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Influence of Nitrogen Nutrition Form and Drought Cycles on the Fine Root Respiration of Two Tree Species

Master thesis

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Declaration of Originality

I certify, that the master thesis was written by me, not using sources and tools other than quoted and without use of any other illegitimate support. Furthermore, I confirm that I have not submitted this master thesis either nationally or internationally in any form.

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Abstract

Root respiration plays an essential role in forest ecosystem carbon cycling and plant carbon budgets. However, knowledge on the environmental and root system intrinsic factors influencing root respiration is rare. In this thesis, root respiration measurements were carried out to test related impacts from abiotic factors and root inherent morphology. The first experiment, on *Acer platanoides* in the lab, determined root respiration reaction to five nitrogen forms (nitrate, ammonium, glycine, diglycine and triglycine), and four concentrations (0mM (N-free), 5mM, 15mM and 45mM) of nitrate and ammonium. Additionally data were analyzed to examine the relationship between root respiration and morphology in two root order classes, terminal orders 1+2 and the coarser root order 4. A second experiment was conducted on roots of *Fagus sylvatica* in the Lehrforst. The aim was to estimate how root respiration change under three different drought stress regimes (control (i.e. ambient), moderate and severe drought periods). Root morphology was highly correlated with respiration. Root order 1+2 exerted high respiration rates per dry weight (RR_{DWT}) compared to order 4. RR_{DWT} tended to decline with increasing root diameter and tissue densities (RTD), thus being positively related with root morphological traits (SRA and SRL). No immediate significant influence of N-forms and N concentration was found. In the experiment of drought cycle effects on root respiration, *Fagus* fine root biomass did not differ from drought treatments, but reduced as time changed. Roots respired more in the drought stressed treatments (moderate and severe) in comparison with the control treatment at the peak drought stress time period (R2). When roots were thought to recover (after rewetting the plots), a significant reduction ($P<0.05$) in RR_{DWT} was detected in drought stressed treatments—corresponding to the drought intensity. With regards to soil layer, roots appeared to respire more in organic O-horizon than Aeh-horizon ($P<0.001$). In conclusion, woody plants roots possessed a low plasticity in respiration when exposed to abiotic factors such has nitrogen sources and soil moisture regimes. Root morphology inherently determined respiration in a more apparent way. Future studies are necessary to determine long term adaptations of root respiration to different N nutrition forms and concentrations, and root respiration measurements should be continued on serious drought stressed tree individuals to determine the influence of future climates on plant and ecosystem C budgets.

Zusammenfassung

Wurzelatmung spielt eine wesentliche Rolle für den Kohlenstoffkreislauf im Ökosystem Wald und im Kohlenstoffbudget der Pflanzen. Unser Wissen über die ökologische und Wurzelsystem interne Einflussfaktoren auf die Wurzelatmung ist bislang jedoch begrenz. In dieser Arbeit wurden Experimente durchgeführt, um die Auswirkungen von abiotischen Faktoren und intrinsischen, wurzelmorphologischen Faktoren auf die Wurzelatmung zu untersuchen. Der erste Versuch, an Acer platanoides Sämlingen im Labor durchgeführt, bestimmt die Wurzelatmung unter fünf Stickstoffformen (Nitrat, Ammonium, Glycin, Diglycin und Triglycin) und unter vier Konzentrationen (0 mM (N-frei), 5 mM, 15 mM und 45 mM) von Nitrat und Ammonium. Zusätzlich wurde die Beziehung zwischen Wurzelatmung und Morphologie zweier Wurzelordnungen, Wurzelspitzen der Ordnungen 1 + 2 und gröbere Wurzel der Ordnung 4, untersucht. Ein zweiter Versuch wurde Altbäumen von Fagus sylvatica im Lehrforst geführt. Ziel war es hier, wie Wurzelatmung unter drei verschiedenen Trockenstress Regime (Kontrolle, mittelschwere und lange Dürreperioden) zu messen. Die Wurzelmorphologie war stark mit der Atmung korreliert. Die Wurzelordnungsklasse 1 + 2 wie deutlich höhere Atmungsraten pro Trockengewicht (RR_{DWT}) im Vergleich zu Wurzeln der Ordnung 4 auf. RR_{DWT} nimmt tendenziell mit zunehmendem Durchmesser und Gewebedichten ab während es positiv mit SRA und SRL korreliert ist. Überraschenderweise konnte kein unmittelbarer signifikanter Einfluss der N-Formen und N-Konzentration auf die Wurzelrespiration der beiden Wurzelordnungen festgestellt werden. Bei den Feldmessungen zeigte sich das die Feinwurzelbiomasse von Fagus sylvatica im Oberboden nur wenig auf die angewendeten Trockenheitsregime reagiert, sondern das jahreszeitliche Schwankungen von größere Bedeutung sind. Die Wurzelatmung wird jedoch deutlich beeinflusst, mit geringeren Respirationsraten unter langandauernder Trockenheit. Wurzeln aus der organischen Auflage wiesen eine höhere Respirationsrate als Wurzeln im Mineralboden auf. Zusammenfassen betrachtet weisen die untersuchten Gehölzen eine eher geringe Plastizität in der Wurzelatmung gegenüber abiotischen Faktoren wie Stickstoffquellen und Bodenfeuchteregime auf. Die Wurzelmorphologie bestimmt die Atmung in deutlichem größerem Ausmaße. Zusätzliche Studien sind notwendig, um langfristige Anpassungen der Wurzelatmung an verschiedene N Ernährungsformen und Konzentrationen zu bestimmen. Wurzelatmungsmessungen sollten an trockenheitsgeschädigten Bäumen durchgeführt werden um den Einfluss des zukünftigen Klimas auf pflanzliche und ökosystemare Kohlenstoffhaushalte zu bestimmen.

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Abbreviations

°C	Celsius Degree
μmol	Micromole
AA	Amino acids
B	Boron
Ca	Calcium
cm	Centimeter
cm^2	Square centimeter
cm^3	Cubic centimeter
CO$_2$	Carbon dioxide
Cu	Copper
D	Root average diameter
DWT, d.wt.	Root dry weight
Fe	Iron
FWT, f.wt.	Root fresh weight
g	Grams
Gly	Glycine, aliphatic amino acid
GLY-GLY	Diglycine, dimer of glycine
GLY-GLY-GLY	Triglycine, short peptides
K	Potassium
KNO$_3$	Potassium nitrate
L	Liter
Mg	Magnesium
min	Minutes
mL	Milliliter

mM	Millimolar
mm	Millimeter
Mn	Manganese
Mo	Molybdenum
N-free	Nitrogen deficient Hoagland nutrient solution
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
nmol	Nanomolar
NO_3^-	Nitrate
NO_x	Mono-nitrogen oxides NO and NO2 (nitric oxide and nitrogen dioxide)
O_2	Oxygen
P	Phosphor
pH	Power of Hydrogen, degree of acidity or alkalinity
Q_{10}	Root respiration temperature coefficient
Q_{10DWT}	Average Root respiration temperature coefficient calculated from RR_{DWT}
RDMC	Root dry matter content
RL	Root length
RR	Root respiration rates
$RR_{(t)}$	Root respiration at given temperature
RR_{DWT}	Root respiration rates per dry weight
RR_{FWT}	Root respiration rates per fresh weight
RR_{SA}	Root respiration rates per surface area
RR_{Vol}	Root respiration rates per volume
RTD	Root tissue density
S	Sulphur
s	Second

SA	Root surface area
SRA	Specific root surface area
SRL	Specific root length
t	Mean temperature in situ
Vol	Root volume
Zn	Zinc

1. Introduction

The increasing CO_2 emission (Pachauri et al. 2014) and nitrogen deposition (Bobbink et al. 2010) from anthropogenic activities are of increasing interests nowadays. Under the issue of climate change, on one hand more regions are likely to suffer from heavy precipitation events while on the other hand longer drought periods would contribute more to the ecosystem vulnerability (Pachauri et al. 2014). All these potentially changing abiotic factors will alter vegetation composition and may result in plant acclimation. These abiotic factors are also responsible to alter plant strategies in respiration. Root respiration is involved both in carbon and nitrogen flux, in plant carbon catabolism, and in nitrogen fixation. Root respiration accounts for 25-60% of total soil respiration (Pregitzer et al. 1997, Epron et al. 1999, Dannoura et al. 2006b) and thus contributes significantly to global carbon cycle. Root respiration regulates carbon allocation in underground parts of plants, representing a major source of CO_2 loss from plants (Rodeghiero and Cescatti 2006, SUBKE et al. 2006). It was estimated 8-75% of the carbohydrates that plants produce daily in photosynthesis respired by roots (Lambers et al. 1996, Majdi et al. 2007). Respiration provides energy trapped in ATP and carbon skeleton in metabolic intermediates for plant maintenance, growth, nutrient absorption and transport. Therefore, factors that influence the metabolic activity of roots and associated microbes are an important component of ecosystem and plant C budgets.

Root respiration varies among species, growth conditions and individual ontogeny (Lambers et al. 1996); in addition, RR rates can be strongly affected by abiotic conditions such as temperature, moisture, and rooting medium (Zogg et al. 1996, Burton et al. 2012), and biotic factors (Trocha et al. 2010). For example, temperature is the major factor for enzyme activity in TCA cycle, controlling respiration and thus playing a major role as abiotic factor influencing root respiration. It would be even more relevant because temperature might increase as a result of progressing climate change (Raich and Schlesinger 1992). The resulted elevated root respiration will change plant carbon allocation strategy. Similar, soil moisture influences root respiration rates (RR), proving optimal or restricted soil conditions for root maintenance and functioning (Atkin et al. 2000). For example, Burton and colleagues (1998) found that soil moisture potentials ≤ 0.2 MPa can greatly reduce fine root respiration in sugar maple forest. While in general there have been decreases in precipitation in the subtropics and tropics outside of the monsoon trough, and increases in land precipitation at higher latitudes, an increased

seasonal variability of precipitation events, with longer summer drought periods in central Europe, on ecosystem functioning became one focus of climate change studies (Trenberth 2011). Temporal distribution of rain events is important, because e.g. steady moderate rains soak into the soil and benefit plants, while the same rainfall amounts in a short period of time may cause local flooding and runoff, leaving soils much drier. Unfortunately, information of rain fall characteristics on soil and root respiration is still scarce.

It is widely accepted a positive relationship exists between root tissue nitrogen (N) concentration and root respiration, with higher RR correlated to higher N tissue concentrations (Burton et al. 2002, Wang et al. 2010). Thus, soil nitrogen availability is able to affect RR by causing higher or lower N concentrations of root tissues (Burton et al. 2012). Plants can absorb nitrogen in both inorganic and organic forms but the ability of a plant to capture a specific N type from the soil depends on soil type, environment and species. Generally, plants adapted to low pH values as found in mature forests tend to take up ammonium (NH_4^+) or amino acids, whereas plants adapted to higher pH and more aerobic soils prefer nitrate (NO_3^-; for a review see Maathuis (2009)). While most previous studies concentrated on comparing ammonium and nitrate, there is increasing proof of the importance of organic N forms like amino acids and peptides for N nutrition of plants (Näsholm et al. 2000, Näsholm and Persson 2001, Jones et al. 2005, Soper et al. 2011). Root respiration is providing energy for nutrient uptake, assimilation, translocation and remobilization (Masclaux-Daubresse et al. 2010). The energy requirements of NH_4^+ and NO_3^- absorption and assimilation constitutes a significant proportion of root respiration (Bloom et al. 1992). With regards to the relationship of nitrogen supply and root respiration, there are disagreements in perspectives related to effects of N-supply on root respiration. Van Der Werf et al. (1993) showed a higher rate of root respiration with high nitrogen supply. The respiration process is responsible for energy supply for nitrogen up-taking and assimilation. The root respiration may cost more energy for nitrogen absorption. It was also found that nitrogen fertilization had a significant negative effect on soil respiration in cottonwood, and no effect was observed in Loblolly pine stands (Lee and Jose 2003). But it was seldom studied how root respiration responses instantly to different nitrogen forms in woody plants.

Except for environmental factors, root respiration is inherently associated with root morphology and mass related traits. Within branched root systems, a distinct heterogeneity of root traits is present between single root units (Rewald et al. 2011a). Thus understanding knowledge of the traits of individual root segments is key to comprehend the functioning of whole root systems. Whiles several systems are currently used to classify individual roots within a branching system,

it is important that selected classes are matched as closely as possible to functional categories. In practice, root diameter has been most frequently used as root system classification in studies addressing respiration measurement due its easy use (Zogg et al. 1996, Wang et al. 2010, Rewald et al. 2014b). Fine roots are referred as roots with diameter ≤ 2mm, and coarse roots are those have diameter >2mm. However, there is also another increasingly popular classification system—based on root branching hierarchy. It includes two principal ways to number segments in a consecutive order according to branching hierarchy: centrifugal (i.e. basal to distal) (Fitter 1991, Rewald et al. 2014b) or centripetal (i.e. distal to basal) ordering (Uylings et al. 1975). The centripetal system was also known as Strahler's ordering system (Strahler 1957). RR is also known to differ according to root morphology and root age (Rewald et al. 2014b). Respiration rates for roots < 0.5mm in diameter found to possess 2.4 to 3.4 times higher respiration rates than larger diameter classes (Pregitzer et al. 1998). Terminal roots in lower ordering classes were reported to respire significantly more than the higher order classes per dry weight as well (Rewald et al. 2014b).

2. Objectives

The study aims to determine the influence of abiotic factors on woody plants' root respiration and to relate the measured respiration rates to morphological root traits. In doing so, two experiments were conducted, the first to determine root respiration responses of *Acer platanoides* seedlings to different nitrogen sources and/or concentrations, the second to determine the influence of varying precipitation regimes on root respiration of mature *Fagus sylvatica* trees.

The hypothesis are：

> ➢ Root morphology determines root respiration according to corresponding root traits (root order class, diameter, SRA, etc.). RR is hypothesized to decrease as diameter and RTD increase, thus being positively correlated to SRA. Because of differentiated development and function, most distal root orders (root tips) are hypothesized to be distinguishable from higher, more coarse root orders by RR. Because the most distal root parts are thought to be major places for ion uptake, they are hypothesized to react physiologically fast to changes in soil nutrient types and concentrations–a fact that should be evidenced by modified respiration rates.

> ➢ Plants have different preferences with regard to N sources. I hypothesize that energy consumption for uptake and assimilation, as evidenced by respiration rates may depend on different nitrogen sources and concentrations. The supplied N sources are inorganic N (nitrate and ammonium), and organic N (amino acids (AA), dimer and peptides). When comparing ammonium with nitrate, the later may consume more O_2 thru the assimilation process. Although the absorption process of AA and peptides might require more energy, roots may not have high absorption ability so the respiration rates are hypothesized to be low. Therefore, the root respiration in these N source absorption were assumed as orders $NO_3^->NH_4^+>AA=dimer=peptides$.

> ➢ Plant roots respond to soil water content. It is assumed, that fine root biomass will be reduced under drought stress, and the amount of root loss and subsequent necromass accumulation will be related to the intensity of drought stress. It is hypothesized that extended drought periods modify fine root respiration besides leading to changes in fine

root biomass. Root respiration rates (RR) are thought to be reduced under moderate and severe drought events compared to the control, dependent on the available soil moisture, and will change during the vegetation period.

3. Materials and Methods

3.1 Nitrogen sources effects on roots of *Acer platanoides*

3.1.1   Species and Growth conditions

Acer platanoides L. is a common European species, also known as Norway maple. It is an easily transplanted and fast growing species, widely adapt to various soils. Its root system is dense and shallow. It is often used as an urban street tree, but also referred as invasive species in North America (Martin 1999, Webb et al. 2000, Wangen and Webster 2006). Seedlings of *A. platanoides* were obtained from a nursery (Murauer Forstpflanzen, Ort im Innkreis, Austria). Three years-old seedlings were planted in 7-L plots (diameter = 22 cm) during dormancy period (April 2013). The growing substrate were made of mixture of nutrient-poor peat and sand as a proportion of 1:3. The washed quartz sand (grain size distribution: 25%, 0.2-1 mm and 75%, 1-2 mm in diameter) allows highly controlled water and nutrient supply and non-destructive root system rinsing (Rewald et al. 2014b). On December 19th, 2014, *A. platanoides* seedlings were distributed equally in two growth chambers (HGC 1514; Weiss Gallenkamp, UK). Subsequently, 48 seedlings were growing in the chambers constantly at a temperature of 20°C and relative humidity of 50%/60% during the day/night respectively. This temperature is the mean of general species-specific optimal soil temperature (i.e. 15°C–25°C) reported earlier (Lyr 1996). The photoperiod was set as 14 h at 350-400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (fluorescent). The seedlings started to sprout on Jan. 5th, 2015. They were initially irrigated twice per day before sprouting. Then they were watered once per day and fertilized every second day with 80mL of nitrogen deficient Hoagland nutrient solution (**Table 1**). Insecticides had to be used two times for pest control during the growing period.

3.1.2   Root Respiration Measurement

Ten different nutrient solutions, varying in N source and concentrations, were used to measure root respiration with a liquid-phase oxygen electrode system. An adjusted nitrogen deficient Hoagland solution (Smith et al. 1983) was used as control and basic solution for making other solutions (**Table 1**). As treatments nine different nitrogen sources or concentrations (**Table 2**) were added to the N-free Hoagland solution and the Hoagland was used itself as treatment number 10. The solutions were thereafter used as nitrogen treatments in this experiment. Five

different sources of N were used, two inorganic nitrogen (NH_4^+, NO_3^-) and three organic nitrogen sources (the aliphatic amino acid Glycine (GLY), the dimer GLY-GLY and the short peptide GLY-GLY-GLY). Nitrate and ammonium were applied in three concentrations of 5, 15 and 45 mM, while the organic N sources were used at 15 mM. In total, ten nutrient solutions were prepared from the same stock solutions on the same day added with different nitrogen forms or concentrations, see **Table 2**. Then they were stored separately in 10 mL bottles in the freezer. Every other day, one bottle of each solution was taken and unfrozen for respiration measurements. Thence RR were measured separately with 10 different nitrogen treatment every day.

Table 1 Nitrogen-deficient Hoagland solution used for the respiration experiment after amending it with different concentrations and sources of N (see Table 2).

Nutrient	Ready solution	Molarity	pH
	$mg·L^{-1}$	mM	
P	31	1	6
S	64	2	
K	254	6	
Mg	48	2	
Ca	160	4	
B	0.5		
Cu	0.02		
Fe	6		
Mn	0.5		
Mo	0.01		
Zn	0.05		

Table 2 The ten nitrogen sources/concentrations in solutions used for root respiration measurement.

