Doctoral Thesis

Fine root growth and vitality of European beech in acid forest soils with a low base saturation

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Fine root growth and vitality of European beech in acid forest soils with a low base saturation

A dissertation submitted to
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Doctor of Sciences

presented by

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>Abs&lt;sub&gt;520&lt;/sub&gt;</td>
<td>absorbance at 520 nm</td>
</tr>
<tr>
<td>AMS</td>
<td>accelerator mass spectrometry</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>BS</td>
<td>base saturation [%]</td>
</tr>
<tr>
<td>BC</td>
<td>basic cations (sum of K&lt;sup&gt;+&lt;/sup&gt;, Mg&lt;sup&gt;2+&lt;/sup&gt;, Ca&lt;sup&gt;2+&lt;/sup&gt;)</td>
</tr>
<tr>
<td>CE</td>
<td>curdlan equivalents</td>
</tr>
<tr>
<td>CEC</td>
<td>cation exchange capacity [mmol·kg&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>DOC</td>
<td>dissolved organic carbon</td>
</tr>
<tr>
<td>DW</td>
<td>dry weight</td>
</tr>
<tr>
<td>EN</td>
<td>Entlebuch</td>
</tr>
<tr>
<td>ETH</td>
<td>Eidgenössische Technische Hochschule</td>
</tr>
<tr>
<td>FM</td>
<td>fresh mass</td>
</tr>
<tr>
<td>n</td>
<td>number (tips, branches)</td>
</tr>
<tr>
<td>NI</td>
<td>Nierdererlinsbach</td>
</tr>
<tr>
<td>NPP</td>
<td>net primary production</td>
</tr>
<tr>
<td>KR</td>
<td>Krauchtal</td>
</tr>
<tr>
<td>PSI</td>
<td>Paul Scherrer Institute</td>
</tr>
<tr>
<td>RBA</td>
<td>root branching abundance [n g&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>RBF</td>
<td>root branching frequency [n cm&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>RGR</td>
<td>relative grow rate [d&lt;sup&gt;-1&lt;/sup&gt;µg&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>RTA</td>
<td>root tip abundance [n g&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>RTD</td>
<td>root tissue density [mg cm&lt;sup&gt;-3&lt;/sup&gt;]</td>
</tr>
<tr>
<td>SE</td>
<td>standard error</td>
</tr>
<tr>
<td>SLA</td>
<td>specific leaf area [cm&lt;sup&gt;2&lt;/sup&gt; mg&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>SOM</td>
<td>soil organic matter</td>
</tr>
<tr>
<td>SRL</td>
<td>specific root length [cm g&lt;sup&gt;-1&lt;/sup&gt;]</td>
</tr>
<tr>
<td>TF</td>
<td>triphenyl formazan</td>
</tr>
<tr>
<td>TTC</td>
<td>triphenyltetrazolium chloride</td>
</tr>
<tr>
<td>VO</td>
<td>Vordemwald</td>
</tr>
<tr>
<td>WA</td>
<td>Walchwil</td>
</tr>
<tr>
<td>WL</td>
<td>Walterswil</td>
</tr>
<tr>
<td>WSL</td>
<td>Swiss Federal Institute for Forest, Snow and Landscape Research</td>
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<tr>
<td>ZO</td>
<td>Zofingen</td>
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Summary
Fine roots are of major importance in below ground nutrient and water acquisition of forest trees. Their dynamic growth, development, and turnover also contribute distinctly to carbon fluxes belowground. It is generally believed that high soil solution Al$^{3+}$ in acid soils with low soil matrix base saturation (BS), negatively influences the growth and vitality of fine roots of forest trees and, therefore, belowground carbon fluxes. In this study, acid forest soils with a low BS (<15%) were chosen to investigate the impact of soil chemical parameters on the fine roots of European beech (*Fagus sylvatica* L.). Fine roots of mature trees as well as roots of young seedlings growing in soil monoliths were analysed on their growth, development, morphology, physiology, and chemistry.

Fine roots of mature trees were sampled down to 1 m depth at seven (2005) and six (2006) forest sites located on the Swiss Plateau. These forests were grouped in sites with a BS < 5% and with a BS 5-10% in the B-horizon. The turnover and estimated age of the fine roots was determined with sequential coring and the age was measured with radiocarbon (only two sites). Seeds of European beech were sown in soil monoliths of four forest sites containing soils with low BS (1.2-6.5%) and different nutrient availability (Ah- and B-horizon) and placed in the greenhouse. Root growth dynamics were also observed in rhizoboxes containing soils from Ah- and B-horizons. From soil monoliths the free Al$^{3+}$ concentration and nutrient availability (basic cations and nitrate) in the soil solution were also analysed. Additionally, the effect of 14 days of drought treatment on the seedlings was examined.

The fine root growth and development was negatively influenced in forest sites with a BS < 5% in the B-horizon as the fine root live/dead ratio was decreased. The turnover of the fine roots examined in this study was not affected due to low pH or BS. But together with published data with a wider scale of soil chemistry (pH 2.9-7.8 and BS 1.9-99.9%) the turnover increased with decreasing pH and slightly with decreasing BS. However, low BS or high Al$^{3+}$ concentration in the soil solution did not impair other fine root or seedling root growth and development properties. A much stronger decrease of the mature fine root biomass, necromass, productivity, mortality, and turnover and increase of the fine root age was due to soil depth. The two methods for fine root age measurement (sequential coring, radiocarbon) were significantly correlated with each other.

The morphology of the fine roots of mature beech trees was negatively affected in the forest sites with a BS < 5%, as the root tip abundance, root branching abundance, and specific root length was decreased. The specific root length of the seedling roots was also lower in the B-horizon where the BS and nutrient availability in the soil solution was lower compared to
the Ah-horizon. In contrast the root branching frequency, length growth, and branches growth of the seedling roots increased in the B-horizons.

The fine root physiology of both, the mature trees and seedlings, was negatively affected in soils with a low BS. The $O_2$-consumption of the fine roots of mature trees was decreased in the forest sites having a BS $<$ 5\% and in seedling roots growing in monoliths having a BS $<$ 3\%. Also the callose concentration in the root apices of the seedling roots was higher in the roots of seedlings growing in monoliths with a BS $<$ 3\%. The Ca/Al molar ratio in the fine root tissue of mature trees was decreased in the BS $<$ 5\% but not in the seedling root tissue. Decreases in the morphological properties and in the $O_2$-consumption in the fine roots of mature trees were related to decreases in the Ca/Al molar ratio of the fine root tissues.

This study shows indications, especially in situ, that the fine root properties are negatively influenced due to low BS and pH. However, there was no strong evidence that beech seedling roots suffered from high concentrations of Al$^{3+}$ in the soil solution. Seedling root properties were more affected due to low nutrient availability in the B-horizon as due to low soil matrix BS or high soil solution Al$^{3+}$ concentration. Also in the drought treatment the decreasing water content most likely affected the seedling root properties more than the soil chemical parameters. The fine root systems of European beech in their natural ecological environment seem to be able to compensate adverse effects of low BS. But it can be hypothesised that further acidification and additional stress, e.g. drought, can potentially decrease the health status of fine roots in future.
Zusammenfassung
Zusammenfassung


In dieser Studie wurden saure Waldböden mit einer geringen BS (<15%) ausgewählt, um den Einfluss von bodenchemischen Parametern auf die Feinwurzeln der Rotbuche (Fagus sylvatica L.) zu ermitteln. Wachstum und Entwicklung, Morphologie, Physiologie und Chemie der Feinwurzeln von Altbäumen sowie Wurzeln von Keimlingen, welche in Bodenmonolithen angezogen wurden, wurden analysiert.


Das Wachstum und die Entwicklung der Feinwurzeln in den Waldbeständen mit einer BS < 5% wurde negativ beeinflusst, indem das Verhältnis der Feinwurzeln lebend/tot verringert war. Die Umsatzrate war weder durch den pH noch die BS beeinflusst. Doch zusammen mit publizierten Studien, welche eine breitere Spanne von bodenchemischen Parametern abdeckten (pH 2.9-7.8 und BS 1.9-99.9%), nahm die Umsatzrate mit abnehmenden pH zu. Ebenfalls war eine geringfügige Zunahme mit der Abnahme der BS zu verzeichnen. Dennoch wurden keine anderen Feinwurzel oder Keimlingswurzel Eigenschaften durch die geringe BS oder die hohe Al\(^{3+}\) Konzentration in der Bodenlösung beeinträchtigt. Eine eher starke Abnahme der Biomasse, Necromasse, Produktivität, Mortalität und Umsatzrate und Zunahme des Alters der Feinwurzeln war auf Grund der zunehmenden Bodentiefe zu sehen. Die zwei
Zusammenfassung

Methoden, um das Alter der Feinwurzeln zu bestimmen (sequential coring, radiocarbon) waren signifikant korreliert.

Die Morphologie der Feinwurzeln der Altbäume war in den Beständen mit einer BS < 5% negativ beeinflusst. So war die Wurzelspitzenhäufigkeit, die Wurzelverzweigungshäufigkeit und die spezifische Wurzellänge geringer, als in den Beständen mit einer BS 5-10%. Die spezifische Wurzellänge der Keimlinge war ebenfalls geringer im B-Horizont, in welchem die BS und Nährstoffversorgung geringer waren im Vergleich zum Ah-Horizont. Demgegenüber waren die Wurzelverzweigungs frequenz, das Längenwachstum und das Verzweigungswachstum der Keimlingswurzeln im B-Horizont höher.

Die Feinwurzelphysiologie der Altbäume und der Keimlinge waren gleichfalls in Böden mit einer geringen BS beeinträchtigt. Der O₂-Verbrauch der Feinwurzeln der Altbäume war geringer in den Beständen mit einer BS < 5% und der Verbrauch der Keimlinge war geringer in den Monolithen mit einer BS < 3%. Ebenso war die Callose Konzentration im Apex der Wurzeln in den Monolithen mit einer BS < 3% erhöht. Das molare Verhältnis von Ca/Al war in den Feinwurzelgeweben der Altbäume geringer bei einer BS < 5% aber nicht in den Wurzel geweben der Keimlinge. Die Abnahme des molaren Verhältnisses von Ca/Al und der O₂-Verbrauch der Feinwurzeln der Altbäume waren miteinander korreliert.

General introduction
Anthropogenic impact to forest ecosystems, such as acidic depositions and nutrient removal due to forest harvesting, may affect the forests’ natural environment and its health. Especially the health of the fine root system may be hampered. In this study the health status of fine roots in naturally grown European beech (*Fagus sylvatica* L.) forest stands and beech seedling roots growing in acid soil with a low base saturation were examined.

**Fine roots**

Fine root systems in forest ecosystems are the most important structural and functional components belowground (Grier et al., 1981; Kolek and Kozinka, 1992; Waisel et al., 1991). They cover a major function in belowground carbon and nutrient cycle as they contribute a large proportion of root biomass production (Hendrick and Pregitzer, 1993). Fine roots represent up to 50% of the global total belowground net primary production (Nadelhoffer and Raich, 1992) and fine root production has been estimated to account as much as 33% on the scale of the global annual net primary production (Jackson et al., 1997). Thus, fine roots have an important implication for plant growth.

Root systems of forest trees consist of coarse roots (> 2 mm in diameter) and fine roots (usually < 2 mm in diameter; Böhm, 1979). The fine roots exhibit a major part of the surface area of the tree root system and are most important for water and nutrient acquisition (Pauliz, 2002). For example Hendrick and Pregitzer (1993) showed that roots of sugar maple with a diameter of < 0.5 mm are the physiologically most active part of the root system, responsible for water and nutrient uptake. Also Atkinson (2000) reported that root length is a direct indicator of the potential for nutrient and water uptake.

A vital fine root system is of primary importance for maintaining the health of forest ecosystems (Bakker, 1999). Aboveground growth is dependent on the health of the root system. A reduced root system leads to a chronic water stress and reduced nutrient availability for stem, branches and leaves (Ulrich, 1982). Nevertheless, above ground stress symptoms are often not easy to identify. Fine roots instead could be used as sensitive indicators of stress caused by environmental changes. Several reviews focused on tree roots as indicators of environmental changes in European and North American countries (Vogt et al., 1993; Cronan and Grigal, 1995; Bakker, 1999; Puhe, 2003). These studies revealed that the monitoring of the root parameters could be useful to detect the responses to a variety of stresses on forest ecosystems (Vogt et al., 1993; Bakker, 1999; Puhe, 2003). Studying fine roots may allow
detecting changes in the environment prior to the appearance of visible aboveground symptoms (Vogt et al., 1993). The following characteristics of fine roots are helpful for this purpose: (1) fine roots are typically directly in contact with the soil and will be the first plant organ reacting to chemical or physical changes of the soil, (2) they have evolved to buffer changes in the soil so that these changes are not reflected in the aboveground parts (e.g. accumulation of toxic metals such as Al, Ni, Cu, and Zn in the cell-walls), (3) changes in the fine roots’ associated microbial communities could reflect stress, (4) fine roots have a relatively short life span, can respond rapidly to stress and, therefore, reflect the current state of the environment, and (5) they are early influenced due to changes in the carbon allocation within plants (Vogt et al., 1993).

Several kinds of stress could affect the fine roots: e.g. soil acidity, drought, water logging. However, to use fine roots as stress indicators, methods to assess their health status have to be developed, and the reaction of fine roots to stress must be understood.

**Acidification of forest soils as stress to fine roots**

A major stress to fine roots is soil acidification (Matzner and Murach, 1995). The natural weathering process of soils, which is a process lasting over thousands of years, is one process leading to acidic soils. The acidity of any soil varies according to the type of bedrock, the length of the weathering time, and the local climate. Hence, some soils are naturally acidic while others are more alkaline. Plants and microorganisms also contribute to soil acidification as the CO\(_2\) produced by respiration leads to accumulation of carbonic acid in the soil solution. Additionally, plant roots exude organic acids and H\(^+\) ions to assure the uptake of nutrients (e.g. Fe, Zn, P, and NH\(_4^+\)) or to detoxify toxic elements (e.g. Al) by chelation (Marschner, 1998; Heim et al., 2001; Jones et al., 2004). However, anthropogenic impact such as acidic deposition and nutrient removal can accelerate the rate of acidification. The atmospheric reactions of NO\(_x\) and SO\(_2\) with water and oxygen lead to nitric and sulphuric acid formation and to an increased inflow of anions and protons into soils. Additionally, the deposition of NH\(_4^+\) from agriculture enhanced this effect. “Soil acidification” is a general term that is used to cover these harmful chemical changes in soils due to acidic deposition. The importance of acidification effects has lead to the definition of critical loads for acid deposition (UNECE, 1993). A critical load is a quantitative estimate of the exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do
not occur (UNECE, 1993). In Europe through the 1980s and 1990s the emissions of NO\textsubscript{x} and SO\textsubscript{2} were reduced due to air pollution abatements. Especially SO\textsubscript{2} emissions have levelled off and decreased in most European countries. Although, the rates of acidic deposition declined, they are still today on a high level (Ferrier et al., 2001; Erisman et al., 2003). Nitrogen depositions are only slowly declining (Grennfelt et al., 2001) and will continue to cause a problem in future. In Switzerland, for example, N deposition (including NO\textsubscript{x} and NH\textsubscript{4}\textsuperscript{+}) from road traffic, agriculture, industry, and heating systems, ranges from 4.5 kgN ha\textsuperscript{-1} y\textsuperscript{-1} (above 1800 m a.s.l.) to 29 kgN ha\textsuperscript{-1} y\textsuperscript{-1} (Southern Switzerland), which is for some measured values above the range of the empirical critical load (Thimonier et al., 2005) which was set by Bobbink et al. (1996) for deciduous forests at 15-20 kgN ha\textsuperscript{-1} y\textsuperscript{-1}. However, Falkengren-Grerup and Diekmann (2003) proposed lower values of N deposition above 7-10 kgN ha\textsuperscript{-1} y\textsuperscript{-1} to be critical and harmful for plant growth. Therefore, it is not astonishing that several field studies have shown that soil acidification is still progressing (Alewell et al., 2000; Moffat et al., 2002). Also Waldner et al. (2007) recently reported in the framework of the Swiss Long-Term Forest Ecosystem Research (LWF) that on several sites the loads of acidity and nitrogen still exceed the critical values and, therefore, represent a long-term ecological risk. This was obvious e.g. in the forest site Vordemwald (one site included in this present study) where the loads of acidity (2 keq ha\textsuperscript{-1} y\textsuperscript{-1}) are in the critical range and the loads of nitrogen (17 kgN ha\textsuperscript{-1} y\textsuperscript{-1}) exceeds the critical values by about 25%.

The high amount of H\textsuperscript{+} ions negatively influences plant growth, e.g. low biomass increment of beech seedlings with brittle and few fine roots reported by Ljungström et al. (1993). Due to the increased H\textsuperscript{+} concentration on the soil exchanger with decreasing pH an increased leaching of basic cations (BC = K\textsuperscript{+}, Mg\textsuperscript{2+}, Ca\textsuperscript{2+}) occurs. A pH < 5 is also accompanied with an increased release of Al from soil minerals. Additionally, Al competes with essential BC on the soil exchanger leading to a larger fraction of exchange sites being occupied by Al at the expense of sites occupied by Ca and Mg and, therefore, to an increased BC leaching and a decreased base saturation (BS; % of Ca, K, Na, Mg of the cation exchange capacity) in the soil matrix (Jönsson et al., 2003; Walthert et al., 2004). De Vries et al. (2003) showed that nitrogen depositions of 13 to 25 kgN ha\textsuperscript{-1} y\textsuperscript{-1} lead to increasing soil acidity and decreasing BC pool in the soil. Falkengren-Grerup and Eriksson (1990) reported that many forest soils have become increasingly acidified and have, therefore, high levels of Al in the soil solution. This two processes result in bases poor soils with high content of free Al species in the soil solution. Al in the soil solution plays a key roll for plant health in acidified soils, as it has a toxic action to roots.
Al is the third most abundant element in the Earth’s crust (8.1%). In living tissues under normal conditions Al is found only in low concentrations. In soils at neutral and weakly acid soil conditions, Al occurs as insoluble and harmless aluminosilicates or oxides in the mineral matrix of the soil. At a pH > 5 mononuclear hydrolysis products are formed (Kinraide, 1991; Marschner, 1995). But, in soils with pH < 5, free Al is occurring. Al is released from soil minerals to the soil solutions and mainly occurring as Al(H$_2$O)$_6^{3+}$ and AlOH$_2^{2+}$ (in the following designated as Al$_{3+}$; Marschner, 1995; Matsumoto, 2000). Large amounts of free Al$_{3+}$ in soil solutions generally acts highly phytotoxic to plant roots (Matsumoto, 2000; Rengel and Zhang, 2003; Kochian et al., 2005). Nevertheless, in humus rich soil horizons, most of the free Al$_{3+}$ is bound to the dissolved organic carbon (DOC) and is no longer phytotoxic (Jönsson et al., 2003; Parker, 2005; Lange et al., 2006). Other notable organic ligands for Al are products of plant and microbial metabolism (organic acid anions such as citrate, oxalate, malate, succinate, propionate) and to a lesser extent phenolic groups (such as phenolic acids such as salicylic acid, caffeic acid, gallic acid; Parker, 2005). Thus Al$_{3+}$ toxicity seems to be a major problem of acidic mineral soils, which naturally have low DOC concentration in the soil solution.

From greenhouse experiments it is well known that the health of fine roots is negatively influenced by high Al$_{3+}$ concentrations in the treatment solutions (Brunner, 2001; van Schöll et al., 2004; Yamamoto et al., 2002; Zyssset et al., 1996). Al$_{3+}$ inhibits root-cell elongation and division in the root tips. Al$_{3+}$ associates with cell-wall and cell components such as pectins and polynucleotides, leading to structural alteration and causing subsequently to plant growth decline (Matsumoto, 2000; Kochian et al., 2005). These damages result in a change in the fine root morphology with the formation of short and stubby roots, dieback of root tips, decline in root elongation, and cessation of the formation of lateral roots (Göransson and Eldhuset, 1991; Hirano and Hijii, 2000). As a competition effect Al$_{3+}$ inhibits the nutrient absorption on the cell surface in the fine roots as the cell-wall and plasma membrane have a up to 700 times higher affinity for Al than for Ca (Kinraide, 2003). This mainly leads to deficiencies of essential BC such as Ca and Mg and elevates the content of Al in the fine root tissue (Göransson and Eldhuset, 1995; Zyssset et al., 1996). As a result in acidic soils plant available Al$_{3+}$ in the soil solution probably become a big threat for the health of fine roots of forest trees. Overall, the result of the toxicity of Al$_{3+}$ is a reduced and damaged root system. This leads also to a limited water and mineral nutrient uptake (Matsumoto, 2000).
Methods to measure the condition of fine roots

Several methods exist to measure the condition of fine roots. Root properties such as the fine root mass, turnover, morphology, physiology, and tissue chemistry are often used to evaluate the health status of fine roots.

One approach is to analyse the fine roots’ growth and development. It is well established that the biomass, necromass, productivity, mortality, and turnover of the fine root systems of forest trees is affected by environmental factors. For example several studies showed that forests growing on acidic and nutrient poor sites have a higher fine root turnover, density and mass of both, bio- and necromass, compared to forests growing on less acidic, nutrient rich sites (Godbold et al., 2003; Jentschke et al., 2001; Vanguelova et al., 2005). Especially, Finer et al. (2007) and Leuschner and Hertel (2003) showed that beech have a higher biomass on nutrient poor sites. This was considered to be due to the lack of nutrients. Also a higher necromass is observed on acidic and nutrient poor sites. This probably shows that fine root systems on these sites have a higher fine root mortality and probably also a higher fine root turnover rate due to the adverse soil chemical conditions (Leuschner et al., 2004). In addition, fine roots from soils with a low BS may have a decreased longevity due to nutrient deficiencies (low BS) as observed by Vanguelova et al. (2005) in a Scots pine stand. Nevertheless, the longevity is not only decreased due to nutrient deficiencies, but also, as reported by Gaudinski et al. (2001), dependent on the depth in the soil profile. It was shown that the age of fine roots measured by radiocarbon increased with increasing depth.

A second approach is to study the fine root’s morphology (Clemensson-Lindell and Persson, 1995; Godbold et al., 2003). The morphology is an indirect measurement of the health status of fine roots. Morphological changes reflect more the physiological or chemical responses, as they are a direct effect of the environment. Some morphological parameters, e.g. specific root length (SRL [cm g\(^{-1}\) \(D W\)]) and root tip frequency (RTF [tips g\(^{-1}\) \(D W\)]), are used to evaluate the reactions of fine roots to different soil conditions and different nutrient supplies (Ostonen et al., 1999; Hodge, 2004). For example Ostonen et al. (2007) reported that the SRL of fine roots with a diameter < 0.5 mm was decreased under fertilization and Al-stress. Different morphological patterns of fine roots reflect the ability of the trees for resource acquisition (“benefit”) relative to the construction and maintenance of root tissue (“cost”) (Fitter, 1991; Espeleta and Donovan, 2002). The morphological plasticity can be considered as the root functional status to provide a rapid acquisition of nutrients and water (Ostonen et al., 1999; Paulitz, 2002). Therefore, the whole root system can be influenced by the chemical
General introduction

status of the soil. For example seedlings invest more in building a larger root biomass in nutrient poor soils where a larger root system provides a better nutrient maintenance of the roots (Coleman and McConnaughay, 1995; Espeleta and Donovan, 2002).

A third approach is to assess the fine roots’ physiological and root tissue chemical responses on environmental factors, as this is one direct approach towards the fine root health status. Commonly used physiological tests for the status of fine roots is the determination of the fine roots’ respiratory activity (Comas et al., 2000) and callose formation (Hirano et al., 2004). Two methods exist to measure the respiratory activity: one method is the TTC-test. Colourless triphenyltetrazolium chloride (TTC) accepts electrons from the electron transport chain in the mitochondria and is reduced to the red-coloured triphenyl formazan (TF; Clemensson-Lindell, 1994; Comas et al., 2000; Ruf and Brunner, 2003). The second method directly measures the \( \text{O}_2 \)-consumption of the root with a Clark type electrode (Comas et al., 2000). The formation of callose in root cells in the apices of fine roots, which are exposed to high \( \text{Al}^{3+} \) concentrations, is a third physiological method (Hirano et al., 2006). Callose is a linear 1,3-\( \beta \)-glucan with some 1,6-branches (Kauss, 1989; Verma and Hong, 2001). Callose is formed in response to wounding, pathogen infection or physiological stress (Kauss, 1989, 1996) and acts as a wound occlusion or protection agent in sieve pores. In agricultural crops, callose formation in roots in response to Al toxicity has been demonstrated and suggested as a physiological indicator of Al toxicity (Wissemeier et al., 1987; Zhang et al., 1994; Rengel and Zhang, 2003). The callose synthesis is a reaction on the intracellular Al induced increase of \( \text{Ca}^{2+} \) and, as callose is mainly found at the plasmodesmata, callose may inhibit the symplastic intercellular transport (Sivaguru et al., 2000). One method to measure callose is the fluorescence intensity of callose, after binding to aniline blue (Hirano et al., 2006).

