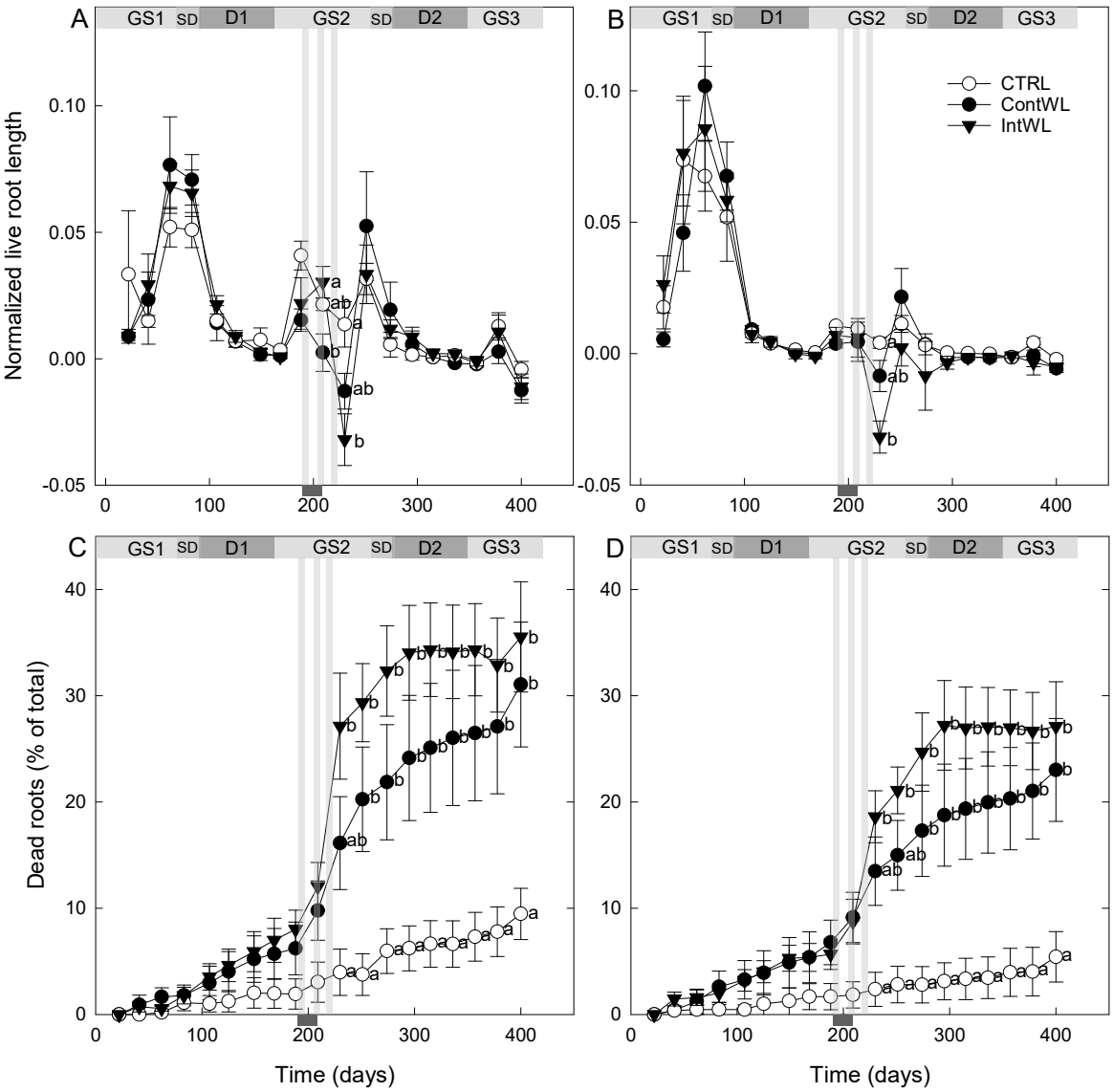
909



910

911 **Figure 4.** Mean biomass of needles, stems, stumps and roots (N=4 ± SEM of total above- and
912 belowground, respectively) of the saplings at the end of the experiment. Different lowercase
913 letters indicate statistically significant differences between treatments for needle biomass
914 (P<0.05).