Nitrogen sources	Nitrogen concentration			
	mM			
N-free	0			
NH$_4$Cl		5	15	45
KNO$_3$		5	15	45
Glycine			15	
Di-glycine			15	
Tri-glycine			15	

Two different parts of the branching root system were chosen for root respiration measurements (see below), i.e. root orders 1+2 and root order 4. It was estimated that those root order classes would widely differ in the physiological and anatomical traits and could thus serve as example for the other orders. As shown in **Figure 1**, the terminal/most distal tips were named order 1, the ordering number increases when two same order roots meet. If unequal root order meet, the higher order number retained for the next segment. However, the explorer root (marked as '1*' in **Figure 1**) visually differed in size and colour to "feeder roots" and was thus not used for respiration measurements.
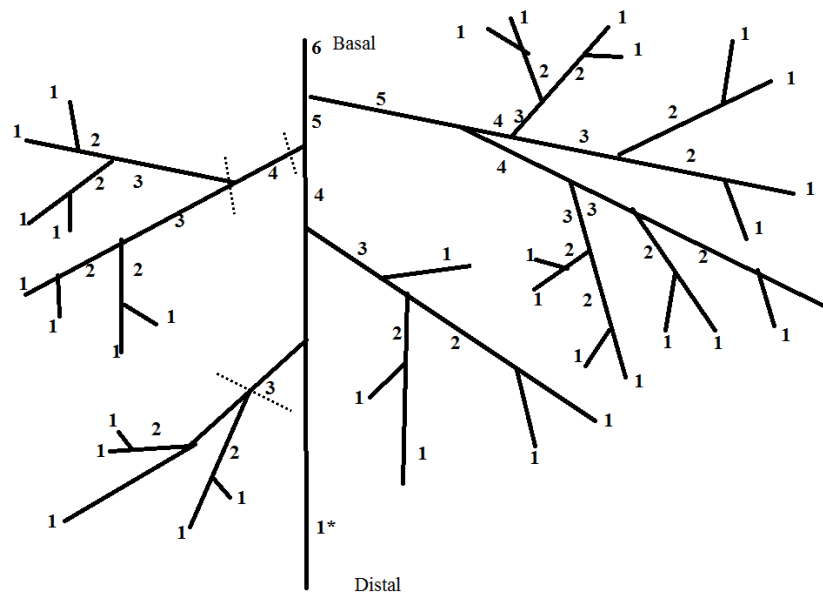
Figure 1 Centripetal root ordering method based on Strahler's method.

'1*' stands for the distinguished explorer root at the very terminal part of the root system, which was excluded from the respiration measurements. The dashed line shows the typical cutting points for getting root segments from order 1+2 and 4. The size is not according to the scale.

Figure 2 Liquid-phase Oxy-graph System, Hansatech Instruments Ltd, UK

Respiration can be estimated as O_2 consumption or CO_2 release, in which the former was applied in this study. Oxygen consumption was measured with Clark type oxygen electrode system (Hansatech Instruments Ltd, UK; **Figure 2**). Roots were placed in 2ml of aerated nutrient solution in a cuvette with controlled temperature and the decrease of O_2 was measured. Prior to measurements, roots were rinsed carefully with water before being entered into the oxygen electrode system. The stirrer was set as 100% speed in the cuvette. Because of that and the high stirrer speed, a depletion of the oxygen boundary layer at the root surface, restricting

respiration, seems unlikely (Rewald et al. 2014b). The temperature was constantly kept as 20℃ by a water circulation bath (F12-ED; Julabo Labortechnik. Germany). The system was calibrated every day at 20℃. Oxygen depletion rate ($nmol \cdot mL^{-1} \cdot min^{-1}$) was measured directly by the instruments software. Respiration rate of each measured root segment was derived from linear regression of O_2 depletion rate ($nmol \cdot mL^{-1} \cdot min^{-1}$) over a 15 minute time-span. Prior to measurements, the roots were allowed to equilibrate for 5 min to the respective N solution and the temperature. The cuvettes were rinsed every time before the next measurement. Two measurements were separately conducted on two complete oxygen electrode systems simultaneously. Only heathy roots were chosen for respiration measurements; root status was judged by eyes with relatively smooth surface, fresh colour (white, yellow or light brown), and no obvious damage (**Figure 3**).

Normally 40 respiration measurements were conducted every day for one plant individual within 10 nitrogen treatments on root order 1+2 and order 4. In case of systematic bias, it was rotated the measurements on a daily base how the 10 different Hoagland solutions were used. Totally 480 root respiration measurements were conducted on the 12 *A. platanoides* seedlings. It is showed in **Figure 4** how *A. platanoides* roots were protected during the measuring time. The measurement period were limited from 9:00 a.m. to 6:00 p.m. which was within the plants' photosynthesis period. Special spectrum light were also used to ensure the plant photosynthesis continuing even indoor (Lipp and Andersen 2003). Besides, roots were kept in moisture conditions and undisturbed in the soil, with wet paper towel covered on the top. In addition, the root sampling time was restricted within 30 min which started from the root segments cutting time until the respiration measurements finished, during which root activity has been proved to be roughly stable (Fukuzawa et al. 2012).

The oxygen consumption rates were expressed per second and standardized by root mass (fresh weight and dry weight) or morphological traits (surface area and volume) to determine root respiration rate per unit. Respiration rates were then estimated from O_2 consumption and expressed as root respiration rate per fresh weight (RR_{FWT}, $nmol \cdot g^{-1} \cdot s^{-1}$), root respiration rate per dry weight (RR_{DWT}, $nmol \cdot g^{-1} \cdot s^{-1}$), root respiration rate per surface area (RR_{SA}, $nmol \cdot cm^{-2} \cdot s^{-1}$), and root respiration rate per root volume (RR_{Vol}, $nmol \cdot cm^{-3} \cdot s^{-1}$). See below for root scanning and weighing procedure.

Figure 3 Roots of *Acer platanoides.*

Healthy roots were judged as with smooth surface, fresh colour (white, yellow or light brown), and no obvious damage (see the left root segments), vice versa (the right one).

Figure 4 Methods to protect *A. platanoides* roots during the measuring time.

Plants were under special spectrum light to ensure continuous plant photosynthesis. roots were kept in moisture conditions and undisturbed in the soil, with wet paper towel covered on the top

3.1.3 Root morphology and architecture of *Acer platanoides*

After respiration measurements, all root samples were scanned with WinRhizo 2012 pro (Regent Instruments Inc., Canada) to determine morphological data such as root surface area (cm^2), average root diameter (mm), root volume (cm^3), etc. These data were then used to standardize the respiration data (see above). The fresh weight was determined after the root surface was gently blotted dry. The dry weight of was determined after 48 hours under 65°C. All the weights were determined to a precision of 0.1 mg. Root mass and morphological parameters were used to calculate below root tissue density (RTD), root dry matter content (root dry mass per fresh mass, RDMC), specific root area (SRA) and specific root length (SRL), using the following formulae:

$$RTD = \frac{d.wt.}{Vol};$$ **(a)**

$$RDMC = \frac{d.wt.}{f.wt.} \quad ;$$ **(b)**

$$SRA = \frac{SA}{d.wt.};$$ **(c)**

$$SRL = \frac{RL}{d.wt.};$$ **(d)**

Where d.wt. is the root dry weight and f.wt. is the root fresh weight. Vol, SA, and RL stand for root volume, root surface area and root length respectively.

Six additional root branches from 3 randomly chosen *A. platanoides* seedlings were harvested for estimating biomass allocation according to root orders. Root segments were taken from root orders 1+2, 3, 4, 5, and 6 separately. They were weighted after drying (65°C, 48 h). Finally data was used to upscale to the root respiration of each order in *A. platanoides*.

3.2 Drought Period Effects on RR in *Fagus Sylvatica* Forest

3.2.1 Species and Site Conditions

Fagus Sylvatica L. is also known as European beech. It is a shade tolerant species common in most parts of Europe, often being regarded as dominant species of the potential natural vegetation in European mixed deciduous forests. The root system is rather shallow with large roots spreading out in all directions; European beech roots bear ectomycorrhizal symbionts. The experimental site was situated at Lehrforst in the Rosalian Mountains, Lower Austria, forest demonstration centre of the University of Natural Resources and Life Sciences, Vienna. The site was located on a west-oriented slope in a mature beech forest (*Fagus sylvatica* L.). The soil type is a Podsolic Cambisol according to the FAO WRB (IUSS, 2007), with organic matter O-horizon (0-0.07 m) and a humus, slightly alluvial Aeh-horizon (0.07-0.25 m) (Schwen et al. 2014). The local climate is characterized by an annual average precipitation of 796 mm, and a mean air temperature of 6.5℃ (Schwen et al. 2014). A weather station was installed, recording climate data and soil temperature and moisture at a soil depth of 10 cm. In 2014, the average soil temperature was 9.7℃ and soil moisture was about 21.1% (Zimmermann et al, 2014).

3.2.2 Experimental Design

Twelve 2m x 2m plots were established for estimating drought effects (**Figure** 5). They were continuously measured in regard to soil temperature, soil moisture, CO_2 flux and NO_x flux. Treatments were three 3 irrigation strategy for the plots as severe drought (n = 4), moderate drought (n = 4) and control (n = 4). The severe drought treated plots were irrigated with 150mm water with 8 weeks of drought period in between, and the moderate stressed plots were watered with 75mm in 4 week intervals. The drought stress treatments were protected from natural precipitation with plastic roofs. Unroofed plots served as control, being subjected to natural precipitation. All the instruments were established as described previously (Zimmermann et al, 2014).

Figure 5 Treatment plots layout (Zimmermann et al., 2014).

Twelve plots were set up with three drought treatments: C(control), 1(moderate), 2(Severe). Sampling grids were used to take soil cores on to the lower part of each plot. The size is not according to scale.

It shows in **Table 3** the sampling dates and irrigation events. Root respiration measurements were conducted three times, i.e. in the beginning of May, end of June and beginning of July. The first time (R1) was from April 29th to May 3rd, 2015, one week before the establishment of the roofs. It was considered as the time before the drought treatment. Secondly it was after 8 weeks of drought stress treatment (R2), which regarded as the peak drought stressed time. Thirdly one week after the rewetting event (R3) was thought to be recovery time for both drought stressed treatments. The control treatment was 4 plots with only ambient precipitation. The moderate was irrigated twice during the measurement period with 75mm water of 4 weeks interval. The severe treatment was irrigated with 150 mm water with 8 weeks interval. So in all the two stress treatments were irrigated with the same amount of water (150 mm) during the

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experiment period. The irrigation events (H14 and H15) were on May 26th and June 23rd respectively. Except for the three measurements, root respiration was measured under temperature of 10°C and 20°C to determine Q10 (see below) at the beginning of June.

Table 3 Sampling Dates and Irrigation Events.

R1: the first RR measurement campaign; R2: the second RR measurement campaign; R3: the third RR measurement campaign; H14, H15: irrigation events.

Harvest Grids	Sampling Dates	Events	Drought Interval (Weeks)		Amount of Irrigation (mm)	
			Moderate	Severe	Moderate	Severe
R1	30/04/2015	Roof Mounted; RR measurement	0	0	0	0
R1	01/05/2015	RR measurement	0	0	0	0
R1	02/05/2015	RR measurement	0	0	0	0
R1	03/05/2015	RR measurement	0	0	0	0
H14	26/05/2015	Irrigation	4	0	75	0
Q10	04/06/2015	Q10 Measurement	0	0	0	0
Q10	05/06/2015	Q10 Measurement	0	0	0	0
R2	19/06/2015	RR measurement	0	0	0	0
R2	20/06/2015	RR measurement	0	0	0	0
R2	21/06/2015	RR measurement	0	0	0	0
R2	22/06/2015	RR measurement	0	0	0	0
H15	23/06/2015	Irrigation	4	8	75	150
R3	01/07/2015	RR measurement	0	0	0	0
R3	02/07/2015	RR measurement	0	0	0	0
R3	03/07/2015	RR measurement	0	0	0	0

3.2.3 Root Morphology of *Fagus sylvatica*

After measurements in the forest, root samples were preserved in ethanol and stored at low temperature in cooling box before root scanning with WinRhizo 2012 pro (Regent Instruments

Inc., Canada) was conducted in the lab **(Figure 6).** Root morphological parameters were obtained as root surface area (cm^2), average root diameter (mm), root volume (cm^3), etc.
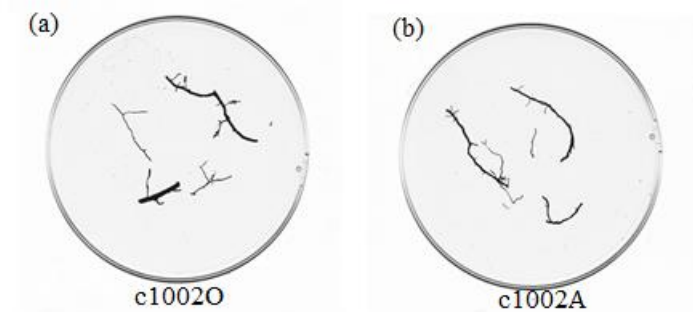
Figure 6 Scanned images illustrating *Fagus* root samples.

'c': roots were from the third measurement campaign; '1002': from plots 10 and core 2; 'O' (a) and 'A' (b) stands for the soil layers.

3.2.4   Root Sampling and Respiration Measurements *in Situ*

Measurements were conducted three times on site as described above. Sampling grids were used to take soil cores on the lower part of each plot. Root sampling were carried out according to the scheme in **Figure 7** in each plot. This sampling frame was put in the position showed as green rectangle in **Figure 5**. The soil cores were taken from Grids marked with R and the number stands for the measurement time period. Soil cores were 4 cm in diameter and 10 cm in depth, so all root samples were collected from the first 10 cm of soils. Each time, four soil samples were taken from each plot with soil cores. The holes were refilled with sand after sampling to mark the sampling points and minimize disturbance.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A																				R3
B																				
C	R2																			R2
D																				
E		R3																		
F																				
G			R1													R2		R3		R1
H												R3								
I	R1																R1			
J											R2									

	Root sampling 2015		Irrigations	
R1	April/May		H13	29.04.2015
R2	mid-June		H14	26.05.2015
R3	Begin-July		H15	23.06.2015

Figure 7 Sampling grid scheme used to take soil cores (according to Zimmermann et al., 2014).

Soil cores were taken from grids marked with R: R1 (First RR measurement in end of April and beginning of May), R2 (2nd RR measurement in mid-June), R3 (3rd EE measurement in beginning of July. The size is not according to scale.

Root respiration rates were measured separately for O-horizon and Aeh-horizon. The measurement were accomplished within 30min after sampling *in situ*. RR rates were regressed from the oxygen depletion curve as described above. Again two complete Oxygraph systems were operated to measure the root respiration at the same time. After five minutes of temperature equilibrium, rates of oxygen depletion were recorded for the next 10 minutes. Healthy roots were judged by eyes and selected for RR measurements. Ectomycorrhiza were commonly found on roots especially in O-horizon. In case mycorrhiza would affect O_2 depletion rates, mycorrhiza infected roots were excluded from the RR measurements. All the roots were thereafter taken to the lab and rinsed with tap water. Roots were sorted into fine roots (<2mm), Coarse roots (>2mm) and necrosis tissue. Dry weight was determined (65℃, 48 h) to a precision of 0.1 mg.

On May 19th and 20th, root samples were taken to estimate root respiration sensitivity to temperature. The Q_{10} temperature coefficient is a measure of the rate of change of a biological

or chemical system as a consequence of increasing the temperature by 10°C. Twenty samples were taken outside of the plots (i.e. 'control' conditions). RR were measured under 10℃ and 20℃ with 10 replicates for each temperature and soil horizon. As root respiration would decrease with time after cutting, root segments from different samples were used for measurements under 10℃ and 20℃. In order to be applicable, roots were measured separately from O-horizon and Aeh-horizon. In order to minimize root morphological effects on respiration when calculating Q_{10}, root respiration rates were treated separately according to diameter classes. Roots were divided into four groups with diameter intervals of 0.1 mm (diameter: 0.2-0.6). Root respiration rates at 10℃ ($RR_{O(10)}$ and $RR_{A(10)}$) and 20℃ ($RR_{O(20)}$ and $RR_{A(20)}$) were achieved averaging the four diameter groups for O-horizon and Aeh-horizon respectively. Q_{10} of each soil layer was hence calculated (equations (e) and (f)) from root respiration rates in O-horizon (Q_{O10}) and Aeh-horizon (Q_{A10}).

$$Q_{O10} = \frac{RR_{O(20)}}{RR_{O(10)}}; \tag{e}$$

$$Q_{A10} = \frac{RR_{A(20)}}{RR_{A(10)}}; \tag{f}$$

$$Q_{10} = \frac{Q_{O10} + Q_{A10}}{2}; \tag{g}$$

$$RR_{(t)} = RR_{20} Q_{10}^{\frac{t-20}{10}}; \tag{h}$$

The average Q_{10} of the two layers (Q_{O10} and Q_{A10}, equation (g)) was used for the calculation of root respiration ($RR_{(t)}$) at real temperature t (equation (h)). RR_{20} is the root respiration measurements under 20℃ in the drought stress experiment (see above). All the respiration rates above could be expressed separately based on mass (dry weight) and morphological parameters (surface area and volume). Finally the Q_{10} value from RR_{DWT} were used for further calculation (Equation (h)). Root respiration was calculated and expressed as root oxygen consumption per square meter (nmol · $m^{-2} \cdot s^{-1}$). It was the result of average fine root biomass multiplied by RR_{DWT} of each plot, and used as RR_{20} in Equation (h). The real RR in given temperature ($RR_{(t)}$) were derived by Equation (h) afterwards.

3.3 Statistical analysis

All the data were processed with the PC program SPSS Statistics 21 (IBM Corp., USA). Some extreme outliers were excluded from the data set and 446 samples out of 480 were retained. Morphological data of *A. platanoides* was not normally distribution so a non-parametric test (Mann-Whitney) was used to test the significance of root order and nitrogen effects differences. Multiple regression were done on to examine relationship between SRA and diameter, SRA and RTD, SRA and RDMC, and for RTD and RDMC. Turkey test was used for testing significance of root order biomass distribution. RR were correlated with fresh weight, dry weight, surface area and volume with linear correlation matrix and regression. RR per unit were analyzed with Spearman non-parametric correlations to root traits order, diameter, SRA, RTD, SRL and RDMC. Exponential regression were fitted with RR_{DWT} to root mean diameter and RTD respectively. N-form and N concentrations effects were test with T-test. The data from the drought experiment was normally distributed and had equal variances, all three factor ANOVA was used to test the difference significance for factors as drought treatments, sampling time and soil layers. LSD multiple comparison were performed to test for significant differences among three drought treatments. Values in this thesis are expressed as Mean±S.E.