The \( \text{Ca}/\text{Al} \) molar ratio in the fine root tissue is a chemical response on the chemical status of the soil and soil solution. Sverdrup and Warfinge (1993) and Cronan and Grigal (1995) proposed the \( \text{Ca} \) to \( \text{Al} \) molar ratio of the soil solution as one ecological indicator of thresholds below that the risk of forest damage due to Al toxicity and nutrient imbalance increases. The \( \text{Ca}/\text{Al} \) molar ratio in the soil solution below 1.0 nearly implies a 50% risk of adverse impact on tree growth (Cronan and Grigal, 1995). Ca is known to ameliorate Al-induced rhizotoxicity and mitigate other adverse effects of Al described above (Cronan and Grigal, 1995; Rengel and Zhang, 2003). The root tissue \( \text{Ca}/\text{Al} \) molar ratio also gives a direct indication on soil solution \( \text{Al}^{3+} \) impact on BC uptake and Al accumulation in fine roots (Nygaard and de Wit, 2004; Vanguelova et al., 2005). Vanguelova et al. (2007) reported in a review that fine root \( \text{Ca}/\text{Al} \) molar ratio was strongly negatively related to Al stress in fine
General introduction

roots.

However, the difficulties associated with studying fine roots have resulted in a rareness of data for plant roots. Up to now reliable methods to measure the fine root condition of adult trees have been inadequately investigated.

Background of the study

An increasing soil acidification leads to an altered soil chemical status. This alteration has ecological consequences for forest ecosystems which could be seen e.g. in the enhanced uprooting of forest trees and in a decreased rejuvenation of forest seedlings. For example after the storm “Lothar” in 1999 Mayer et al. (2005) reported that uprooting was more frequent on acidic soils and Braun et al. (2003) observed a higher uprooting rate on soils with a low BS. The uprooting of the trees seems to be attributed to the fact that tree roots in acidified soils are more concentrated to the upper soil layers (Godbold et al., 2003; Jentschke et al., 2001). Additionally the coarse roots in deeper layers have only few fine roots and also might be reduced in diameter. As a consequence these trees lose more easily their anchorage in the soil (Jentschke et al., 2001). Already several years before, Ljungström and Stjernquist (1993) observed a decreased rejuvenation or seedling growth of forest trees on strongly acidified soils due to a reduction of the root system. Thornton et al. (1989) and van Praag et al. (1991) described a concentration of \( \text{Al}^{3+} > 500 \, \mu\text{mol l}^{-1} \) as critical due to Al toxicity to beech seedlings. Rost-Siebert (1983) and Thornton et al. (1989) observed in hydroponics and Neitzke and Runge (1985) in acidic soils root damage of beech seedlings due to high \( \text{Al}^{3+} \) and low Ca/Al molar ratio. All these studies demonstrate that beech fine roots and seedling roots potentially are negatively affected by soil acidification and high \( \text{Al}^{3+} \) concentration in the soil solution.

Objectives, hypothesis, and structure of the thesis

In this thesis the main objective was to analyse the consequences of acidic soils with a low BS on fine roots and seedlings of European beech. One aim was also to establish methods for fine root health examinations \textit{in situ} and to link root biochemical and physiological properties with
soil chemical parameters of acidified soils. A special attention was given to the impact of Al\(^{3+}\) toxicity on root health status.

This study mainly deals with fine roots of European beech *Fagus sylvatica* (L.) as beech is one of the most important tree species in the submontane and montane zone in northern Switzerland. According to Leuschner et al. (2006), the growth of European beech can be limited below BS 3.3% (data from organic and the first 20 cm of the mineral horizon). The forest sites were chosen on the bases of the WSL soil data-base (Swiss Federal Institute for Forest, Snow and Landscape Research; see Chapter II Table 3.1). The forest sites of the investigation areas are located on the Swiss plateau (Figure 1.1).

![Figure 1.1: Location of the forest sites on the Swiss Plateau investigated in this study.](image)

The soils of these sites were all extremely acidic (pH 2.4-4.7), and were deeply decalcified soils with a lime threshold in the soil profile deeper than 1.50 m depth. They were classified as acidic brown soils, had weak compaction in the deeper mineral layers. Only some profiles show a few characteristics of water logging in the lower profile, but none were classified as extremely water logged. The sites showed all critical values for stress on the trees, which was expressed by a BS < 15% (0.9%-15.0%; Cronan and Grigal, 1995). According to Graf Pannatier et al. (2004), there is a trend for Al\(^{3+}\) concentration, beside of the pH dependency, to increase in mineral soils with decreasing BS. For example, at the forest site Vordemwald, the mean values of Al\(_{\text{tot}}\) decreased from 23.6 µmol l\(^{-1}\) at a depth of 10-20 cm, 18.9 µmol l\(^{-1}\) at 40-60 cm, and 3.8 µmol l\(^{-1}\) at 60-80 cm as the BS increased from 3.1, 3.8, to 5.3%, respectively (Graf Pannatier et al., 2004). About one third of Al\(_{\text{tot}}\) is phytotoxic Al\(^{3+}\) (Graf Pannatier et al., 2004). Five of the nine forest sites belong to the alliance *Luzulo-Fagion* (KR, NI, TR, WL,
ZO), two sites belong to the alliance Abietion where European beech is secondary among Silver fir (Abies alba Mill.; EN, VO), and two sites belong also to the alliance Abietion but European beech is absent (ME, WA), although some adult beech trees occur in neighbouring forest stands (Ellenberg and Klötzli, 1972).

The study was divided into two main experimental parts: One part monitored fine roots of adult beech trees growing in highly acidified forest soils with low BS and the second part was conducted in the greenhouse and analysed the growth and development of beech seedlings in natural soil monoliths from the same forest sites and in rhizoboxes containing the same forest soils.

The main research hypotheses addressed in this work are:

- European beech fine root and seedling root properties (growth, development, morphology, physiology, and chemistry) are negatively affected due to low BS and high Al$^{3+}$ concentration in the soil solution in acidic soils.
- Low BS in the soil matrix and high Al$^{3+}$ concentration in the soil solution are the most important drivers affecting fine root and seedling root health of European beech in acidic soils.
- Acidity and low BS affects the belowground carbon cycle related to fine roots.

The thesis is divided in four main experimental sections approaching further research hypothesis and questions (Chapter I – IV).

The first chapter is an investigation of two methods that could be used for determine the health condition (vitality) of fine roots. The chapter mainly deals with the method that uses triphenyltetrazolium chloride (TTC) to measure the dehydrogenase activity of the mitochondrial respiratory chain. The other method determines the O$_2$-consumption of the fine roots using a Clark-type O$_2$ electrode. The major aim of this study was to investigate which substances in fine roots of adult trees interfere with the TTC and, therefore, hampers correct measurements, and if the reduction of TTC correlates with the O$_2$-consumption measured with the Clark-type O$_2$ electrode.

The second chapter presents the results of a field experiment on the comparison of European beech fine roots of seven forest sites growing in soils with low BS. The sites were grouped into two BS-groups of < 5% and 5-10% in the mineral soil layers. This study was conducted under the hypothesis that low BS, as a predictor for Al$^{3+}$ toxicity, can negatively influence the amount of bio- and necromass, the morphology, the physiology, and the
chemistry of the fine roots. The fine root properties in both BS-groups were compared to each other.

In the third chapter the field experiment was expanded for an additional year. The bio- and necromass, productivity, mortality, and turnover rate of the fine roots of six European beech forests was analysed. Additionally, the age of the fine roots was measured in two exemplary sites with radiocarbon. The main hypothesis of this study was that these root properties were affected by soil acidity and/or low BS. To approach this hypothesis these fine root properties were compared to several other studies on the turnover of European beech in middle Europe.

The fourth chapter presents the results of an experiment carried out in a greenhouse, where European beech seedlings were sown in soil monoliths taken from forest sites with low and extremely low BS. The seedling growth, root system development, root physiology, and root chemistry were related to soil solution chemistry. The main objective of this study was to evaluate if in soils, where the BS is very low and Al$^{3+}$ soil solution content possibly is very high, seedling root morphology and physiology and the growth of the root and shoot are inhibited or limited.
Chapter I
Polyphenols in the woody roots of Norway spruce and European beech reduce TTC

Chapter I

Summary

One common method to determine the vitality of fine root tissue is the measurement of respiratory activity using triphenyltetrazolium chloride (TTC). The colourless TTC is reduced to the red-coloured triphenyl formazan (TF) as a result of the dehydrogenase activity of the mitochondrial respiratory chain. However, measurements with woody fine roots of adult Norway spruce and European beech trees showed that dead control roots still had a high potential to react with the TTC. Such a high reactivity was found in boiled fine roots and the bark of coarse roots, but not in the boiled wood of coarse roots. By sequential extraction of dried and ground adult Norway spruce fine roots the reactivity with TTC was reduced by about 75% (water), 93% (water/methanol) and 94% (water/acetone). The water extract reacted with TTC similarly to polyphenols like lignin, catechin, and epicatechin. Boiling did not affect the extent to which fine roots of adult trees reduced TTC, whereas it greatly reduced TTC reduction by seedling roots. Application of the TTC test to roots of spruce seedlings subjected to increasing drought showed a progressive decrease in TTC reduction. The decrease in TTC reduction was paralleled by a reduction in O$_2$ consumption, thus supporting the conclusion that for roots with a low polyphenol content the TTC test provides a valid assessment of tissue vitality. Our results suggest, however, that the TTC-test should not be applied to the fine roots of adult trees because of their high content of polyphenolic compounds whose reaction with TTC masks changes in TTC reduction due to changes in the respiratory capacity of the tissue.

Introduction

The vitality of fine roots is commonly taken to reflect the effects of such factors as frost, drought or soil acidity on forest tree health (Clemensson-Lindell and Persson, 1995; Bakker, 1999; Stattin and Lindström, 1999; Zhu et al., 2002; Vanguelova et al., 2005). For example, acidic deposition has caused acidification of many forest soils (Blaser et al., 1999; Graf Pannatier et al., 2005), which may have affected fine root activity, turnovers (Godbold et al., 2003; Vanguelova et al., 2005) and growth (Jentschke et al., 2001). However, methods for assessing the physiological state of the fine roots of forest trees have been little investigated.

One way of assessing the condition of fine roots is by their colour and brittleness (Helmisaari and Hallbäcken, 1999; Comas et al., 2000). However, this method allows only
the distinction between living and dead fine roots. Another approach is to measure root morphological parameters, such as specific root length (cm g\(^{-1}\) DW) and specific root tip density (root tips g\(^{-1}\) DW) (Clemensson-Lindell and Persson, 1995; Godbold et al., 2003). These parameters are often used to evaluate the reactions of fine roots to different soil conditions or nutrient supplies (Hodge, 2004), but do not reflect physiological vitality.

A commonly used physiological measure of tissue vitality is the triphenyltetrazolium chloride (TTC) test. Colourless TTC accepts electrons from the electron transport chain of mitochondria reducing it to the red-coloured triphenyl formazan (TF) (Clemensson-Lindell, 1994; Comas et al., 2000; Ruf and Brunner, 2003). Under favourable conditions, the reaction is directly linked to the rate of respiration. However, our tests with boiled (dead) tree roots revealed that not only the respiratory chain, but also some reactive substances within the root tissues, can reduce TTC. These substances prevent accurate measurement of respiratory activity by the TTC reaction. The aim of the present study was to investigate: (1) in which part of the woody tree roots TTC-reactive substances occur; (2) which substances in the fine roots of the two main tree species of the Swiss Plateau, Norway spruce (Picea abies (L.) Karst.) and European beech (Fagus sylvatica L.), react with TTC; (3) whether the fine roots of adult trees react to TTC in the same way as roots of tree seedlings; and (4) whether the TTC test correlates with O\(_2\) consumption.

**Material and methods**

*Fine root sampling and preparation*

Roots of Norway spruce and European beech were sampled at a forest site at Vordemwald (634/236), Switzerland (for details, see Brunner et al. 2004). Soil cores including fine roots were taken with a soil corer (5 cm diameter) in the Ah-horizon (0-5 cm) 1 m away from the tree stems. The samples were immediately wrapped in plastic bags to prevent water loss and stored at 1 °C in the laboratory for no longer than 1 week.

The soil cores were sieved and the roots collected and gently washed with tap water. The roots were assayed visually. Roots with flexible cell walls, a white stele and turgid and unbroken root tips were classified as alive (Hertel and Leuschner, 2002). Living roots were divided into fine roots (< 2 cm) and coarse roots (2-5 cm) and stored in water at 0 °C for no longer than 1 h.
Living fine roots were cut into segments 1-2 mm long, mixed and divided into three parts. One part was immediately used for the TTC test (see below). The other parts served as controls. For one control, 100 mg fresh mass was boiled in 150 µl of distilled water for 20 min in 2-ml Eppendorf tubes. For the other control, the roots were dried for 48 h at 60 °C and ground for 5 min at 90% speed in a swing mill (MM 2000; Retsch, Haan, Germany).

For tissue-specific measurements, the bark and wood of the coarse roots were separated and cut into segments 1-2 mm long. These tissues were also divided into three parts and treated in the same way as the three fractions of fine roots.

**TTC-test**

To prepare the samples, 100 mg fresh tissue, 100 mg fresh tissue boiled, and 23 mg dried and ground tissue (the dry mass of 100 mg fresh fine roots) was transferred into 2-ml Eppendorf tubes containing 1.5 ml of TTC buffer (0.1 M potassium phosphate buffer (pH 7.0) with 0.6% TTC and 0.05% Tween 20; according to Ruf and Brunner (2003)). The samples were placed under reduced pressure (300 hPa) for 15 min to remove air from the intercellular spaces and support the infiltration of the TTC buffer. The samples were incubated for 20 h at 30 °C in darkness. During incubation, TTC was reduced to TF. After incubation, the TTC-buffer was decanted and the root pieces were washed twice with 2 ml distilled water. Two steel balls (2 mm diameter) were added to each sample and the root pieces were milled for 5 min at 90% speed in the swing mill. In the case of the dried samples, the TTC buffer was decanted after centrifugation for 15 min at 10,000 g.

To extract the alcohol-soluble TF from each sample, 1 ml of 96% alcohol was added and the samples were vortexed for 10 s. The samples were centrifuged for 2 min at 10,000 g and the absorption of 300 µl alcohol containing dissolved TF was measured photometrically at 520 nm with a microplate reader (VERSA max; Molecular Devices). Samples were dried for 48 h at 60 °C and weighed. The reactivity of the samples with TTC was measured as absorption of TF per g dry mass [A$_{520}$ g$^{-1}$ DM].

**Extractions of fine roots and the bark of coarse roots**

Roots were extracted to investigate the group of substances that react with TTC in the control samples. Samples of 5 g of dried and ground Norway spruce and European beech fine roots and coarse root bark were extracted for 2 h under continuous shaking with 160 ml of a 1:1 (v/v) water/methanol mixture (Matthews et al., 1997). The extraction residue was filtered from the root powder (filter Nr. 589², Schleicher and Schuell AG, Feldmeilen) under reduced...
pressure (300 hPa). The extraction residue was dried for 48 h at 60 °C, and 23 mg of each residue were tested for their reactivity with TTC (see above).

**Sequential extraction of the fine roots**

Fine roots of adult Norway spruce were sequentially extracted as described by Peng et al. (1991) and Matthews et al. (1997). First, 6 g of dried and ground fine roots was extracted with 240 ml distilled water, filtered and dried. The extract was lyophilised and stored at 1 °C. Second, 4 g aliquots of the water extraction residue were further extracted with 160 ml of a water/methanol mixture (1:1; v/v), filtered and dried. Finally, 3 g of the water/methanol extraction residue were extracted with 120 ml water/acetone mixture (3:7), filtered and dried. The dried fine root material and the extraction residues (each 23 mg) were tested for reactivity with TTC.

**Reactivity of extract and cell components**

To compare the reactivity of the lyophilised water extract with the fine root powder of Norway spruce and other common cell constituents of roots, samples were tested for their reactivity with TTC. Other constituents included: cellulose (for column chromatography; Macherey, Nagel and Co., Düren, Germany), pectin (from apples; Fluka, Buchs, Switzerland), lignin (from fir wood (*Pseudotsuga menziesii* (Mirbel) Franco); Bender and Hobein AG, Zürich), tannic acid (from oaks; Fluka), ellagic acid (Fluka), epicatechin (Fluka) and catechin (Fluka). As controls, the assay was run with fine quartz sand (washed; Merck, Darmstadt, Germany) and with water. One mg of each of these substances were added to 1.5 ml TTC-buffer in reaction tubes and their reactivity observed.

**Effect of the age of tree roots on the TTC reduction**

Living fine roots of different ages were collected from the forest site in Vordemwald. Fine roots from adult Norway spruce and European beech trees, which were assumed to be older than six months, were sampled in soil cores. Seedlings of beech and spruce (1-6 months), which germinated in the same forest site, were excavated and taken with an intact root ball to the laboratory. In addition, seeds (Tägerwilen, #845, seed collection WSL) of Norway spruce were sown in pots containing forest soil of the Ah-horizon from Vordemwald. The seedlings were grown for either one or two months in the greenhouse. For vitality measurements with TTC, fine roots of adult trees were treated as described above. For the measurements of seedling roots, the soil was removed and the fine roots were gently washed with tap water.
Boiled roots from each age class served as controls (see above). The fine roots (alive or boiled) were tested for reactivity with TTC.

Effect of drought on the fine root vitality

To investigate the effect of drought on seedling root vitality under different soil conditions, samples were collected from the Ah-horizon (base saturation (BS) 6.5%, carbon concentration 7.9%) and from the B-horizon (BS 3.1%, carbon concentration 1.0%). Soil monoliths were taken with a HUMAX soil corer (5 cm diameter; Lucerne, Switzerland) in plastic tubes. Monoliths were taken from six sampling sites 2 m apart with three replicates each.

In the laboratory, the plastic tubes containing the soil monoliths were divided into the Ah- (0-8 cm) and the B- (8-16 cm) horizons. Each horizon was sown with 20 seeds of Norway spruce (Tägerwilen, #845, seed collection WSL) and placed in the greenhouse. The seedlings were watered three times weekly with about 10 ml of distilled water. After six weeks, watering was stopped. Reactivity with TTC and O₂ consumption of the seedlings in each sample was tested 1, 4, 8, 15 and 22 day(s) after the watering ended.

Soil water losses were calculated by subtracting the dry mass (after 4 days of drying at 50 °C) from the fresh mass. Samples without watering for 18 days were dried for 4 days at 50 °C and served as controls. For the TTC-measurements, 100 mg fresh seedling roots of one sample (three replicates) of each horizon were used (see above). For the consumption of O₂ measurement, seedling roots were measured for 20 min with a Clark-type O₂ electrode (Hansatech, King’s Lynn, UK). For this measurement 25-100 mg fresh roots were placed in 2.5 ml of aerated 1 mM CaSO₄ + 5 mM MES buffer (adjusted with KOH to pH 5.5; Comas and Eissenstat, 2004). The temperature of the whole system was kept constant at 25 °C. Roots were then dried for 48 h at 60 °C and weighed. Respiration was expressed as O₂ consumption per g dry mass (nmol O₂·g⁻¹·s⁻¹).

Statistical analyses

three replicates were measured For each test. The data were subjected to two-way analysis of variance (ANOVA). The significance level was $P < 0.05$ by Fisher’s PLSD test. All tests were calculated with StatView 5.0 (SAS Institute, Cary, NY)
Chapter I

Results

Reactivity with TTC

The TTC reactivity of living and dead fine roots, and of the bark and wood of coarse roots of both Norway spruce and European beech is summarised in Table 2.1. Except for the wood of European beech, the reactivity of the living samples was relatively high. There were almost no significant decreases in the reactivity when the fine root and bark were dried and (except in the case of bark) boiled. In contrast the reactivity of the dead wood of coarse roots significantly decreased. The reactivity of the extraction residues with TTC, however, decreased significantly after a water/methanol extraction (for 79% to 87% (fine roots) and 92% to 97% (bark); Table 2.1).

Table 2.1: Reactivity (absorbance of triphenyl formazan (TF) ($\text{Abs}_{520} \, \text{g}^{-1} \text{Dw}$)) of living and dead root tissues and of the extraction residues of dead root tissues after a water/methanol (1:1; v/v) extraction. Different letters indicate significant differences ($P < 0.05$; n.d. = not determined)

<table>
<thead>
<tr>
<th>Root tissues</th>
<th>Norway spruce</th>
<th>European beech</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fine roots</td>
<td>Coarse roots</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Bark</td>
</tr>
<tr>
<td>Living</td>
<td>196 a</td>
<td>108 b</td>
</tr>
<tr>
<td>Dead (boiled)</td>
<td>156 a</td>
<td>48 c</td>
</tr>
<tr>
<td>Dead (dried/ground)</td>
<td>203 a</td>
<td>252 a</td>
</tr>
<tr>
<td>Residues after extraction</td>
<td>27 b</td>
<td>7 d</td>
</tr>
</tbody>
</table>

When dried and ground Norway spruce fine root samples were sequentially extracted, a stepwise decrease in TTC reactivity was observed (Figure 2.1).

![Figure 2.1: Mean reduction of the triphenyltetrazolium chloride ($\pm$ SE) of dried and ground Norway spruce fine roots and their dried extraction residues after different extraction steps. Abbreviations: Me = Methanol; and Ac = Acetone. Different letters indicate a significant difference ($P < 0.05$).](image-url)
After the water extraction and the water/methanol extraction the reactivity of the dried extraction residue decreased 75% and 93%, respectively. The last extraction (water/acetone) had no effect on TTC reactivity.

A comparison of the TTC reactivity of different cell-wall substances, dried and ground fine roots, the lyophilised water extract, and controls revealed that some cell-wall constituents such as cellulose and pectin showed almost no reactivity with the TTC (Table 2.2), whereas lignin and substances belonging to the group of water-soluble polyphenols reacted strongly with TTC. In particular, the reactivities of epicatechin and catechin were high, as were those of the dried and ground fine root material and its water extract (Table 2.2). Almost no reactivity was recorded with the control substances (Table 2.2).

Table 2.2: Mean reactivity (absorbance of triphenyl formazan (TF) \( \text{Abs}_{520 \text{ g}^{-1} \text{ DM}} \) ± SE) of different root cell-wall constituents, polyphenols, dried and ground spruce fine roots, the water extract of the dried and ground spruce fine roots, and two controls (1 mg).

<table>
<thead>
<tr>
<th>Substance class</th>
<th>Substance</th>
<th>Absorbance (Abs( _{520 \text{ g}^{-1} \text{ DM}} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-wall constituents</td>
<td>Cellulose</td>
<td>35 ± 16</td>
</tr>
<tr>
<td></td>
<td>Pectin</td>
<td>31 ± 14</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>310 ± 22</td>
</tr>
<tr>
<td>Polyphenols</td>
<td>Tannic acid</td>
<td>176 ± 111</td>
</tr>
<tr>
<td></td>
<td>Ellagic acid</td>
<td>187 ± 14</td>
</tr>
<tr>
<td></td>
<td>Catechin</td>
<td>293 ± 57</td>
</tr>
<tr>
<td></td>
<td>Epicatechin</td>
<td>360 ± 65</td>
</tr>
<tr>
<td>Dried/ground fine roots</td>
<td>Powder</td>
<td>402 ± 71</td>
</tr>
<tr>
<td></td>
<td>Lyophilised water extract</td>
<td>357 ± 126</td>
</tr>
<tr>
<td>Controls</td>
<td>Quartz sand</td>
<td>0.01 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>0.2 ± 0.23</td>
</tr>
</tbody>
</table>

Effect of the ages of tree roots on TTC reduction

Living fine roots of seedlings of Norway spruce and European beech were two to three times more reactive with TTC than the living fine roots of the adult trees (Table 2.3). Boiling reduced the reactivity of seedling roots to about 11 - 13%, whereas boiling had little effect on the reactivity of fine roots of adult trees (80%).
Table 2.3: Triphenyltetrazolium chloride reactivity (absorbance of triphenyl formazan (TF) (Abs$_{520}$ g$_{DM}^{-1}$)) of Norway spruce and European beech fine roots of different ages (age in month in parentheses) and origins, and the remaining percentage of the reactivity of the living roots. Different letters indicate a significant difference ($P < 0.05$).

<table>
<thead>
<tr>
<th>Roots</th>
<th>Norway spruce</th>
<th>European beech</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Forest</td>
<td>Greenhouse</td>
</tr>
<tr>
<td></td>
<td>Adult (6)</td>
<td>Seedlings (6)</td>
</tr>
<tr>
<td></td>
<td>Seedlings (2)</td>
<td>Seedlings (1)</td>
</tr>
<tr>
<td>Living</td>
<td>196 a</td>
<td>364 a</td>
</tr>
<tr>
<td>Dead (boiled)</td>
<td>156 a</td>
<td>43 b</td>
</tr>
<tr>
<td>% of living</td>
<td>80%</td>
<td>12%</td>
</tr>
</tbody>
</table>

$^{1}$ n = 2

**Effect of drought on fine root vitality**

Significant water losses in the soil monoliths of the two horizons were observed 22 days after watering was stopped (Figure 2.2a). The decrease in soil monolith water content was highly significant for both the time period and the horizon.