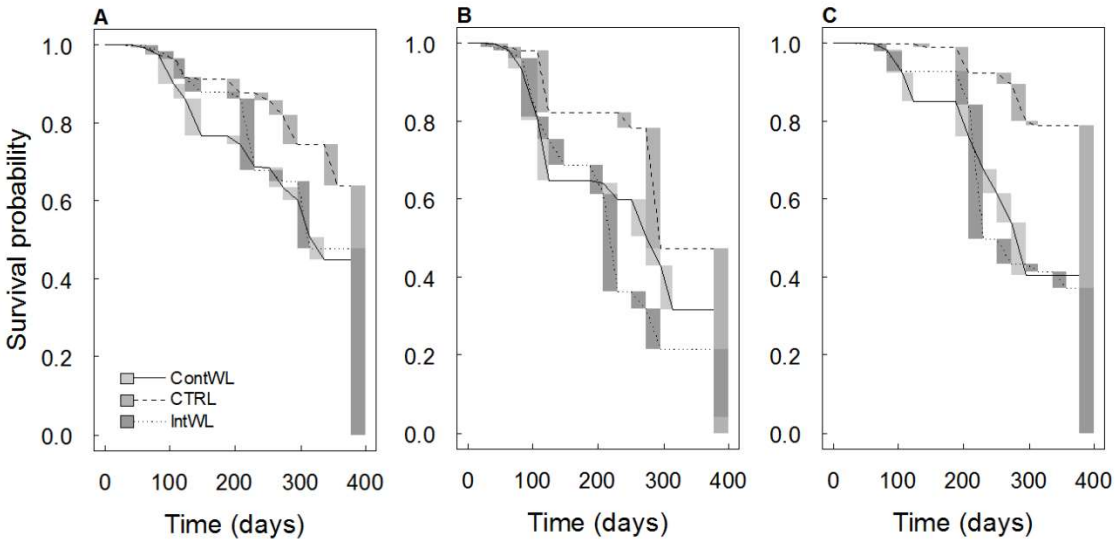
915



916

917 **Figure 5.** (A) Short and (B) long root length production (in Euclidian normalized scale, see
918 text for more details) and proportion of dead (C) short roots and (D) long roots (out of all
919 roots present at respective imaging sessions) during the experiment (N=4, ±SEM).
920 Continuous waterlogging (ContWL) is indicated by the dark grey bar below the x-axis and
921 interrupted waterlogging (IntWL) by light grey columns. Different lowercase letters indicate
922 statistically significant differences between the treatments within sampling times (P<0.05).
923 Different phases of the experiment are indicated in the upper part of the figures (see Table1).
924 Time indicates days from the beginning of the experiment.