4. Results

4.1 Root respiration and Root Morphology

4.1.1 Root Morphological Traits of *Acer platanoides*

There are significant differences ($P < 0.001$) in morphological traits between the two root order classes of *Acer platanoides* (**Figure 8**). Generally, root orders 1+2 have smaller root average diameter, root tissue density (RTD) and root dry matter content (RDMC) than root order 4. But order 4 roots hold lower values when it comes to specific root area (SRA) and specific root length (SRL). Root orders 1+2 have on average a root diameter less than half the diameter of root order 4. As for mass related parameters, root orders 1+2 have almost 8 times larger SRL than root order 4. Root orders 1+2 also have a significantly larger SRA (1358.41 ± 16.63 cm$^2 \cdot$g^{-1}) than root order 4. The descriptive statistics are in appendix.
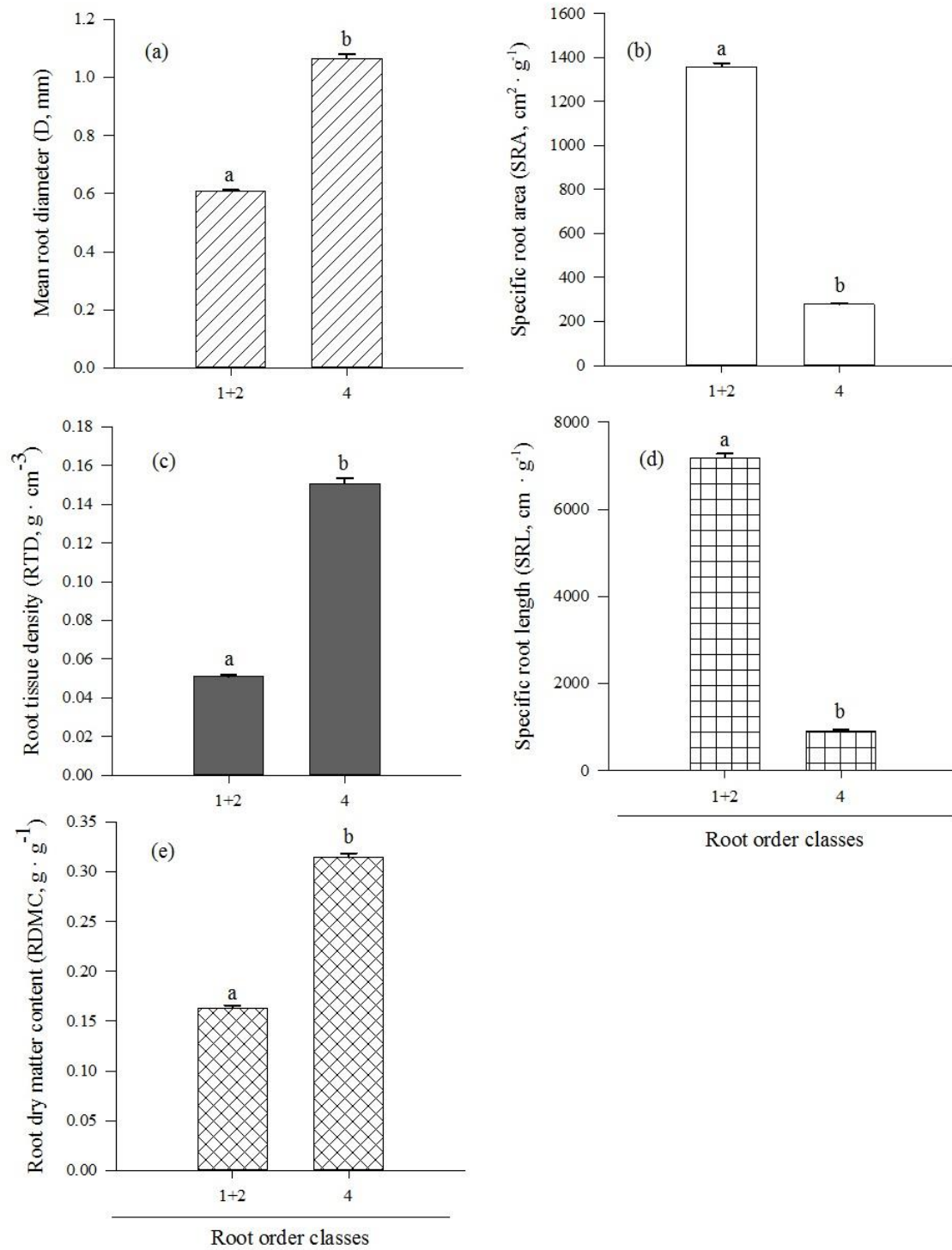
Figure 8 Root morphological traits of *A. platanoides* on root order 1+2 and order 4: root average diameter(a), specific root area (SRA, (b)), root tissue density (RTD, (c)), Specific root length (SRL, (d)), and Root dry matter content (RDMC, (e)).

Small letters indicate significant differences of parameters between root order classes 1+2 and 4 (mean ± S.E.; Mann-Whitney Test, n=23, $P < 0.001$).

The relationship of SRA and root mean diameter is shown in **Figure 9**. Power function is fitted with R square as 0.63 (**Figure 9**). The regression equation is SRA = 487.01*Diameter$^{-1.75}$. SRA decreased with increasing diameter. In spite of that, exponential decay regression analysis is performed for SRA and RTD (**Figure 10**). The results show a high R square which indicates approximately 95% of the variance in SRA is exponentially related to RTD. SRA is declining in an exponential way with RTD increasing. Root order 1+2 has a low root diameter and RTD, but has the higher SRA with comparison with order 4.
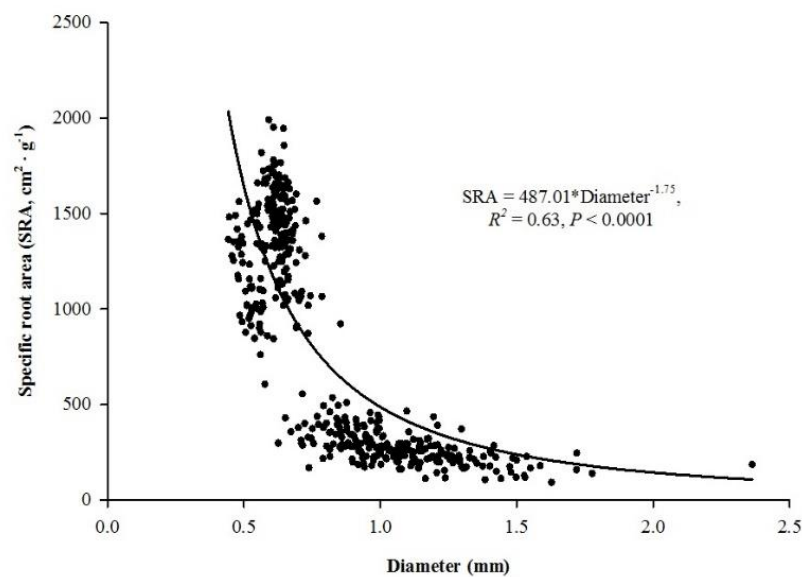
Figure 9 Specific root area (SRA) was negatively related to root diameter of *A. platanoides*.

Power function curve was fitted to all the data points from both root order classes. The R^2 results (P <0.0001) indicate that about 63% of the variance in SRA is accounted for by root diameter (n=446).
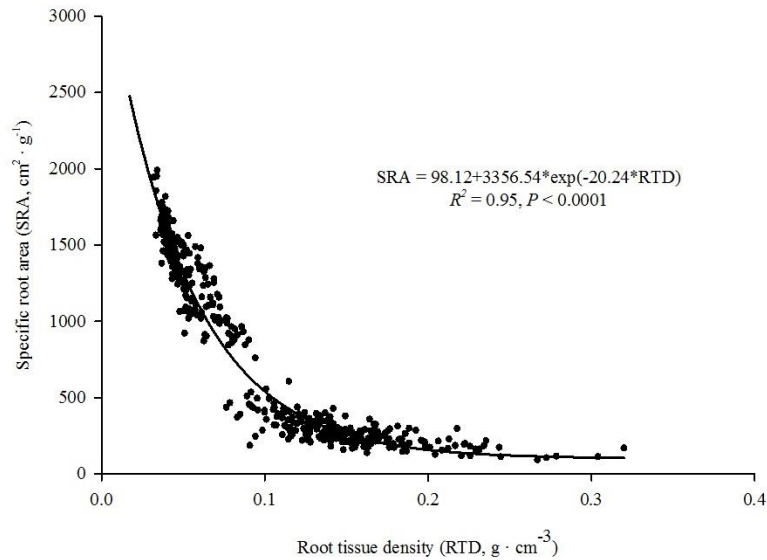
Figure 10 Specific root area (SRA) was negatively related to Root tissue density (RTD) of *A. platanoides*.
Exponential curve was fitted to all the data points from both root order classes. The R^2 results (P <0.0001) indicate that about 95% of the variance in SRA is accounted for by RTD (n=446).

RTD is positively related to RMDC with root order 1+2 changing more in RDMC than RTD (**Figure 1**). The equation RTD = -0.02+0.50*RDMC indicates a liner relation of RTD as a function of RDMC. The R square demonstrates RTD was 63% related to RDMC for *Acer* root samples in this experiment. SRA is linearly related to RDMC in a negative way (**Figure 12**). Roots with higher dry matter content (root order 4) has a smaller specific surface area. R square was equal to 0.63, and the equation was SRA = 2029.92-5060.36*RDMC. However it is noticeable that data dots from root order 1+2 were rather scattered with low RDMC, see **Figure 12**. SRA of root order 4 varies in the range roughly from 0 to 500 $cm^2 \cdot g^{-1}$ with RDMC increasing. The relationship would not follow linear regression if analyzing two root order classes separately. But if fitting an exponential decay curve for SRA and RDMC, the resulted regression equation would be SRA = 3704.04*$exp^{(-7.67*RDMC)}$. R^2 increases to 0.65 which became more representable.

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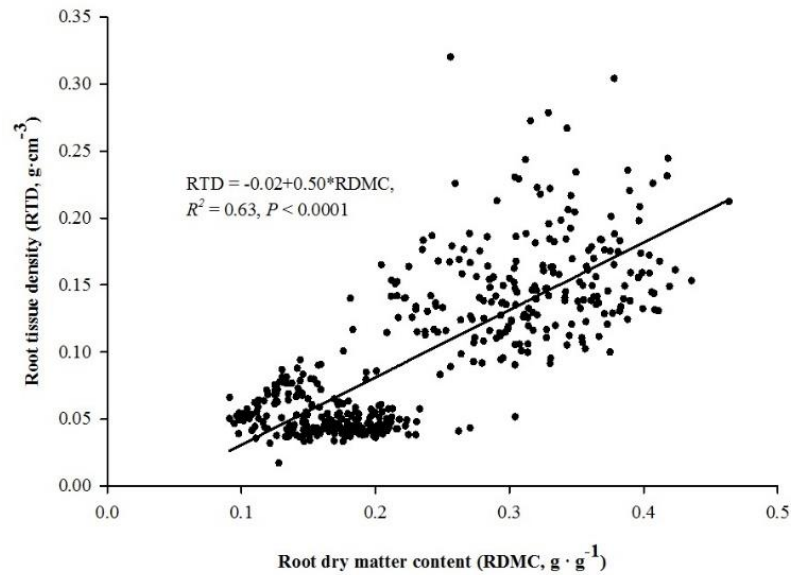
Figure 11 Root tissue density (RTD) was in positive linear relation to root dry matter content (RDMC) of *A. platanoides*.

The R^2 results (P <0.0001) was 0.63 in the relation between RTD and RDMC (n=446).
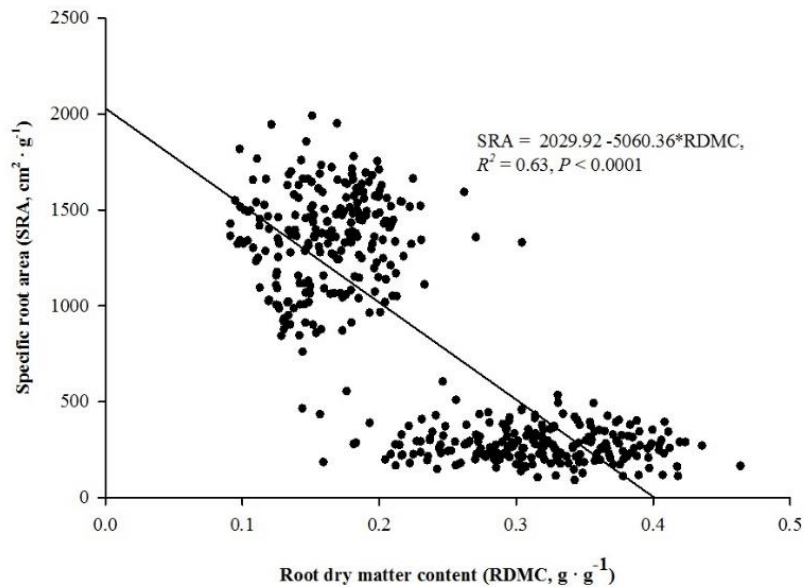
Figure 12 Specific root area (SRA) was negatively related to root dry matter content (RDMC) in linear regression of *A. platanoides*.

The R^2 results (P <0.0001) was 0.63 in the relation between SRA and RDMC (n=446).

The biomass of the entire root system is composed most by root orders 1+2 (25.35%) and 6 (36.44%) while root order 3 contributing less than 10% (**Table 4**). Root orders 1+2 are most

terminal orders while order 6 is located closest to the tap root. Root order 6 holds significantly higher biomass than root order 3 and root order 4.

Table 4 Absolute plant biomass per order (grams dry weight) and relative biomass distribution between the five extended root order classes 1+2, 3, 4, 5 and 6 of *A. platanoides*.

Small letters indicate significant differences of the root biomass between root order classes (mean ± SE, n = 6; Tukey test, P < 0.05).

Root Order	Biomass	Relative Root Biomass
	g	%
1+2	0.13±0.04ab	25.35
3	0.04±0.01a	8.04
4	0.06±0.02a	10.59
5	0.10±0.03ab	19.58
6	0.19±0.07b	36.44

4.1.2 Root Respiration Standardization

Root respiration rates are presented as respiration per root fresh weight (RR_{FWT}, nmol g^{-1} s^{-1}), respiration per root dry weight (RR_{DWT}, nmol g^{-1} · s^{-1}), respiration per root surface area (RR_{SA}, nmol · cm^{-2} · s^{-1}) and respiration per root volume (RR_{Vol}, nmol · cm^{-2} · s^{-3}). I can show that respiration rates have linear relation with the four root characteristics (**Figure 13**). It is only between dry weight and surface area that the correlation was not significant (P<0.01). The other root parameters are correlated significantly with different correlation coefficients.

The RR is strongly correlated to root fresh weight, volume and surface but less to root dry weight looking at both root order 1+2 and 4. Root dry weight is also poorly correlated to the other root morphological parameters (**Appendix Table 3**). However, looking at the two root orders separately, also dry weight is correlated to respiration rate and the other root parameters (**Appendix Table 4** and **Appendix Table 5**). RR is strongly correlated to the root volume (**Figure 13** d) in a very similar way for both root order 1+2 and root order 4. Also for fresh weight there is a similar slope for order 1+2 and order 4. For dry weight the RR increases much faster with increasing root weight for root order 1+2 than for root order 4 which RR is not so dependent on the weight of the sample. On the other hand RR increases faster with increasing root surface area in order 4 than in order 1+2.

RR of root order class 1+2 is significantly higher than the order 4 with root mass as standards, vice versa for RR with root morphological traits as standards. The following four figures show relationship of RR to root fresh weight (a), dry weight (b), surface area (c) and volume (d) (**Figure 13**). Linear regression lines are fitted to overall data (solid lines), and data in root order subgroups (dotted lines). There might be some root segments from order 4 which has high dry weight but respired low. So overall R square is low as 0.08 between RR and dry weight. The data dots of two root order classes go to different directions. R square for root order subgroups are higher than the overall value as 0.506 for order 1+2 and 0.219 for order 4. There are no clear separate root order trend with functions as fresh weight and volume. RR is well linearly related to root surface area and volume with overall R^2 as 0.592 and 0.582 respectively. But there seem to be a gap between root order 1+2 and order 4 in **Figure 13** (c).
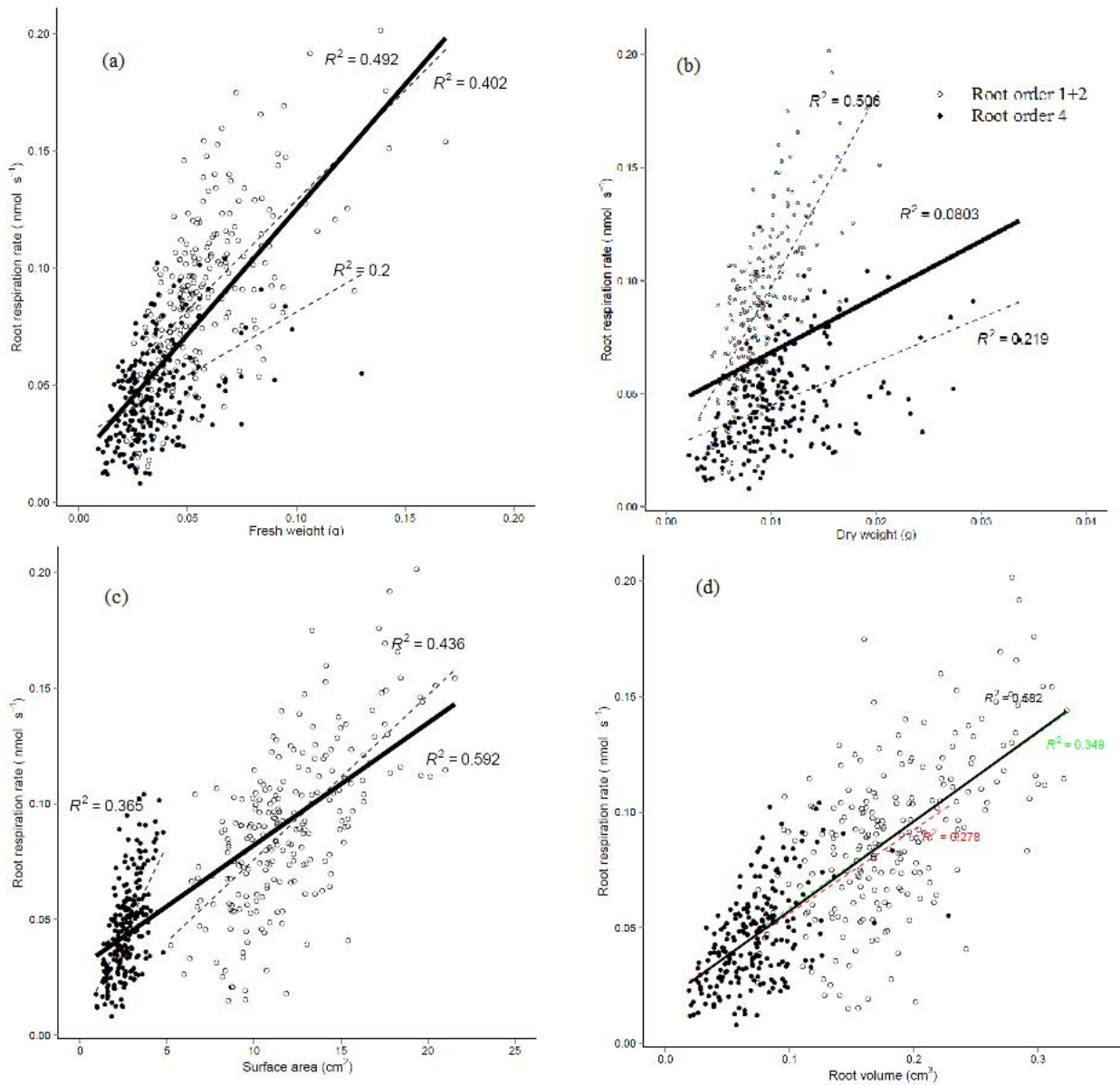
Figure 13 Relations between root respiration and different standards, fresh weight (a), dry weight (b), surface area (c) and volume (d) of *A. platanoides*.