Root vitality measurements based on the reduction of TTC and the consumption of O$_2$ (Figures 2.2b, 2.2c) showed a slight increase in the vitality of the roots from Days 1-4 of drought. This increase was significant only in the case of the O$_2$ consumption. Four days after watering stopped, fine root TTC reactivity decreased continuously and significantly and reached minimum values after 22 days of drought. There were no difference in TTC reactivity with soil type.

Analysis of the relationship between seedling root TTC reactivity and the soil water content revealed a highly significant correlation (Figure 2.3a). Figure 2.3b shows that the relationship between the TTC reduction and the O$_2$ consumption of the seedling roots was also highly significant.
Figure 2.2: Changes over the course of a 22-day drought in the water content of Ah and B horizons of soil monoliths with Norway spruce seedlings (a); and in the triphenyltetrazolium chloride (TTC) reactivity (b) and O$_2$ consumption (c) of Norway spruce seedling roots. Different letters indicate a significant difference ($P < 0.05$) and bars indicate standard error. Abbreviations: C = control after 18 days of drought and 4 days drying at 50 °C; **** = $P < 0.0001$; bars indicate standard error; n.s. = not significant.

Figure 2.3: Relationship between triphenyltetrazolium chloride (TTC) reactivity of Norway spruce seedling roots after different lengths of drought: (a) with the water content of soil monoliths, and (b) with the consumption of O$_2$ of Norway spruce seedling roots. Probability level for the analysis of variance (ANOVA): **** = $P < 0.0001$. 

Chapter I

Discussion

The TTC-test is commonly used to distinguish living from dead fine roots, and to assess the vitality classes of the fine roots of woody and non-woody plants (Clemensson-Lindell, 1994; Comas et al., 2000; Ruf and Brunner, 2003; Sturite et al., 2004). In these investigations, boiled roots often served as controls. The reduction of TTC by the boiled roots was often found acceptably low and comparable to the reactivity of fine roots that were defined as dead (Ruf and Brunner, 2003, Sturite et al., 2004). However, Clemensson-Lindell (1994) and Comas et al. (2000) observed that the reduction of TTC by boiled roots remained high. Our tests revealed that the boiled fine roots of adult trees reduced TTC as much as living fine roots. Dried and ground fine roots of adult trees showed the same reactivity with TTC.

By separating the bark from the wood of coarse roots, we were able to demonstrate that reactive substances occur in higher quantities in the bark than in the wood. It was shown that these substances could be largely eliminated by water extraction and, for smaller quantities, with a water:methanol extraction. Water and alcohol extractions are commonly used to extract plant polyphenolic substances (Matthews et al., 1997; Peng et al., 1990; Kraus et al., 2003). In wood-processing, boiling is even one common method to extract polyphenols (Roffael et al., 2000). Pan and Lundgren (1995) detected polyphenols like epicatechin and catechin and other proanthocyanidins (= condensed tannins) in the root bark of Norway spruce. Our data also revealed that the extractable components from spruce fine roots (15 - 29% dry mass; data not shown) were comparable in quantity to the extractable polyphenols measured by Matthews et al. (1997) (21% dry weight). Kraus et al. (2003) reported that polyphenols reach up to 35% of the dry mass in roots.

To test if these polyphenolic components in the fine roots are able to reduce TTC, we compared the reactivity of the lyophilised water extract of spruce fine roots with several cell-wall constituents. The results demonstrated that root polyphenols, especially epicatechin and catechin, are as reactive with TTC as the fine root water extract. Due to their antioxidant and radical scavenging ability, polyphenols are extremely reactive (Rice-Evans et al. 1997, Hättenschwiler and Vitousek 2000, Kraus et al. 2003, Karonen et al., 2004, Dixon et al., 2005). This has also been reported to be determined with tetrazolium salts (Galato et al., 2001; Aehle et al., 2003; Franke et al., 2004). For example, Vyas et al. (2002) observed that XTT (sodium, 3’-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene-sulfonic acid hydrate) is reduced by polyphenols, especially by catechin.
Nevertheless, reflecting their low polyphenol content, we found that boiling seedling roots resulted in a measurable reduction in their TTC reactivity. Beyeler and Heyser (1997) measured an increase in polyphenols in European beech seedlings with age. As a consequence, the TTC test can give a false indication of vitality in fine roots of mature trees.

To demonstrate the link between respiration (O\textsubscript{2} consumption) and TTC reduction, we monitored TTC reduction by Norway spruce seedling roots during the imposition of drought while simultaneously measuring root oxygen consumption with a Clark-type O\textsubscript{2}-electrode. Both measurements indicated a significant decrease in root vitality with increasing drought stress. The measurements were significantly correlated and thus appeared to be equally valid measures of root respiration. These results also support the findings of Comas et al. (2000), that a good correlation exists between TTC reduction and O\textsubscript{2} consumption in the fine roots of young grape vines. That TTC reactivity of seedling roots, unlike O\textsubscript{2} consumption, did not approach zero towards the end of the 22-day drought can be explained by the presence of some polyphenolic substances even in seedling roots. Kirakosyan et al. (2004) reported a 6 to 10-fold increase in epicatechin and catechin in two hawthorn species (Crataegus spp.) in response to 10 days of drought.

In conclusion, our results indicate that the TTC-test can be applied to estimate the vitality of fine roots of tree seedlings but not of adult tree fine roots, which have a high concentration of polyphenols. The boiled controls indicate that the TTC reactivity of living fine roots with high polyphenolic content is not solely due to the respiratory activity of the tissue, but in part, depends on the presence of polyphenols. In this case, measurements of O\textsubscript{2} consumption with a Clark-type electrode may be a valuable alternative to indicate the vitality of fine roots.
Chapter II
Does low soil base saturation affect fine root properties of European beech (*Fagus sylvatica* L.)?

Summary

It is generally believed that high soil solution Al\textsuperscript{3+} in acidic soils with low base saturation (BS), negatively influences the properties of fine roots. Fine roots from European beech (\textit{Fagus sylvatica} L.) trees growing in highly acidic soils with very low BS and potentially high Al\textsuperscript{3+} concentration in the soil solution were analysed and the dependency of fine root properties on soil BS was measured. The fine roots were sampled down to 1 m depth at seven forest sites located on the Swiss Plateau. These sites varied in their BS from 1.4\% to 11.4\% in the mineral layers. We evaluated relationships between the BS of these mineral layers and fine root properties, such as ratio between bio- and necromass (live/dead ratio), specific root length (SRL), root tip abundance (RTA), root branching abundance (RBA), O\textsubscript{2}-consumption, and the Ca/Al molar ratio in the fine root tissue. The fine root properties were compared not only with the BS of the soil, but also with the Ca/Al molar ratio in the fine root tissues. Significant relations of fine root properties occurred when the soils of the seven sites were grouped into two BS groups (< 5\% and 5-10\%). The live/dead ratio, the RTA, the RBA, the O\textsubscript{2}-consumption, and Ca/Al molar ratio were lower in the group of BS < 5\% than in the group 5-10\%. Decreases in the morphological properties and in the O\textsubscript{2}-consumption were related to decrease in the Ca/Al molar ratio of the fine root tissues. There is evidence that the fine root properties are negatively influenced, nevertheless, fine root systems of mature European beech in their natural ecological environment seem to be able to compensate adverse effects of low BS.

Introduction

Since high depositions of acidifying pollutants over the last decades have accelerated soil acidification (Blaser et al., 1999; Graf Pannatier et al., 2004), this may have ecological consequences for forest ecosystems. For example uprooting of forest trees in storms has been found to be more frequent on acidic soils (Mayer et al., 2005) and on soils with a low base saturation (BS; Braun et al., 2003). Soil acidification beyond a pH of 5 is accompanied by an increase in free aluminium (Al) species content in the soil solution and contributes to a further decrease in BS (Walthert et al., 2004). Free Al\textsuperscript{3+} is the most important rhizotoxic Al species in the soil solution (Kochian et al., 2004).
Al\(^{3+}\) has toxic effects on plants as it hampers fine root growth by inhibiting root-cell elongation and cell division (Matsumoto, 2000; Kochian et al., 2004). This results in a change in fine root morphology with the formation of short and stubby roots, dieback of root tips, decline in root elongation, and cessation of the formation of lateral roots (Göransson and Eldhuset, 1991; Hirano and Hijii, 2000). As a consequence tree fine root systems become degraded and their water and mineral nutrient uptake is, therefore, limited (Matsumoto, 2000). Additionally, Al\(^{3+}\) competes with essential basic cations such as Mg\(^{2+}\) and Ca\(^{2+}\) on the soil exchanger and inhibits nutrient absorption to cell walls of fine roots (Göransson and Eldhuset, 1995). This can lead to deficiencies in these elements and elevates the Al content in the fine root tissues (Zysset et al., 1996). One indicator for Al\(^{3+}\) toxicity is when the Ca to Al molar ratio in the fine root tissues decreases below 0.2 (Cronan and Grigal, 1995). Both low basic cation availability (expressed in BS) and high concentrations of Al\(^{3+}\) in the soil solution could potentially affect the ‘vitality’ of the fine roots.

Several methods exist to measure the fine root properties to assess the ‘vitality’ of the fine roots. One first approach is to analyse the occurrence of fine roots. In acidified soils the mass of living fine roots is decreased and that of dead fine roots is increased, and, thus, the turnover rates are influenced (Jentschke et al., 2001; Godbold et al., 2003; Leuschner et al., 2004; Vanguelova et al., 2005). A second approach is to study fine root morphology (Clemensson-Lindell and Persson, 1995; Godbold et al., 2003). Some morphological features, e.g. specific root length (cm g\(^{-1}\)DW) and root tip abundance (tips g\(^{-1}\)DW), are used to evaluate the reactions of fine roots to different soil conditions and different nutrient supplies (Ostonen et al., 1999; Hodge, 2004). A third approach assesses fine roots’ physiological and chemical responses by determining the respiration activity (Comas et al., 2000; Richter et al., 2007a) and the Ca/Al molar ratio of fine roots (Cronan and Grigal, 1995). Ca is known to ameliorate Al-induced rhizotoxicity and mitigate other adverse effects of Al (Cronan and Grigal, 1995; Rengel and Zhang, 2003).

However, little is known about the changeability of fine root properties in relation to soil parameters in situ. One threshold parameter often used to estimate potential fine root damage due to Al\(^{3+}\) toxicity is a BS < 15% (Cronan and Grigal, 1995). According to Leuschner et al. (2006), the growth of European beech (Fagus sylvatica L.) can be limited at BS below 3.3%. Hence, in the present study, the main objective was to evaluate whether BS can be used to predict fine root ‘vitality’ of European beech in soils where Al\(^{3+}\) toxicity potentially occurs. We, therefore, hypothesise that (i) fine root properties such as fine root mass, morphology, physiology, and chemistry are related to low soil BS at different depths of forest soils, (ii)
morphological and physiological properties are related to the Ca/Al molar ratio in the fine root tissue, and (iii) fine root properties have the potential to be used to assess plant root stress in soils with a very low BS.

Materials and methods

Forest sites

Fine roots of European beech (*Fagus sylvatica* L.) were sampled in seven forest sites on the Swiss Plateau (Table 3.1).

| Table 3.1: Description of the seven study sites (in order of increasing mean base saturation). |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Coordinates                     | Entlebuch (EN)                  | Walterswil (WL)                 | Vordemwald (VW)                  | Zofingen (ZO)                    | Niedererlbach (NI)               | Triengen (TR)                    | Krauchtal (KR)                  |
| Coordinates                     | Entlebuch (EN)                  | Walterswil (WL)                 | Vordemwald (VW)                  | Zofingen (ZO)                    | Niedererlbach (NI)               | Triengen (TR)                    | Krauchtal (KR)                  |
| Elev. [m a.s.l.]                 | 3.2                             | 3.9                             | 4.6                             | 7.1                             | 9.3                             | 12.9                            | 18.2                            |
| pH                              | 3.6                             | 3.6                             | 3.8                             | 3.7                             | 3.5                             | 3.7                             | 3.7                             |
| CEC [mmol, kg⁻¹]                | 83                              | 84                              | 103                             | 67                              | 125                             | 62                              | 142                             |
| Layer                           | 7-15                            | 5-12                            | 6-15                            | 5-10                            | 4-9                             | 4-8                             | 6-11                            |
| [cm]                            | 15-100                          | 12-100                          | 15-100                          | 10-100                          | 9-100                           | 8-100                           | 11-100                          |

1) according to Ellenberg and Klötzli (1972); Abietetum=Bazzanio-Abietum, Fagetum a=Millio-Fagetum, Fagetum b=Galio odorati Fagetum, Fagetum c=Galio odorati Fagetum luzuletosum

2) Classification after FAO (1997); Gleysol=Distric Gleysol, Cambisol=Distric Gleyic Cambisol

3) Mean Base Saturation (not weighted between the horizons; based on element content in milliequivalents per kg fine earth)

4) Mean pH (CaCl₂) (not weighted between the horizons)

5) Mean Cation Exchange Capacity (not weighted between the horizons; based on element content in milliequivalents per kg fine earth)

6) according to Walthert et al. (2004)

The forest sites were chosen from the WSL soil data-base (Swiss Federal Institute for Forest, Snow and Landscape Research; for methodical details, see Walthert et al. (2004) and Graf Pannatier et al. (2004)). Deeply decalcified soils with a BS < 15% in the mineral soil
matrix and a lime threshold deeper than 1.50 m depth were selected. The soil types of all sites could be classified as acidic brown soils (Distric Gleysol, Gleyic Cambisol, distric, and Luvisol), with a BS from 1.6% to 24.2% in the Ah-layers and 1.4% to 11.4% in the B-layers (Table 3.2). The pH (CaCl$_2$) varies between 2.3 and 3.6 in the Ah-layers and 3.4 and 4.2 in the mineral layers (Table 2.2). All soils have only weak compaction in the deeper mineral layers. Some profiles show a few characteristics of water logging in the lower profile, but none were classified as extremely water logged. Five forest sites belong mainly to the alliance Luzulo-Fagion, whereas the other two sites belong to the alliance Abietion, where the European beech is secondary (Ellenberg and Klötzli, 1972).

Table 3.2: Base saturation (BS) and pH (CaCl$_2$) of the soil matrix of each layer of the seven forest sites (abbrev. according to Table 3.1).

<table>
<thead>
<tr>
<th>Layer</th>
<th>EN</th>
<th>WL</th>
<th>VW</th>
<th>ZO</th>
<th>NI</th>
<th>TR</th>
<th>KR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ah1</td>
<td>2.2</td>
<td>8.7</td>
<td>6.5</td>
<td>12.1</td>
<td>21.7</td>
<td>8.3</td>
<td>24.2</td>
</tr>
<tr>
<td>Ah2</td>
<td>1.6</td>
<td>3.3</td>
<td>3.4</td>
<td>12.1</td>
<td>11.3</td>
<td>4.1</td>
<td>4.7</td>
</tr>
<tr>
<td>B, -25 cm</td>
<td>1.6</td>
<td>1.4</td>
<td>3.1</td>
<td>3.9</td>
<td>5.8</td>
<td>5.1</td>
<td>3.2</td>
</tr>
<tr>
<td>B, 25-50 cm</td>
<td>2.7</td>
<td>3.3</td>
<td>3.8</td>
<td>4.3</td>
<td>5.8</td>
<td>5.3</td>
<td>3.9</td>
</tr>
<tr>
<td>B, 50-75 cm</td>
<td>4.9</td>
<td>3.7</td>
<td>5.3</td>
<td>4.3</td>
<td>4.8</td>
<td>8.2</td>
<td>11.4</td>
</tr>
<tr>
<td>B, 75-100 cm</td>
<td>6.0</td>
<td>3.7</td>
<td>5.3</td>
<td>5.2</td>
<td>6.4</td>
<td>47.5$^1$</td>
<td>61.9$^1$</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ah1</td>
<td>3.3</td>
<td>2.8</td>
<td>3.3</td>
<td>3.1</td>
<td>3.1</td>
<td>3.2</td>
<td>2.9</td>
</tr>
<tr>
<td>Ah2</td>
<td>3.4</td>
<td>3.0</td>
<td>3.6</td>
<td>3.1</td>
<td>3.2</td>
<td>3.6</td>
<td>2.3</td>
</tr>
<tr>
<td>B, -25 cm</td>
<td>3.4</td>
<td>3.4</td>
<td>3.9</td>
<td>3.8</td>
<td>3.8</td>
<td>3.8</td>
<td>4.1</td>
</tr>
<tr>
<td>B, 25-50 cm</td>
<td>3.8</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>3.8</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>B, 50-75 cm</td>
<td>3.9</td>
<td>4.1</td>
<td>4.0</td>
<td>4.0</td>
<td>3.9</td>
<td>3.8</td>
<td>4.0</td>
</tr>
<tr>
<td>B, 75-100 cm</td>
<td>3.9</td>
<td>4.1</td>
<td>4.0</td>
<td>4.0</td>
<td>3.7</td>
<td>3.9</td>
<td>4.1</td>
</tr>
</tbody>
</table>

$^1$ values were excluded from the calculation of the base saturation groups

**Fine root sampling**

At each forest site four individual European beech trees, belonging to the dominant individuals in the stands, were chosen in the vicinity of the soil profile. Each tree was sampled twice with soil cores 1 m away from the stem, once North and once South of the tree. Soil cores containing the fine roots were taken with a HUMAX soil corer (Ø 10 cm) down to 1 m depth consisting of four segments. Each core segment consisted of a plastic tube 25 cm long with the soil left undisturbed during sampling and storage. The core segments were
stored in a fridge at 1°C in the laboratory for no longer than 1 week. All forest sites were sampled within five weeks.

The first, uppermost soil core segments, containing the Ah- and the upper B-layers, were separated into two Ah-layers, Ah1 and Ah2, and one mineral B-layer. There was more organic matter content in the Ah1-layer than in the Ah2-layer, so, these two layers were separated (according to Walthert et al., 2004). The other three soil core segments of the mineral B-layer were left undivided.

For the fine root (⌀ < 2 mm) measurements, the soil samples were sieved and the fine roots gently washed in a 1 mm sieve with tap water. All the fine root fragments down to 0.5 cm length were collected and stored in iced water. The roots were examined under a microscope to separate living from dead roots (bio- and necromass). Roots with turgescent cells and flexible cell walls, a white stele, and turgescent unbroken root tips were classified as living roots. Brownish, inflexible and air-filled roots were classified as dead (Hertel and Leuschner, 2002). The dead roots were dried for 48 h at 60°C and then weighed. Two aliquots of the living fine roots were separated. The first one of an average of five living fine root fragments of 10 cm length was stored in iced water until further morphological measurements (see below). The second aliquot of approx. 0.5 g fresh weight was left in iced water for no longer than 1 h for further physiological measurements (see below). The remaining living fine roots were dried for 48 h at 60°C. Bio- and necromass in each soil layer were expressed in g (dry weight) per volume soil and the ratio between bio- and necromass (live/dead ratio) was measured. For the biomass the dry weight of the two aliquots (morphological measurements and O₂-consumption measurements) and the remaining rest of the whole sample were summarised.

**Morphological measurements**

For the morphological measurements, the representative aliquots of each sample, were analysed with WinRhizo (V4.1c; Regent Instruments INC., Quebec, Canada), dried for 48 h at 60°C, and weighed. Length [cm], surface area [cm²], diameter [cm], number of tips [n] and branches [n] were analysed. The recorded properties were root tip abundance (RTA [n g⁻¹]), root branching abundance (RBA [n g⁻¹]), and specific root length (SRL [cm g⁻¹]), which were expressed on a fine root dry weight basis.
Respiration measurements and element concentrations

For respiration measurements, the aliquots of approx. 0.5 g of living European beech fine roots of each mineral layer were tested to assess the roots’ O$_2$-consumption. Because all roots were washed and freed from the attached soil particles, it is assumed, that the microorganisms, which live on the root surface and within the roots, contribute to the O$_2$-consumption more or less equally per root. The consumption of O$_2$ was measured for 20 min with a Clark-type O$_2$-electrode (Hansatech, King’s Lynn, UK). For this measurement, the roots were submersed in 2.5 ml of stirred 1 mmol CaSO$_4$ + 5 mmol MES buffer (adjusted with KOH to pH (H$_2$O) 5.5; Comas and Eissenstat, 2004; Richter et al., 2007). The whole system was kept at a constant temperature of 25°C. After the measurements, roots were dried for 48 h at 60°C and weighed. Respiration was expressed as O$_2$-consumption per g dry weight and time [nmol O$_2$ g$^{-1}$ s$^{-1}$]. In addition, the O$_2$-consumption of dead fine roots was also measured in order to compare the two different root conditions. It can be assumed, that the microorganisms which colonise these dead root tissues, account for a major part of the O$_2$-consumption. In comparison to living roots, however, the O$_2$-consumption of dead roots was very small (see results).

For element analyses, the fine roots previously used for the O$_2$-consumption measurements were ground for 1 min at 70% speed in a swing mill MM 2000 (Retsch, Haan, Germany). Elements were measured after digestion of the ground material in a high-pressure microwave (Milestone MLS Ultraclave) with an inductively coupled plasma atomic-emission spectrometer (ICP-AES Optima 3000, Perkin Elmer; see Brunner et al., 1999). The fine root element concentrations were expressed as [mol kg$_{DM}^{-1}$] and the molar ratio between Ca/Al was calculated. In a few cases, also the element concentration of dead fine roots was analysed.

Statistical analyses

The fine root measurements were averaged per North and South soil core of the tree and soil layers. Per forest site, the tree data of the four sampled trees were averaged per soil layers and compared with the soil chemical parameters. Soil chemical data were taken from a single soil profile per forest site. The data were subjected to one- and two-way analysis of variance (ANOVA). The significance level was $P < 0.05$ by Fisher's PLSD test throughout the text unless stated otherwise. All tests were conducted with StatView 5.0 (SAS Institute, Cary, NY, USA). PCA was conducted with Canoco 4.5 (Biometris-Plant Research International, Wageningen, The Netherlands) and cluster analyses with SYSTAT 10 (Systat Software Inc., California, USA).
Chapter II

Results

Relation of the fine root properties to the soil chemical parameters

Our analyses revealed few relations between fine root properties and soil parameters, which occurred especially in the B-layer (Table 3.3). In particular, the live/dead ratio and the Ca/Al molar ratio were related with BS or pH within the B-layers (Table 3.3). No relation between the fine root properties and the water regime in the soil profiles could be found (data not shown). The principal component analyses (PCA; Figure 3.1) applied in the B-layers showed negative correlations between soil parameters and RTA, SRL, and O$_2$-consumption. The fine root properties (except live/dead ratio) appeared to be all strongly related to each other. No PCA for the Ah-layers were made because this study focuses on differences in the fine root properties in the B-layers, where free Al$^{3+}$ in the soil solution can be expected in higher concentrations (Graf Pannatier et al., 2004; Parker, 2005).

Table 3.3: P-values of one-way ANOVA for the mean values of biomass, necromass, live/dead ratio, root tip abundance (RTA), root branching abundance (RBA), specific root length (SRL), O$_2$-consumption, and Ca/Al molar ratio in the fine root tissues versus base saturation (BS) and pH (n.s. = not significant; $P < 0.05$).

<table>
<thead>
<tr>
<th>Layer</th>
<th>Bio-mass [g l$^{-1}$]</th>
<th>Necro-mass [g l$^{-1}$]</th>
<th>Live/dead ratio</th>
<th>RTA [n g$^{-1}$]</th>
<th>RBA [n g$^{-1}$]</th>
<th>SRL [cm g$^{-1}$]</th>
<th>O$_2$- consumption [nmol O$_2$ g$^{-1}$s$^{-1}$]</th>
<th>Ca/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ah</td>
<td>BS</td>
<td>n.s.</td>
<td>0.006</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-$^1$</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>n.s.</td>
<td>n.s.</td>
<td>0.04</td>
<td>n.s.</td>
<td>n.s.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>BS</td>
<td>n.s.</td>
<td>0.03</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>0.03</td>
<td>0.04</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$^1$not determined

Figure 3.1: Principal component analysis (PCA) of the soil chemical parameters and the mean fine root properties in all B-layers: base saturation (BS: [%]), pH, biomass [g l$^{-1}$], necromass [g l$^{-1}$], live/dead ratio, root tip abundance (RTA; [n g$^{-1}$]), root branching abundance (RBA; [n g$^{-1}$]), specific root length (SRL; [cm g$^{-1}$]), O$_2$-consumption [nmol O$_2$ g$^{-1}$s$^{-1}$], and Ca/Al molar ratio. Total explained variance by the 1, 2, and 3 principal axes: 99.8%.
Two groups of BS

To analyse the trends apparent from the PCA of the B-layers, the seven forest sites were subjected to a cluster analysis with the BS from the soils of the B-layers (Table 3.2) as the dependent variable (Figure 3.2).