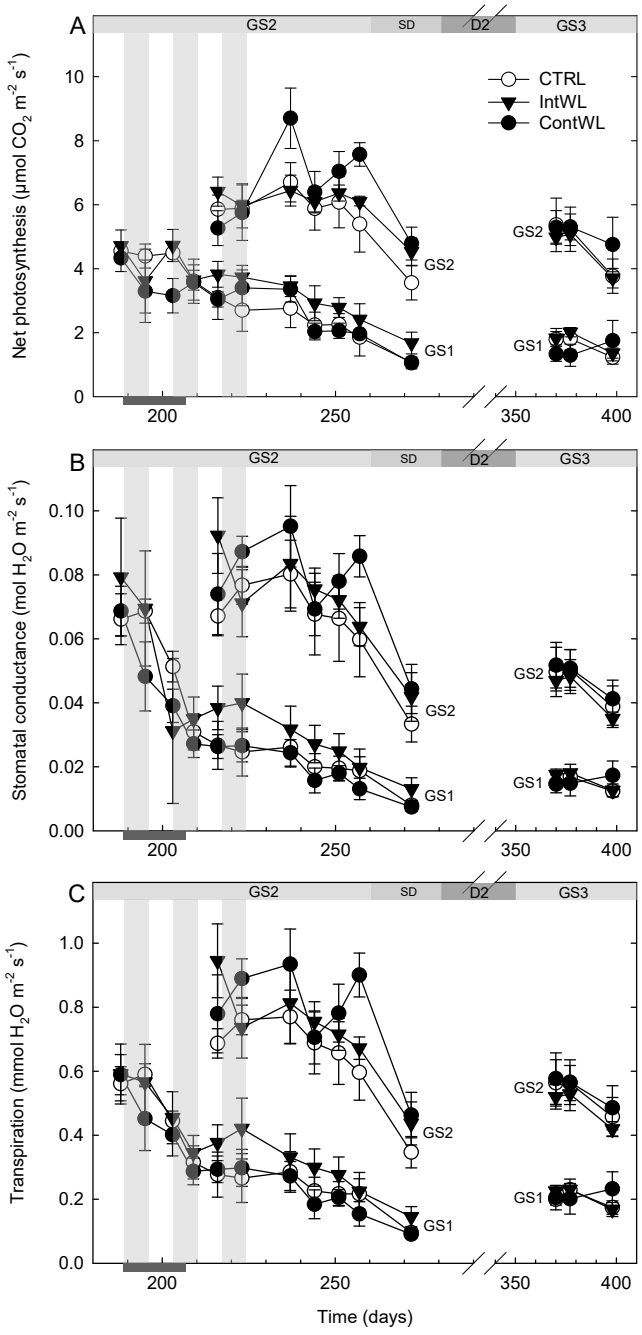
925



926

927 **Figure 6.** Survival curves of (A) short and (B) long roots and (C) root tips for control
928 (CTRL), continuous waterlogging (ContWL) and interrupted waterlogging (IntWL) during
929 the experiment by Kaplan-Meier statistics (N=4). Median longevity is achieved at a survival
930 probability of 0.5. The grey shadings indicate the undefined intervals.