Data points are: empty circles for root order 1+2 and filled circles for order 4. Linear regression lines were fitted to overall data (solid lines), and data in root order subgroups (dotted lines). R^2 are shown for each trend line (n=446).

4.1.3 Root Respiration Rates and Morphological Traits

After standardization, non-parameter correlation are conducted for root respiration per unit and root characteristics (root order, average diameter, SRA, RTD, SRL and RDMC). The coefficients are shown in **Table 5**; correlations are mostly highly significant ($P < 0.01$) except for RR_{FWT} to RDMC, and RR_{Vol} to diameter. Generally, coefficients are higher for RR_{DWT}. Root respiration rates per mass unit (RR_{DWT} and RR_{FWT}) were positively correlated with SRA and

SRL, while respiration rates per morphological unit (RR_{SA} and RR_{Vol}) were positively correlated with root order classes, diameter, RTD and RDMC.

Table 5 Spearman non-parameter correlation between RR and root traits of *A. platanoides*.

RRFWT, root respiration rate per fresh weight; RRDWT, root respiration rate per dry weight; RRSA, root respiration rate per surface area; RRVol , root respiration rate per root volume. **Correlation significant at the 0.01 level (2-tailed) (n=446).

	RR_{FWT}	RR_{DWT}	RR_{SA}	RR_{Vol}
Order	-0.137**	-0.749**	0.771**	0.358**
Diameter	-0.232**	-0.731**	0.636**	0.08
SRA	0.301**	0.754**	-0.765**	-0.366**
RTD	-0.241**	-0.716**	0.771**	0.478**
SRL	0.297**	0.768**	-0.733**	-0.264**
RDMC	0.06	-0.699**	0.578**	0.213**

In **Figure 14** relations between RR_{DWT} and diameter. RR_{DWT} were fitted with an exponential decay curve as a function of diameter. R square was 0.50 which indicates half of the change in RR_{DWT} to can be based on root diameter changes. Roots with smaller diameter (e.g. root order 1+2) present higher RR than the ones with larger diameter (e.g. root order 4).

$$RR_{DWT} = 1.38 + 25.98*\exp(-1.90*Diameter)$$
$$R^2 = 0.50, P < 0.0001$$
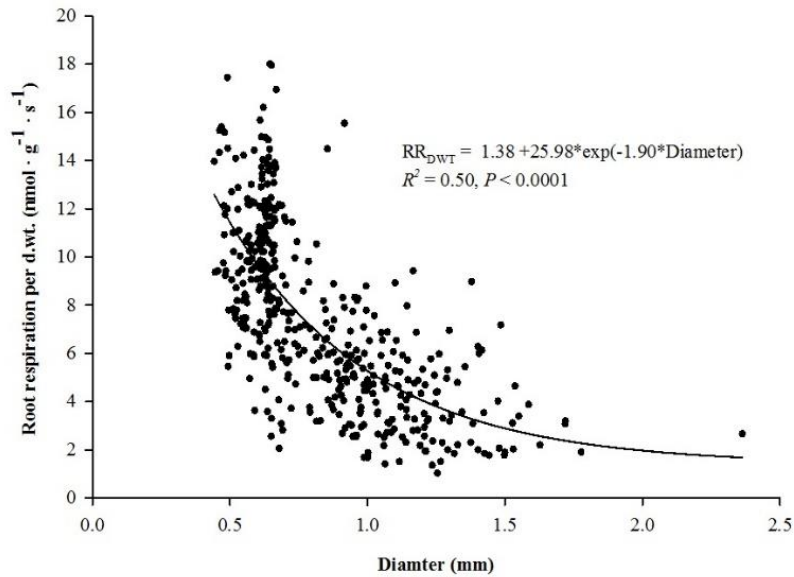
Figure 14 RR_{DWT} was negatively related to root average diameter in *A. platanoides*.

Exponential curve was fitted to all the data points from both root order classes. The R^2 results (P <0.0001) indicate that about 50% of the variance in RR_{DWT} is accounted for by root average diameter (n=446).

RR_{DWT} as a function of root tissue density was fitted with exponential decay curve and R^2 was about 0.5 (**Figure 15**). Roots with higher tissue density respire less.

$$RR_{DWT} = 1.53 + 13.32*\exp(-1.90*Diameter)$$
$$R^2 = 0.50, P < 0.0001$$
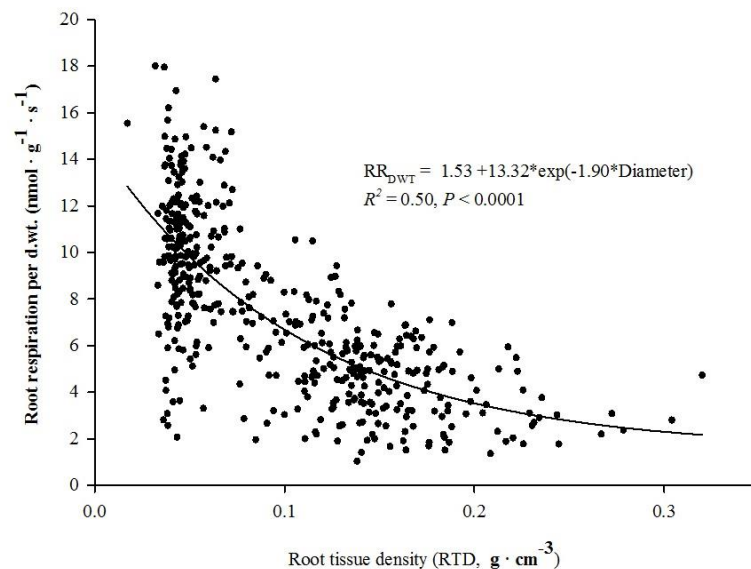
Figure 15 RR_{DWT} was negatively related to root tissue density (RTD) in *A. platanoides*.

Exponential curve was fitted to all the data points from both root order classes. The R^2 results (P <0.0001) indicate that about 50% of the variance in RR_{DWT} is accounted for by RTD (n=446).

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4.2 Nitrogen & Root Respiration Rates

4.2.1   N-form Effects on Root Respiration

RR per unit are shown in **Figure** 17 for the six nitrogen treatment groups including two mineral nitrogen sources, three organic ones and nitrogen free as control. For comparison only the 15 mM concentration for each N-source is presented. In summary, N-form had no clear significant effect on RR, neither in root order 1+2 nor root order 4. When RR expressed per root volume, root segments of order 1+2 respire most with nitrogen sources as ammonium, in which RR_{Vol} is significantly greater than that under di-glycine treatment ($P<0.05$). In root order 4 RR are again lowest in di-glycine treatment, but only significant lower compared to the N-free treatment.
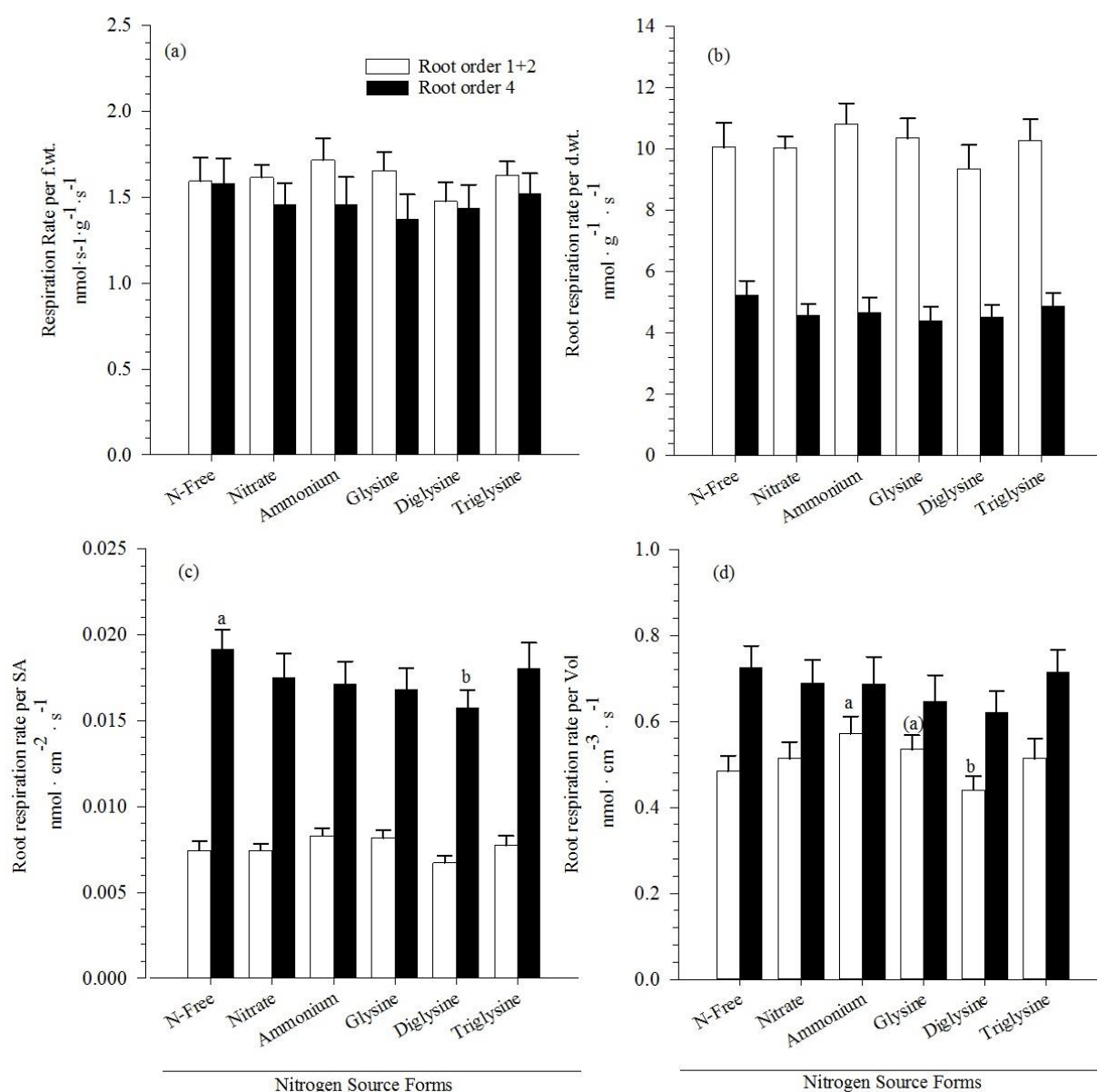
Figure 16 Nitrogen form effects on root respiration rate of *A.platanoides*.

N concentration was 15mM for all N forms. Small letters (P < 0.05) and letters in parentheses (P < 0.1) indicate significance of RR differences between N-forms (Mean ± SE, n = 23, T-test).

Results of non-parameter correlation demonstrate no correlation of RR and nitrogen forms (**Table 6**). All the coefficients are not significant (P<0.05) regardless of standards used for RR expression. Two-Way ANOVA are used to test the influence of N-forms and root orders on RR_{DWT} and RR_{SA}. It is noticeable root orders have extremely significant different effects on RR (P<0.01). However, it indicates no significant N-form effects (**Table 7**).

Table 6 Spearman Correlations of root respiration rates and N-forms of *A.platanoides*.

N-forms were nitrate and ammonium, glycine, di-glycine and tri-glycine. The nitrogen concentration was set as 15mM for all treatments. (n = 23)

		RR_{FWT}	RR_{DWT}	RR_{SA}	RR_{Vol}
N-form	**Correlation Coefficient**	-0.01	-0.02	-0.03	-0.05
	Sig. (2-tailed)	0.91	0.76	0.68	0.46

Table 7 Two-Way ANOVA analysis of RR (on root dry weight and surface area basis) for Nitrogen form (nitrate and ammonium, glycine, di-glycine and tri-glycine) and Root Orders (1+2 and 4).

SS, Type III Sum of Squares; Df, Degree of freedom; MSE, Mean square (n=23).

Source	RR_{DWT}					RR_{SA}				
	SS	Df	MSE	F	Sig.	SS	Df	MSE	F	Sig.
N-forms	18.93	5	3.79	0.55	0.737	0.00	5	0.00	1.05	0.389
Order	1937.09	1	1937.09	282.54	0.000	0.01	1	0.01	313.99	0.000
N-forms x Order	15.94	5	3.19	0.47	0.802	0.00	5	0.00	.71	0.619
Model	1984.39	11	180.40	26.31	0.000	0.01	11	0.00	29.24	0.000
Error	1727.71	252	6.86			0.01	252	0.00		

4.2.2 Nitrogen Concentration (NO_3^- and NH_4^+) Effects on Root Respiration

Measurements were conducted to analyze root respiration associated with different nitrate concentrations. Root segments were measured in solutions with four different nitrate concentrations (0 mM, 5 mM, 15 mM, and 45 mM N). As consequences of the Spearman correlation analysis, all the coefficients are not significant between RR and nitrate concentrations ($P<0.05$).

RR_{DWT} and RR_{SA} are shown in **Figure 17** under given nitrate concentrations. RR show no significant difference over the concentration levels. RR_{DWT} are highest without nitrogen sources. Among treatments root segments are in Hoagland solutions with nitrate, the highest RR_{DWT} is in treatment of nitrogen concentration as 15 mM on root orders 1+2. RR_{DWT} is also found to be highest in the control treatments as for root order 4. RR_{SA} hardly shows any difference among nitrate concentration treatments on root order 1+2. The pattern of RR_{SA} on root order 4 is similar as RR_{DWT}, where N-free treatment possessed the highest RR_{SA}. Nevertheless, RR_{SA} values of

order 4 are more than two times larger than RRSA of order 1+2 which is on the contrary capered with RR_{DWT} values.

To test the influence of nitrate concentration and root order on RR_{DWT} and RR_{SA}, two-way ANOVA test (**Table 9**) is performed and the results illustrated a significant difference ($P<0.05$) in root order effects. There is also no significant effect of N concentration taking both root orders into account.
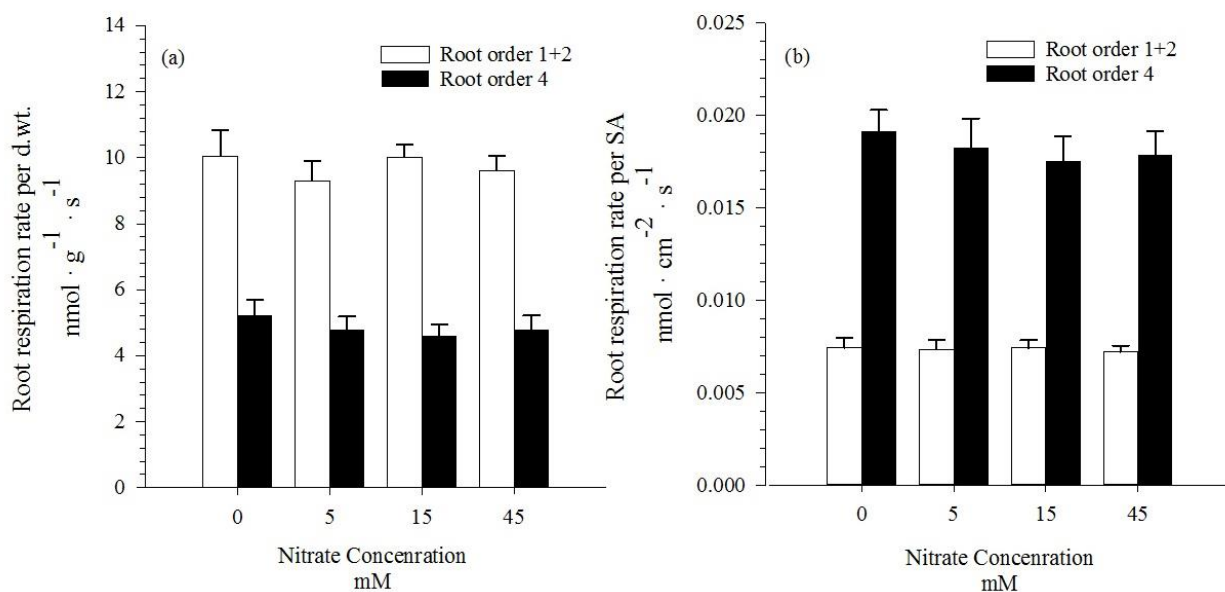
Figure 17 Nitrate Concentration Effects on Root Respiration Rate of *A.platanoides*.

The measuring solutions were with nitrogen concentrations as 0mM, 5mM, 15mM and 45mM. Nitrate source was KNO_3. (Mean ± SE, n = 23, T-test, $P < 0.05$).

Table 8 Spearman's Correlations of RR and nitrate concentrations in *A.platanoides*.

The measuring solutions were with nitrogen concentrations as 0mM, 5mM, 15mM and 45mM. Nitrate source was KNO_3. (n = 23).

		RR_{FWT}	RR_{DWT}	RR_{SA}	RR_{Vol}
Nitrate concentration	**Correlation Coefficient**	0.06	0.03	-0.01	-0.03
	Sig. (2-tailed)	0.52	0.77	0.93	0.72

Table 9 Two-Way ANOVA analysis of RR_{DWT} and RR_{SA} for Nitrate concentrations (0 mM, 5 mM, 15 mM and 45 mM) and Root Order 1+2 and 4.

SS, Type III Sum of Squares; Df, Degree of freedom; MSE, Mean square (n=23).