![Cluster analysis](image)

Figure 3.2: Cluster analysis of the mean soil base saturation (BS [%]) in the B-layers of the seven forest sites (abbrev. according to Table 3.1).

The deepest layers from the sites at Triengen and Krauchtal were excluded because the BS there was extremely high due to the influence of the high lime content in layers deeper than 1.50 m. The analysis resulted in two clear clusters of sites in two BS-groups. The first group had a very low BS < 5% (EN, WL, VW, ZO), and the second group had BS 5-10% (NI, TR, KR; Figure 3.2). The variability of the pH between the forest sites and soil layers was too small to show a natural clustering between the forest sites.

Standing mass and morphological fine root properties

The fine root standing bio- and necromass did not differ between the two BS-groups (data not shown). The live/dead ratio, however, showed slight, but significantly smaller values in the BS-group < 5%, in particular in the deepest layers (Figure 3.3). The live/dead ratio increased with increasing depth of the soil profile. The increase in the ratio with depth within the BS-group < 5% was about 5 times elevated and that of the group 5-10% about 10 times elevated.
Chapter II

Figure 3.3: Live/dead ratio of the fine roots of European beech in the two mean base saturation groups (BS; ■ < 5%, □ 5-10%) in the B-layers. Probability levels for the one- and two-way analyses of variance (ANOVA): * = $P < 0.05$, ** = $P < 0.01$; bars indicate standard error; n.s. = not significant.

The morphological properties of the fine roots also differed between the two BS-groups. The RTA was significantly (10-60% according to soil layer) lower in the BS-group < 5% than in the group 5-10% (Figure 3.4a). The most distinct differences occurred in the deeper layers (50-100 cm depth). The RBA was significantly (10-40% according to soil layer) lower in the BS-group < 5% than in the group 5-10% (Figure 3.4b). The soil depth did not appear to affect the RTA and RBA. The SRL, however, was not affected by the BS-groups (Figure 3.4c), as the trend towards the lower SRL values (by 5-20%) in lower BS-group as compared to the higher BS-group was not significant.

Figure 3.4: Root tip abundance (RTA) a), root branching abundance (RBA) b), and specific root length (SRL) c) of the European beech fine roots in the two mean base saturation groups (BS; ■ < 5%, □ 5-10%) in the B-layers. Probability levels for the one- and two-way analyses of variance (ANOVA): * = $P < 0.05$, ** = $P < 0.01$; bars indicate standard error; n.s. = not significant.
The other morphological properties, such as length, surface area, and average diameter, did not vary with the BS-groups nor with soil depth (data not shown).

**Physiological and chemical fine root properties**

There were significant differences between the two BS-groups in the O$_2$-consumption of the fine roots in the B-layers (Figure 3.5a). The O$_2$-consumption in the BS-group < 5% was decreased by about 25% in the uppermost B-mineral layer and by about 5% in the deeper layers. With increasing depth, there was a distinct and significant decrease in both BS-groups from the uppermost layer to the deeper layers. Dead fine roots had a very low O$_2$-consumption of 0.2 ± 0.03 nmol O$_2$ g$^{-1}$s$^{-1}$.

The Ca/Al molar ratio in the fine roots was significantly smaller in the BS-group < 5% throughout the whole soil profile (Figure 3.5b). In both BS-groups, there was a distinct decrease from the uppermost layer to the deeper layers. Within the group < 5% this decrease continued constantly through the profile until it was by about 50% decreased. In the BS-group 5-10% the decrease occurred mainly between the uppermost B-layer and the subsequent layer, after which almost no further decrease occurred with depth (25-100 cm). The dead fine roots had a very low Ca/Al molar ratio of 0.5 ± 0.1. The Ca/Al molar ratio of the fine root tissue was very positively correlated to RTA, RBA, and SRL (Figure 3.6), and to the O$_2$-consumption (Figure 3.7).

![Figure 3.5: O$_2$-consumption a) and Ca/Al molar ratio b) of the European beech fine roots in the two mean base saturation groups (BS; □ < 5%, □ 5-10%) in the B-layers. Probability levels for the one and two-way analyses of variance (ANOVA): * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$; bars indicate standard error; n.s. = not significant.](image-url)
Figure 3.6: Relationships between a) root tip abundance (RTA), b) root branching abundance (RBA), and c) specific root length (SRL) of the European beech fine roots and the Ca/Al molar ratios in European beech fine root tissues. Fine roots from the B-layers were analysed. Probability levels for the analyses of variance (ANOVA): *** = $P < 0.001$, **** = $P < 0.0001$

Figure 3.7: Relationship between the O$_2$-consumption of the European beech fine roots and the Ca/Al molar ratio in the European beech fine root tissues. Fine roots from the B-layers were analysed. Probability level for the analyses of variance (ANOVA): *** = $P < 0.001$.

Discussion

In greenhouse experiments under controlled conditions in hydroponics, fine roots showed a strong response to elevated Al$^{3+}$ concentrations in the nutrient solutions (Godbold and Jentschke, 1998; van Schöll et al., 2004). The fine root growth was always heavily impaired
immediately after applying $\text{Al}^{3+}$ rich solutions. Fine root cell elongation was inhibited and after prolonged exposure to high $\text{Al}^{3+}$ concentrations, roots showed an impaired development due to inhibited cell division (Matsumoto, 2000; Kochian et al., 2004). However, in *in situ* measurements, most studies found no inhibition of fine root growth and development. Leuschner et al. (2004) could not find any marked differences in the fine root morphology of European beech stands with variations in soil acidity (pH 2.9 – 6.7) and fertility (BS 3.9 – 98.9%). Ostonen et al. (1999) identified several morphological relationships between the short roots of Norway spruce and soil conditions (humus content, field capacity, and specific soil surface area), but not with the Ca/Al ratio in the soil. Wargo et al. (2003) also found no changes in red spruce root parameters, such as the living root tips per branch and the percentage of dead roots, along a gradient of exchangeable Al/Ca ratios in the forest floor. Nygaard and de Wit (2004) failed to detect any changes in fine root properties, such as decreased biomass or elevated necromass, after applying $\text{Al}^{3+}$ *in situ* during a three-year experiment in a Norway spruce stand with BS 6% and pH 4.3 in the soil matrix. Eldhuset et al. (2006) also found no prominent changes in the same fine root properties after a further four years in the same experiment.

We were, nevertheless, in our study able to identify a series of changes in the characteristic root properties due to the extreme low BS in the soil, e.g. a decreased live/dead ratio and decreased root-tip and root-branching abundance. The fine root properties differed according to the forest site group: the group with low BS (5-10%) or the group with very low BS (< 5%). According to Graf Pannatier et al. (2004), there is a trend for $\text{Al}^{3+}$ content, beside of the pH dependency, to increase in mineral soils with decreasing BS; therefore, higher $\text{Al}^{3+}$ concentrations can be expected at BS < 5% than at BS 5-10%. At the forest site Vordemwald, the mean values of $\text{Al}_{\text{tot}}$ decreased from 23.6 $\mu$mol l$^{-1}$ at a depth of 10-20 cm, 18.9 $\mu$mol l$^{-1}$ at 40-60 cm, and 3.8 $\mu$mol l$^{-1}$ at 60-80 cm as the BS increased from 3.1, 3.8, to 5.3%, respectively (Graf Pannatier et al., 2004). About one third of $\text{Al}_{\text{tot}}$ is phytotoxic $\text{Al}^{3+}$ (Graf Pannatier et al., 2004).

Commonly used fine root properties to assess the ‘vitality’ of the fine roots are the amount of biomass, necromass, and live/dead ratio, as well as the productivity and mortality of the fine roots. It has often been reported that forests growing on acidic sites have a higher fine root density and fine root mass of both bio- and necromass than forests growing on less acidic sites (Godbold et al., 2003; Jentschke et al., 2001; Vanguelova et al., 2005). In particular, a higher necromass is attributed to the fact that fine root systems on acidic and poor sites have a higher mortality rate (Leuschner et al., 2004). In addition, fine roots from soils with a low BS
may have a decreased longevity due to nutrient deficiencies (low BS) and/or Al$^{3+}$ toxicity, as observed by Vanguelova et al. (2005) in a Scots pine stand. Nevertheless, Gaudinski et al. (2001) showed an increase in the age of fine roots measured by radiocarbon with increasing depth (4-6 years at 0-15 cm depth and 15-18 years at 15-30 cm depth), which may also explain the increased live/dead ratio with increasing depth.

Some morphological properties of fine roots appeared to be influenced by very low BS. One significant change was that fine roots had fewer root tips. This may be due to the inhibition of development and growth or to death caused by Al$^{3+}$ toxicity or nutrient deficiencies, as reported in the study of Göransson and Eldhuset (1991). This finding is supported by the study of Leuschner et al. (2004), who showed a lower RTA in the first 5 cm of the mineral layer of forest sites with a low BS (9.4% and 3.9%; RTA 390 n g$^{-1}$ and 560 n g$^{-1}$) and higher at sites with a high BS (98.9% and 45.9%; RTA 620 n g$^{-1}$ and 640 n g$^{-1}$). Not only the dieback of root tips but also a cessation of formation of lateral roots were observed in several studies in hydroponics (Göransson and Eldhuset, 1991; Hirano and Hijji, 2000). This characteristic development could also be seen in our study as the branching abundance was decreased at lower BS. This change in morphology most likely originates from the ability of the trees to use less energy and nutrients for fine root growth when the BS is very low. SRL reflects, for example, the benefits in resource acquisition relative to the cost of resources used for the construction and maintenance of root tissue (Fitter, 1991; Espeleta and Donovan, 2002). Nevertheless, SRL was only slightly, but not significantly, decreased in sites with very low BS. Morphological plasticity can be considered as a root functional status to provide rapid acquisition of nutrients and water (Ostonen et al., 1999; Paulitz, 2002). As a result, we hypothesize that roots with fewer tips and lateral roots (branches) could be less effective in fulfilling these functions.

Very low BS in the soil affects not only the morphology, but also the physiology. The O$_2$-consumption was also lower at very low BS, as was the Ca/Al molar ratio of the fine root tissue. This ratio shows not only a direct interference of Al with nutrient accumulation in fine roots (Nygaard and de Wit, 2004; Vanguelova et al., 2005), but it may also be the result of the fine root system being reduced as the organ for nutrient uptake (see decreased RTA, RBA and SRL; Rengel, 1992). In the study of Yamamoto et al. (2002) the O$_2$-consumption of cultured tobacco cells was also decreased with increasing Al$^{3+}$ in the cultivation medium. This decrease was correlated with a decrease of ATP production and cell growth. Therefore, it can be hypothesized that fine roots growing under very low BS conditions will have a decreased energy potential for their functions, as they have a lower O$_2$-consumption and an adverse
Ca/Al molar ratio in the tissues. However, the fact that O₂-consumption is comparatively high in the first mineral layers may go against this interpretation. The higher O₂-consumption might be due to better soil conditions, e.g. temperature, aeration, or compaction.

All the three morphological properties RTA, RBA, and SRL, as well as the O₂-consumption are strongly related to the Ca/Al molar ratio in the fine root tissues. As this ratio reflects the nutrient status and Al content in the fine roots based on the BS in the soil (Nygaard and de Wit, 2004; Vanguelova et al., 2005), this suggests that these properties could be potentially useful in assessing stress to the plants in soils with a very low BS.

Conclusions

This work provided evidence that the fine root properties in the B-layers are negatively influenced in stands with a BS < 5% relative to those with a higher BS. Fine roots in such layers have fewer root tips and laterals, a lower live/dead ratio, a slightly decreased SRL and a decreased respiratory activity. Furthermore, the morphological and physiological properties are also related to the Ca/Al molar ratio in the root tissue. However, according to our data, the most suitable parameter for ‘vitality’ measurements is probably the measurement of the O₂-consumption. As a physiological parameter, the measurement of the O₂-consumption is a more direct way to analyse the ‘vitality’ of the fine roots than the standing mass or the morphological and chemical parameters. Additionally, the measurement of the O₂-consumption is an easy and fast method to analyse a physiological feature of fine roots.

Nevertheless, the differences were not that distinct, and significances between the BS-groups did not occur in every soil layer. Nygaard and de Wit (2004) suggested that fine roots seem to be able to compensate adverse soil chemical effects much better under natural conditions than under artificial conditions. They hypothesized that the fine roots are more resistant when they grow within their natural ecological balance with normal soil heterogeneity and microbial community structure. Therefore, we also conclude that fine roots of European beech might have mechanisms (e.g. growth of roots in chemically more suitable parts of the heterogeneous soil) to compensate adverse effects.
Chapter III

Chapter III
Turnover and age of European beech fine roots in acid forest soils
Summary

- Fine roots of forests contribute with their turnover to carbon fluxes within soils. In order to examine the impact of soil chemical parameters and soil depth on fine roots, six European beech (*Fagus sylvatica*) stands with acidic soils and low base saturation were sampled and the results compared with published data.

- Living and dead fine roots were sampled by sequential coring down to 1 m depth. Productivity, mortality, turnover, and estimated age were calculated. The age of fine roots from two stands were measured by radiocarbon.

- Weak relationships were observed between soil chemical parameters and fine root properties. A negative correlation between fine root turnover and soil pH was observed when considering published data. Fine root biomass, necromass, productivity, mortality, and turnover strongly declined with soil depth, from 2.8 to 0.5 g l⁻¹, 0.8 to 0.1 g l⁻¹, 1.0 to 0.2 g l⁻¹, 1.1 to 0.3 g l⁻¹, and 1.9 to 0.7 y⁻¹, respectively. The age of fine roots estimated with turnover ranged between 0.1-10 years and with radiocarbon between 3-26 years.

- The two fine root age measurements correlated strongly with each other, but differed by about a factor of 4, indicating possibly different fine root pools.

Introduction

Estimates of fine root productivity and turnover are important to assess carbon budgets and fluxes belowground. Fine roots represent up to 50% of the global total belowground Net Primary Production (NPP; Nadelhoffer & Raich, 1992). Gill & Jackson (2000) estimated that global fine root production accounts for as much as 33% of the global annual NPP. With the Kyoto Protocol there is an increasing need to define present and future carbon budgets and fluxes on regional and continental scales (Steffen *et al.*, 1998; Schulze *et al.*, 2000). Estimates of belowground carbon budgets and fluxes are given by biogeochemical models such as the Biome-BGC model, which include fine root parameters (Pietsch *et al.*, 2005). In this model, it is assumed that the fine roots of European beech (*Fagus sylvatica* L.) trees have a turnover of 1 [y⁻¹] (Pietsch *et al.*, 2005). However, different environmental conditions, such as the soil type and depth, influence the growth and development of fine roots (Leuschner & Hertel, 2003, Finer *et al.*, 2007, Richter *et al.*, 2007). In particular, Finer *et al.* (2007) and Leuschner
& Hertel (2003) showed that European beech trees growing on nutrient-poor sites have a higher fine root biomass than those growing on nutrient-rich sites, as they probably invest more carbon in their fine roots to compensate for the loss due to a rapid rate of root death. Leuschner et al. (2004) also showed an increased necromass due to an enhanced mortality and turnover under low pH. In addition, these fine roots may have a decreased longevity due to nutrient deficiencies (low BS), which Vanguelova et al. (2005) observed in a Scots pine stand. Therefore, changes in the soil chemistry induced by acidifying pollutants could change the productivity and mortality of fine root systems of forest trees and thus change the carbon fluxes belowground.

The turnover and age of fine roots can be examined by several methods, e.g. sequential coring. The age of fine roots can also be measured on the basis of the radiocarbon ($^{14}\text{C}$) content in the structural carbon (cellulose). This method relies on the fact that the atmospheric radiocarbon concentration strongly increased in the early 1960s when thermonuclear weapons were tested in the atmosphere. Since then, the $^{14}\text{C}$ concentration of the atmosphere has decreased and the rate of decrease today is about 4‰ per year (Levin & Hesshaimer, 2000). Experiments conducted by Gaudinski et al. (2001) showed that fine roots growing in 1999 in root screens had the same radiocarbon signal in the cellulose as the atmospherical signal present in 1999. Several studies have used the radiocarbon method to estimate the age of fine roots (Tierney & Fahey, 2002; Trumbore, 2006; Trumbore et al., 2006).

In the present study the fine roots of European beech forests growing in highly acidic soils with a very low base saturation (BS) were examined. This narrow range was selected to examine fine root productivity and turnover under conditions critical for fine root growth (Cronan & Grigal, 1995). The following hypotheses were investigated: (i) soil chemical parameters (pH, BS) and soil depth strongly influence the biomass, necromass, productivity, and mortality of fine roots; (ii) soil chemistry and depth greatly influence the turnover and age of fine roots; (iii) measurements of the turnover and age of fine roots depend greatly on the used method; and (iv) estimates and measurements of the age of fine roots are correlated.

Material and methods

Forest sites

Fine roots of European beech ($Fagus sylvatica$ L.) were sampled on the Swiss Plateau in six forest sites (Table 1) where the BS is < 15%. This threshold was chosen as it is reported to be
critical for root growth (Cronan & Grigal, 1995). The forest sites were selected from the WSL (Swiss Federal Institute for Forest, Snow and Landscape Research) soil data-base (see Walthert et al. (2004) and Graf Pannatier et al. (2004) for details). The soil chemical data were taken from one soil profile per forest site. The soil types of all sites could be classified as acidic brown soils (Distric Gleyso, Distric Gleyic Cambisol, and Luvisol), with a BS from 3.2% to 18.2% and a pH (CaCl$_2$) between 3.5 and 3.8 (Table 4.1).

Table 4.1: Description of the six study sites (in order of increasing mean base saturation).

<table>
<thead>
<tr>
<th></th>
<th>Entlebuch (EN)</th>
<th>Walterswil (WA)</th>
<th>Vordemwald (VO)</th>
<th>Zofingen (ZO)</th>
<th>Niedererinsbach (NI)</th>
<th>Krauchtal (KR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates</td>
<td>8°04'N</td>
<td>7°47'N</td>
<td>7°53'N</td>
<td>7°59'N</td>
<td>8°00'N</td>
<td>7°34'N</td>
</tr>
<tr>
<td>Elev. [m a.s.l.]</td>
<td>46°58'E</td>
<td>47°06'E</td>
<td>47°16'E</td>
<td>47°18'E</td>
<td>47°23'E</td>
<td>47°02'E</td>
</tr>
<tr>
<td>Prec. [mm yr$^{-1}$]</td>
<td>1603</td>
<td>1324</td>
<td>1100</td>
<td>1186</td>
<td>1036</td>
<td>1095</td>
</tr>
<tr>
<td>Temp. [$^\circ$C yr$^{-1}$]</td>
<td>6.7</td>
<td>7.4</td>
<td>8.8</td>
<td>8.2</td>
<td>8.7</td>
<td>8.2</td>
</tr>
<tr>
<td>Vegetation type$^{1)}$</td>
<td>Abietum</td>
<td>Fagetum a</td>
<td>Abietum</td>
<td>Fagetum b</td>
<td>Fagetum b</td>
<td>Fagetum c</td>
</tr>
<tr>
<td>Soil type$^{2)}$</td>
<td>Gleysol</td>
<td>Cambisol</td>
<td>Cambisol</td>
<td>Cambisol</td>
<td>Luvisol</td>
<td>Cambisol</td>
</tr>
<tr>
<td>pH$^{3)}$</td>
<td>3.6</td>
<td>3.6</td>
<td>3.8</td>
<td>3.7</td>
<td>3.5</td>
<td>3.7</td>
</tr>
<tr>
<td>BS$^{4)}$ [%]</td>
<td>3.2</td>
<td>3.9</td>
<td>4.6</td>
<td>7.1</td>
<td>9.3</td>
<td>18.2$^{5)}$</td>
</tr>
</tbody>
</table>

$^{1)}$ according to Ellenberg and Klötzli (1972): Abietum=Bazzanio-Abietum, Fagetum a=Mellio-Fagetum, Fagetum b=Gale odorati Fagetum, Fagetum c=Gale odorati Fagetum luzuletosum

$^{2)}$ Classification according to FAO (1997): Gleysol=Distric Gleyso, Cambisol=Distric Gleyic Cambisol

$^{3)}$ Mean pH (CaCl$_2$) (not weighted between the horizons; compare Richter et al., 2007)

$^{4)}$ Mean Base Saturation (not weighted between the horizons; based on the elements content in milliequivalents per kg fine earth; compare Richter et al., 2007)

$^{5)}$ B-layer 75-100 excluded because of high lime content (compare Richter et al., 2007)

The means of the horizons of all six forest stands varied between 3.1-4.0 (pH) and 5.3-9.6% (BS) from the Ah1- to the deepest B-layer (Figure 4.1a,b). The deepest B-layer in the forest site Krauchtal (KR) was excluded because of the high lime content and in the site Vordemwald (VO) because of water logging. The deeper mineral layers of nearly all soils were only weakly compacted. The exception was in Entlebuch (EN) where soil compaction occurred at a depth of 75-100 cm. Four of the sites were classified as belonging to the alliance Luzulo-Fagion and the other two sites to the alliance Abietion, where European beech is a secondary tree species (Ellenberg & Klötzli, 1972).
Figure 4.1: Mean pH (a) and base saturation (BS) (b) of the different horizons of six soil profiles under European beech forest stands. Different letters indicate significant differences between forest sites. Probability level for the one-way analyses of variance (ANOVA): $P < 0.05$; bars indicate standard errors ($n = 6$).

**Fine root sampling**

At each site four individual European beech trees, belonging to the dominant individuals in the stands, were chosen in the vicinity of the soil profiles. Each tree was sampled once at the beginning of the summer in 2005 and twice (beginning of summer and end of summer) in 2006. Sampling times were chosen to correspond with the growth peaks and cessations of fine roots recorded by Burke & Raynal (1994). The fine roots were sampled with one soil core 1 m to the North of the tree stem. In order to minimize the effect of disturbance to the fine roots due to the previous sampling, a space of 40 cm was left between each soil core. Soil cores containing the fine roots were taken with a HUMAX soil corer (diameter of 10 cm) down to 1 m depth in four segments. Each segment consisted of a plastic tube 25 cm long with the soil left undisturbed during sampling and storage. The segments were stored in a fridge at 1°C for no longer than 1 week. All six forest sites were sampled within a period of five weeks in each sampling period.

The uppermost soil core segments, containing the Ah- and the upper B-layer, were separated into two Ah-layers, Ah1 and Ah2, and one B-layer. The organic matter content in the Ah1-layer was higher than in the Ah2-layer, so that these two layers could be clearly separated by hand (according to Walthert et al., 2004). The other three soil core segments of the B-layers were left undivided.

For the fine root (diameter < 2 mm) analyses, the soil samples were sieved and the fine roots gently washed in a 1 mm sieve with tap water. All the fine root fragments down to 0.5 cm length were collected and examined under a microscope to separate living from dead roots (bio- and necromass). Roots with turgescent cells and flexible cell walls, a white stele, and turgescent unbroken root tips were classified as living roots. Brownish, inflexible and air-
filled roots were classified as dead (Hertel & Leuschner, 2002). The fine roots were dried for 48 h at 60°C and then weighed.

In order to calculate the productivity and mortality, the differences in the biomass and necromass between these two sampling points were determined. Productivity and mortality were then calculated according to the decision matrix of Fairley & Alexander (1985), where \( B \) is biomass, \( N \) is necromass, \( P \) is productivity, and \( M \) is mortality:

\[
\begin{array}{c|c|c}
\text{Biomass increase} & |AB|> |AN| & |AB|< |AN| \\
\hline
\text{Necromass increase} & P=\Delta B+\Delta N; M=\Delta N & P=0; M=-\Delta B \\
\text{Necromass decrease} & P=\Delta B; M=0 & P=0; M=-\Delta B \\
\end{array}
\]

In the calculations we also included not statistically significant different values. Turnover was calculated as the productivity divided by the mean fine root biomass (= standing crop) per year (Gill & Jackson, 2000):

\[
\text{Turnover} = \frac{\text{Productivity}}{\text{Standing crop}} \quad [2]
\]

The mean age of the fine roots was estimated as the inverse of the turnover. These root properties at the different forest sites were compared as the sum (biomass, necromass, productivity, and mortality) and average (turnover, mean age) of the six layers at each forest site. In order to compare the biomass, necromass, productivity, and mortality per layer throughout the soil profiles, the values were expressed in dry weight per soil volume or dry weight per soil volume and year, respectively.

**Cellulose extraction**

For radiocarbon dating the structural cellulose was extracted as proposed by Gaudinski et al. (2001). In order to extract the structural cellulose, we followed the method of Böttger et al. (2007) adapted by A. Kress (personal communication) to European beech fine roots. Approximately 100-120 mg dry weight of 5-10 individual beech fine root fragments were weighed and filled into filter bags (F57, ANKOM Technology, Macedon, NY, USA). Samples of living and dead roots from each soil layer of two root diameter classes (< 1.5 mm and 1.5-2 mm, main and side branches) from the forest site Vordemwald (VO) and from Entlebuch (EN; only living roots) were used. The filter bags were closed tightly and then exposed to 0.5 l of 0.77 M sodium chlorite (NaClO₂) solution, adjusted with pure acetic acid to pH 4 and heated to 70°C, under constant stirring for 30 min in one-litre Erlenmeyers. This procedure was then repeated five times, each time with a new sodium chlorite solution. One
additional treatment was conducted over night for about 10 h. Afterwards the samples were treated with 4.25 M NaOH for 45 min at 25°C. In order to neutralize the samples, they were treated three times with 0.27 M HCl at 25°C and afterwards washed three times with hot deionised water. Finally, the filter bags containing the samples were dried at 50°C for 24 h. Then the bags were opened and the cellulose was stored in 2 ml plastic tubes. For the living roots 13-30% of the initial dry mass was left after extraction and for the dead roots 6-11%.