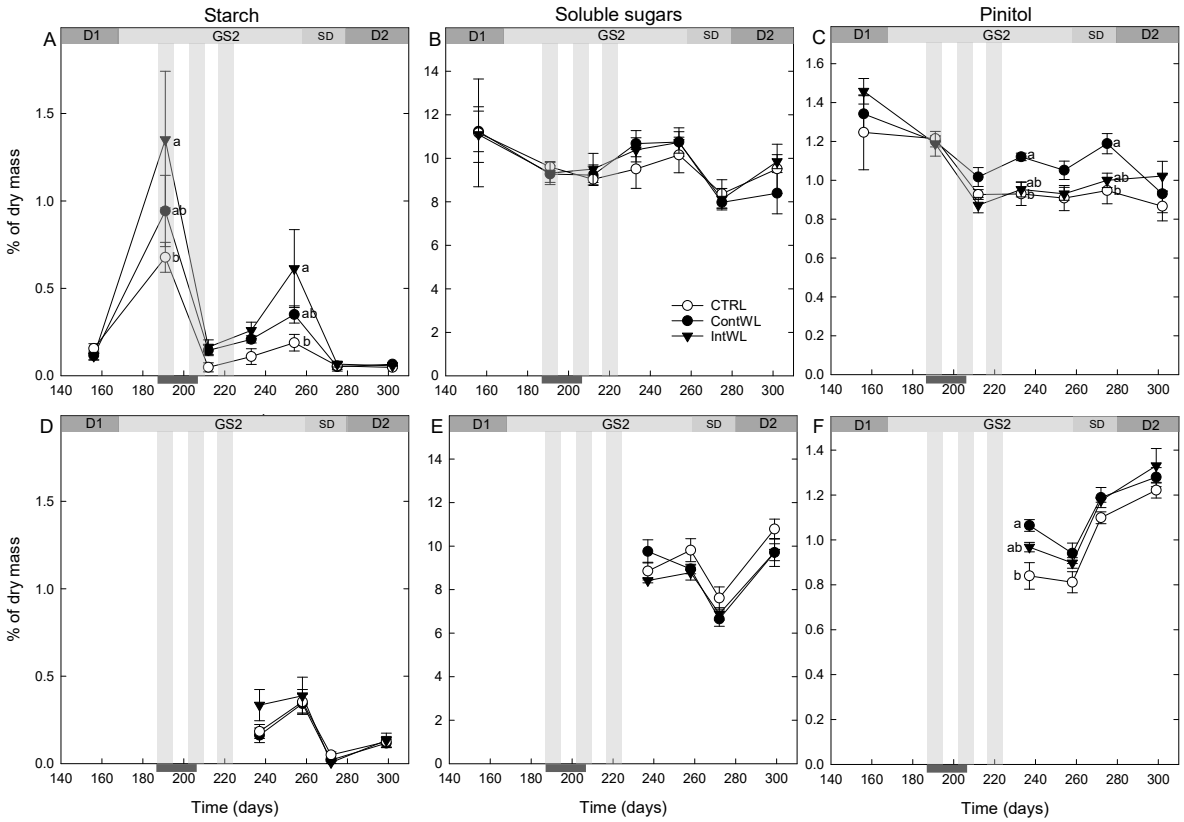
931



932

933 **Figure 7.** (A) Net photosynthesis, (B) stomatal conductance and (C) transpiration of needles
934 developed during GS1 and GS2 (N=4, ±SEM). Continuous waterlogging (ContWL) is
935 indicated by the dark grey bar below the x-axis and interrupted waterlogging (IntWL) by light
936 grey columns. Different phases of the experiment are indicated in the upper part of the figures
937 (see Table1). Time indicates days from the beginning of the experiment.

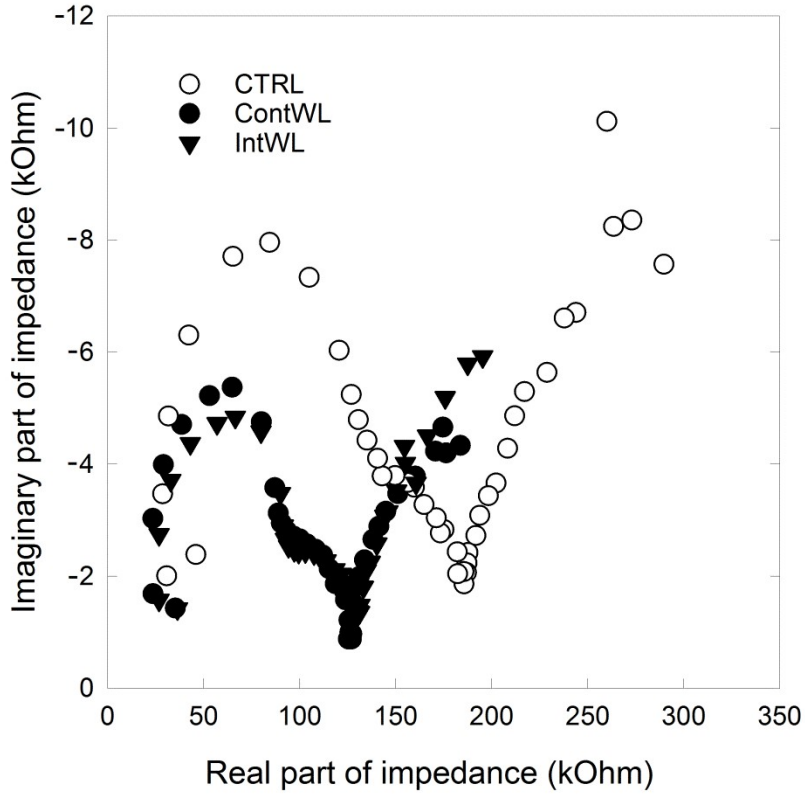
938



939

940 **Figure 8.** Concentrations (% of dry mass) of starch, soluble sugars and pinitol in needles
941 developed during GS1 (A, B and C) and GS2 (D, E and F) (N=4, ±SEM). Continuous
942 waterlogging (ContWL) is indicated by the dark grey bar below the x-axis and interrupted
943 waterlogging (IntWL) by light grey columns. Different lowercase letters indicate statistically
944 significant differences between treatments within sampling times (P<0.05). Different phases
945 of the experiment are indicated in the upper part of the figures (see Table 1). Time indicates
946 days from the beginning of the experiment.