Source	RR_{DWT}					RR_{SA}				
	SS	Df	MSE	F	Sig.	SS	Df	MSE	F	Sig.
Concentration (NO$_3$$^-$)	7.80	3	2.60	0.49	0.687	0.00	3	0.00	0.26	0.855
Order	1046.01	1	1046.01	198.55	0.000	0.01	1	0.01	229.61	0.000
Concentration x Order	4.68	3	1.56	0.30	0.828	0.00	3	0.00	0.22	0.885
Model	1063.15	7	151.88	28.83	0.000	0.01	7	0.00	32.92	0.000
Error	879.80	167	5.27			0.00	167	0.00		

With regards to different ammonium concentrations (0 mM, 5 mM, 15 mM, and 45 mM), RR_{DWT} and RR_{SA} do not significantly differ. The 15 mM treatment present the largest RR_{DWT} on order 1+2, then followed by 5 mM, 0 mM and 45 mM. RR_{DWT} is declining on order 4 with ammonium concentration increasing. RR_{SA} is in similar pattern as RR_{DWT}. Namely, RR_{SA} is high under 15 mM ammonium treatment in root order 1+2, and under 0 mM treatment in order 4.

Results of Spearman correlations indicate no significant correlations between RR and ammonium concentration (P<0.05); the significance values are lower compared to those of N-form and nitrate concentration. For instance, RR_{Vol} is correlated with ammonium concentration with significance value as 0.24, which is the most significant correlation among RR and nitrogen source treatments. Two-way ANOVA results indicate no significance between ammonium concentration and RR with dry weight and surface area as basis (**Table 11**).
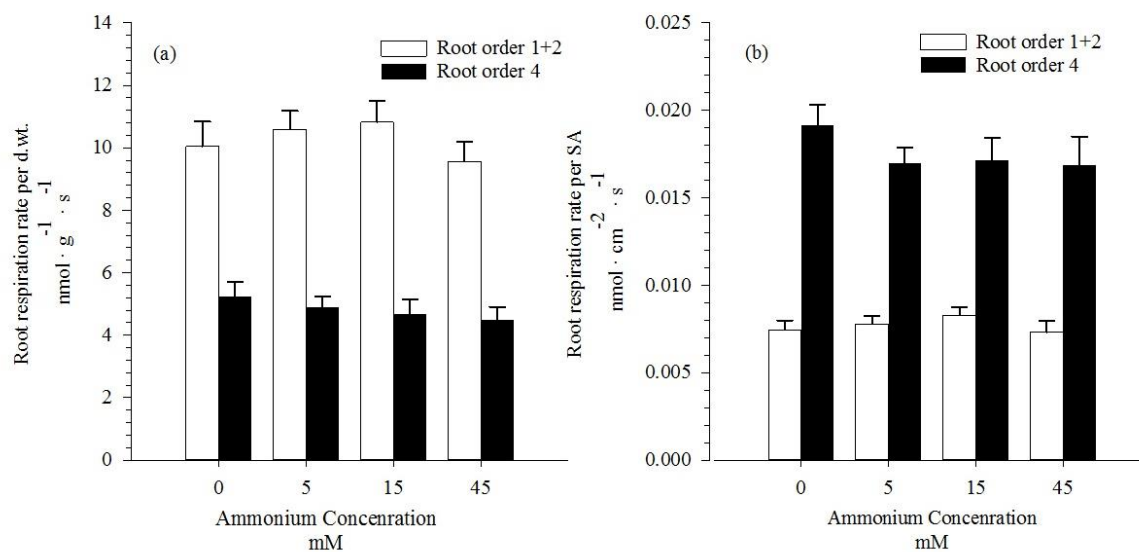
Figure 18 Ammonium Concentration Effects on Root Respiration Rates of *A.platanoides*.

The measuring solutions were with nitrogen concentrations as 0mM, 5mM, 15mM and 45mM. Ammonium source

was NH_4Cl. (Mean ± SE, n = 23, T-test, $P < 0.05$).

Table 10 Spearman Correlations of RR and ammonium concentrations of *A.platanoides*.

The measuring solutions were with nitrogen concentrations as 0mM, 5mM, 15mM and 45mM. Ammonium source was NH_4Cl. (n = 136).

|		RR_{FWT}	RR_{DWT}	RR_{SA}	RR_{Vol}
Ammonium concentration	**Correlation Coefficient**	-0.07	-0.08	-0.07	-0.10
	Sig. (2-tailed)	0.42	0.38	0.44	0.24

Table 11 Two-Way ANOVA analysis of RR_{DWT} and RR_{SA} for ammonium concentrations (0, mM, 5 mM, 15 mM and 45 mM) and root orders (1+2 and 4).

SS, Type III Sum of Squares; Df, Degree of freedom; MSE, Mean square (n=23).

Source	RR_{DWT}					RR_{SA}				
	SS	Df	MSE	F	Sig.	SS	Df	MSE	F	Sig.
Concentration (NH_4^+)	16.73	3	5.58	0.81	0.488	0.00	3	0.00	0.54	0.657
Order	1291.31	1	1291.31	188.38	0.000	0.00	1	0.00	201.05	0.000
Concentration x Order	11.33	3	3.78	0.55	0.648	0.00	3	0.00	0.81	0.487
Model	1329.63	7	189.95	27.71	0.000	0.00	7	0.00	29.08	0.000
Error	1151.61	168	6.85			0.00	168	0.00		

4.2.3 Root Order Effects on Respiration within Different Nitrogen Sources

In general, root order classes have significant effects on root respirations. Especially RR_{DWT} and RR_{SA}, in all 10 measured treatments, were all significantly different ($P<0.001$) for root order 1+2 and 4. Root respiration showed more fluctuations on root order 1+2 over the 10 nitrogen treatments. Respiration were greater in root orders 1+2 when RR was standardized by tissue mass (dry weight or fresh weight). But it goes on the other way around if RR are related to morphological parameters (surface area or volume).

It is noticeable that RR_{FWT} does not differ significantly ($P<0.05$) as a function as root orders. Only a slight significance ($P<0.1$) are found in glycine treatment. RR_{DWT} is significantly twice more than that on root order 1+2 (10.00 ± 0.20 nmol$\cdot$g$^{-1}\cdot$s^{-1}) than root order 4 (4.72 ± 1.28 nmol$\cdot$g$^{-1}\cdot$s^{-1}). RR_{DWT} is fluctuating in order 1+2 but is rather stable in order 4 over the 10 treatments. On the contrary, RR_{SA} is greater ($P<0.01$) in root order 4 (0.02 ± 0.00 nmol$\cdot$cm$^{-2}\cdot$s^{-1}) than in order 1+2 (0.01 ± 0.00 nmol$\cdot$cm$^{-2}\cdot$s^{-1}). RR_{Vol} is generally greater in root order 4. It shows significant differences ($P<0.01$) between root order classes in N-free treatment. In the three nitrate and tri-glycine treatments, RR_{Vol} is greater in order 4 ($P <0.05$).
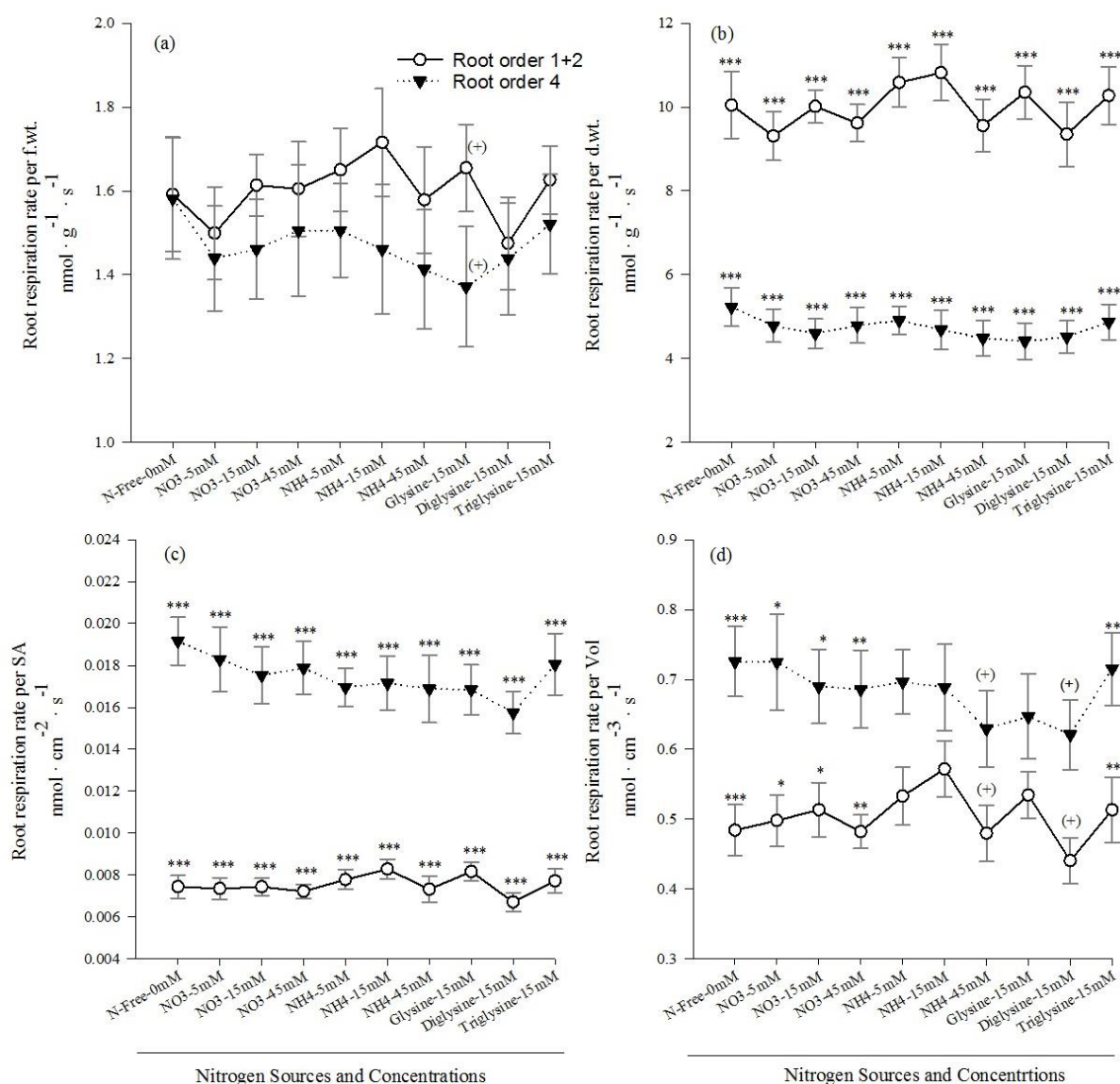
Figure 19 Root Respiration Rate as functions of different N-Sources on root order 1+2 and order 4 of *A.platanoides*.

The nitrogen sources were N-free (0mM), nitrate and ammonium (5mM, 15mM and 45mM), glycine (15mM), diglycine (15mM) and triglycine (15mM). RR of root order 1+3 are in empty circle, and RR of order 4 are in filled inverted triangles. The asterisks and plus in parentheses indicate significance of RR differences between root order classes of each nitrogen source. ***, $P < 0.001$; **, $0.001 \le P < 0.01$; *, $0.01 \le P < 0.05$; (+), $0.05 \le P < 0.1$ (Mean $\pm$ SE, n = 23; Mann-Whitney Test).

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4.3 Drought Effects on Root Respiration

4.3.1 Abiotic Soil Condition

The figures show soil temperature (°C) and soil volumetric water content (%) in soil depth of 10 cm for the three sampling periods (**Figure 20**). Soil temperature increased from around 7.7°C in the beginning of May for the first measurement (R1) to about 11.6°C in the end of June for the second measurement (R2), and finally reached above 12.5°C in the beginning of July during the last measurement (R3). It is slightly warmer in the control treatment (approximately 13.9°C at R3) than in the drought treatments (around 13.0°C at R3) especially in the later sampling periods. Compared with temperature, soil volumetric water content varies more among the three drought treatments. At the first sampling date the moderate stress treatment has the highest water content as 22.7%, the control is slightly lower as 20.1% and the severe stressed treatment plots has the lowest water content 15.6%. During the seconds sampling (R2), which is during the peak drought stress time, both moderate and severe stress plots have reduced water content of approximately 12.8%. Water content decreases down to around 14.7% for the control treatment. One week after irrigation (R3) the water content in both moderate and severe drought plots increase to about 14.6% whereas the control water content drop down further to 12.3%.
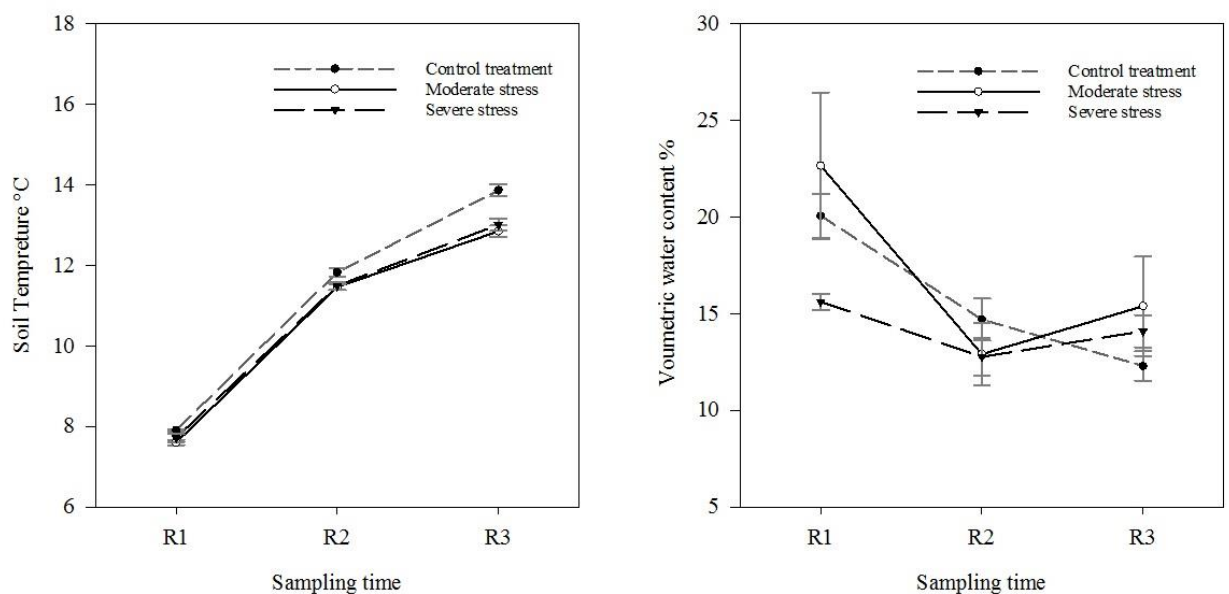
Figure 20 Volumetric water content (%) and soil temperature (°C) at depth of 10 cm.

R1, R2, R3 are the three sampling periods. The control, moderate and severe stress treatment are displayed as short dashed lines, long dashed lines and solid lines respectively (Mean±S.E., n = 4).

4.3.2 Drought Effects on Root Biomass

Root biomass and necromass change during the experiment period as shown in **Figure 21**. In general, fine root biomass density decline from around 1.4 g·L^{-1} to 0.8 g·L^{-1} for all the three treatments as in **Figure 21**, (a). The control always possess the largest amount of fine root biomass in each sampling time. Moderate and severe stressed plots exhibit roughly the same root biomass at sampling times R1 and R2; the control increase a little (non-significant) at the last sampling date. The non-significant increase in root biomass from R2 to R3 in the control treatment caused the fine root biomass at the last sampling to be marginally significant higher in the control than in the moderate drought-stressed plots (P<0.1).

The necromass increase during the experiment at a similar scale as the biomass decrease (**Figure 21**, (a)). The necromass data illustrates a clear pattern among three drought treatments. In the beginning, necromass density values ranked opposite to drought degree without significance. After 8 weeks of drought treatments, the drier the treatment plots were, the greater necromass densities are found in the plots at the peak drought stress time R2. After irrigation, at the last sampling time, necromass density come to roughly the same level for all three treatments, 0.3 g·L^{-1} (**Figure 21**, (b)).
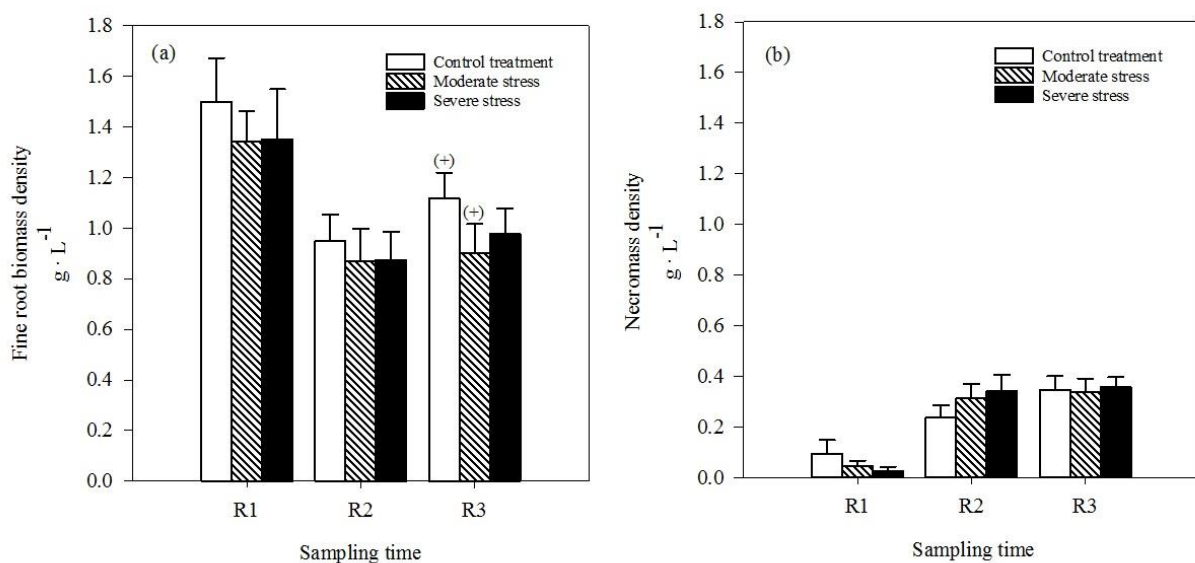
Figure 21 Fine root biomass density (a) and necromass density (b).

R1, R2, R3 are the three sampling periods (Mean ± SE, n = 12; Mann-Whitney Test; (+), P < 0.1.).