Radiocarbon measurement
The radiocarbon content of the structural cellulose, expressed in $\Delta^{14}C$ [‰], was analysed at the ETH/PSI AMS facility (Eidgenössische Technische Hochschule / Paul Scherrer Institute Accelerator mass spectrometry facility). In order to transfer the samples into measurable pure graphite, they had to undergo several sample preparation steps (Hajdas et al., 2004). An aliquot of 7-8 mg cellulose was weighed into small quartz glass tubes and placed, together with CuO wires and Ag powder, in larger quartz glass tubes. The tubes were evacuated and closed with a torch. After that the quartz tubes were combusted for 2 h at 950°C. For graphitisation the processing described by Vogel et al. (1984) was followed. Cobalt powder was used as a catalyst for the reaction of CO$_2$ and H$_2$, in which graphite is produced. The samples were transferred to a reactor. An oven was placed over the reactor, and the samples were heated to 625°C. The reaction was completed after 3-4 h when there was hardly any change in the gas pressure (Hajdas et al., 2004). The graphite and Co powder was then pressed into a pre-roughened surface of a Cu sample holder and analysed by AMS (Bonani et al., 1987).

![Figure 4.2: $\Delta^{14}C$ content of the atmosphere since 1950 (redrawn from Gaudinski et al., 2001). Open circles = $\Delta^{14}C$ content of living roots of VO and EN, closed circles = $\Delta^{14}C$ content of dead roots of VO.](image)

The age of the fine roots was calibrated with the online calibration program CALIBomb
(Reimer et al., 2004) using the data bases from (Levin & Hesshaimer, 2000) included in this program (see also Fig. 4.2). This program uses the $\Delta^{14}C$ and F$^{14}C$ (fraction modern) values for age estimates (Reimer et al., 2004). Negative values implied ages of about 150 y and were, therefore, excluded.

Fine root biomass, productivity, and turnover of European beech:

In order to obtain an overview of the biomass, productivity, and turnover of the fine roots in European beech forests, published data were collected. If in the published studies data about the productivity and turnover of fine roots were lacking, they were calculated with the decision matrix. In general, the published data were derived from roots collected using the soil coring method. In most cases the roots with a diameter < 2 mm were collected and classified as fine roots. Only Stober et al. (2000) considered roots with a diameter < 1 mm. The accuracy of the fine root selection varied from author to author. Hertel (1999), Hertel & Leuschner (2002) and Richter (2004) used the microscope to acquire all root fragments including fine root detritus (≥ 1 mm length). Wu (2000) included all fine root fragments of a length ≥ 1 cm. In this study we included a length ≥ 0.5 cm. No information about the minimal root fragment length is given in the publications of Cassens-Sasse (1987), Raben (1988), and Rapp (1991). All beech stands referred to in the published data were located in the temperate zone of Central Europe. The soil characteristics of these forest stands covered a broad range of pH (2.9-7.8) and BS (1.9-99.9%) values.

In order to compare the data, including the data from the present study, the values of the O-, A-, and B-horizons were averaged. In the B-horizon only the values down to 50 cm soil depth were included. The biomass, productivity, and turnover of the O- and A-horizons were compared with those of the B-horizon by averaging all values.

Statistical analyses

The fine root measurements were averaged per tree, soil layer, and sampling time point at each site. These data were used for the calculations with the decision matrix. In order to compare the fine root properties and soil depth, the data of the six sampled sites were averaged per soil layers. Soil chemical data were taken from a single soil profile per site. Per forest stand, the values of the four trees were averaged per horizon. The values included in the literature comparison were analysed as means of the O-, A-, and B-horizons. The data were subjected to one-way analysis of variance (ANOVA). The significance level was $P < 0.05$ by
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Fisher's PLSD test throughout the study unless stated otherwise. All tests were conducted with StatView 5.0 (SAS Institute, Cary, NY, USA).

Results

Fine root properties

The biomass, necromass, productivity, mortality, average turnover and estimated age of the fine roots are summarised for each of the six forest sites in Table 4.2. Only a few differences between the forest sites were found. The main exceptions were the significantly lowest biomass and productivity at WA and the significantly highest biomass at VO.

Table 4.2: Sum of biomass, necromass, productivity, mortality, and average of turnover and estimated age of each forest site (abbreviations according to Table 1). Different letters indicate significant differences between forest sites. Probability level for the analysis of variance (ANOVA): $P < 0.05$

<table>
<thead>
<tr>
<th>Site</th>
<th>Biomass [g m$^{-2}$]</th>
<th>Necromass [g m$^{-2}$]</th>
<th>Productivity [g m$^{-2}$y$^{-1}$]</th>
<th>Mortality [g m$^{-2}$y$^{-1}$]</th>
<th>Turnover [y$^{-1}$]</th>
<th>Estimated age [y]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EN</td>
<td>998 ab</td>
<td>235 a</td>
<td>490 ab</td>
<td>406 a</td>
<td>1.45 a</td>
<td>0.69 a</td>
</tr>
<tr>
<td>WA</td>
<td>642 b</td>
<td>244 a</td>
<td>247 b</td>
<td>245 a</td>
<td>1.15 a</td>
<td>0.87 a</td>
</tr>
<tr>
<td>VO</td>
<td>1250 a</td>
<td>344 a</td>
<td>551 a</td>
<td>368 a</td>
<td>0.82 a</td>
<td>1.22 a</td>
</tr>
<tr>
<td>ZO</td>
<td>1041 ab</td>
<td>203 a</td>
<td>521 ab</td>
<td>288 a</td>
<td>1.12 a</td>
<td>0.89 a</td>
</tr>
<tr>
<td>NI</td>
<td>868 ab</td>
<td>281 a</td>
<td>374 ab</td>
<td>330 a</td>
<td>0.76 a</td>
<td>1.31 a</td>
</tr>
<tr>
<td>KR</td>
<td>847 ab</td>
<td>286 a</td>
<td>408 ab</td>
<td>273 a</td>
<td>0.70 a</td>
<td>1.43 a</td>
</tr>
</tbody>
</table>

The change in the fine root properties with soil depth showed mainly a decrease in values with depth (Figure 4.3a-f). The bio- and necromass decreased significantly with depth from 2.8 to 0.5 g l$^{-1}$ and 0.8 to 0.1 g l$^{-1}$, respectively. The necromass was about one third of the biomass in each layer. The productivity and mortality of the roots also decreased with depth from 1.0 to 0.2 g l$^{-1}$ and 1.1 to 0.3 g l$^{-1}$, respectively. These values were almost equal, which implies that these forest sites were most likely in equilibrium. The turnover was significantly lower in the B-layer located between 25 and 75 cm than in the Ah-layer (from 1.9 to 0.7 y$^{-1}$). In the deepest layer the turnover again increased significantly (2.3 y$^{-1}$). The estimated age of the fine roots was highest in the upper three layers of the B-layer and lowest in the Ah-layers and the deepest B-layer (from 0.8 to 1.7 y).
Figure 4.3: Mean biomass (a) and necromass (b), productivity (c) and mortality (d), and turnover (e) and estimated age (f) of fine roots in six European beech forest stands according to the soil depth of each layer. Different letters indicate significant differences between forest sites. Probability level for the one-way analyses of variance (ANOVA): \( P < 0.05 \); bars indicate standard errors (\( n = 6 \)).

The age of fine roots measured with radiocarbon

The \( \Delta^{14} \text{C} \) values of the radiocarbon measurements and the corresponding age of fine roots of VO and EN are given in Table 4.3. The age of the living fine roots in both forest sites increased markedly with soil depth (VO: from 6 to 26 y; EN: from 5 to 25 y). The fine roots of EN were slightly younger, except in the deepest layer (75-100 cm) where they were very young (3 y), probably due to soil compaction. The comparison of the two diameter classes of the living roots showed almost no differences between these classes, when the deviation of ±1 y implied in the method was taken into consideration. The ages of the living and dead roots at the site VO also did not seem to differ distinctly. Only the age of the dead fine roots in the deepest layer were lower than the living fine roots.
Table 4.3: $\Delta^{14}C$ values and corresponding age (±1y) of living and dead fine roots at Vordemwald and Entlebuch at different soil depths and two root diameter classes. Fine roots were sampled at the beginning of Summer in 2005, dead fine roots from Entlebuch were not measured.

<table>
<thead>
<tr>
<th>Soil depth [cm]</th>
<th>Root Ø [mm]</th>
<th>Living roots</th>
<th>Dead roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Vordemwald (VO)</td>
<td>Entlebuch (EN)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\Delta^{14}C$ [%]</td>
<td>Age [y]</td>
</tr>
<tr>
<td>Ah1 0-4</td>
<td>&lt; 1.5</td>
<td>100.7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>87.7</td>
<td>6</td>
</tr>
<tr>
<td>Ah2 4-12</td>
<td>&lt; 1.5</td>
<td>106.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>158.3</td>
<td>15</td>
</tr>
<tr>
<td>B 12-25</td>
<td>&lt; 1.5</td>
<td>190.5</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>166.3</td>
<td>17</td>
</tr>
<tr>
<td>B 25-50</td>
<td>&lt; 1.5</td>
<td>150.4</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>143.3</td>
<td>14</td>
</tr>
<tr>
<td>B 50-75</td>
<td>&lt; 1.5</td>
<td>209.1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>249.7</td>
<td>24</td>
</tr>
<tr>
<td>B 75-100</td>
<td>&lt; 1.5</td>
<td>305.4</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>1.5-2</td>
<td>282.8</td>
<td>24</td>
</tr>
</tbody>
</table>

The age of the fine roots (averaged between the two diameter classes) measured with radiocarbon and those estimated from the turnover calculation were positively correlated with each other (Figure 4.4), and the estimated age was about four times lower than the measured age.

Figure 4.4: Relationship of estimated age versus radiocarbon ($^{14}C$) age of living European beech fine roots from Vordemwald and Entlebuch. The radiocarbon age of both fine root diameter classes was averaged. The value marked with (1) was excluded from calculations. Probability level for the analysis of variance (ANOVA): *** = $P < 0.001$.

The data point of the 75-100 cm layer in VO was excluded as we assumed that it was difficult to obtain correct age estimates with the sequential coring and radiocarbon method due to possible water logging in the lower profile. This can also be seen in Table 4.3, as the radiocarbon age of the dead fine roots in VO was younger than the age of the living fine roots.
This indicates a high turnover, but the living fine roots were very old, which shows the ambivalence of this value.

Comparison of fine root properties of different European beech stands in Central Europe

The comparison of data from the literature with the data from the present study showed a high variability of each fine root property (Table 4.4a, 4.4b). The biomass ranged between 9.1 and 526.4 g m\(^{-2}\), the productivity between 6.6 and 408.1 g m\(^{-2}\), and the turnover between 0.1 and 14.1 y\(^{-1}\). The biomass and productivity values were almost the same in the different horizons, but the turnover values were about twice as high in the O- and A-horizons (Table 4.4a) as in the B-horizons (Table 4.4b).

Table 4.4a: Biomass, productivity, and turnover of European beech fine roots in O- and A-horizons from published studies conducted with sequential coring together with horizon, BS, and pH in alphabetical order.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Horizon</th>
<th>pH</th>
<th>BS</th>
<th>Biomass</th>
<th>Productivity</th>
<th>Turnover</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Göttinger Wald</td>
<td>O</td>
<td>5.9</td>
<td>96.2</td>
<td>9.1</td>
<td>32.2</td>
<td>3.5</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Hann. Münden</td>
<td>O</td>
<td>4.8</td>
<td>61.8</td>
<td>182.9</td>
<td>225.4</td>
<td>1.2</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Harste</td>
<td>A 0-5</td>
<td>3.6</td>
<td>34.3</td>
<td>77.3</td>
<td>82.4</td>
<td>1.1*</td>
<td>Cassens-Sasse (1987)</td>
</tr>
<tr>
<td>Hils</td>
<td>O</td>
<td>3.3</td>
<td>7.0</td>
<td>74.7</td>
<td>56.2</td>
<td>0.8*</td>
<td>Raben (1988)</td>
</tr>
<tr>
<td>Lüneburger Heide</td>
<td>O</td>
<td>3.1</td>
<td>-</td>
<td>526.4</td>
<td>282.5</td>
<td>3.6</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Lüneburger Heide</td>
<td>O</td>
<td>3.1</td>
<td>-</td>
<td>95.0</td>
<td>305.0</td>
<td>3.1</td>
<td>Hertel &amp; Leuschner (2002)</td>
</tr>
<tr>
<td>Solling</td>
<td>O</td>
<td>3.3</td>
<td>-</td>
<td>319.3</td>
<td>172.8</td>
<td>14.1</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Solling</td>
<td>A 0-5</td>
<td>3.0</td>
<td>6.0</td>
<td>51.7</td>
<td>90.8</td>
<td>1.8*</td>
<td>Rapp (1991)</td>
</tr>
<tr>
<td>Solling</td>
<td>O</td>
<td>4.4</td>
<td>52.9</td>
<td>185.8</td>
<td>323.5</td>
<td>1.7</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Solling</td>
<td>O</td>
<td>4.2</td>
<td>28.1</td>
<td>86.0</td>
<td>-</td>
<td>-</td>
<td>Wu (2000)</td>
</tr>
<tr>
<td>Voremberg</td>
<td>O</td>
<td>5.7</td>
<td>96.4</td>
<td>12.0</td>
<td>42.3</td>
<td>3.5</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Ziegelroader Forst</td>
<td>O</td>
<td>3.8</td>
<td>-</td>
<td>18.3</td>
<td>6.6</td>
<td>1.9</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Switzerland</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entlebuch (EN)</td>
<td>A 0-12</td>
<td>3.4</td>
<td>1.9</td>
<td>126.0</td>
<td>58.0</td>
<td>4.1</td>
<td>This study</td>
</tr>
<tr>
<td>Krauchtal (KR)</td>
<td>A 0-10</td>
<td>2.9</td>
<td>44.3</td>
<td>113.7</td>
<td>55.6</td>
<td>1.3</td>
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</tr>
<tr>
<td>Niedererlinsbach (NI)</td>
<td>A 0-8</td>
<td>3.2</td>
<td>16.5</td>
<td>84.8</td>
<td>76.6</td>
<td>5.8</td>
<td>This study</td>
</tr>
<tr>
<td>Vordemwald (VO)</td>
<td>A 0-12</td>
<td>3.5</td>
<td>3.1</td>
<td>298.2</td>
<td>176.9</td>
<td>1.5</td>
<td>This study</td>
</tr>
<tr>
<td>Walterswil (WA)</td>
<td>A 0-10</td>
<td>2.9</td>
<td>6.0</td>
<td>55.0</td>
<td>66.3</td>
<td>1.2</td>
<td>This study</td>
</tr>
<tr>
<td>Zofingen (ZO)</td>
<td>A 0-11</td>
<td>3.1</td>
<td>12.1</td>
<td>152.9</td>
<td>105.7</td>
<td>3.1</td>
<td>This study</td>
</tr>
</tbody>
</table>

* calculated on the basis of data in the literature

Mean in A/O-horizon

|                  | Mean   | 33.3  | 133.7 | 123.5 | 3.0 |

65
Table 4.4b: Biomass, productivity, and turnover of European beech fine roots in B-horizons from published studies conducted with sequential coring together with horizon, BS, and pH in alphabetical order.

<table>
<thead>
<tr>
<th>Site name</th>
<th>Horizon</th>
<th>pH</th>
<th>BS</th>
<th>Biomass</th>
<th>Productivity</th>
<th>Turnover</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Germany</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Göttinger Wald</td>
<td>B 0-15</td>
<td>6.8</td>
<td>97.8</td>
<td>140.3</td>
<td>32.1</td>
<td>0.5</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Göttinger Wald</td>
<td>B 0-20</td>
<td>6.5</td>
<td>99.9</td>
<td>151.2</td>
<td>211.7</td>
<td>1.4</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Göttinger Wald</td>
<td>B 0-20</td>
<td>7.8</td>
<td>97.4</td>
<td>120.7</td>
<td>330.3</td>
<td>0.7</td>
<td>Wu (2000)</td>
</tr>
<tr>
<td>Hann. Münden</td>
<td>B 0-20</td>
<td>4.3</td>
<td>18.3</td>
<td>136.4</td>
<td>408.1</td>
<td>2.9</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Harste</td>
<td>B 5-40</td>
<td>4.1</td>
<td>42.9</td>
<td>113.2</td>
<td>92.2</td>
<td>0.8*</td>
<td>Cassens-Sasse (1987)</td>
</tr>
<tr>
<td>Hils</td>
<td>B 0-70</td>
<td>3.6</td>
<td>6.0</td>
<td>162.2</td>
<td>110.7</td>
<td>0.7*</td>
<td>Raben (1988)</td>
</tr>
<tr>
<td>Lüneburger Heide</td>
<td>B 0-5</td>
<td>2.9</td>
<td>8.2</td>
<td>270.3</td>
<td>170.3</td>
<td>2.2</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Lüneburger Heide</td>
<td>B 0-5</td>
<td>2.9</td>
<td>8.2</td>
<td>21.0</td>
<td>79.0</td>
<td>3.8</td>
<td>Hertel &amp; Leuschner (2002)</td>
</tr>
<tr>
<td>Solling</td>
<td>B 0-5</td>
<td>2.9</td>
<td>9.4</td>
<td>276.0</td>
<td>287.7</td>
<td>8.9</td>
<td>Hertel (1999)</td>
</tr>
<tr>
<td>Solling</td>
<td>B 5-40</td>
<td>3.7</td>
<td>3.7</td>
<td>106.2</td>
<td>86.1</td>
<td>0.8*</td>
<td>Rapp (1991)</td>
</tr>
<tr>
<td>Solling</td>
<td>B 0-20</td>
<td>3.8</td>
<td>14.4</td>
<td>37.3</td>
<td>121.8</td>
<td>3.1</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Solling</td>
<td>B 0-40</td>
<td>4.2</td>
<td>5.6</td>
<td>28.3</td>
<td>114.8</td>
<td>0.3</td>
<td>Wu (2000)</td>
</tr>
<tr>
<td>Voremberg</td>
<td>B 0-20</td>
<td>5.7</td>
<td>88.5</td>
<td>94.5</td>
<td>118.2</td>
<td>1.3</td>
<td>Richter (2004)</td>
</tr>
<tr>
<td>Ziegelroader Forst</td>
<td>B 0-10</td>
<td>3.5</td>
<td>45.9</td>
<td>148.9</td>
<td>126.0</td>
<td>5.0</td>
<td>Hertel (1999)</td>
</tr>
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<td><strong>France</strong></td>
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<tr>
<td>Aubure</td>
<td>B 0-30</td>
<td>4.0</td>
<td>9.2</td>
<td>26.0</td>
<td>137.0</td>
<td>2.4</td>
<td>Stober et al. (2000)</td>
</tr>
<tr>
<td><strong>Switzerland</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Entlebuch (EN)</td>
<td>B 12-50</td>
<td>3.6</td>
<td>2.2</td>
<td>249.0</td>
<td>108.3</td>
<td>0.5</td>
<td>This study</td>
</tr>
<tr>
<td>Krauchtal (KR)</td>
<td>B 10-50</td>
<td>4.2</td>
<td>3.6</td>
<td>192.5</td>
<td>100.7</td>
<td>0.7</td>
<td>This study</td>
</tr>
<tr>
<td>Niedererlinsbach (NI)</td>
<td>B 8-50</td>
<td>3.5</td>
<td>8.6</td>
<td>222.1</td>
<td>110.8</td>
<td>1.7</td>
<td>This study</td>
</tr>
<tr>
<td>Vordenwald (VO)</td>
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<td>4.0</td>
<td>3.5</td>
<td>221.6</td>
<td>85.2</td>
<td>0.8</td>
<td>This study</td>
</tr>
<tr>
<td>Walterswil (WA)</td>
<td>B 10-50</td>
<td>3.7</td>
<td>2.4</td>
<td>216.3</td>
<td>114.7</td>
<td>0.5</td>
<td>This study</td>
</tr>
<tr>
<td>Zofingen (ZO)</td>
<td>B 11-50</td>
<td>3.9</td>
<td>4.1</td>
<td>191.7</td>
<td>152.9</td>
<td>1.0</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Mean in B-horizon</strong></td>
<td>4.2</td>
<td>22.4</td>
<td>163.7</td>
<td>139.7</td>
<td>1.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* calculated on the basis of data in the literature

No relationships between pH or BS and the fine root biomass and productivity were observed (data not shown). The turnover of fine roots in the B-horizons was negatively correlated to the pH and slightly negatively related to the BS (Figure 4.5).
Discussion

In this study we examined the growth, turnover, and age of fine roots in European beech forests growing in very acidic soils (pH < 4.5) with a low BS (<15%). From earlier studies it could be expected that the fine roots of forests growing under these conditions would be affected by the increased concentration of phytotoxic Al$^{3+}$ in the soil solution and by lower nutrient availability (Graf Pannatier et al., 2004; Cronan & Grigal, 1995). Additionally, it has often been reported that the fine root density and mass (bio- and necromass) is higher in forests growing on acidic and nutrient-poor sites than in forests growing on basic and nutrient-rich sites (Godbold et al., 2003; Leuschner & Hertel, 2003; Finer et al., 2007).

Our results, however, do not show any relation between the standing bio- and necromass of fine roots and pH or BS. Similarly the productivity and mortality of fine roots were not related to these soil parameters. This may be due to the very small range of variability in the soil parameters of the data set in this study. However, together with the data from the literature also no trends could be detected between pH and BS related to the biomass and productivity of European beech fine roots. This goes against the findings of Leuschner & Hertel (2003) and Finer et al. (2007). Nevertheless, the examined fine root properties did vary with the depth of the soil profile as they decreased with increasing depth. This might be due to a decrease in soil temperature, soil compaction, and/or oxygen availability (Jentschke et al., 2001). The root mass and productivity in the present study were, however, especially reduced at the border between the humus rich Ah-layers and B-layers. Moreover Jentschke et al.
Chapter III

(2001) and Godbold et al. (2003) observed a reduced subsoil fine root mass in acidic and nutrient-poor soils. This change in rooting pattern is probably due to a change in carbon partitioning in a larger root system and, therefore, a better nutrient acquisition in layers which are more nutrient rich (Jentschke et al., 2001).

The change in biomass due to adverse chemical conditions in the soil is, in the literature, mostly attributed to an enhanced mortality and, probably, turnover as well (Leuschner & Hertel, 2003; Leuschner et al., 2004). In this study the variability of the turnover in the B-layers depending on the soil’s chemical status became apparent when the data from the present study was compared with the published data. It could be shown that the turnover of beech fine roots was slightly negatively affected by low pH in the soil, which suggests that fine roots under critical soil conditions may have to be reproduced more often as they die earlier (Leuschner et al., 2004). This coherence of high turnover and low pH was also found in the Norway spruce stands described in Godbold et al. (2003). In the present study a negative trend in the fine root turnover was also obvious with decreasing BS. This suggests that fine roots are affected negatively by low BS (Cronan & Grigal, 1995). Vanguelova et al. (2005) also observed that the age of fine roots in soils with a low BS decreased due to nutrient deficiencies (low BS) in a Scots pine stand.

Not only the pH and BS influenced the turnover and age of the fine roots but also the depth of the soil profile. The age measured on the basis of the turnover as well as with the radiocarbon dating method was higher in the B-layer (see also Joslin et al., 2006). The correlation between the ages determined with these two methods was positive and highly significant, but the radiocarbon data estimated the age to be about 4 times older than the classical method of sequential coring. This difference might be due to the extracting procedure for the structural carbon. Only 6-30% of the initial dry weight was left after the cellulose extraction. During this procedure it is most likely that the cellulose of the very thin and very young fine roots was probably dissolved by the sodium chlorite, as stems and roots of woody species contain about 60% of structural carbon (cellulose, hemicellulose, and pectin; Poorter & Villar, 1997). Comparable values for each of the two methods were found in the literature, although there were discrepancies. The turnover conducted by sequential coring from published data was comparable with the values measured in the present study. In addition, other methods, such as the minirhizotron method, investigated the age of European beech fine roots to be about 1.4 years old (turnover 0.71±0.17 y⁻¹; Mainiero & Kazda, 2006) and those of spruce in the mineral soil in northern Sweden to be around 1 years old (Andersson & Majdi, 2005). With radiocarbon dating Gaudinski et al. (2001) estimated
comparable ages for tree fine roots in temperate forests in the eastern United States (4-6±1-2 years old at 0-15 cm depth and 15-18±1-2 years old at 15-30 cm depth). Tierney & Fahey (2002) found fine roots about 4.5±1 years old in the organic horizon of a northern hardwood forest.