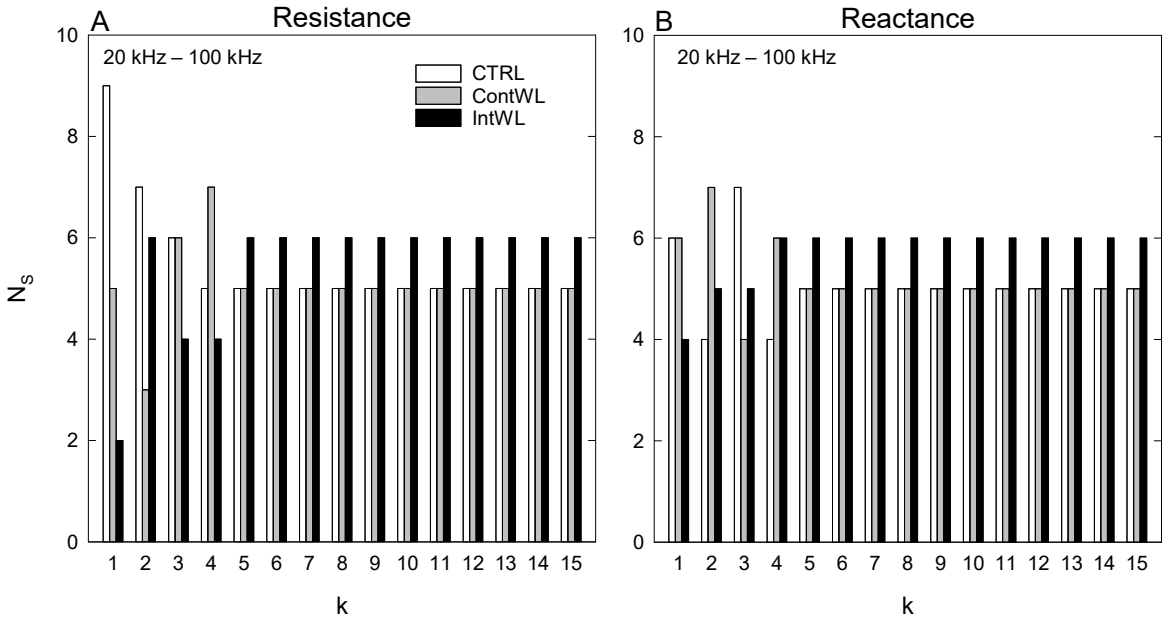
947



948

949 **Figure 9.** Mean electrical impedance spectra (Wessel diagram) of the roots of Scots pine
950 saplings exposed to continuous waterlogging (ContWL) and interrupted waterlogging
951 (IntWL) measured at the end of the experiment (N=4). The frequency increases from right
952 (20 Hz) to left (100 kHz).

953



954

955 **Figure 10.** Class-Featuring Information Compression (CLAFIC) analysis of the (A) real
956 (resistance) and (B) imaginary (reactance) part of the impedance spectra (data from 20 kHz to
957 100 kHz) for the roots of Scots pine saplings exposed to control (CTRL, N=5), continuous
958 (ContWL, N=5) and interrupted waterlogging (IntWL, N=6). N_s indicates the number of the
959 spectra in each classification group of CTRL, ContWL and IntWL by subspace k-values.

960

961 **SUPPLEMENTARY TABLES AND FIGURES**

962

963 **Table S1.** Results of linear mixed models, analysing the effects of waterlogging treatments
964 and time on needle length during the three growing seasons (needles developed during GS1,
965 GS2 and GS3, respectively). 'Time' was used as a repeated factor. Statistically significant
966 effects (P<0.05) are depicted in bold (N=4).

967

	Numerator df	Denominator df	*F*	*P*
		GS1		
Treatment	2	10.224	1.074	0.377
Time	12	105.979	97.701	**<0.001**
Time*Treatment	24	105.978	1.108	0.348
		GS2		
Treatment	2	9.753	0.546	0.596
Time	11	94.530	91.699	**<0.001**
Time*Treatment	22	94.529	0.635	0.888
		GS3		
Treatment	2	9.493	0.251	0.783
Time	4	34.904	500.058	**<0.001**
Time*Treatment	8	34.900	0.111	0.999

968

969

970 **Table S2.** Results of liner mixed models, analysing the effects of waterlogging treatments
971 and time on net photosynthesis, stomatal conductance and transpiration during GS1 and GS2
972 for needles developed during GS1 and GS2, respectively. 'Time' was used as a repeated
973 factor. Statistically significant effects (P<0.05) are depicted in bold (N=4).