4.3.3 Drought Effects on Root Respiration

Root respiration were measured in the same way as in the first experiment (*A. platanoides*) for *F. sylvatica*. RR_{DWT} at 20°C with both soil layers combined are shown in **Figure** 22. RR_{DWT} has notably increased with time, from around 3.0 nmol $\cdot g^{-1} \cdot s^{-1}$ to above 4.0 nmol $g^{-1} \cdot s^{-1}$ in R2 and R3 after the drought period. At the first sampling (R1), RR_{DWT} is significantly higher in the control treatment than in the drought treatments (P<0.05). Eight weeks later, at the drought stress peak, there is no difference in root respiration between treatments. One week after irrigation (R3), roots once again show an influence from drought—with severe stress treatment possessing the lowest respiration level, which was (marginally) significantly lower compared to the control (P<0.05) and the moderate drought stress treatment (P<0.1).
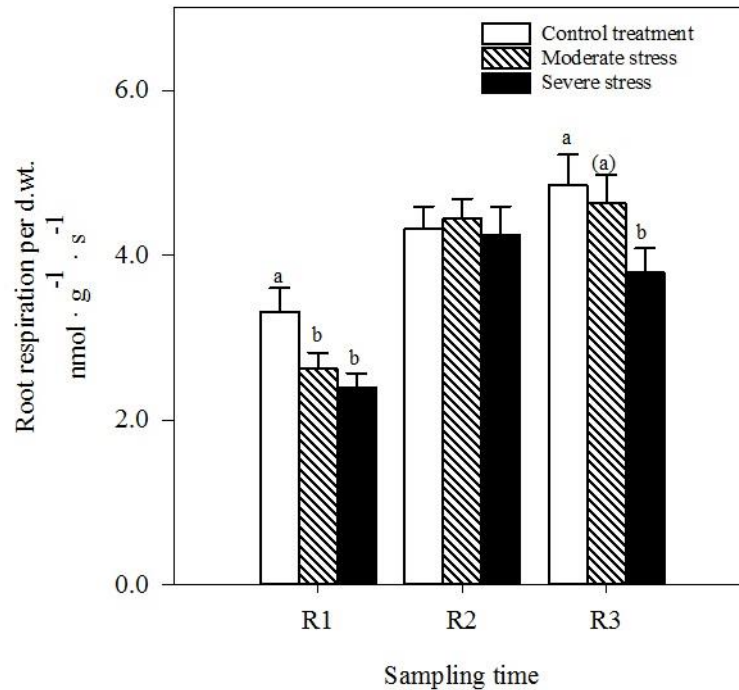
Figure 22 Root respiration per dry weight (d.wt.) at 20 °C for *Fagus sylvatica*.

R1, R2, R3 are the three sampling periods. Small letters (P < 0.05) and letters in parentheses (P < 0.1) indicate significance differences of RR between drought stress treatments (Mean ± S.E., n = 12; LSD multiple comparisons; P < 0.05).

The six column graphs in **Figure 23** shows RR_{DWT}, RR_{SA} and RR_{Vol} at 20°C differentiate according to two soil layers (O-horizon and Aeh-horizon) and the three treatments over the

experiment period. Roots respire more in the organic O-horizon than in the mineral Aeh-horizon (**Table 12**). RR_{DWT}, RR_{SA} and RR_{Vol} are following similar trends changing with factors as time, drought treatment and soil layer. RR_{DWT} just give faintly higher significances in comparison with RR_{SA} and RR_{Vol}. RR is increasing over time no matter in which soil layer roots were found (**Figure 23**). RR of moderate stress treatment increase in O-horizon but dropped down in Aeh-horizon at time R3. Comparably, the severe drought treatment has in general highest RR in R2 and lowest RR in R1 for both soil layers. At R1 the higher RR of the control mainly come from O-horizon ($P < 0.05$). There is no significant difference among three treatments in Aeh-horizon. As shown by the measurements from peak stress period R2, moderate stress treatment hold the lowest RR in O-horizon but highest in Aeh-horizon (non-significant). After rewetting (R3), RR_{DWT} of the severe drought treatment is the least ($P < 0.1$) compared to the other two treatments in O-horizon.
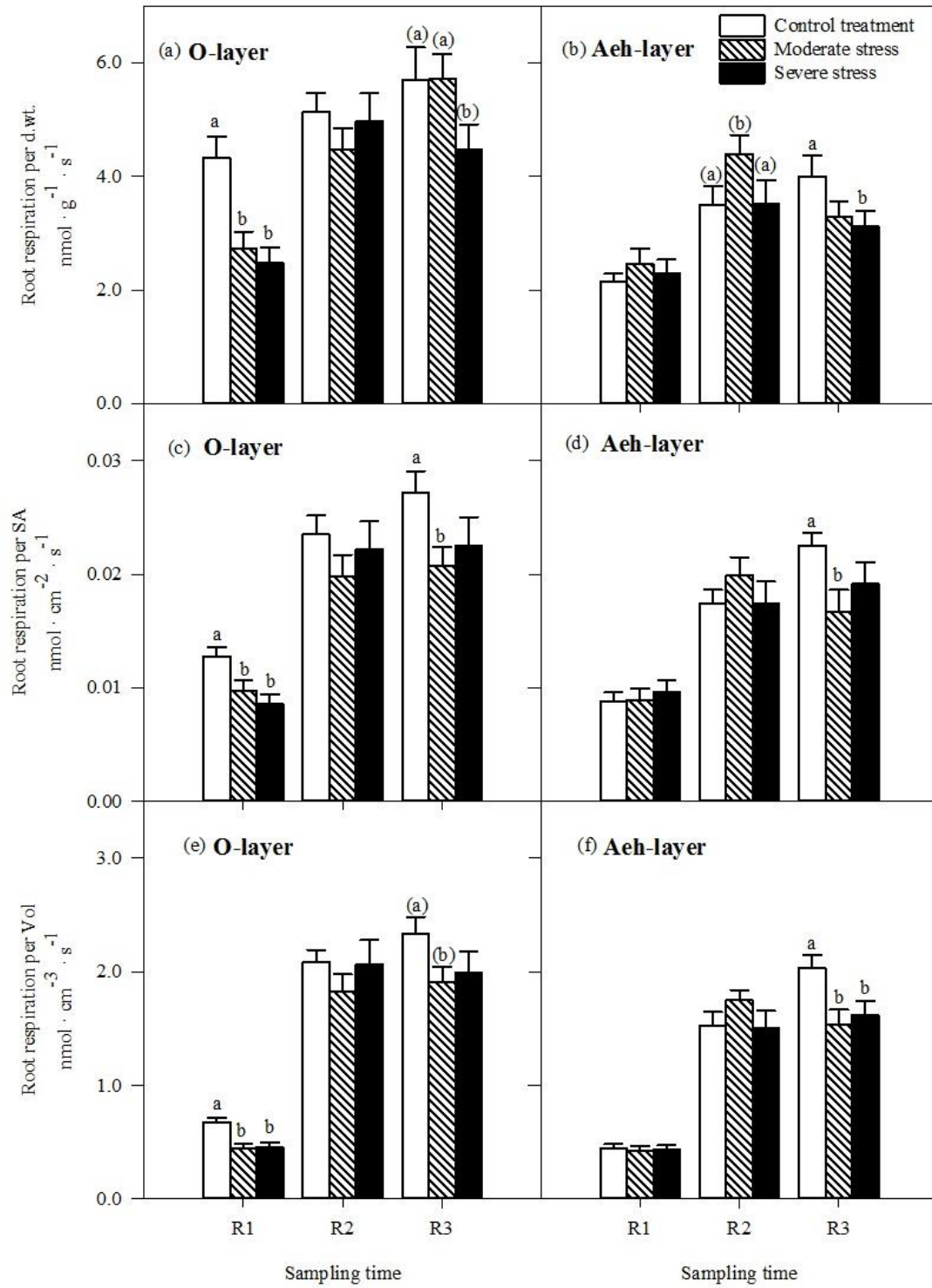
Figure 23 Root respiration rates per root unit (RR_{DWT}, RR_{SA} and RR_{Vol}) in two soil layers O-horizon and Aeh-horizon.

RR was measured at 20 °C for *Fagus sylvatica*. R1, R2, R3 are the three sampling period in the drought experiment. (a) & (b): Root respiration per root dry weight (RR_{DWT}, d.wt., nmol g^{-1} s^{-1}); (c) & (d): Root respiration per root surface area (RR_{SA}, SA, nmol cm^{-2} s^{-1}); (e) & (f): Root respiration per root volume (RR_{Vol}, Vol, nmol cm^{-3} s^{-1}). Small letters ($P < 0.05$) and letters in parentheses ($P < 0.1$) indicate significance of RR differences between drought stress treatments (Mean ± S.E., n = 12; LSD multiple comparisons; $P < 0.05$).

Table 12 All Three Way ANOVA results on RR$_{DWT}$ to test influence of sampling time, soil layer (O-horizon and Aeh-horizon) and drought stress treatments (control, moderate and severe) on root respiration rates in *Fagus sylvatica* forest.

SS, Type III Sum of Squares; Df, Degree of freedom; MSE, Mean square (n = 12).

Source	RR$_{DWT}$				
	SS	Df	MSE	F	Sig.
Sampling time	158.127	2	79.063	37.912	0.000
Layer	109.189	1	109.189	52.357	0.000
Treatment	20.506	2	10.253	4.916	0.008
Sampling time * Layer	11.493	2	5.746	2.755	0.065
Sampling time * Treatment	10.552	4	2.638	1.265	0.284
Layer * Treatment	12.102	2	6.051	2.901	0.057
Sampling time * Layer * Treatment	23.226	4	5.806	2.784	0.027
Model	346.042	17	20.355	9.761	0.000
Error	544.305	261	2.085		

The Q_{10} is calculated from RR$_{DWT}$, RR$_{SA}$ and RR$_{Vol}$ measurements at 10°C and 20°C for O-horizon and Aeh-horizon separately. The average value Q_{DWT10} = 1.43 measured from RR$_{DWT}$ were finally used to calculate root respiration at actual soil temperatures.

Table 13 Q_{10} values calculated from RR$_{DWT}$, RR$_{SA}$ and RR$_{Vol}$ measured at 10°C and 20°C.

The average values of Q_{10} were calculated from Q_{10} of O-horizon and Aeh-horizon.

Q_{10}	Q_{SA10}	Q_{Vol10}	Q_{DWT10}
Q_{O10}	1.35	1.42	1.94
Q_{A10}	1.24	1.33	0.92
Mean Q_{10}	1.30	1.38	1.43

Root respiration rates (RR $_{(t)}$) are finally expressed according to real Q_{DWT10} and temperature *in situ* in **Figure** 24, (a). RR $_{(t)}$ stay more or less in the same level at around 600 nmol $\cdot m^{-2} \cdot s^{-1}$ for R1 and R2 in the control treatment and tend to increase in R3 to about 872 nmol $\cdot m^{-2} \cdot s^{-1}$ (non-significant). The RR $_{(t)}$ of the control tend to be higher than in the drought treatments during R1 and R2 but the difference only became significant in R3 (P<0.05) (**Figure 24**).

The percentages of the total soil respiration being root respiration is calculated by dividing the RR $_{(t)}$ by the total soil respiration (**Figure** 24, (b)). At R1, root respiration is account for about 30% to the total soil respiration in all treatments. At the peak stress time (R2), RR proportion in the severe drought treatment increase to 52% of soil respiration, whereas it remains at about 30% in control and moderate drought stress treatments (non-significant). At the last sampling date (R3), RR in the control remains the same or tended to increase whereas both moderate and severe drought stressed plots tended to decrease the proportion of RR on the total soil respiration. The two drought stress treatments show opposing trends from R2 to R3. Specifically, the autotrophic respiration percentage in moderate treatment decreases about 8% to 26% of total soil respiration, which is the lowest at R3. The mean value of autotrophic respiration percentage in the severe drought stress treatment declines from 52% to 37%. Overall, there are no significance in drought treatments with regards to root respiration in instant temperature. As shown in the results of Two-Way ANOVA, RR $_{(t)}$ and autotrophic proportion neither significantly response to drought treatment regime nor to sampling time periods (**Table 14**).
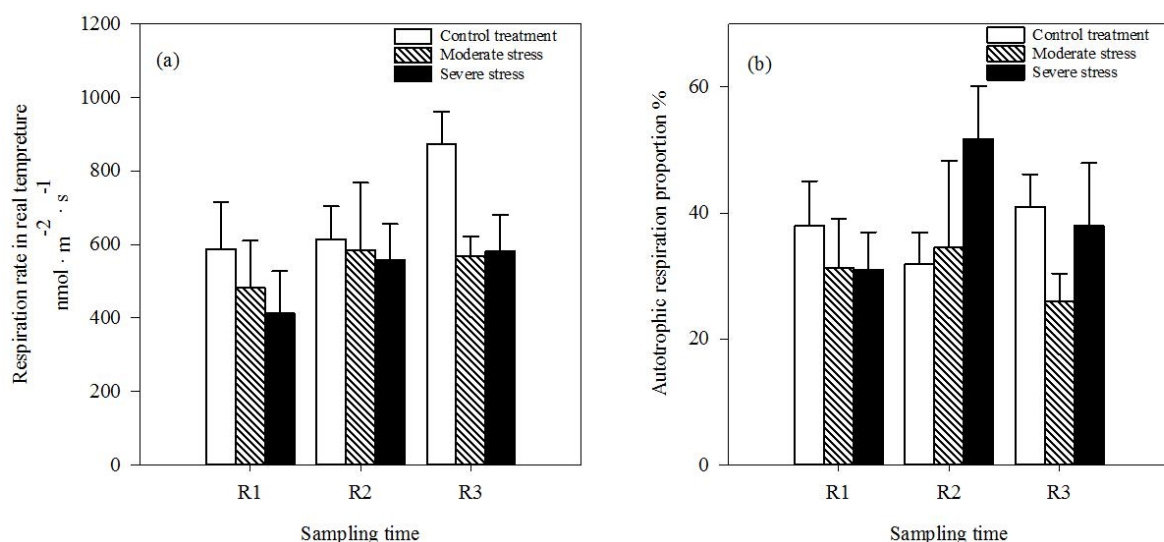
Figure 24 Root respiration rate at real soil temperature derived from Q_{10} (RR $_{(t)}$, (a)) and its proportion to CO_2 soil efflux *in situ* (b).

Small letters (P < 0.05) indicate significance of RR differences between drought stress treatments (Mean ± S.E., n = 4; LSD multiple comparisons; P < 0.05).

Table 14 Two-Way ANOVA of RR (on root dry weight and surface area basis) for upscaled root respiration at real temperature calculated from Q10 (RR(t), nmol · m-2 · s-1) and autotrophic Respiration proportion.

SS, Type III Sum of Squares; Df, Degree of freedom; MSE, Mean square (n = 4).

Source	RR(t)					Autotrophic RR proportion %				
	SS	Df	MSE	F	Sig.	SS	Df	MSE	F	Sig.
Time	193658.829	2	96829.415	1.844	0.178	0.023	2	0.012	0.449	0.643
Treatment	209409.852	2	104704.926	1.993	0.156	0.058	2	0.029	1.122	0.34
Time * Treatment	95611.887	4	23902.972	0.455	0.768	0.098	4	0.025	0.957	0.447
Model	498680.568	8	62335.071	1.187	0.343	0.179	8	0.022	0.871	0.552
Error	0.694	27	0.026			0	168	0		

5. Discussion

5.1 Root Morphology of *A. platanoides*

More persistent root segments in seedlings of the tree species *A. platanoides*, *C. betulus*, *T. cordata* and *Q. robur* have been found before to occur at root order ≥3. In this study, impressive differences in morphological traits between root order 1+2 and order 4 of *A. platanoides* have been found. Distal root segments order 1+2 possess larger SRA and SRL, with much lower value in diameter, RTD and RDMC in comparison with root order 4. These morphological traits are consistent with the supposed physiological functions of the root orders. For example, higher SRA are thought to increase the root system interface with the soil environment and thus interchange of water and nutrients, root order 1+2, i.e the most terminal root segments, are known to be the most active root system parts for ion up-take ((Hishi 2007)). RTD is approximately 0.05 and 0.15 g·cm^{-3} in root order classes 1+2 and 4 respectively. It is relatively lower compared to previous studies as roughly 0.20 and 0.40 g·cm^{-3} (Valenzuela-Estrada et al. 2008, Rewald et al. 2014b). This might be consequences of irrigation with tap water and nitrogen deficient nutrient solution. The low RTD and RDMC indicate a high water content and thus high tissue 'sponginess' in root orders 1+2. RTD is a fundamental trait in comparative root ecology, being increasingly used as an indicator of plant species' resource use strategy (Birouste et al. 2013). Because of its high ecological importance, tissue density, or the easier to determine RDMC, is now measured routinely in many studies world-wide comparing species and growth plasticity under environmental conditions (Kembel and Cahill Jr 2011). Rewald et al. (2014b) have recently shown that RTD is a parameter outlining convergent patterns (Meinzer 2003) of root respiration among different tree species much better than root diameter. Root orders 1+2 of other woody species have been found previously to possess a shorter longevity than root segments of higher orders (Valenzuela-Estrada et al. 2008). Vessel numbers are reported to be more than two times higher in order 4 than 1+2 (Valenzuela-Estrada et al. 2008). Being in secondary development, the mature xylem of order 4 contributes thus most to the mass but may not play important roles in most physiological processes such as respiration. Higher root orders are previously thought to have predominantly conducting functions, but questions remain (Rewald et al. 2011a). The measured specific root area is fairly high compared with previous results (Rewald et al. 2014b) and declines exponentially with increasing root diameter. Moreover, SRA and RTD are also related with each other in an exponentially negative trend. RTD are furthermore associated with RDMC positively in linear relation as both of then presenting root mass characteristics with somehow scattered data dots. So it could be expectable

SRA is in exponential decay relation with RDMC, with rather scattered data dots compared to **Figure 10**. However, plant morphological traits are commonly correlated with mass traits. Shipley (2002) and Prieto (2015) previously found negative linear relations between plant specific morphological traits and dry matter content with R^2 values similar to the values in this study (**Figure 12**). Interestingly, within each single root order class the correlations disappear; this phenomenon requires further attention in future studies.

Preciously Rewald et al. (2014b) could show that the frequency of root order classes is tree species-specific. This study found that root branch biomass is built to large proportions by orders 1+2 and 6 in *A. platanoides* seedlings (**Table 4**). Previously, Valenzuela-Estrada (2008) reported high biomass percentage in high root orders 4,5 and 6 but lowest in order 1 and 2. It is understandable high root orders hold more mass because of the higher woody xylem proportions. Nonetheless, in this study results illustrate more mass allocation to order 1+2 from *A. platanoides* seedlings, which could be predictable by high number of terminal root segments in the measurement. This might be results of nutrient, especially nitrogen, deficiency that causes root system to 'seek' for more nutrient rich soil patches.