To explain the differences in age obtained with the various methods, Joslin et al. (2006) made following suggestions: They found in a $^{14}$C-labeled hardwood forest living fine roots aged 3.8±2.4 years together with dead fine root material just a few month old. Therefore, it seem that some fine roots live for several years but others live only for some month. On the basis of this observation they hypothesised that the fine root system of trees consists of pools of fine roots with different ages. One pool is very dynamic, “short-lived” (< 1 year) that contributed to minirhizotron observations. The other pool consists of older fine roots, which can be seen as “long-lived” (> 1 year) fine roots measured by radiocarbon. For example, Andersson & Majdi (2005) showed that the seasonal timing of production might influence fine root age. Fine roots produced in spring before the trees developed their leaves have shorter life spans than those produced later. This might be due to the fine roots produced earlier having lower carbohydrate reserves. The radiocarbon method probably overestimates the age of fine roots at least in the northern hardwood forest studied by Tierney & Fahey (2002). This method does not take into account the highly heterogeneous age distribution in fine root systems. Trumbore & Gaudinski (2003) and Majdi et al. (2005) both pointed out that the “long-lived” fine roots affect age estimates by radiocarbon because there is a heterogeneous age distribution within one fine root population. Despite the findings in literature in our study, the dead fine roots seemed to be similar in age to the living roots, except at the depth of 75-100 cm, where the age of the dead roots was distinctly younger than that of the living roots. Our data on dead fine roots indicates that they have a decomposition rate of about one year as they all seem to be almost the same age as the living fine roots. It is not possible to specify the exact time of death, so the decomposition could be very slow. Evidence for a slow decomposition rate is provided in Heim & Frey (2004), who found about half of the beech fine roots in a litterbag experiment in VO remained after the first year of decomposition. However, if the hypothesis of Joslin et al. (2006) is correct, there should have been at least some young dead fine roots.

Both methods to measure the turnover and age of fine roots have their advantages and disadvantages. The sequential coring method cannot be used to observe all changes in fine root dynamics as it depends on punctual observations of the fluent processes of production and mortality of fine roots (Majdi et al., 2005). Nevertheless, this method provides reliable
data about the distribution of fine roots and total biomass and necromass related to soil volume (Hertel & Leuschner, 2002). Additionally, the sequential coring method is often used in several studies to compare and discuss the recorded data. The radiocarbon method provides reliable data on the age of the carbon used to build the roots based on the assumptions that fine roots use recently fixed carbon for growth and that fine root populations fit in a steady-state homogeneous pool model (Gaudinski et al., 2001). However, as reported above, it is likely, that fine root populations have a heterogeneous age distribution. Moreover, the assumption that fine roots use recently fixed carbon for growth is discussed. Carbon may be added from storage reserves, via secondary growth, from carbon stored in the tree for some time or mycorrhizal symbiosis (Tierney & Fahey, 2002, Trumbore et al., 2006). However, Trumbore et al. (2006) observed that the known age of fine roots from root screens fitted with the radiocarbon age.

It can be stated, in conclusion, that the turnover and age of fine roots vary greatly with depth and soil conditions. In our study the turnover depended on the soil depth, ranging between 1.6 (Ah1-layer) and 0.7 y\(^{-1}\) (B50-75 cm layer). The radiocarbon age ranged between 5-26 y in the same layers. In the present study and in published studies of European beech forests, the variation of the turnover was between 3.0 (O- and A-horizon) and 1.7 y\(^{-1}\) (B-horizon). The turnover varied depending on the pH from 2.6 y\(^{-1}\) (pH 2-5) and 1.8 y\(^{-1}\) (pH 5-8). This variability should be taken into account in models such as the Biome-BGC model that considers a turnover for European beech of 1 (Pietsch et al., 2005) and 1.023 (Cienciala & Tatarinov, 2006; Tatarinov & Cienciala, 2006), as it influences carbon fluxes and budgets belowground.

Methods to observe “long-lived” and “short-lived” fine root pools are needed. Trumbore & Gaudinski (2003) suggest comparing the results of different methods to approach the real age distribution of the fine roots. Tierney & Fahey (2002) recommend, with respect to these two pools observing at least fine roots in minirhizotrons for longer than 2 years. Majdi et al. (2005) proposed using new methods like functional genomics together with traditional field experiments to answer the question about the turnover and age of fine roots. In order to have reliable data on fine root turnover and age, several methods should be applied and correlated with each other to identify the effective age of the fine root systems.
Chapter IV
Root growth and physiology of European beech (*Fagus sylvatica* L.) seedlings are affected by very low base saturation
Summary

To assess the potential effects of Al toxicity on the roots of young European beech (Fagus sylvatica L.), seedlings were sown in soil monoliths taken from the Ah- and B-horizons of forest soils with very low base saturation (1.2-6.5%). The Ah-horizons offered a larger supply of exchangeable cationic nutrients than respective the B-horizons. The monoliths were placed in the greenhouse. After eight weeks of growth under optimal soil moisture conditions, the seedlings were further treated for 14 days under drought conditions. The root growth dynamics were observed in rhizoboxes containing soils from the Ah- and B-horizons. The concentrations of labile Al$^{3+}$, base cations, and nitrate in the soil solution, as well as element concentrations in the root tissue were compared with above- and below-ground growth parameters, root growth dynamics, and root physiological parameters. There was no strong evidence that beech seedling roots suffered from the high concentrations of Al$^{3+}$ in the soil solution. However, there were indications that root physiological parameters such as O$_2$-consumption decreased and callose concentration increased in soils with a base saturation < 3%. Seedlings growing in the Ah-horizons had a higher relative growth rate of the shoot, specific root length, and length and branching increments, but a lower root/shoot ratio and root branching frequency than those growing in the B-horizons. We conclude that these differences in growth patterns were most likely due to greater nutrient availability in the Ah-horizons.

Introduction

Acid depositions have accelerated the acidification process of forest soils for many years, although their rates declined in Europe through the 1980s and 1990s (Blaser et al. 1999, Jönsson et al. 2003, Graf Pannatier et al. 2004). This enhanced acidification can lead to an altered habitat for forest trees and, therefore, to ecological consequences for forest ecosystems. Acidification may result in a decreased rejuvenation and seedling growth of forest trees (Ljungström and Stjernquist 1993). Soil acidification below a pH of 5 is accompanied, on the one hand, by increased weathering of Al from soil minerals and, on the other hand by a decrease in base saturation (BS) in the soil matrix as Al competes with essential base cations (BC = K, Mg, Ca) on the soil exchanger (Jönsson et al. 2003, Walthert et al. 2004). Mineral soils are more likely to have a high free Al content in the soil solution as
they contain smaller concentrations of dissolved organic carbon (DOC) that can form stable complexes with Al (Jönsson et al. 2003, Parker 2005, Lange et al. 2006).

The most important rhizotoxic Al species in the soil solution is free Al\(^{3+}\) as it hampers root growth by inhibiting root-cell elongation and cell division (Matsumoto 2000, Kochian et al. 2005). These damages result in a formation of short and stubby roots, die back of root tips, decline in root elongation, and lateral root formation stops (Godbold et al. 1988, Göransson and Eldhuset 1991, Hirano and Hijii 2000). Several studies under controlled conditions in hydroponics using tree seedlings showed that their root growth and development have a distinct dependency on free Al\(^{3+}\) in the nutrient solution (e.g. Godbold and Jentschke 1998, van Schöll et al. 2004, Hirano et al. 2007, Vanguelova et al. 2007b). The lower concentrations of BC in soil solution and the degraded root system mainly lead to deficiencies in essential elements and elevated Al contents in the root tissues (Göransson and Eldhuset 1995, Zysset et al. 1996). Both low BC and high Al\(^{3+}\) concentrations in the soil solution could potentially affect the root morphology (Godbold et al. 1988, Clemensson-Lindell and Persson 1995, Godbold et al. 2003). Moreover low BS in the soil solid phase could affect the root physiology and chemistry (Hirano and Brunner 2006, Richter et al. 2007b). Physiological properties such as the roots’ respiratory activity (Comas et al. 2000, Richter et al. 2007a,b), their reduced uptake of essential elements (Göransson and Eldhuset 1991, Nygaard and de Wit 2004, Vanguelova et al. 2005, Richter et al. 2007b) and the callose concentration in the root apices (Hirano et al. 2004, 2006) can serve as indicators of the health status of roots.

The changed root morphology, physiology, and chemistry may also affect shoot growth. Baligar and Fageria (2005) showed that aboveground biomass development was altered due to Al\(^{3+}\) toxicity and the leaching of BC from the soil. An adverse soil chemical status can lead to changes in biomass partitioning between the shoot and the root as different nutrient availabilities may require different nutrient acquisition strategies (Ljungström and Stjernquist 1993, Coleman and McConnaughay 1995). A larger root system is able to acquire more nutrient elements from nutrient-poor soils than a smaller root system.

The relation between root and shoot properties of European beech (\textit{Fagus sylvatica} L.) seedlings to levels of high free Al\(^{3+}\) and low BC concentrations in the soil solution has been poorly investigated. According to Leuschner et al. (2006), the growth of European beech can be limited with a BS below 3.3%. It could be shown that the growth of beech seedlings was inhibited due to high Al concentrations in hydroponics (Thornton et al. 1989). Neizke and Runge (1985) had similar results for acidic soils. Rost-Siebert (1983) also showed that a low Ca/Al molar ratio in the nutrient solution of hydroponic experiments causes Al toxicity
symptoms. Additional factors, e.g. drought, can also enhance the toxicity. Vangelova et al. (2005) observed that the fine root growth of Scots pine was not affected by adverse chemical soil conditions if the soil was sufficiently moist to ensure adequate root growth.

In our study, we intended to test the hypotheses that (i) biomass production, nutrient status and the vitality of European beech seedlings are related to increasing Al\(^{3+}\) concentration and decreasing Ca/Al molar ratios in the soil solution, as well as to decreasing BS in the soil solid phase; and (ii) drought stress enhances the negative impact of Al\(^{3+}\) in the soil solution on root health. To this end, the root and shoot parameters of beech seedlings grown in soil monoliths with low BS between 1 and 7% should be investigated as well as the reactions of the seedlings grown under drought stress.

**Materials and methods**

**Forest sites**

Soils were sampled at four forest sites on the Swiss Plateau. The forest sites were chosen based on information from the WSL (Swiss Federal Institute for Forest, Snow and Landscape Research) data-base on soil profiles in forest stands. For methodical details, see Walthert et al. (2004) and Graf Pannatier et al. (2004). The site Triengen (TR; 47°15′N, 8°06′E) belongs to the potential natural vegetation type *Millio-Fagetum* with European beech (*Fagus sylvatica* L.) as the dominant tree species, whereas Vordemwald (VO; 47°16′N, 7°53′E), Meggen (ME; 47°03′N, 8°22′E), and Walchwil (WA; 47°07′N, 8°33′E) belong to the *Bazzanio-Abietum* (Ellenberg and Klötzli 1972). In ME and WA European beech is absent, although some adult beech trees occur in neighbouring forest stands. In VO, European beech is secondary among Silver fir (*Abies alba* Mill.).

The soil type of all sites was a Gleyic Cambisol (distric), with a pH (CaCl\(_2\)) varying between 3.0 and 3.4 in the Ah- and 3.8 and 4.2 in the mineral B-horizons (Table 5.1). The BS ranged from 2.4% to 6.5% in the Ah- and 1.2% to 5.2% in the B-horizons and, therefore, the BC content in the soil matrix was 3-14 times higher in the Ah-horizons than in the corresponding B-horizons. The Al content was higher in the B-horizons, but the BC/Al molar ratio did not differ much between the horizons (Table 5.1). The C and N contents were also about 3 times higher in the Ah-horizons than in the B horizons, but the C/N ratio did not vary within a profile.
Table 5.1: Chemical characterisation of the soils at the four forest sites from the soil profiles in terms of: pH, base saturation (BS), organic carbon content (C), total nitrogen content (N), C/N ratio, basic cations content (Mg, K, and Ca), aluminium content, total basic cations content (BC: sum of Mg, K, and Ca), and BC/Al molar ratio of the Ah- and B-horizons.

<table>
<thead>
<tr>
<th>Site</th>
<th>pH</th>
<th>BS</th>
<th>C</th>
<th>N</th>
<th>C/N</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Al</th>
<th>BC</th>
<th>BC/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ah-horizon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triengen (TR)</td>
<td>3.4</td>
<td>6.5</td>
<td>4.51</td>
<td>0.23</td>
<td>19.6</td>
<td>6.6</td>
<td>0.4</td>
<td>1.4</td>
<td>54</td>
<td>8.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Vordemwald (VO)</td>
<td>3.3</td>
<td>6.5</td>
<td>6.22</td>
<td>0.31</td>
<td>20.0</td>
<td>3.9</td>
<td>0.9</td>
<td>2.0</td>
<td>107</td>
<td>6.8</td>
<td>0.06</td>
</tr>
<tr>
<td>Meggen (ME)</td>
<td>3.0</td>
<td>2.4</td>
<td>11.84</td>
<td>0.61</td>
<td>19.3</td>
<td>16.8</td>
<td>1.2</td>
<td>3.9</td>
<td>143</td>
<td>21.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Walchwil (WA)</td>
<td>3.3</td>
<td>2.7</td>
<td>4.91</td>
<td>0.43</td>
<td>11.4</td>
<td>0.8</td>
<td>0.6</td>
<td>1.8</td>
<td>95</td>
<td>3.2</td>
<td>0.03</td>
</tr>
<tr>
<td>B-horizon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triengen (TR)</td>
<td>4.2</td>
<td>5.2</td>
<td>0.81</td>
<td>0.05</td>
<td>16.0</td>
<td>0.9</td>
<td>0.4</td>
<td>0.5</td>
<td>35</td>
<td>1.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Vordemwald (VO)</td>
<td>3.9</td>
<td>3.1</td>
<td>1.98</td>
<td>0.10</td>
<td>20.0</td>
<td>1.0</td>
<td>0.4</td>
<td>0.7</td>
<td>63</td>
<td>2.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Meggen (ME)</td>
<td>3.8</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
<td>0.2</td>
<td>0.6</td>
<td>100</td>
<td>1.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Walchwil (WA)</td>
<td>3.8</td>
<td>1.2</td>
<td>1.54</td>
<td>0.13</td>
<td>11.5</td>
<td>0.3</td>
<td>0.2</td>
<td>0.4</td>
<td>76</td>
<td>0.9</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Growth of beech seedlings in soil monoliths

Soil samples were collected in monoliths from the Ah-horizons and the B-horizons within a distance of 3 m away from the soil profile in each forest stand. Soil monoliths were taken with a HUMAX soil corer (Ø 5 cm, 25 cm depth) in transparent plastic tubes remaining undisturbed during sampling.

For each forest stand 24 monoliths were taken. Each plastic tube with the soil monolith was then cut into halves about 12 cm in length corresponding to the Ah- and B-horizons, and covered with aluminium foil to prevent interference from daylight. Ten European beech seeds (seed collection WSL, seeds from forest stand Auw near Falk, Switzerland) were sown in the soil monoliths and placed in the greenhouse (17-24°C, 70% humidity, daylight during spring and early summer with 50% shading). Four soil monoliths of each horizon and field site were weighed afterwards two to three times per week in order to record the water loss by evapotranspiration. In order to keep the moisture constant throughout the experiment, the monoliths were then watered with the same amount of deionised water they had lost since the last recording.

After ten days the beech seedlings germinated. Due to the very low germination rate of the beech seedlings (30-40%), germinating seedlings from unused soil samples were additionally planted directly into the soil monoliths. Ten days after the first germination each monolith thus contained two to three seedlings. After eight weeks of growth, the first third of the
monoliths (eight monoliths from each horizon and forest site) were harvested (day 0 of control and of drought treatment). After this first harvest, one half of the remaining pots were further watered (controls) and for the other half of the pots, watering was stopped in order to apply a drought stress to the seedlings. After one week of treatments (control, drought), the second third of the monoliths were harvested (7 days of treatments), and after one additional week the last third were harvested (14 days of treatments).

For seedling root analyses, the plants were carefully taken out of the soils, washed under tap water, and placed in iced water for not longer than 30 min until they were used for further measurements. The roots of one seedling of each monolith were taken to measure the O$_2$-consumption, to determine the element concentrations in the root tissue, and to analyse their morphology. The roots of the second seedling were taken to measure the callose concentration in the root apices. Root apices, 1 cm in length, were excised from the second seedling and stored in 96% (v/v) ethanol to determine callose concentration. The above-ground part of the beech seedlings was cut into different sections according to growth stadiums. The seedlings were divided into primary growth (cotyledons and first two leaves) and secondary growth (growth after the first two leaves). Each part was measured for its morphology and dry weight (see below).

After the plant harvest, the fresh and dry weights of the soil monoliths were measured in order to identify the % of the water content, the % of water loss, and the dry weight density [g cm$^{-3}$] of the soil. The water content of the soils was calculated by subtracting the dry weight (after four days drying at 60°C) from the fresh weight.

Soil solution sampling and soil solution analysis in the soil monoliths

Soil solution samples were taken from four monoliths for each forest site, horizon, and treatment at the beginning of the experimental treatment (day 0). In order to obtain the soil solution, two micro suction cups were positioned in each plastic tube containing the soil monolith. Cups were installed opposite each other, one 3 cm below the upper rim and one 3 cm above the bottom of the monolith. One millilitre syringes (Norm-Ject, Henke Sass Wolf, Tuttlingen, Germany) connected to the micro suction cups were used to collect the soil solution. A vacuum was applied once by pulling the piston of the syringe to its end position and fixing the piston with adhesive tape (Dessureault-Rompré et al. 2006). Ceramic capillaries (pure aluminium oxide produced by PI ceramic, Lederhose, Germany) were used for the micro suction cup materials (Göttlein et al. 1996). The suction cup materials were conditioned by rinsing them with up to 10 ml of autoclaved soil solution obtained from a
batch soil-water extract (soil/water ratio 1/10) to minimise adsorption of metals. Soil solutions were sampled three times for 24 h during a period of three days. After these 3 days the yield of soil solution in the drought treatment was zero or dare to zero. Soil solution sampling was then stopped and the yield of the third sampling date was analysed (day 3).

Solutions were analysed for major cations, nitrate, and phosphate by capillary electrophoresis using a BioFocus 3000 (BioRad) on a 40 cm x 50 µm fused silica capillary. For the analysis of cations (NH$_4^+$, K$^+$, Na$^+$, Ca$^{2+}$, Mg$^{2+}$, Al$^{3+}$), a buffer system consisting of 5 mM Metol (4-methylamino-phenole-sulfate), 1 mM ascorbic acid, and 2 mM 18-crown-6 was used. Separation was done at 20 °C with +15 kV voltage at a detection wavelength of 220 nm (Göttlein and Blasek 1996). For the analyses of the anions (NO$_3^-$, PO$_4^{3-}$), a buffer system consisting of 3 mM pyromelitic acid adjusted to pH 8.0 with TEMED (N,N,N',N'-Tetramethylethylenediamine) was used. Separation was done at 30 °C with -20 kV voltage at a detection wavelength of 230 nm (Göttlein and Blasek 1996). With this method it was possible to detect nitrate in the soil solution, but the concentration of phosphate was below the detection limit of 10 µmol l$^{-1}$.

The dissolved organic carbon (DOC) concentration in the soil solution was determined with a spectrophotometer using a wavelength of 260 (Cary 50, Varian, Palo Alto, CA; Dilling and Kaiser 2002). In order to calibrate the absorption of the soil solution in terms of DOC concentration, dilution curves for the pooled soil solution material of each forest site and horizon were recorded. The DOC concentration in the pooled samples was determined with a TOC-V analyser (Shimadzu, Kyoto, Japan).

The pH of soil solutions was measured with an ion-sensitive field effect transistor electrode (ISFET sensor, Sentron, Roden, The Netherlands).

$O_2$-consumption, callose concentrations in the root apices, and element concentrations in the root tissues

For the $O_2$-consumption measurement, seedling roots were measured for 15 min with a Clark-type $O_2$ electrode (Hansatech, King’s Lynn, UK). For this measurement 75-100 mg fresh roots were submerged in 2.5 ml of stirred 1 mM CaSO$_4$ + 5 mM MES buffer (adjusted with KOH to pH 5.5; Comas and Eissenstat 2004, Richter et al. 2007a,b). The temperature of the whole system was kept constant at 25 °C. After the measurements, the roots were dried for 48 h at 60 °C and weighed. Respiration was expressed as $O_2$ consumption per g dry weight and time [nmol $O_2$ g$^{-1}$s$^{-1}$]. In addition, the $O_2$-consumption of dead (dried) roots was also measured in order to compare the two different root conditions.
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Callose in the root apices of tree roots was assayed according to the modified method described by Hirano and Brunner (2006). Five milligrams (fresh mass, FM) of fixed root apices were homogenized and washed with polyvinylpolypyrrolidone (PVPP; Fluka No. 81385, Buchs; 5% (w/v) in 20% ethanol). After adding 1 M NaOH, the tubes were heated at 80 °C to solubilize the callose. The supernatant was assayed for callose. The callose concentration in the supernatant was quantified fluorometrically at 393 nm excitation and 484 nm emission wavelengths with a spectrofluorometer (Shimadzu RF5000, Kyoto, Japan) using 0.1% (w/v) aniline blue (water soluble, Fluka) as a dye and curdlan (Sigma, Buchs) as a reference substance. Callose concentrations were expressed as mg curdlan equivalents (CE) per mg root fresh weight [µgCE mg⁻¹FW]. For each root sample, the fluorescence intensity without the aniline blue dye (autofluorescence) was subtracted from the intensity in the presence of the dye.

For element analyses, the fine roots previously used for O₂-consumption measurements were measured after digestion of the ground material in a high-pressure microwave reactor (Milestone MLS Ultraclave, Schelton, CT, USA) with an inductively coupled plasma optical-emission spectrometer (ICP-OES Optima 3000, Perkin Elmer, Waltham, USA; see Brunner et al. 1999). Fine root element content was expressed as [mg g⁻¹DM] and the Ca/Al molar ratio was calculated.

**Morphology and relative growth rate (RGR) of the roots and shoots**

For morphological measurements, roots were analysed with WinRhizo (Ver. 4.1c; Regent Instruments INC., Quebec, Canada), dried for 48 h at 60°C, and weighed. Parameters were expressed in terms of length and dry weight. The recorded parameters were specific root length (SRL [cm mg⁻¹]), root branching frequency (RBF [n cm⁻¹]), and root tissue density (RTD [mg cm⁻³]).

The aboveground parts of the seedlings were divided into cotyledons, together with the primary leaves and the primary stem, and into secondary leaves and secondary stem. Then the parts were analysed with WinRhizo, dried for 48 h at 60°C, and weighed. The dry weight was analysed for each of these two compartments and the secondary mass was expressed as an increment of the secondary stem and leaf. The specific leaf area (SLA [cm² g⁻¹]) was measured for each of these compartments.

To compare the growth of the beech seedlings between the forest sites and soil horizons, the relative growth rate (RGR) of the root and the shoot was calculated (Hunt 1982):
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\[ RGR = (\ln DW_2 - \ln DW_1) \cdot (t_2 - t_1)^{-1} \]  

Whereas \( DW_1 \) is the dry weight at time point 1, \( DW_2 \) the dry weight at time point 2, \( t_1 \) the time point 1 (three days after germination), and \( t_2 \) the time point 2 (“day 0” before the start of treatment). RGR was expressed as \([\mu g \mu g^{-1} d^{-1}]\). The initial dry weight values of the roots and the shoots (\( t_1 \)) were measured at the beginning of the experiment after germination. For that purpose ten beech seedlings were harvested directly after they defoliated their cotyledons, divided into root and shoot, dried for 48 h at 60°C, and weighed. RGR was calculated from the seedlings in the control for every harvest.

**Rhizoboxes and root length growth**

Soils from two forest sites (VO and WA) were used for a rhizobox experiment. Material was taken from the Ah- and B-horizons and sieved (2 mm mesh). For each horizon at each forest site, three boxes were installed (12 in total).

Each rhizobox had a length of 60 cm, a width of 15 cm and a depth of 1 cm in the main rooting compartment (inner volume around 900 cm\(^3\); see Dieffenbach et al. 1997, Dessureault-Rompré et al. 2006). The front side of the rhizobox was covered with a transparent acrylic glass plate to allow visual observation of the development of the roots. The rhizobox was positioned at an angle of 30° to force the roots to grow along the transparent plate. The transparent side of the rhizobox was covered with a dark plastic foil to prevent the interference of light with root growth. The plastic foil was removed only to observe the root development. The boxes were filled with the soil at a bulk density of about 1.2 g cm\(^{-3}\). The rhizoboxes were placed in the greenhouse at 70% humidity, a temperature of 17-25°C, and daylight during spring and early summer with 50% shading. The boxes were irrigated with deionised water using polyether sulfone hollow fiber wicks (Rhizon irrigators, Rhizosphere research products, Wageningen, The Netherlands; Dessureault-Rompré et al. 2006).