974

Needle age	Treatment	Numerator df	Denominator df	*F*	*P*
		Net photosynthesis			
GS1	Treatment	2	24.642	1.794	0.187
	Time	13	75.093	25.407	**<0.001**
	Treatment*Time	26	77.040	1.043	0.426
GS2	Treatment	2	17.598	2.376	0.122
	Time	9	55.383	16.196	**<0.001**
	Treatment*Time	18	55.383	1.393	0.172
		Stomatal conductance			
GS1	Treatment	2	22.616	1.331	0.284
	Time	13	67.616	24.366	**<0.001**
	Treatment*Time	26	69.431	0.582	0.937
GS2	Treatment	2	11.785	0.966	0.409
	Time	9	60.526	42.834	**<0.001**
	Treatment*Time	18	60.526	1.513	0.117
		Transpiration			
GS1	Treatment	2	20.951	1.213	0.317
	Time	13	76.997	29.306	**<0.001**
	Treatment*Time	26	78.542	0.622	0.913
GS2	Treatment	2	12.387	1.538	0.293
	Time	9	61.699	40.632	**<0.001**
	Treatment*Time	18	61.699	3.884	0.134

975

976

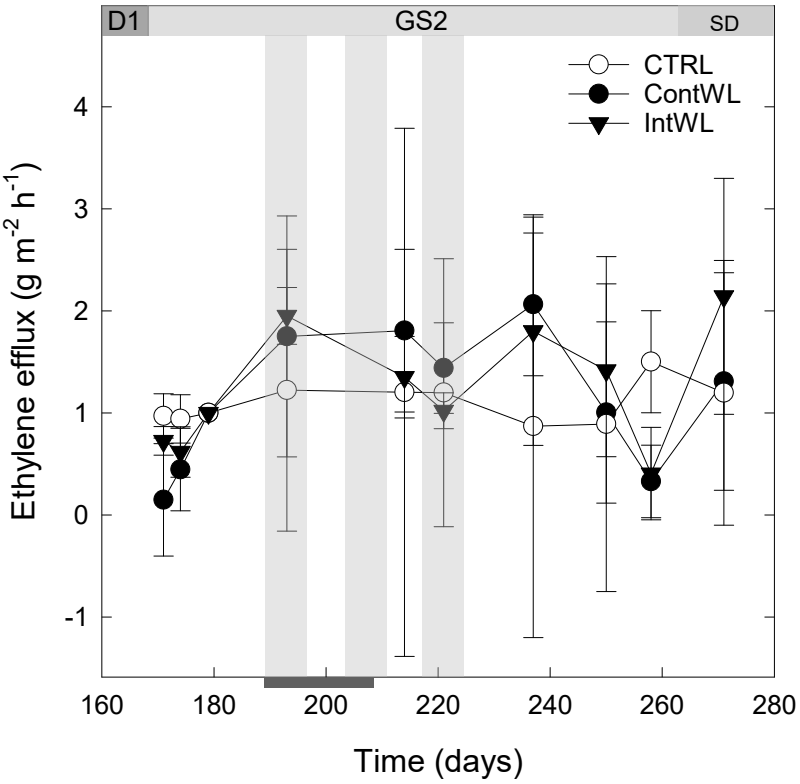
977 **Table S3.** Results of linear mixed models, analysing the effects of waterlogging treatments
978 and time on chlorophyll content index (CCI), maximum photochemical efficiency of dark-
979 adapted needles (F_v/F_m) and effective yield of photosystem II fluorescence ($\Delta F/F_m'$) for
980 needles developed during GS2 and GS2. 'Time' was used as a repeated factor. Statistically
981 significant effects ($P<0.05$) are indicated in bold (N=4).