5.2 Root Respiration Rates and Root Morphology

5.1.1   Root Respiration Rates standardization

RR_{DWT} and RR_{FWT} gives the amount of RR per root mass unit. In higher root orders (order 4), woody root cells hardly respire but hold great amount of absolute mass. RR_{DWT} and RR_{FWT} would be under estimated because of the existence of dead xylem cells. Even though, RR_{FWT} RR could be more under estimated in order 1+2 than in order 4 when considering its high water content. In conclusion, neither dry weight nor fresh weight could be perfect standards in RR expression. But RR_{DWT} reflects root matter respiration in a more reasonable way. But RR cannot be perfectly expressed by morphological traits as root surface area and volume either. Especially RR_{SA} may a valuable expression of root respiration beside mass-based RR because of its direct interrelationship to water and nutrient uptake per surface area. However, other living cells inside roots respire as well. If all cells in root tissues have more similar physiological activities, volume-based respiration rates might be a valuable standardization. However, this might be roughly assumed only for some homorhiz roots of grass and herb species (but not allorhiz root systems) and probably most basal parts of first order root segments of woody species (which do not show and longitudinal growth anymore but have not started secondary

growth and thus xylem maturation yet). However, morphological standardization approaches are shown to be better correlated with RR in *A. platanoides* in this experiment. Therefore, RR_{SA} and RR_{Vol} might better illustrate root respiration compared to RR_{DWT} and RR_{FWT}. In fact, most respiration results are expressed as RR_{DWT} (Pregitzer et al. 1998, Burton et al. 2002) while RR_{SA} or RR_{VOL} are rather rarely calculated (DesRochers et al. 2002, Dannoura et al. 2006a). Further studies are necessary to determine optimal respiration rates standardization for different tissues types.

5.1.2 Root Respiration Rates and Morphological Traits

The RR_{DWT} of *A. platanoides* roots varies from 1.03 to 18.01 $nmol \cdot g^{-1} \cdot s^{-1}$ in this experiment (**Table 15**). The range of measured root respiration rates are comparable with RR rates of other tree species reported earlier (Pregitzer et al. 1998, Burton et al. 2012, Rewald et al. 2014b). It is slightly lower than in a previous study on *A. platanoides* seedlings, which reported RR_{DWT} from 1.0 to 44.9 $nmol \cdot g^{-1} \cdot s^{-1}$ (Rewald et al. 2014b), likely because white root orders 1+2 with known higher RR were not found in this experiment.

Table 15 Descriptive statistics of root respiration rates (root orders 1+2 and 4 combined) by four different standards.

FWT, fresh weight; DWT, dry weight; SA, surface area; Vol, root volume.

	RR_{FWT}	RR_{DWT}	RR_{SA}	RR_{Vol}
	$nmol \cdot g^{-1} \cdot s^{-1}$	$nmol \cdot g^{-1} \cdot s^{-1}$	$nmol \cdot cm^{-2} \cdot s^{-1}$	$nmol \cdot cm^{-3} \cdot s^{-1}$
n	446	446	446	446
Minimum	0.29	1.03	0.002	0.09
Maximum	3.20	18.01	0.039	1.51
Mean	1.54	7.36	0.012	0.59
Std. Deviation	0.58	3.62	0.007	0.24

RR is significantly distinguished between the two measured root order classes 1+2 and 4, no matter which standards are used to express RR. Because root order biomass allocation shows that root orders 1+2 account for 25.35% (0.13 g) of the estimated plant root total biomass, while root order 4 present 10.59% (0.06 g) of the root branch biomass, the estimated RR_{DWT} in a root

branch of one g were approximately 1.30 nmol·s^{-1} from root order 1+2 and 0.28 nmol·s^{-1} from order 4 respectively. In other words, terminal root orders 1+2 in root systems of *A. platanoides* seedlings respire almost 5 times as much as root segments of order 4. Hence, it is logical to state that distal roots contribute much more to total root system respiration.

Root morphological traits are highly correlated to root respiration rates regardless of the standardization methods; distinct morphological traits existed in different orders as shown above. In **Table 5**, it is shown that RR$_{DWT}$ and RR$_{SA}$ are all significantly related with selected root traits although holding different coefficients of determination. RR$_{DWT}$ decrease with diameter and as RTD increase. Previously it has been shown, that root respiration of other species and *A. platanoides* is highly related ($R^2 = 0.67\text{-}0.78$) to RTD in an exponential decay fashion (Rewald et al. 2014b). While (Rewald et al. 2014b) stated that root diameter can be useful for the approximation of root respiration ($R^2 = 0.56\text{-}0.79$) only after creating laborious calibration curves, this study found that more simple relations exist if a within root order differentiation into functional classes is absent. Thus, in this study results of RR$_{FWT}$, RR$_{DWT}$ and RR$_{SA}$ are all significantly correlated with both root order and root diameter (P<0.01). RR$_{Vol}$ is not correlated with root average diameter with P-value equal to 0.406 whole it is still in significant correlation with root order (P<0.01). These results are in contrast to previous findings of Rewald and colleagues (2014b) and further studies on other species and environmental conditions are necessary to unravel the interdependency of RR$_{Vol}$ to morphological traits in root systems.

5.3 Nitrogen Nutrition Effects on Root Respiration Rates

Nitrogen plays a major role in plant nutrition (Lambers et al. 2008). Nitrogen oxides emitted during fuel combustion and ammonia volatilized as a result of intensive agriculture have increased atmospheric nitrogen inputs (mostly NO3 and NH4) to temperate forests in the Northern Hemisphere (Nadelhoffer et al. 1999). Plants show different preferences with regards to chemical forms when absorbing nitrogen. Previously, *A. platanoides* was found to absorb more NH$_4^+$ than NO$_3^-$ (Templer and Dawson 2004). It is evident that roots absorb more mineral nitrogen such as ammonium and nitrate, rather than organic nitrogen sources. However, my results indicated not any difference (P-Value < 0.05) in root respiration as functions of various N-sources in this experiments. This is surprising, at least for root orders 1+2, because absorption and assimilation processes need more energy from respiration for AA and peptides ((Jones et

al. 2005)). Regarding differences between inorganic N sources, it has been shown on *Hordeum vulgare* that the respiratory cost of uptake and assimilation of NH_4^+ can be only half that for uptake and assimilation of NO_3^- (Bloom et al. 1992), although opposite or no effects have been found on herbs (Cucumis sativus, Matsumoto and Tamura 1981) and trees (Zogg et al. 1996). Several could have cause the observed absence of respiration differences under different nitrogen sources and concentration in this experiment, the most important being: i) the nitrate absorption process is a negative feedback mechanism, which may not be controlled by roots (von Wirén et al. 1997), and ii) time to measure the respiration change in certain solutions for just approximately 20 min might have been too short. Indeed, (Rewald et al. 2014a) recently found that long-term (one growing period) irrigation with different ammonium:nitrate ratios influenced fine root respiration of Poplar and willow clones , wizth higher NO_3^- fertilisation increasing the fine RR. In contrast, root excision seems not to be a factor; Bloom (1992) reported a similar amount of nitrate absorption but lower assimilation on excised roots in comparison with intact root in barley and Lambers et al. (1993) reported that root excision does not have a substantial effect on root respiration when assessed within half an hour after excision.

While it was recently shown that N uptake might occur also in woody coarse roots (Hawkins et al. 2014), Rewald et al. (2014a) recently claimed that more stable RR under greater NO_3^- supply indicate that coarse roots have only a minor involvement in NO_3^- assimilation (in Salicaceae). I hypothesized no major influences of N form on the respiration of *A. platanoides* root order 4 because the root cortex was previously identified as major nitrate uptake site (Siddiq et al. 1991, Robinson 1996) and woody coarse roots change their anatomy dramatically during secondary growth—shedding the cortex.

5.4 Drought Stress Effects on Root Respiration Rates

Continuing global warming and its potential associated factors threat ecosystems world-wide. Especially, changes in precipitation extremes have increasingly received attention since many regions worldwide have experienced significant variations in climate extremes during the past few decades (Easterling et al. 2000). For example, Tebaldi et al. (2006) reported simulated increases in precipitation intensity and longer dry periods between precipitation events world-wide, and Griffiths and Bradley (2007) predicted increasing precipitation extremes in North America. In most parts of Central Europe, moderate water stress was typically during short rainless periods that occur at irregular intervals while severe droughts are episodic events (Rewald et al. 2011b). However both the frequency and the intensity of weather extremes such

as water restrictions and floods are likely to increase in Europe over the next decades (Pachauri et al. 2014). Biological soil activity, for which water availability is the primary limiting factor in many areas, could be affected severely, since the impacts of drought on soil quality are very severe (Sowerby et al. 2005).

The direction of change, i.e. increase or decrease, and magnitude of root biomass response to drought largely depend on tree species or variety, but also on study duration and/or study design (Rewald et al. 2011b). However, two general trends can be noticed: (i) an increase of root biomass in response to drought have mostly been found in conifer species (Gower et al. 1992, Parker and Van Lear 1996), and (ii) a decrease of root biomass in European deciduous tree species (Fort et al. 1998, Chiatante et al. 2006). In the current study, fine root biomass of mature Fagus sylvatica trees was reduced with proceeding vegetation period (comparing end of May to end of July). However no significant differences are found between the three droughts treatments, although the control treatment tended to hold the highest fine root density at all three measurement periods. Especially more drought sensitive tree species like *Fagus sylvatica* and *Quercus robur* are previously found to retain their root biomass in the upper soil horizons even under severe drought, resulting in high turnover rates (Konôpka et al. 2005, Mainiero and Kazda 2006). In contrast, the fine root biomass of rather drought tolerant, mature *Quercus petraea* trees was significantly reduced in the organic layer after three months of experimentally-induced summer drought (Rewald et al. 2011b). Thus, our finding could be related to the lack of adaptive fine root biomass reaction of *Fagus sylvatica* to drought periods.

Generally root respiration do not response to the drought stress treatments as expected. It is also reported previously that at low soil temperatures (10 °C), respiration was little influenced by soil moisture contents (Huang et al. 2005). With regards to time span, root respiration increased after drought period which could be result of largely increased temperature. RR was highest in the control and lowest in the severe for before and after recovery from the drought stress. Roots had RR reduced after experience of moderate and severe drought period. But at the peak drought time, there was not significant difference among three treatments. This could be explain as the tree might not be under drought stress as a whole. The experiment plots occupied just parts of the total roots distribution region in the *Fagus* forest. Plants are not totally hindered from water source.

In general, roots respire more in the organic O-horizon than the mineral Aeh-horizon. It could mostly because of high infection by ecto-mycorrhiza for the surface roots. Roots also need to be more active to absorb nutrients in organic layer. Besides, high soil porosity in O-horizon

provided well air condition for root respiration. In fact, roots in Aeh-horizon is thought to suffer more from the drought stress. To the control as relative level, it shows an increased RR at the peak drought stress point but reduced a lot after irrigation. This is a more detailed RR change regime with drought stress compared to previous study (Burton et al. 1998). *Fagus* roots may be more active in the start of low water content. For one side, it can provide energy to survive from the drought. And on the other side, high respiration and CO_2 loss could regulate carbon allocation in the plant scale to reduce the input in underground part.

Respiration were thence transformed as $RR_{(t)}$ with the mean temperature on site. $RR_{(t)}$ was significantly high for the control in the 3rd measurement which was in consistent with the high temperature at that time. The drought stressed plots was not in high temperature because of shading effects of the roofs. In the drought treatments especially the severe one, $RR_{(t)}$ goes up at the peak stress time and drop down after recovery irrigation. This pattern was more evident in proportion of root respiration to total soil respiration. At the peak stress time, dry plots hold higher autotrophic respiration percentage. And they came back to be lower than the control after recovery. Therefore, it might be concluded that root respiration strategy to drought regime was to increase respiration. Plant root tissue is supposed to accelerate the lignification or suberization processes by vigorous respiration so as to survive from the adversity. Nevertheless, the high autotrophic respiration proportion may be also for the reason that microbes suffered in a larger extent than the roots. The reduction in microbial respiration is larger than that of the roots. So autotrophic respiration percentage increased in the very drought time. As for the third measurement, microbes might recovered and reacted more rapidly to increased soil water content, which resulted in the declined autotrophic respiration proportion. Results of the autotrophic respiration proportion were in coincidence with other studies. Hanson (2000) found the integrated root respiration contribution to total soil respiration for an entire year or growing season showed mean value of 45.8 percent for forest.

RR values are comparable at around 0.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$ with results of other studies (Burton et al. 2012). In addition, before the drought experiment, the severe treatment was already in lowest water content compared to the other two which indicated an influence of drought experiments from last year. This could affect the results in biomass and RR measurements. The measured $Q10$ values are relatively lower compared to Q_{10} values of about 2.2 in other studies (Tjoelker et al. 2001, Janssens and Pilegaard 2003). The value is determined by many factors like temperature and soil moisture and it also depends on species (Atkin et al. 2000, Tjoelker et al. 2001). Soil water content was low in the $Q10$ measurement time and $Q10$ was measured under

10 ℃, which might be the reasons. Further studies are necessary to determine realistic root Q_{10} values for a range of environmental conditions and species.

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Appendix

**Appendix Table 1** Nitrogen deficient Hoagland Solution recipe used in the study.

	Stock Solution	Dilution	Ready solution	Molarity in Ready Solution
	$g \cdot L^{-1}$	Times	$mg \cdot L^{-1}$	mM
Major Nutrient Solution				
KH_2PO_4	68.05	500	136.09	1
$MgSO_4$	120.00	500	240.00	2
KCl	168.10	500	336.21	5
$CaCl_2*2H_2O$	294.00	500	588.00	4
Micro Nutrient Solution				
H_3BO_3	2.86	1000	2.86	
$CuCl_2*2H_2O$	0.05	1000	0.05	
$FeNaETDA$	45.01	1000	45.01	
$MnCl_2*4H_2O$	1.81	1000	1.81	
$Na_2MoO_4*2H_2O$	0.03	1000	0.03	
$ZnSO_4*7H_2O$	0.22	1000	0.22	

Appendix Table 2 Mean root diameter, specific root area (SRA), root tissue density (RTD), Specific root length (SRL), and Root dry matter content (RDMC) of two root order classes 1+2 and 4 in *A. platanoides*.

Small letters indicate significant differences of average diameter, SRA, RTD, SRL and RDMC between root order classes 1+2 and 4 (mean ± S.E.; Mann-Whitney Test, P < 0.001).

| Root Order	n	Mean Root Diameter	SRA	RTD	SRL	RDMC
		mm	$cm2 \cdot g^{-1}$	$g \cdot cm^{-3}$	$cm \cdot g^{-1}$	$g \cdot g^{-1}$
1+2	223	0.61±0.00a	1358.41±16.63a	0.05±0.00a	7176.00±97.41a	0.16±0.00a
4	223	1.07±0.02b	278.72±6.08b	0.15±0.00b	909.00±29.92b	0.31±0.00b
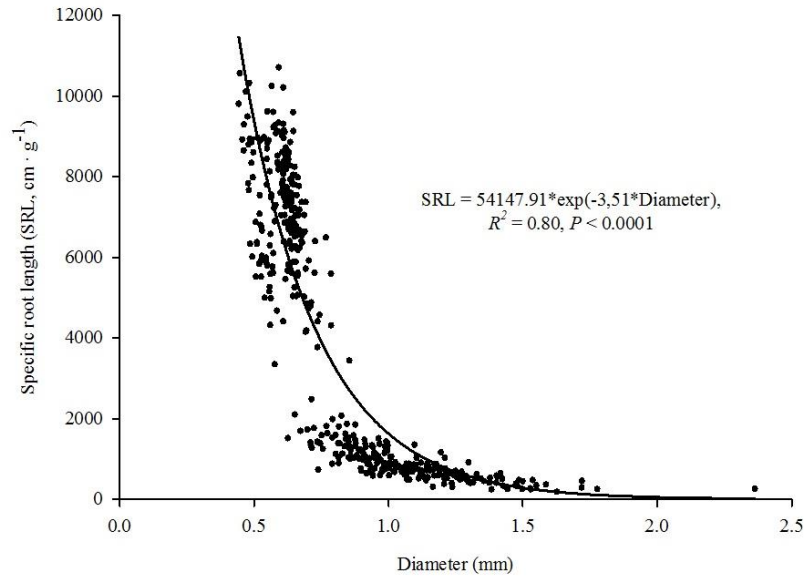
Appendix Figure 1 Specific root length (SRL) was negatively related to root diameter of A. platanoides.

Exponetial function curve was fitted to all the data points from both root order classes. The R2 results (P <0.0001) indicate that about 90% of the variance in SRL is accounted for by root diameter (n=446).
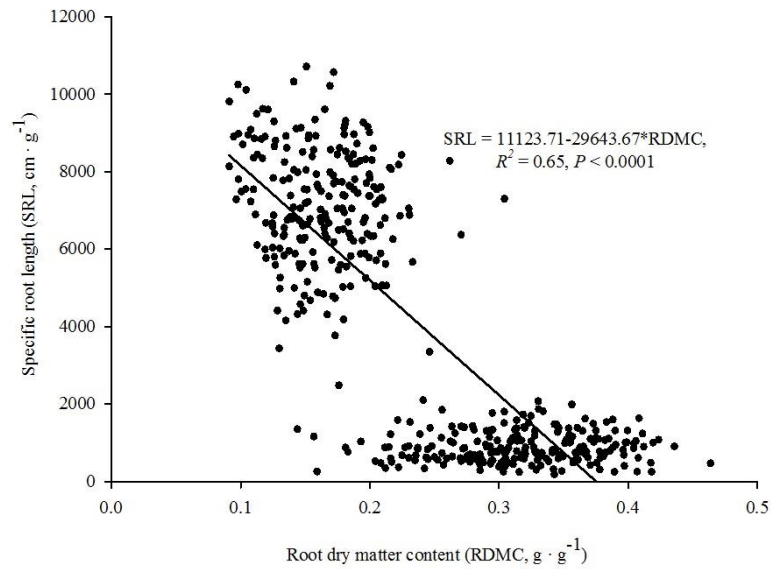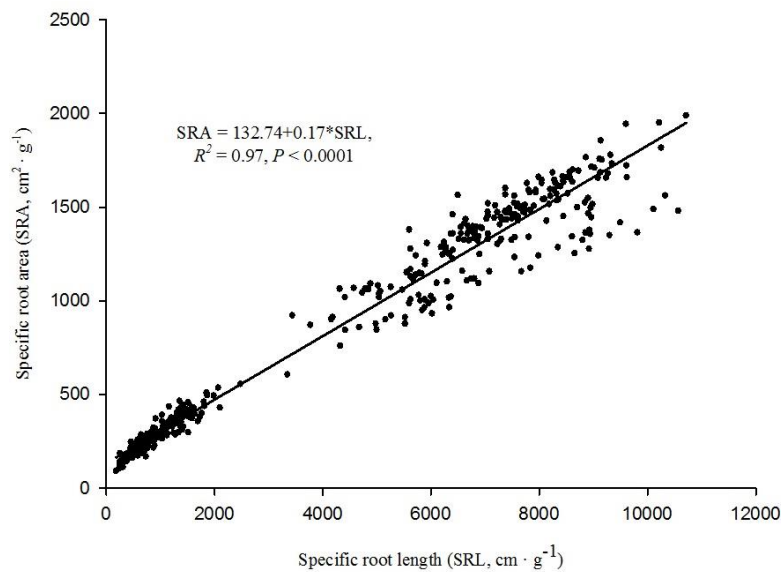

Appendix Figure 2 Specific root length (SRL) was in positive negative relation to root dry matter content (RDMC) of A. platanoides.