Each rhizobox was planted with one beech seed (seed collection WSL, seeds from forest site Auw near Falk, Switzerland). The growth of the seedling roots was monitored once a week from germination onwards for 14 weeks by copying the root onto a transparent overhead foil. The drawn pictures of the roots were analysed with WhinRhizo for the length growth and the branching development of the roots.
Beech seedlings grown in hydroponics

In order to clarify whether European beech roots induce callose, which is suggested to indicate Al toxicity, the following hydroponics experiment with a high Al concentration in the nutrient solution was conducted: European beech seeds (seed collection WSL, seeds from forest site Auw near Falk, Switzerland) were sown in a substrate composed of a 1:1 mixture of sand and peat without fertiliser. After germinating, the seedlings grew for additional three weeks. After these three weeks, 20 seedlings were carefully taken out of the soil, the roots were gently washed, and all attached soil particles were removed. The seedlings were then transferred into hydroponics and pre-cultured for two hours in a 2 mM CaCl$_2$ solution. After this, five different treatments with increasing concentrations of Al (0 = control, 0.05, 0.1, 0.5 and 1 mM; added as AlCl$_3$) were conducted (pH = 4.0). As basis for the treatment solution and control, the composition of the pre-culture solution was used. The treatment time was 24 h and four seedlings were analysed in each treatment. The callose concentrations in the root apices were analysed as described above. In order to analyse the free Al$^{3+}$ concentrations in the nutrient solution, capillary electrophoresis was conducted as described above.

Statistical analyses

For each harvest, treatment, soil horizon, and forest site, the root and soil solution measurements were averaged for four seedlings, each from a different monolith. The data were subjected to one- and two-way analysis of variance (ANOVA). The significance level was $P < 0.05$ by Fisher's PLSD test throughout the study unless stated otherwise. All tests were conducted with StatView 5.0 (SAS Institute, Cary, NY, USA).

Results

Soil and soil solution parameters from soil monoliths

The soil density was the highest by far at the forest site TR (Table 4.2). Comparing the two soil horizons, the values of the B-horizons were about 1.5 – 2 times higher than those of the Ah-horizons.

In the soil solution there were almost no differences in pH and DOC, NO$_3^-$, and NH$_4^+$ concentrations for the four forest sites (Table 5.2), but significant differences were observed between the Ah- and B-horizons. The pH, and DOC and NO$_3^-$ concentrations were
significantly higher in the Ah-horizons than in the B-horizons. No differences were visible in the $\text{NH}_4^+$ concentrations.

Table 5.2: Soil bulk density and soil solution parameters (pH, dissolved organic carbon (DOC), nitrate concentrations, and ammonium concentration of the Ah- and B-horizons) from soil monoliths at the four forest sites eighth weeks after germination of beech seedlings. Abbreviations for the forest sites: see Table 1. Different letters indicate significant differences between the forest sites ($P < 0.05$). Probability level for the analysis of variance (ANOVA): $** = P < 0.01$, $**** = P < 0.0001$; n.s. = not significant.

<table>
<thead>
<tr>
<th>Site</th>
<th>Horizon</th>
<th>Soil density [g cm$^{-3}$]</th>
<th>pH</th>
<th>DOC [mg l$^{-1}$]</th>
<th>NO$_3^-$ [mmol l$^{-1}$]</th>
<th>NH$_4^+$ [mmol l$^{-1}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Ah</td>
<td>1.25 a</td>
<td>2.96 a</td>
<td>65.8 ab</td>
<td>5.23 a</td>
<td>1.10 a</td>
</tr>
<tr>
<td>VO</td>
<td>Ah</td>
<td>0.79 b</td>
<td>3.15 a</td>
<td>112.0 a</td>
<td>1.74 b</td>
<td>0.59 a</td>
</tr>
<tr>
<td>ME</td>
<td>Ah</td>
<td>0.61 c</td>
<td>3.03 a</td>
<td>44.7 b</td>
<td>3.12 b</td>
<td>0.94 a</td>
</tr>
<tr>
<td>WA</td>
<td>Ah</td>
<td>0.74 b</td>
<td>2.87 a</td>
<td>25.9 b</td>
<td>2.04 b</td>
<td>0.73 a</td>
</tr>
<tr>
<td>TR</td>
<td>B</td>
<td>1.66 a</td>
<td>2.50 a</td>
<td>27.7 ab</td>
<td>1.06 a</td>
<td>0.05 b</td>
</tr>
<tr>
<td>VO</td>
<td>B</td>
<td>1.37 b</td>
<td>2.62 a</td>
<td>42.4 a</td>
<td>1.80 a</td>
<td>1.43 a</td>
</tr>
<tr>
<td>ME</td>
<td>B</td>
<td>1.16 c</td>
<td>2.78 a</td>
<td>13.6 b</td>
<td>0.84 a</td>
<td>0.36 b</td>
</tr>
<tr>
<td>WA</td>
<td>B</td>
<td>1.38 b</td>
<td>2.93 a</td>
<td>16.0 b</td>
<td>1.56 a</td>
<td>0.46 b</td>
</tr>
</tbody>
</table>

$P$  Horizon **** ** ** **** n.s.

Differences in the base cation (BC) concentrations of the soil solutions at the forest sites mainly occurred in the Ah-horizons, where one from TR had by far the highest BC concentration (Table 5.3), mainly due to the elevated Ca$^{2+}$ and Mg$^{2+}$ concentrations. Overall the BC concentrations were two to six times higher in the Ah-horizons than in the B-horizons. The Al$^{3+}$ concentration in the soil solution varied greatly. At two sites, it was higher in the Ah-horizons than in the B-horizons, and in two sites it was higher in the B-horizons. There were no differences in the BC/Al molar ratio either between the forest sites or the horizons.

The elemental composition from treatment day 0 to day 3 did not change during the three-day observation of the soil solution either for the control or the drought treatment (data not shown).
Table 5.3: Soil solution parameters (base cation (BC) concentrations (sum of Mg$^{2+}$, K$^+$, and Ca$^{2+}$), aluminium concentrations, and BC/Al molar ratio of the Ah- and B-horizons) from soil monoliths at the four forest sites eighth weeks after germination of beech seedlings. Abbreviations for the forest sites: see Table 1. Different letters indicate significant differences between the forest sites ($P < 0.05$). Probability level for the analysis of variance (ANOVA): $* = P < 0.05$, ** = $P < 0.01$; n.s. = not significant.

<table>
<thead>
<tr>
<th>Site</th>
<th>Horizon</th>
<th>Ca$^{2+}$</th>
<th>K$^+$</th>
<th>Mg$^{2+}$</th>
<th>Al$^{3+}$</th>
<th>BC</th>
<th>BC/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Ah</td>
<td>634 a</td>
<td>305 a</td>
<td>333 a</td>
<td>581 a</td>
<td>1271 a</td>
<td>2.69 a</td>
</tr>
<tr>
<td>VO</td>
<td>Ah</td>
<td>149 b</td>
<td>168 a</td>
<td>121 b</td>
<td>59 b</td>
<td>437 b</td>
<td>7.29 a</td>
</tr>
<tr>
<td>ME</td>
<td>Ah</td>
<td>192 b</td>
<td>456 a</td>
<td>119 b</td>
<td>151 b</td>
<td>766 ab</td>
<td>5.65 a</td>
</tr>
<tr>
<td>WA</td>
<td>Ah</td>
<td>115 b</td>
<td>139 a</td>
<td>46 b</td>
<td>42 b</td>
<td>300 b</td>
<td>7.44 a</td>
</tr>
<tr>
<td>TR</td>
<td>B</td>
<td>153 a</td>
<td>27 b</td>
<td>55 a</td>
<td>108 b</td>
<td>234 a</td>
<td>3.92 ab</td>
</tr>
<tr>
<td>VO</td>
<td>B</td>
<td>100 a</td>
<td>123 a</td>
<td>76 a</td>
<td>231 a</td>
<td>298 a</td>
<td>2.28 ab</td>
</tr>
<tr>
<td>ME</td>
<td>B</td>
<td>119 a</td>
<td>65 b</td>
<td>41 ab</td>
<td>41 b</td>
<td>291 a</td>
<td>7.27 a</td>
</tr>
<tr>
<td>WA</td>
<td>B</td>
<td>99 a</td>
<td>45 b</td>
<td>27 b</td>
<td>121 ab</td>
<td>170 a</td>
<td>1.80 b</td>
</tr>
</tbody>
</table>

$P$ Horizon ** * ** * ** n.s.

*Root tissue elemental composition*

The forest sites differed little with respect to the root tissue elemental concentrations of beech seedlings grown in the monolith soils for eight weeks (Table 5.4). Only the values for K and Mg were higher at TR than at the other three forest sites. There were very significant differences in the Al concentrations and the Ca/Al molar ratio of the plants grown in the Ah- and B-horizons. Plants grown in the B-horizons Al concentrations were two to seven times higher and the Ca/Al molar ratio two to ten times lower than in plants grown in the Ah-horizons.
Table 5.4: Root tissue elemental concentrations (base cation (BC) concentrations (sum of Mg, K, and Ca), aluminium concentrations, and Ca/Al molar ratios of the European beech seedlings grown in the Ah- and B-horizon) in the soil monoliths at the four forest sites eight weeks after germination. Abbreviations for the forest sites: see Table 1. Different letters indicate significant differences between the forest sites ($P < 0.05$). Probability level for the analysis of variance (ANOVA): $* = P < 0.05$, $** = P < 0.01$, $**** = P < 0.0001$; n.s. = not significant.

<table>
<thead>
<tr>
<th>Site</th>
<th>Horizon</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>Al</th>
<th>BC</th>
<th>Ca/Al</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Ah</td>
<td>3.48 ab</td>
<td>8.34 a</td>
<td>1.73 a</td>
<td>3.90 a</td>
<td>13.6 a</td>
<td>0.61 b</td>
</tr>
<tr>
<td>VO</td>
<td>Ah</td>
<td>4.01 a</td>
<td>5.01 b</td>
<td>1.23 b</td>
<td>4.49 a</td>
<td>10.3 b</td>
<td>0.77 b</td>
</tr>
<tr>
<td>ME</td>
<td>Ah</td>
<td>3.79 ab</td>
<td>5.95 ab</td>
<td>1.18 b</td>
<td>2.17 ab</td>
<td>10.9 b</td>
<td>1.26 ab</td>
</tr>
<tr>
<td>WA</td>
<td>Ah</td>
<td>3.09 b</td>
<td>8.19 a</td>
<td>0.93 b</td>
<td>1.00 b</td>
<td>12.2 ab</td>
<td>3.12 a</td>
</tr>
<tr>
<td>TR</td>
<td>B</td>
<td>3.62 a</td>
<td>8.07 a</td>
<td>1.60 a</td>
<td>7.89 a</td>
<td>13.3 a</td>
<td>0.32 a</td>
</tr>
<tr>
<td>VO</td>
<td>B</td>
<td>3.60 a</td>
<td>5.47 b</td>
<td>1.66 a</td>
<td>7.99 a</td>
<td>10.7 ab</td>
<td>0.33 a</td>
</tr>
<tr>
<td>ME</td>
<td>B</td>
<td>3.01 a</td>
<td>5.66 ab</td>
<td>1.46 a</td>
<td>7.25 a</td>
<td>10.1 ab</td>
<td>0.33 a</td>
</tr>
<tr>
<td>WA</td>
<td>B</td>
<td>3.11 a</td>
<td>5.26 b</td>
<td>1.24 a</td>
<td>7.75 a</td>
<td>9.6 b</td>
<td>0.30 a</td>
</tr>
</tbody>
</table>

$P$ Horizon n.s. n.s. $*$ **** n.s. **

Seedling root and shoot properties

The root properties of plants grown in soil from the forest site ME were different from those grown in soil from the other sites (Table 5.5). Roots grown in soil from ME had the highest root dry weight, RBF, RTD, and callose concentration and the lowest root length, SRL and $O_2$-consumption. Differences between plants grown in different horizons occurred only in the root dry weight, SRL, and RBF. SRL was up to twice as high in roots grown in the Ah-horizons as in the B-horizons. By contrast, root dry weight and RBF were significantly higher in roots growing in the B-horizons. Dried (=dead) seedling roots had a very low $O_2$-consumption of just $0.18 \pm 0.02$ nmol $O_2$ g$^{-1}$ s$^{-1}$ (data not shown).
Table 5.5: Seedling root parameters (root dry weight, root length, specific root length (SRL), root branching frequency (RBF), root tissue density (RTD), relative growth rate (RGR) of the root, O₂-consumption, and callose concentration in the root apices of the roots of European beech seedlings grown in the Ah- and B-horizons) in the soil monoliths at the four forest sites eight weeks after germination. Abbreviations for the forest sites: see Table 1. Different letters indicate significant differences between the forest sites ($P < 0.05$). Probability level for the analysis of variance (ANOVA): * = $P < 0.05$, *** = $P < 0.001$; n.s. = not significant.

<table>
<thead>
<tr>
<th>Site</th>
<th>Horizon</th>
<th>Root dry weight [mg]</th>
<th>Root length [cm]</th>
<th>SRL [cm·mg⁻¹]</th>
<th>RBF [n·cm⁻¹]</th>
<th>RTD [mg·cm⁻³]</th>
<th>RGR root [µg·µg⁻¹·d⁻¹]</th>
<th>O₂ consumption [nmol·g⁻¹·s⁻¹]</th>
<th>Callose concent. [µg·CE·mg⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Ah</td>
<td>59 b</td>
<td>52 b</td>
<td>2.41 a</td>
<td>2.09ab</td>
<td>128 b</td>
<td>25.1 a</td>
<td>37.6 ab</td>
<td>0.14 b</td>
</tr>
<tr>
<td>VO</td>
<td>Ah</td>
<td>77 b</td>
<td>98 a</td>
<td>2.10 ab</td>
<td>2.17ab</td>
<td>66 b</td>
<td>30.1 a</td>
<td>44.7 a</td>
<td>0.10 b</td>
</tr>
<tr>
<td>ME</td>
<td>Ah</td>
<td>134 a</td>
<td>52 b</td>
<td>1.44 c</td>
<td>2.48 a</td>
<td>289 a</td>
<td>30.4 a</td>
<td>22.0 b</td>
<td>0.19 a</td>
</tr>
<tr>
<td>WA</td>
<td>Ah</td>
<td>62 b</td>
<td>110 a</td>
<td>1.84 bc</td>
<td>1.88 b</td>
<td>55 b</td>
<td>24.3 a</td>
<td>29.9 ab</td>
<td>0.17 ab</td>
</tr>
<tr>
<td>TR</td>
<td>B</td>
<td>99 a</td>
<td>46 c</td>
<td>1.41 a</td>
<td>2.39 b</td>
<td>221 ab</td>
<td>30.4 a</td>
<td>36.4 b</td>
<td>0.15 a</td>
</tr>
<tr>
<td>VO</td>
<td>B</td>
<td>104 a</td>
<td>99 ab</td>
<td>1.33 a</td>
<td>2.53ab</td>
<td>106 bc</td>
<td>28.4 a</td>
<td>43.3 a</td>
<td>0.14 a</td>
</tr>
<tr>
<td>ME</td>
<td>B</td>
<td>144 a</td>
<td>53 bc</td>
<td>1.29 a</td>
<td>2.93 a</td>
<td>299 a</td>
<td>30.9 a</td>
<td>21.2 c</td>
<td>0.15 a</td>
</tr>
<tr>
<td>WA</td>
<td>B</td>
<td>97 a</td>
<td>132 a</td>
<td>1.47 a</td>
<td>2.74ab</td>
<td>54 c</td>
<td>26.4 a</td>
<td>26.5 bc</td>
<td>0.14 a</td>
</tr>
</tbody>
</table>

No differences were detected in the callose concentrations at the different sites and between the horizons (Table 5.5). However, in hydroponics, a significant positive correlation occurred between the free $\text{Al}^{3+}$ content in the nutrient solution and the callose concentration in the root apices (Figure 5.1).

![Figure 5.1: Relationship between the callose concentration in the root apices of European beech seedlings and the $\text{Al}^{3+}$ concentration of the treatment solution in hydroponics. Probability level for the analyses of variance (ANOVA): ** = $P < 0.01$.](image-url)
Differences in the shoot properties of plants grown in soil from different forest sites only occurred in those grown in soil from the Ah-horizons (Table 5.6). Again plants grown in soil from ME had the highest shoot dry weight, RGR of the shoot, increment of the secondary stem and leaves, and root/shoot ratio. All parameters for plants grown in different horizons differed except for SLA. Shoot dry weight, RGR, and increment of the secondary stem and leaves were higher in plants grown in the Ah-horizons. By contrast, the root/shoot ratio was twice as small for plants grown in the Ah-horizons as for those grown in the B-horizons.

Table 5.6: Seedling shoot parameters (shoot dry weight, specific leaf area (SLA), relative growth rate (RGR) of the shoot, increment of secondary stem and leaf, and root/shoot ratio of European beech seedlings grown in the Ah- and B-horizons) in the soil monoliths at the four forest sites eight weeks after germination. Abbreviations for the forest sites: see Table 1. Different letters indicate significant differences between the forest sites ($P < 0.05$). Probability level for the analysis of variance (ANOVA): $* = P < 0.05$, $** = P < 0.01$, $**** = P < 0.0001$; n.s. = not significant.

<table>
<thead>
<tr>
<th>Site</th>
<th>Horizon</th>
<th>Shoot dry weight [mg]</th>
<th>SLA $[cm^2/mg^{-1}]$</th>
<th>RGR shoot $[µg µg^{-1}d^{-1}]$</th>
<th>Increment sec. stem [mg]</th>
<th>Increment sec. leaf [mg]</th>
<th>Root / shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Ah</td>
<td>240 b</td>
<td>1.29 a</td>
<td>7.2 b</td>
<td>5.3 c</td>
<td>3.4 b</td>
<td>0.23 ab</td>
</tr>
<tr>
<td>VO</td>
<td>Ah</td>
<td>257 b</td>
<td>1.13 a</td>
<td>7.9 ab</td>
<td>11.4 bc</td>
<td>7.7 b</td>
<td>0.29 ab</td>
</tr>
<tr>
<td>ME</td>
<td>Ah</td>
<td>378 a</td>
<td>1.16 a</td>
<td>11.1 a</td>
<td>21.6 ab</td>
<td>35.9 a</td>
<td>0.34 a</td>
</tr>
<tr>
<td>WA</td>
<td>Ah</td>
<td>295 ab</td>
<td>1.23 a</td>
<td>9.4 ab</td>
<td>15.5 ab</td>
<td>21.6 ab</td>
<td>0.21 a</td>
</tr>
<tr>
<td>TR</td>
<td>B</td>
<td>218 a</td>
<td>1.13 a</td>
<td>5.0 a</td>
<td>1.6 a</td>
<td>2.1 a</td>
<td>0.45 a</td>
</tr>
<tr>
<td>VO</td>
<td>B</td>
<td>248 a</td>
<td>1.18 a</td>
<td>6.6 a</td>
<td>1.4 a</td>
<td>1.3 a</td>
<td>0.40 a</td>
</tr>
<tr>
<td>ME</td>
<td>B</td>
<td>275 a</td>
<td>1.07 a</td>
<td>6.8 a</td>
<td>4.7 a</td>
<td>4.7 a</td>
<td>0.50 a</td>
</tr>
<tr>
<td>WA</td>
<td>B</td>
<td>229 a</td>
<td>1.22 a</td>
<td>6.0 a</td>
<td>3.9 a</td>
<td>6.1 a</td>
<td>0.40 a</td>
</tr>
</tbody>
</table>

$P$ | Horizon | * | n.s. | ** | **** | * | ****

Root growth dynamics in rhizoboxes

The root length and branching dynamics of beech seedlings grown in rhizoboxes containing soil from the Ah- or B-horizons from VO or WA are shown in Figure 5.2. The growth of the root length and branches was significantly higher for plants grown in soil from WA than in soil from VO, in particular for the B-horizons. The growth declined after six weeks of continuous growth. The increase in the number of branches followed the same pattern, except for plants grown in the Ah-horizons, where almost no changes in the increment of number of branches per week over the growing period were observed.
Changes in root elemental and physiological patterns due to drought and seedling age

The water content in the soil [%] was monitored during the drought treatment for each harvest (0, 7, 14 days) and during the soil solution sampling (0, 3 days; Figure 5.3).

The water content in the control treatment decreased only slightly and was not significant. For both horizons the decrease in the drought treatment was not significant for the first 3 days.
of treatment, but the subsequent decrease was significant. The water content in the B-horizons was about half that in the Ah-horizons, except for TR, where the water content in the Ah-horizon was as low as in the B-horizon (due to the high soil density).

In the seedling roots (Table 5.7), the concentrations of Ca, K, and Mg of the root tissues in the control were after 14 days of treatment less than those in roots harvested before the treatment start (0 days). In the drought treatment, only the Ca concentration was decreased after 14 days of treatment. The Al concentration did not differ significantly between the two harvest times in either the control or in the drought treatment. However, the Ca/Al molar ratio was decreased in the control but not in the drought treatment after 14 days.

Table 5.7: Effects of drought treatment on root variables: P-values of root variables – element concentrations of root tissues and physiological root parameters - according to their dependencies on the drought vs. control treatment and treatment duration (14 days). Probability level for the analysis of variance (ANOVA): * = $P < 0.05$, *** = $P < 0.001$, **** = $P < 0.0001$; n.s. = not significant.↓ = decreasing values compared to control; ↑ = increasing values compared to control.

<table>
<thead>
<tr>
<th>Root variable</th>
<th>Drought vs. control after 14 days of treatment</th>
<th>Treatment</th>
<th>0 days vs. 14 days of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>****↓</td>
<td>Control</td>
<td>****↓</td>
</tr>
<tr>
<td></td>
<td>Drought</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>****↓</td>
<td>Control</td>
<td>****↓</td>
</tr>
<tr>
<td></td>
<td>Drought</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>n.s.</td>
<td>Control</td>
<td>*↓</td>
</tr>
<tr>
<td></td>
<td>Drought</td>
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The physiological status of the beech roots differed significantly at the two harvest time points. The O₂-consumption was decreased from the harvest after 0 days to the harvest after 14 days in both the control and drought treatment and the callose concentration was increased.
Nevertheless, the O₂-consumption of the roots from the drought treatment was lower than that of roots from the control, and for the callose formation no difference was found between the two treatments (Table 5.7).

**Discussion**

Acidification and a low BS of forest soils play an important role in the health of forest trees. They also have an impact on the natural rejuvenation of trees (Ljungström and Stjernquist 1993). The concentrations of Al³⁺ and nutrient cations in soil solution and at the cation exchange sites in acidic forest soils seem to be the main criteria affecting the health of tree roots (Ljungström and Stjernquist 1993). Accordingly, it can be assumed that roots growing in the acid B-horizons with a very low BS (e.g. < 3%) face the most “toxic” conditions, as in the acid Ah-horizons Al³⁺ is most likely to form stable complexes with DOC (Jönsson et al. 2003, Parker 2005, Lange et al. 2006), and these complexes are no longer phytotoxic.

The forest sites included in our study had both the lowest BS and belong to the most acidified forest soils in the Swiss Plateau. However, there was no evidence that particularly high Al³⁺ concentrations in the soil solution from the B-horizons from the monoliths at these sites occur. The BC/Al molar ratio in the soil solution of the B-horizons was, although very low, never below the critical value for phytotoxicity of 1.0 (Cronan and Grigal 1995). Critical Al³⁺ concentrations for beech (> 500 µmol l⁻¹; Thornton et al. 1989, van Praag et al. 1991) occurred only in one of the sampled soils (Ah-horizon, TR). The Ca/Al molar ratio in the root tissue was also never below the critical value of 0.2 (Cronan and Grigal 1995). However, seedling roots seemed to have a higher uptake of Al in the B-horizons. The Al concentration in the root tissues in these horizons was twice as high as in the Ah-horizons. This was probably due to the significantly lower amount of DOC occurring in the B-horizons.

Despite the high Al concentrations found in the root tissue, only minor symptoms due to Al toxicity could be detected in the roots. A trend towards less healthy seedlings with a reduced capacity for O₂-consumption and enhanced callose accumulation was only observed in the beech seedlings growing in the soils with the lowest BS (1.2 - 2.7%; ME and WA). Increased callose concentration is a symptom of physiological damage in roots (Hirano et al. 2004, 2006). The hydroponic experiment showed, that significantly higher levels of callose were induced in the root apices of beech seedlings at Al³⁺ concentrations > 350 µmol l⁻¹. This suggests that the beech seedlings were adversely affected by Al toxicity. Additionally, in our
study, the SRL was smaller in the B-horizons. SRL has been reported to be negatively affected due to Al stress, e.g. Ostonen et al. (2007) reported that the growth of fine roots was hampered under high Al concentrations, which caused a thickening of the roots resulting in a smaller SRL.