982

Needle age	Treatment	Numerator df	Denominator df	*F*	*P*
		CCI			
GS1	Treatment	2	66.374	0.924	0.402
	Time	8	15.942	23.803	**<0.001**
	Treatment*Time	16	16.714	1.728	0.138
GS2	Treatment	2	26.798	0.735	0.489
	Time	4	14.942	20.47	**<0.001**
	Treatment*Time	8	14.824	1.898	0.136
		F_v/F_m			
GS1	Treatment	2	7.247	0.644	0.553
	Time	6	33.862	19.541	**<0.001**
	Treatment*Time	12	33.862	0.79	0.657
GS2	Treatment	2	14.046	1.652	0.227
	Time	4	30.147	13.318	**<0.001**
	Treatment*Time	8	30.147	0.504	0.843
		$\Delta F/F_m'$			
GS1	Treatment	2	16.199	1.124	0.349
	Time	6	39.182	18.111	**<0.001**
	Treatment*Time	12	39.182	1.082	0.401
GS2	Treatment	2	10.097	2.444	0.136
	Time	4	32.605	7.523	**<0.001**
	Treatment*Time	8	32.605	3.178	**0.009**

983

984

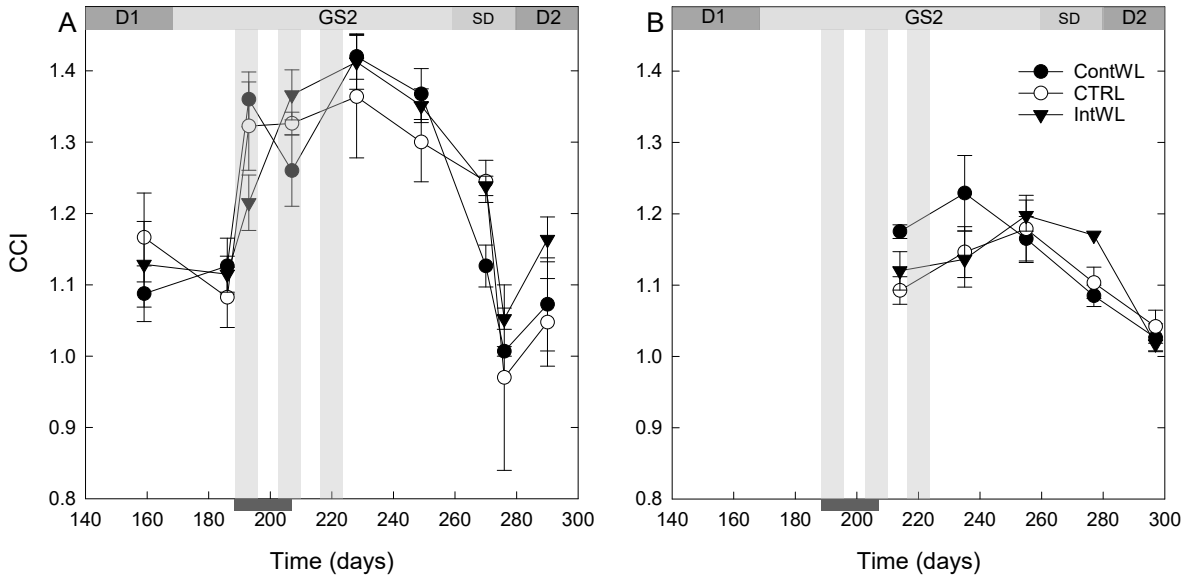


985

986

987 **Figure S1.** Mean (± SEM, N=4) soil ethylene efflux in relation to the respective efflux prior
988 to the WL treatments. Continuous waterlogging (ContWL) is indicated by the dark grey bar
989 below the x-axis and interrupted waterlogging (IntWL) by light grey columns. Different
990 phases of the experiment are indicated in the upper part of the figure (see Table1). Time
991 indicates days from the beginning of the experiment.