The R2 results (P <0.0001) was 0.65 in the relation between SRL and RDMC (n=446).
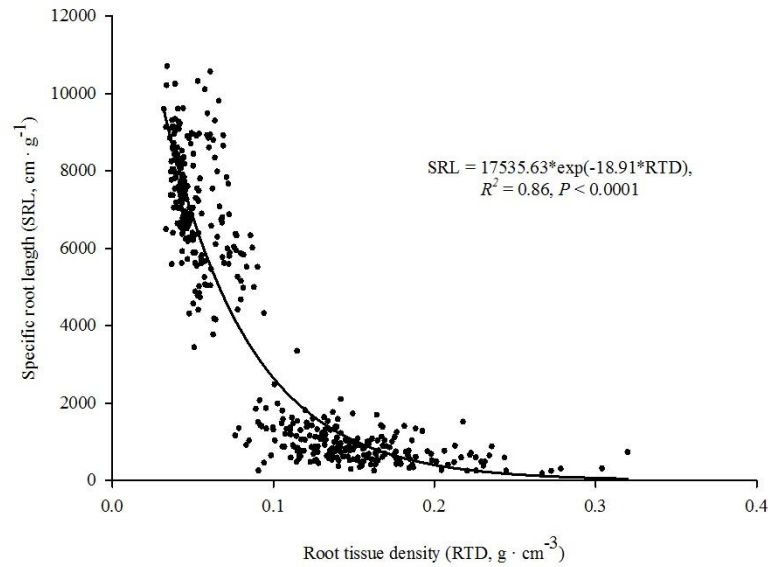
Appendix Figure 3 Specific root length (SRL) was negatively related to Root tissue density (RTD) of A. platanoides.

Exponential curve was fitted to all the data points from both root order classes. The R2 results (P <0.0001) indicate that about 86% of the variance in SRL is accounted for by RTD (n=446).

Appendix Figure 4 Specific root area (SRA) was in positive negative relation to Specific root length (SRL) of A. platanoides.

The R2 results (P <0.0001) was 0.65 in the relation between SRL and RDMC (n=446).

Appendix Table 3 Root respiration rate and morphological traits inter-item correlation matrix on both root order 1+2 and 4 of *A. platanoides*.

The four root traits are fresh weight, dry weight, surface area and volume. (n=446, P < 0.01).

	Respiration rate	Fresh weight	Dry weight	Surface area	Volume
Respiration rate	1.00	0.70	0.28	0.77	0.76
Fresh weight		1.00	0.56	0.64	0.68
Dry weight			1.00	0.04	0.23
Surface area				1.00	0.95
Volume					1.00

Appendix Table 4 RR and morphological traits inter-item correlation on root order 1+2 of *A. platanoides*.

The four root traits are fresh weight, dry weight, surface area and volume. (n = 223, P < 0.01).

	Respiration rate	Fresh weight	Dry weight	Surface area	Volume
Respiration rate	1.00	0.63	0.71	0.66	0.59
Fresh weight		1.00	0.81	0.54	0.42
Dry weight			1.00	0.76	0.68
Surface area				1.00	0.94
Volume					1.00

Appendix Table 5 RR and morphological traits inter-item correlation matrix on root order 4 of *A. platanoides*.

The four root traits are fresh weight, dry weight, surface area and volume. (n = 223, P < 0.01).

|	Respiration rate	Fresh weight	Dry weight	Surface area	Volume
Respiration rate	1.00	0.45	0.47	0.60	0.53
Fresh weight		1.00	0.88	0.49	0.82
Dry weight			1.00	0.57	0.78
Surface area				1.00	0.81
Volume					1.00
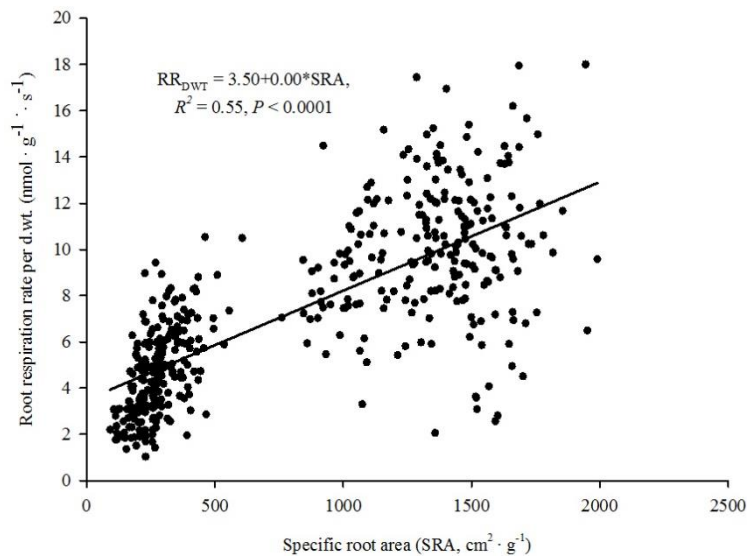
Appendix Figure 5 RR$_{DWT}$ was negatively related specific root area (SRA) in *A. platanoides*.

Exponential curve was fitted to all the data points from both root order classes. The R2 results (P <0.0001) indicate that about 55% of the variance in RR$_{DWT}$ is accounted for SRA (n=446).
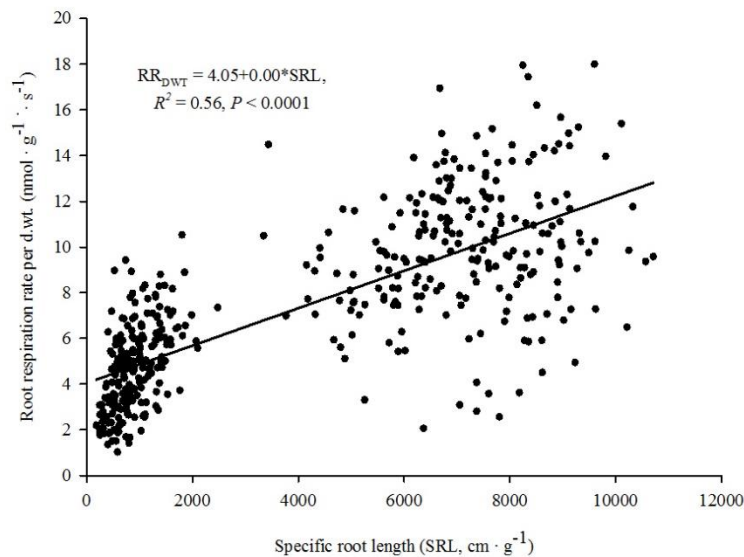

Appendix Figure 6 RR$_{DWT}$ was negatively related specific root length (SRL) in A. platanoides.

Exponential curve was fitted to all the data points from both root order classes. The R2 results (P <0.0001) indicate that about 55% of the variance in RR$_{DWT}$ is accounted for SRL (n=446).
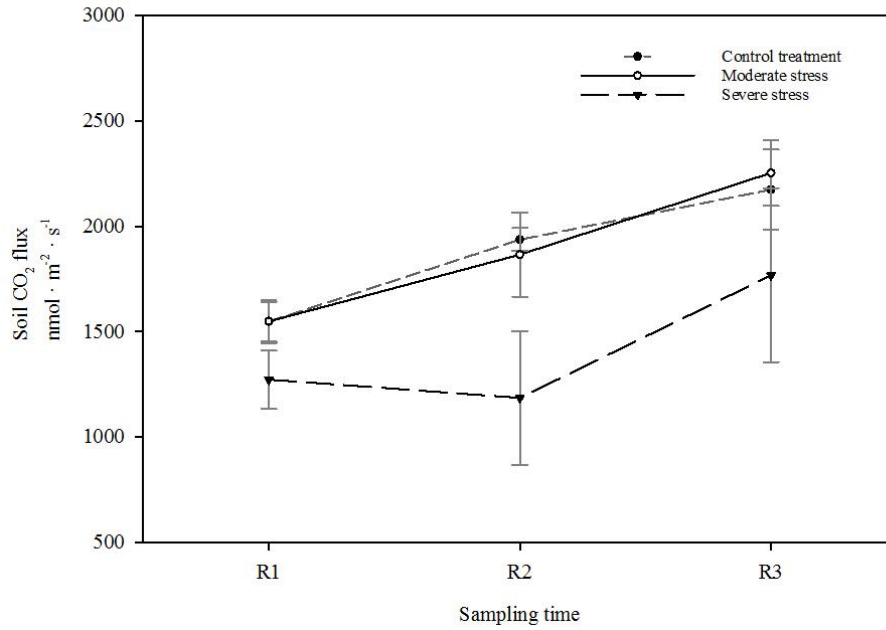
Appendix Figure 7 Soil CO2 flux in Fagus Forest (according to Zimmermann et al, 2015).

R1, R2, R3 are the three sampling period in the drought experiment. The control is shown as filled circles with dashed line. The moderate treatment is shown as empty circles with solid line. The severe treatment is shown as inverted triangles with dashed line.
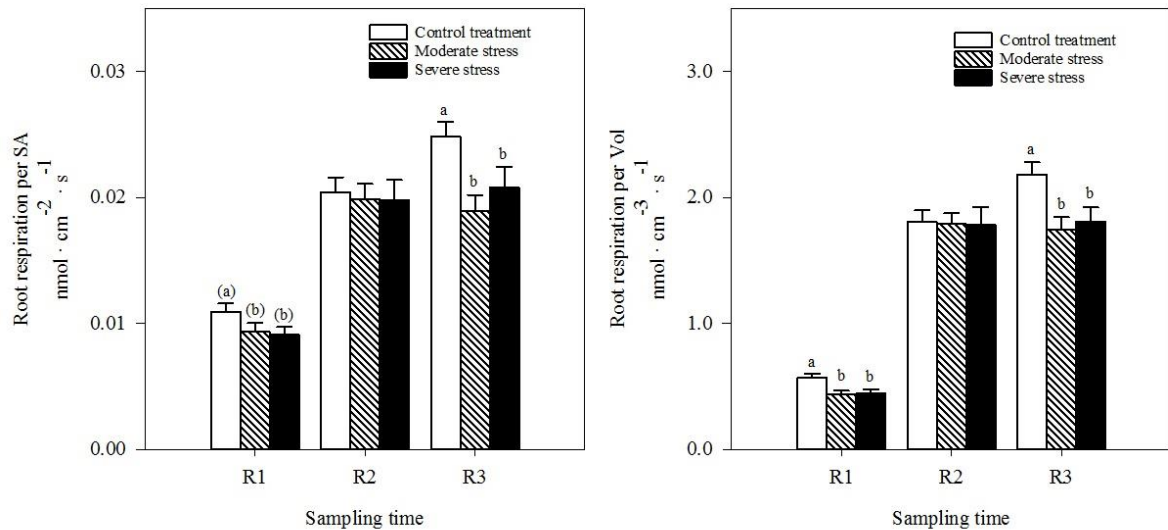
Appendix Figure 8 Root respiration per surface area (SA) and volume (Vol) measured at 20 °C for *Fagus sylvatica*.

R1, R2, R3 are the three sampling period in the drought experiment. Small letters (P < 0.05) and letters in parentheses (P < 0.1) indicate significance of RR differences between drought stress treatments (Mean ± S.E., n = 30-32; LSD multiple comparisons; P < 0.05).
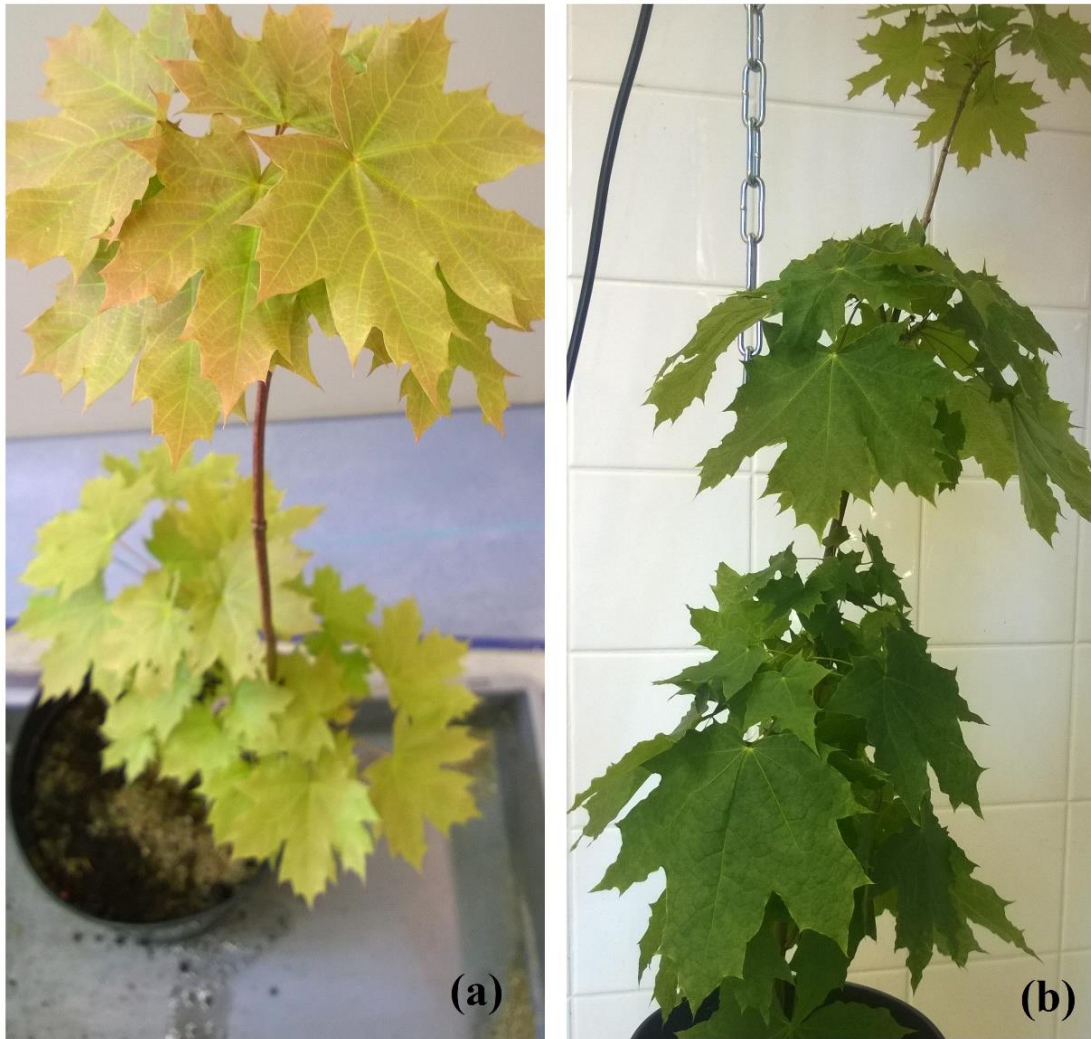
Appendix Figure 9 Seedlings of *Acer platanoides.*

Plant in (a) had yellowish leaves while plant in (b) presented relatively green. This was resulted from irrigation of nitrogen free Hoagland solutions. Acer plant individuals showed different responses to nutrient status.
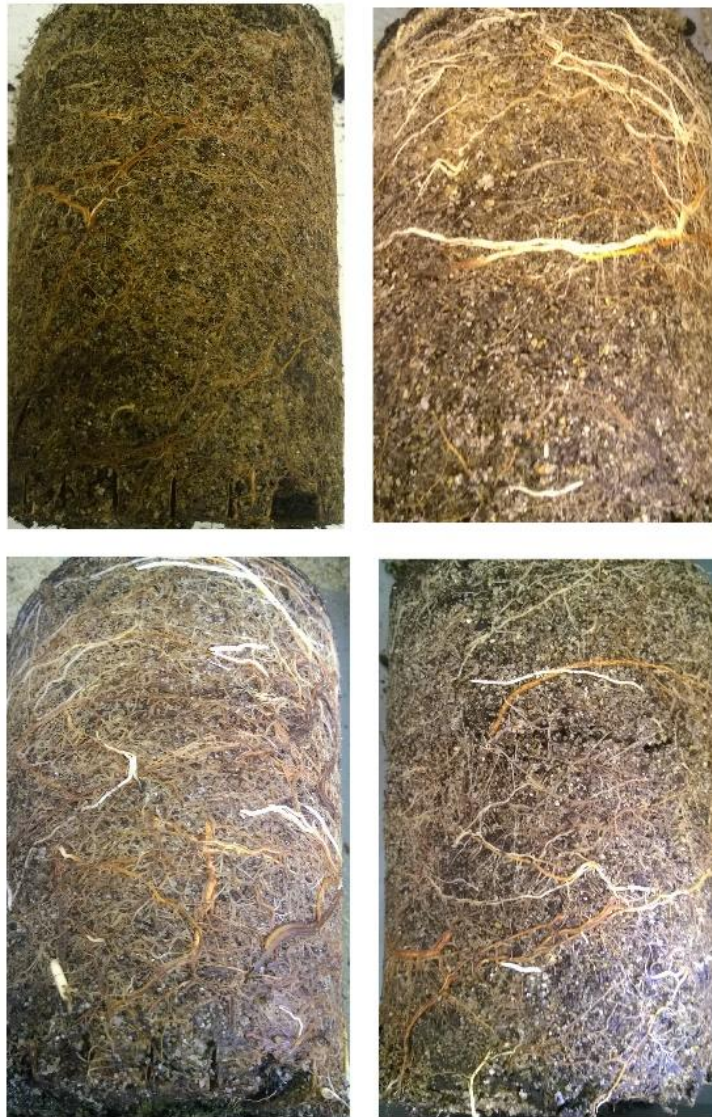
Appendix Figure 10 Examples of recently unpotted root systems of *A. platanoides.*

Appendix Figure 11 Examples of soil cores form *Fagus sylvatica* forest.

Appendix Figure 12 Typical *Fagus* roots from O-horizon and Aeh-horizon respectively in time period R3.

Appendix Figure 13 Root oxygen depletion measurement chamber for *Acer platanoides*.

Appendix Figure 14 Root oxygen depletion measurement chamber for *Fagus sylvatica*.

Appendix Figure 15 Two complete oxygen electrode systems for root reparation measurement on *A. platanoides* in the lab.

Appendix Figure 16 Two complete oxygen electrode systems for root reparation measurement on *F. sylvatica* in the instrument hut *in situ*.