The RGR of the root and root morphological development did not seem to be inhibited by elevated Al concentrations in the root tissue. Indeed the opposite appeared to be the case: In the B-horizons a higher root mass, RBF, length, and branching were observed than in the Ah-horizons. These differences may be due to lower nutrient concentrations being available in the B- than in the Ah-horizons. The differences in seedling root and shoot growth found were most likely the result of having a different nutrient supply. The larger root biomass in the B-horizon was mainly due to branching being more developed and extensive, which led to a higher RBF. The root systems of *Rumex crispus* and *Poa annua* have also been shown to develop more and longer second-order elements (branches) in a low nutrient treatment than in a high nutrient treatment in order to increase their absorbing surface (Fitter 1982).

Similarly differences in the root/shoot ratio indicate that the biomass partitioning is different between the roots and the shoots in plants grown in the different soil horizons, especially in the elevated increment of secondary stem and leaf mass in the Ah-horizon. Considering the different nutrient supply in the Ah- and B-horizons, the root/shoot ratio showed clearly that the seedlings invested more in building a large root biomass in the B-horizons, where rare nutrients can be acquired better than in the Ah-horizons due to the larger root system the B-horizon (Coleman and McConnaughay 1995, Espeleta and Donovan 2002).

The effect of the age of the seedlings, which was evident in the control seedlings of the drought treatment, showed a strong decrease in O$_2$-consumption with seedling age. This demonstrated that the respiratory activity depends on the age of the roots. Evidence can also be found in Richter et al. (2007b), where the respiratory activity of adult beech fine roots was much smaller (1.2 – 2.0 [nmol O$_2$ g$^{-1}$ s$^{-1}$]) than that of the seedlings in this study (21.2 – 44.7 [nmol O$_2$ g$^{-1}$ s$^{-1}$]). The elemental content of BC decreased greatly in the older seedlings, whereas the Al content did not differ in seedlings of different age. This might be due to lower uptake rates of BC, but also to uptake competition with Al as reported by Göransson and Eldhuset (1991), Nygaard and de Wit (2004) and Vanguelova et al. (2005). Therefore, we cannot exclude the possibility that the “age-effect” had a high impact on the root data of the older beech seedlings.

During the observation of the drought treatment we were, unfortunately, not able to sample the soil solution later than three days. Therefore, we were not able to further test our
hypothesis that drought stress enhances the negative impact of Al$^{3+}$ in the soil solution on root health with changes in the soil solution chemistry. The decrease in the water content in the soil was not significant after three days of treatment but it was after seven and 14 days. However, there was a clear decrease in the O$_2$-consumption, which was larger than the decrease in the control, and an increase in the callose content. As these observations can not be related to toxicity factors (Ca/Al molar ratio and Al concentration in the root tissues), it is more likely that these changes in the root physiology arose from the decreased water content in the soil. Richter et al. (2007a) were also able to show a correlation between a decrease in the water content in the soil and a decrease in the O$_2$-consumption of Norway spruce seedling roots. However, the element contents in the root tissue did not vary with the length of drought treatment. Only the Ca concentration decreased and no accumulation of Al in the root tissue or a decrease in the Ca/Al molar ratio could be observed.

We conclude that nutrient availability is more important for European beech root and seedling growth than the Al$^{3+}$ concentration in the soil solution, as the growth patterns of the seedling roots growing in the B-horizons were mostly dependent on the nitrate and BC concentrations in the soil solution. This observation is supported by Vanguelova et al. (2007a), who showed that there was a reduction in the fine root biomass and the density of Scots pine seedlings in an acid treatment, which were more dependent on the deficiency in exchangeable BC and the input of H$^+$ in the soil solution. Moreover Göransson and Eldhuset (1991) suggested that it is not the high concentration of Al$^{3+}$ that is the major cause of the reduced health status of the tree roots, but rather the low Ca and Mg concentrations in the culture solution. However, the decreased SRL and O$_2$-consumption and increased callose concentration at BS < 3% gave a hint for a potential reduction of root health due to the occurrence of toxic Al$^{3+}$ concentrations, under these conditions, Al$^{3+}$ is most likely phytotoxic.
General discussion and conclusion
The main objective of this study was to assess the effects of acidic soils with low base saturation (BS) on the fine root growth, development, morphology, physiology and chemistry of European beech. This species was selected, as it is naturally the dominant tree species in Central Europe. It is remarkably tolerant against a broad range of hydrological and soil chemical factors including high and low soil moisture, high H\(^+\) and Al\(^{3+}\) concentrations, and low nitrogen availability (Leuschner, 1999; Härdtle et al., 2004). Vital mono-specific beech forests are found on highly acidic quartzitic soils and on basic carbonate-rich soils and they occur in regions with less than 550 to more than 2000 mm of annual rainfall (Pinto and Gegout, 2005). Beech forests grow on nearly all geological substrates if drainage is sufficient (Leuschner, 1999). Thus, this species realizes a very broad ecological niche in terms of soil chemical properties and water availability. Nevertheless, in very acidic soils with a very low nutrient availability beech does not seem to be competitive anymore. Leuschner et al. (2006) for example observed among 50 Central European beech stands on 13 acidic to basic bedrock types that beech forests were found on soils with pH values between 3.2 and 7.3 and BS from 3.3 to 99.9% in the organic layer and the first 20 cm of mineral horizon suggesting that, below a pH of 3.2 and a very low BS of 3.3% beech was not competitive anymore. Also in Switzerland on the Swiss Plateau beech is absent on soils with a BS < 6.5% in the Ah-horizon and < 3.1% in the B-horizon. Given the large variation in soil physical and chemical properties across the distribution range of the European beech, one would expect a high degree of morphological and physiological plasticity at the level of individual roots as well as for entire root systems (Fitter, 1991).

The present study focuses on soils with a BS < 15% and their effects on root health condition of European beech trees. The low BS (< 15%) in the soil matrix is seen as an indicator of the presence of phytotoxic Al\(^{3+}\) concentrations in the soil solution and is discussed in relation to its adverse effects on tree root growth (Cronan and Grigal, 1995). The main findings of this study are synthesised and discussed below. The most important results are presented in a schematic way in Figure 6.1. Additionally, an attempt is made to extrapolate the results to other tree species and a conclusion and an outlook are provided.
Variation in fine root and seedling root properties in relation to low soil BS and acidity

Root growth and development

Root system carbon pools are of major importance for estimates of ecosystem carbon pools and fluxes (Jackson et al., 1997). For proper estimates the differences in fine root growth and development in relation to soil chemical parameters is needed (Finer et al., 2007). In this study the fine root biomass, necromass, productivity, and mortality of European beech did not differ between the forest stands studied in 2005 and 2006. This suggests that neither the standing crop (biomass, necromass) nor the fine root dynamic (expressed in productivity and mortality) was different due to the soil chemical parameters pH and BS in this forest sites. However, in the reviews of Leuschner and Hertel (2003) and Finer et al. (2007) it was shown that on a wider scale of soil chemistry (pH 3.2-7.0 and nutrient rich to nutrient poor soils) the
General discussion and conclusion

Fine root biomass of European beech forests increased with decreasing pH and soil nutrient availability. Leuschner and Hertel (2003) stated that the higher biomass and, therefore, the amount of fine roots would ensure sufficient nutrient absorption even in nutrient poor soils. Nevertheless, the comparison of the data of this study with several published data from European beech forests with a wide scale of soil chemistry (pH 2.9-7.8 and BS 1.9-99.9%) did not show these relationships of higher fine root biomass or productivity with low pH and BS. This might be due to the low number of studies included in this study compared to the other study.

However, the biomass/necromass ratio (live/dead ratio) of the fine roots sampled in 2005 was significantly lower on forest sites with a BS < 5% in the B-layers (Figure 3.3). The turnover and age of the beech fine roots measured during the two observation years did not vary between the six forest stands. But together with published data about fine root turnover of European beech in Central Europe a relationship with pH and BS was visible (Figure 4.4). Godbold et al. (2003) reported a higher turnover for Norway spruce fine roots in more acidic soils. This shows that the turnover is probably a more sensitive parameter towards soil chemical conditions than fine root biomass and necromass.

In contrast to the results of the mature tree fine roots, the growth and development of the beech seedling roots was affected neither by the low BS in the B-horizon nor by the high Al$^{3+}$ concentration measured in the soil solution. The RGR of the root did not differ between forest sites or horizons (Table 5.5). However, the RGR of the shoot was significantly lower when the seedlings were growing on the B-horizon leading to a higher root/shoot ratio (Table 5.6). This was rather explained by the lower basic cations (BC) and nitrate availability in the B-horizon than by the Al$^{3+}$ toxicity.

Root morphology

The root morphology is one parameter that directly influences the water and nutrient uptake (Fitter, 1985). Therefore, morphological differences of the fine roots may lead to water stress and reduced nutrient availability of the aboveground plant. Several morphological properties of the fine roots of mature European beech trees studied in situ and root systems of seedlings growing in soil monoliths were investigated and related to soil conditions. The in situ RTA, RBA, and SRL (slightly) were negatively affected in the forest sites with a very low BS < 5% in the B-layers (Figure 3.4). In contrast the root RBF, length growth, and branches growth of the seedling root systems increased in the mineral B-horizons where the BS and nutrient
availability was very low. The SRL of the seedling roots was also lower in the B-horizon (Table 5.5).

These different morphological responses of fine roots and seedling root systems on the chemical soil parameters may be due to Al toxicity symptoms or nutrient deficiency. In the case of the fine roots of the mature forest stand, the low BS and the probable toxic concentrations of Al$^{3+}$ in the soil solution might have caused the decline in the morphological properties. One indicator for Al toxicity and acidity stress to roots is the Ca/Al molar ratio in the fine root tissue (Cronan and Grigal, 1995; Vanguelova et al., 2007). The RTA, RBA, and SRL \textit{in situ} were significantly, positively related to the Ca/Al molar ratio in the root tissue (Figure 3.6). Göransson and Eldhuset (1991) showed that higher Al$^{3+}$ concentrations or lower BC/Al molar ratio in nutrient solution negatively influenced the growth and development of branches of fine roots of Norway spruce and Scots pine. The decrease of the RTA could also be explained by the dieback of tips due to Al toxicity (Göransson and Eldhuset, 1991).

Vanguelova et al. (2007) underlined the negative effect of a low Ca/Al molar ratio on fine root morphology (e.g. length and SRL). In the monolith experiment of the present study the Ca/Al molar ratio in the root tissue and the SRL were significantly lower when plants were grown on a B-horizon with a low BS. The lower SRL can be considered as a symptom of Al toxicity. Ostonen et al. (2007) reported that the SRL decreased under high Al concentrations causing a thickening of the roots. Alternatively Eissenstat et al. (2000) assume that species of stressed environments may need higher investment per root length to attain the longevity generally associated with their roots. Godbold et al. (2003) suggested that, under acidic soil conditions, the fine roots have to be renewed more often to maintain their resource exploiting function. Higher turnover rates, and therefore shorter life spans, may result in smaller SRL in forest stands.

In the monolith experiment most root morphological properties were most likely influenced by the low nutrient availability (BC and nitrate) in the B-horizon. The root properties were significantly increased in the B-horizon leading to a larger and highly structured root system. Due to this larger root system a better maintenance of the rare nutrients is ensured (Coleman and McConnaughay, 1995; Espeleta and Donovan, 2002). For example, Fitter (1982) showed that in a low fertility treatment the root systems of \textit{Rumex crispus} and \textit{Poa annua} developed more and longer second-order elements (branches) compared to a high fertility treatment. Fitter (1985) also stated that highly structured root systems comprising many short branches are initially more effective in depleting soil than
General discussion and conclusion

those of few long roots. However, Fitter (1985) suggested that these highly structured root systems produce local depletion and are subsequently less effective.

The discrepancy between the root morphological response of the fine roots of mature trees and seedlings may be due to contrasting time scales of observations: experiments cover short-term physiological responses of roots; whereas site comparisons reflect long-term adaptive growth and allocation processes of trees (Fahey and Hughes, 1994). In the monolith experiment the nutrient availability seemed to be the main driver for root development and growth and in situ the critical soil conditions (low BS and possibly high Al\(^{3+}\) soil solution concentration). Other parameters like soil density and structure did not seem to affect the fine root growth (see Chapter II and IV).

Root physiology and chemistry

The root physiology and chemistry are parameters that reflect the health status of fine roots (Clemensson-Lindell, 1994; Comas et al., 2000; Ruf and Brunner, 2003). The measurement of the O\(_2\)-consumption of fine roots is a good physiological method to determine their health status (Chapter I). It was shown that a reduced O\(_2\)-consumption in situ was associated with the BS < 5% in the B-layers (Figure 3.5). This was possibly due to the high Al\(^{3+}\) concentration in the soil solution. In the monolith experiment the BS (1.2-6.5%) and the BC/Al molar ratio (1.8-7.4) in the soil solution were very low. But the BC/Al molar ratio was never below the critical value for Al\(^{3+}\) toxicity to roots of 1.0 (Cronan and Grigal, 1995). Seedlings growing in soil monoliths with very low BS (< 3%) showed a trend towards a reduced capacity for O\(_2\)-consumption and an elevated callose formation in the root apices (Table 5.5). These two trends were also slightly enhanced due to drought as additional stress application. However, no direct relationship between soil and soil solution parameters and seedling root properties were obvious. Nevertheless, beech seedlings responded in a hydroponic experiment with a significantly elevated callose formation due to an Al\(^{3+}\) concentration > 350 \(\mu\)mol l\(^{-1}\) (Figure 4.1). This experiment showed that beech seedling roots also potentially respond with callose formation on high Al\(^{3+}\) concentration as earlier shown by Hirano et al. (2004, 2006) for Norway spruce and European chestnut. Therefore, it is likely that the beech seedlings in the soil monoliths responded to Al\(^{3+}\) occurring in the soil solution.

Soil acidity and BS not only affected the fine root physiology, but also the fine root tissue chemistry of beech trees and seedlings. In the in situ study root physiology and chemistry were related with each other, as the O\(_2\)-consumption of the fine roots was significantly related to the Ca/Al molar ratio in the fine root tissues (Figure 3.7). However, the seedling roots
growing in monoliths showed neither a relationship of the root physiology with the Ca/Al molar ratio nor with the Al concentration in the root tissue, although their Ca/Al molar ratio was much lower than in the fine roots of mature trees. The Ca/Al molar ratio in the beech seedling roots with a ratio of about 0.3 (B-horizon TR and VW) was about five times lower compared to the fine roots of mature trees with a ratio between 1.2-1.9 (B-layer -25 cm TR and VW). The lack of correlation between O\textsubscript{2}-consumption and Ca/Al molar ratio might be due to the generally higher O\textsubscript{2}-consumption of the beech seedlings. The O\textsubscript{2}-consumption with 19.0-46.1 nmolO\textsubscript{2} g\textsuperscript{-1}s\textsuperscript{-1} (B-horizon TR and VW) was about 17.5 times higher in the beech seedlings compared to the fine roots of mature trees with 1.26-2.27 nmolO\textsubscript{2} g\textsuperscript{-1}s\textsuperscript{-1} (B-layer -25 cm TR and VW). These results also underline the higher respiratory activity of seedlings. This was also obvious by comparing the reduction of TTC (another method to measure the respiratory activity of fine roots) of European beech seedlings of different ages. Also Fahey and Yavitt (2005) showed in different tree species that young roots respired much more rapidly than older roots.

**Effects of low soil BS and acidity to belowground carbon cycle**

As mentioned above fine root represent 33% of the global annual net primary production. Nevertheless, for this measurement a fine root turnover of 1 y\textsuperscript{-1} was assumed (Jackson et al., 1997). In this study the turnover of beech fine roots was slightly negatively affected due to low pH and BS. Therefore, the age of the fine roots was also decreased with decreasing pH and BS. Remarkable and unexpected were the results of the age measurements with the radiocarbon method, as the age of fine roots is mostly reported to be around 1 year measured with e.g. minirhizotrons (Anderson and Majdi, 2005; Mainiero and Kazda, 2006). Nevertheless, the radiocarbon results of this study were similar to the results of fine root age in a northern hardwood forest measured by Gaudinski et al. (2001). The results of the present study showed that at least a certain amount of fine roots live for as long as 3-25 years depending on the soil layer. That means that also the turnover for European beech fine roots could be much slower than measured with sequential coring in the present study (0.1-10 y\textsuperscript{-1}) and by other authors (0.3-14.1 y\textsuperscript{-1}; Table 4.4a 4.4b). These findings certainly influence models like the Biome-BGC for biogeochemical element cycling in terrestrial ecosystems, where a turnover of 1 (Pietsch et al., 2005) and 1.023 y\textsuperscript{-1} (Cienciala and Tatarinov, 2006; Tatarinov and Cienciala, 2006) is assumed. Trumbore and Gaudinski (2003) suggested if less
carbon was used for the grow of fine roots, as they live longer than expected, then a higher amount of carbon transferred belowground then must go to ephemeral roots, root exudates, fungi, and root respiration. This carbon used for energy generation more quickly returns to the atmosphere than carbon used for construction processes. In this case the carbon storage potential belowground was overestimated. With respect to the carbon budget of mature forest these results may influence the classification for beech forests as carbon sinks or sources. Knohl and Buchmann (2005) estimated a deciduous forest with European beech as a carbon sink. Nevertheless, the results of the present study show that increasing acidity of soils increases the turnover of fine roots of European beech. Therefore, soil acidity increases the carbon flux by enhancing the provision of dead fine root material for decomposition - and, therefore, for heterotrophic respiration - (see also Godbold et al., 2003; Leuschner et al., 2004) and enhancing the need for new fine root growth (see also Leuschner and Hertel, 2003).

Comparability of the results for European beech forests to other tree species

This thesis described the effect of acidic soils with a low BS on European beech trees. However, the results can probably not be directly transferred to other tree species. For example the comparison of the fine root biomass of beech and spruce in relation to soil fertility in Central Europe revealed that beech had a significantly increased and spruce a significantly decreased biomass on low fertility soils (Finer et al., 2007). Pine, instead, did not show any response on changes in soil fertility (Finer et al., 2007). Also Leuschner and Hertel (2003) reported a different response of the fine root biomass of broad-leaved and coniferous tree species of temperate forests towards certain biotic and abiotic variables (pH, temperature, precipitation, elevation, stand age). They showed that Scots pine and oak species were influenced by fewer variables (pH, temperature, elevation) than European beech and Norway spruce (pH, temperature, precipitation, elevation, stand age). Therefore, the results of the present study concerning the fine root biomass, and certainly related parameters like productivity and turnover, are probably not transferable to other tree species especially to coniferous species. However, Godbold et al. (2003) also showed a decreasing turnover of Norway spruce fine roots with decreasing acidity.

The results of the effects of acidic soils with a low BS on the fine root morphology of European beech can be transferred to other tree species. Vangelova et al. (2007) found that decreasing BC availability and increasing acidity decreased the SRL of Scots pine and
Godbold et al. (2003) observed a significant decrease of the SRL and RTA of Norway spruce fine roots in more acidic stands compared to less acidic ones. Also Ostonen et al. (2007) stated in a review including 11 deciduous and coniferous tree species that the effect of stress (e.g. Al-stress) towards the fine roots of these tree species tended to result in a reduction of the SRL. These observations were comparable with the results of the present study for European beech. But Ostonen et al. (2007) also reported, that fine root SRL depended significantly on tree species. In the reviewed dataset the SRL was significantly higher for deciduous trees than for coniferous trees.

The effect of acid soil and Al toxicity on fine root physiology and chemistry could also be transferred to other species. Comas et al. (2000) reported a decrease in O$_2$-consumption of grape roots with decrease of their age and metabolic activity until they reached death. Hirano and Hinjii (2000) observed a decrease in the respiratory activity of Japanese red cedar saplings as a result of probable high Al$^{3+}$ concentrations in the soil solution. Also the results of the callose formation in beech seedling roots in the hydroponical experiment were comparable to other tree species. Hirano et al. (2004) showed a significantly elevated callose concentration of about 0.1 µg$_{CE}$ mg$_{DW}^{-1}$ in Norway spruce seedling root tips in response to 280 µmol l$^{-1}$ Al in simulated soil solution. Also European chestnut responded with significantly elevated callose concentration of about 0.2 µg$_{CE}$ mg$_{DW}^{-1}$ on 168 µmol l$^{-1}$ Al in a simulated soil solution (Hirano et al., 2006). However, European beech seedlings seem to be more resistant as a callose concentration of about 0.2 µg$_{CE}$ mg$_{DW}^{-1}$ was observed at a concentration of 350 µmol l$^{-1}$ Al in the nutrient solution (Figure 5.1). This higher resistance could also be due to the higher nutrient availability, which occurred in the nutrient solution of the beech seedlings. The nutrient solution of the European beech seedlings contained about 100 times more Ca$^{2+}$ than the nutrient solutions used in the experiments of Norway spruce and European chestnut.

The comparability of the fine root tissue chemical parameter Ca/Al molar ratio to several other tree species is also evident. Several other studies, reviewed by Vanguelova et al. (2007), showed a negative relation between low tissue Ca/Al molar ratio and Al toxicity symptoms on roots. However, Vanguelova et al. (2007) stated that this response of tree fine roots under field conditions was not clear because other factors may have interacted.
Main conclusions and outlook

It can be concluded that very low BS in the soil matrix negatively affects the health status and several root properties of fine roots of mature trees and seedlings of European beech. For example in both studies *in situ* and in the greenhouse a decrease of O$_2$-consumption and SRL and additionally in the seedling roots an increase of callose concentration in the root apices in soils with lowest BS could be seen. However, the relationship of reduced health with the occurrence of Al toxicity, seen e.g. in the Ca/Al molar ratio in the root tissue, was only significant in the *in situ* study. Therefore, it can be suggested that the reductions in root health of the fine roots of mature beech trees were due to toxic effects of Al. The seedling root growth and seedling development instead was more dependent on the higher nutrient availability than the Al$^{3+}$ concentration in the soil solution or the high Al concentration or low Ca/Al molar ratio in the root tissue. However, the decreased SRL in the B-layers and decreased O$_2$-consumption and increased callose concentration of the seedling roots growing in soil monoliths with a BS < 3% gave a hint for a potential reduction of root health due to Al toxicity. This may have also influenced the aboveground growth expressed in a lower shoot RGR of the seedlings in the B-horizons. Contrarily, Nadelhoffer and Reich (1992) did not find a relationship between the above- and belowground productivity and root turnover of forest ecosystems. But they concluded that it is unlikely that above- and belowground parameters are not related on each other and pointed on the difficulties in measuring reliable data belowground.

However, the differences in root properties were not that distinct and the health status was only slightly decreased due to low BS. The seedlings for example grew well and did not seem to suffer. Therefore, it can be hypothesised, that roots growing in natural soil are not distinctly affected by low BS, acidity, and, as it was shown in the hydroponical experiment, Al toxicity. Also Nygaard and de Wit (2004) suggested that fine roots most likely compensate adverse soil chemical effects when growing under natural conditions. The roots might be favoured through the microbial community existing under natural conditions. Mycorrhiza, for example, are able to accumulate heavy metals (e.g. Al) and protect the roots from Al toxicity (Brunner 2001). But also the roots might be able to compensate the adverse effects as they are able to grow in chemically more suitable parts of the heterogeneous soil or to detoxify Al by chelating agents exuded by the roots (Marschner, 1998; Heim et al., 2001; Jones et al., 2004). Another possibility for compensation of adverse effects was suggested by Borken et al. (2007). They hypothesised that the fine root system of Norway spruce in soil layers with
adverse chemical conditions may profit from high BS in other layers, as in their study the Al, Mg, and Ca concentration in the fine root tissue was not affected due to changes in soil chemistry in different layers. However, it also is likely that beech trees in forests, which had not been managed for a very long period, have adapted to acidic soils, as Spinnler et al. (2003) reported a different growth response of different beech genotypes on acidic and basic soils. These adapted trees may be more resistant against toxicity symptoms of acidity.

Although the negative effects on fine roots of European beech on the Swiss Plateau were not distinct, it cannot be excluded that in future the problem of soil acidification will affect fine roots of mature trees and seedling roots. Due to the ongoing threat of nitrogen depositions due to road traffic, agriculture, industry, and heating systems in Switzerland and the neighbouring countries, the acidification will accelerate further (Thimonier et al., 2005; Waldner et al., 2007). Under this conditions it can be stated that the observed trends of acidification stress towards fine roots and seedling growth may be enhanced and cause further ecological problems on forest communities in future. Multiple stressors like drought, pests, and storms may cause more damage to trees, as they are weakened due to the soil acidity, as it was shown in the case of the storm “Lothar” in 1999 in studies of Mayer et al. (2005) and Braun et al. (2003).
References


References


References


References


References


References


References

Sverdrup H, Warfinge P (1993) The effects of soil acidification on the growth of trees, grass and herbs as expressed by the (Ca+Mg+K)/Al ratio. In: Reports in Ecology and
References


References


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Curriculum vitae
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Presentations:
Richter A K, Hajdas I, Frossard E, Brunner I: Turnover and age of European beech (Fagus sylvatica L.) fine roots in acid forest soils. COST E38 Wales 2007
Richter A K, Frossard E, Brunner I: Does decreasing soil base saturation affect the vitality of fine roots of European beech? COST E38 Finland 2006