992

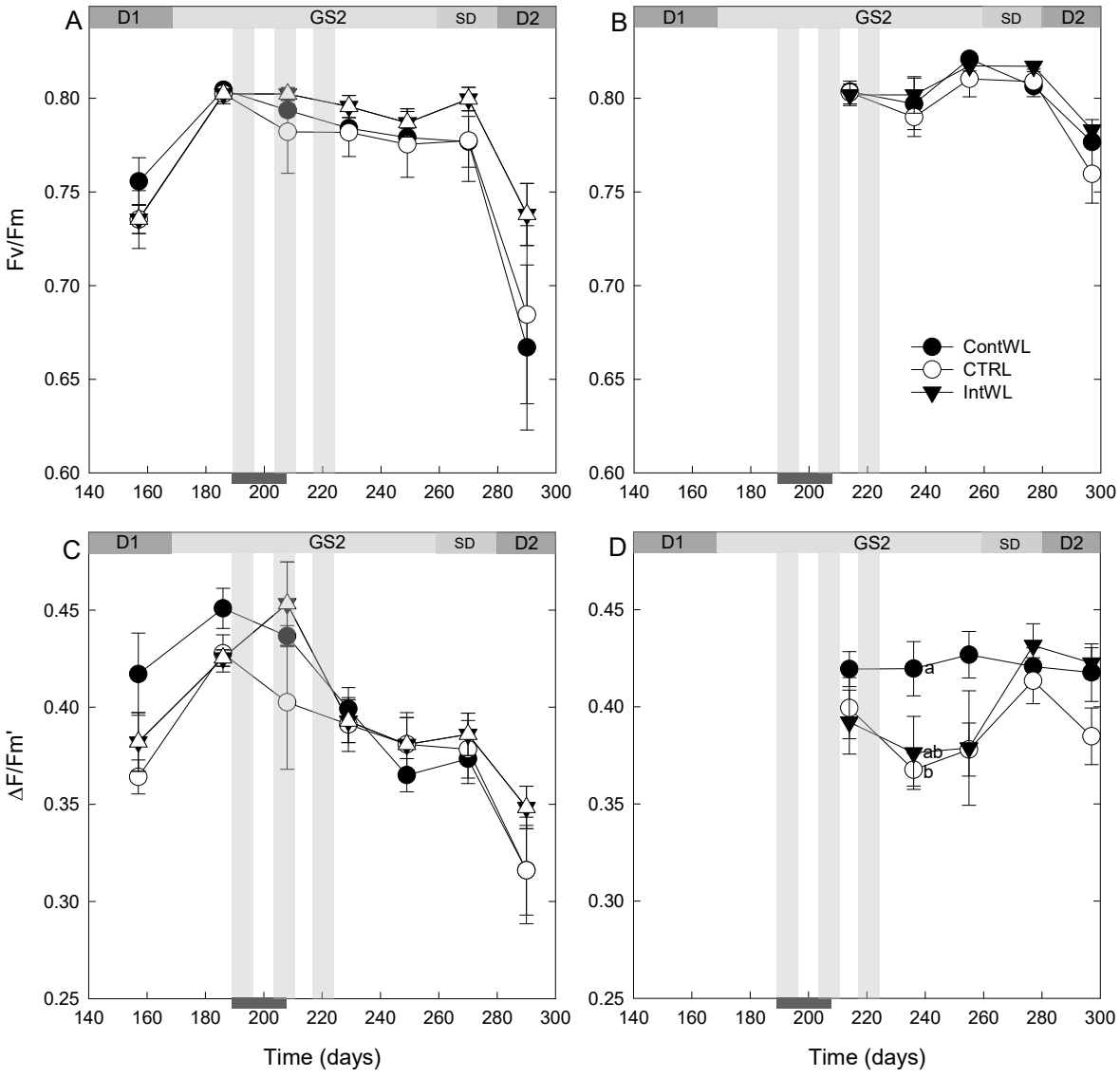


993

994

995 **Figure S2.** Mean (± SEM, N=4) chlorophyll content index (CCI) of needles developed
996 during GS1 (A) and GS2 (B). Continuous waterlogging (ContWL) is indicated by the dark
997 grey bar below the x-axis and interrupted waterlogging (IntWL) by light grey columns.
998 Different phases of the experiment are indicated in the upper part of the figures (see Table1)
999 Time indicates days from the beginning of the experiment.

1000



1001

1002

1003 **Figure S3.** Maximum photochemical efficiency of dark-acclimated needles developed during
1004 (A) GS1 and (B) GS2, and effective yield of photosystem II of needles developed during (C)
1005 GS1 and (D) GS2 (N= 4 ±SEM). Continuous waterlogging (ContWL) is indicated by the dark
1006 grey bar below the x-axis and interrupted waterlogging (IntWL) by light grey columns.
1007 Different lowercase letters indicate statistically significant differences between treatments
1008 within sampling times (P<0.05). Different phases of the experiment are indicated in the upper
1009 part of the figures (see Table1). Time indicates days from the beginning of the experiment.