Doctoral Thesis

Effect of wood ash recycling and liquid fertilisation on the fine roots of Norway spruce

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Effect of wood ash recycling and liquid fertilisation on the fine roots of Norway spruce

MATTHIAS GENENGER

2001
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Effect of wood ash recycling and liquid fertilisation on the fine roots of Norway spruce

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presented by

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# Table of contents

1 Introduction  
  1.1 N in forests ................................................................. 5  
  1.2 Forest management ......................................................... 6  
  1.3 Nitrate reductase ............................................................ 8  
  1.4 Experimental approach .................................................... 10  
    1.4.1 Study site .................................................................. 10  
    1.4.2 Treatments .................................................................. 11  
  1.5 Objectives ........................................................................ 13  
    1.5.1 Root growth and elements ............................................. 13  
    1.5.2 NRA in Norway spruce roots .......................................... 14  
    1.5.3 N uptake ..................................................................... 15  
    1.5.4 Synthesis .................................................................... 16  

2 Fine root growth and element concentrations of Norway spruce as affected by liquid fertilisation and wood ash  
  2.1 Abstract ............................................................................ 18  
  2.2 Introduction ........................................................................ 19  
  2.3 Materials and methods .......................................................... 20  
    2.3.1 Field experiment .......................................................... 20  
    2.3.2 Sampling design ............................................................. 20  
    2.3.3 Analyses ...................................................................... 21  
    2.3.4 Ingrowth-cores .............................................................. 22  
    2.3.5 Harvest ingrowth-cores ................................................. 22  
    2.3.6 Statistics ...................................................................... 23
Table of contents

2.4 Results ........................................................................................................... 24
  2.4.1 Biomass, growth dynamic and soil pH ................................................. 24
  2.4.2 Elements in the fine roots ................................................................. 28
  2.4.3 Element change in current needles ................................................. 31
2.5 Discussion .................................................................................................... 33
  2.5.1 Root growth ....................................................................................... 33
  2.5.2 Elements ............................................................................................ 35
  2.5.3 Foliage elements versus fine root elements ...................................... 38
2.6 Conclusions ................................................................................................. 39

3 Reactions of the nitrate reductase activity in the roots of Norway spruce
  seedlings to nitrate .......................................................................................... 41
  3.1 Abstract ....................................................................................................... 42
  3.2 Introduction ................................................................................................ 43
  3.3 Materials and Methods ............................................................................ 45
    3.3.1 Plant material ................................................................................... 45
    3.3.2 N source experiment ....................................................................... 45
    3.3.3 Time course experiment .................................................................. 46
    3.3.4 Harvest ............................................................................................. 46
    3.3.5 Nitrate reductase activity .................................................................. 46
    3.3.6 Nitrate concentration ....................................................................... 47
    3.3.7 Statistics .......................................................................................... 47
  3.4 Results ......................................................................................................... 48
    3.4.1 Effects of inorganic N sources .......................................................... 48
    3.4.2 Time course of nitrate reductase induction ...................................... 49
  3.5 Discussion .................................................................................................... 54
4 The effects of fertiliser or wood ash on nitrate reductase activity in Norway spruce fine roots

4.1 Abstract ............................................................................................................. 60
4.2 Introduction ........................................................................................................ 61
4.3 Materials and methods ...................................................................................... 62
    4.3.1 Experiment ................................................................................................. 62
    4.3.2 Sample trees .............................................................................................. 62
    4.3.3 Sampling ..................................................................................................... 64
    4.3.4 Soil solution ............................................................................................... 64
    4.3.5 Nitrate reductase activity ............................................................................ 65
    4.3.6 Statistics ..................................................................................................... 65
4.4 Results ............................................................................................................... 66
    4.4.1 Nitrate reductase activity ............................................................................ 66
    4.4.2 Soil ............................................................................................................. 69
    4.4.3 Correlation NRA to soil data ..................................................................... 72
4.5 Discussion .......................................................................................................... 74
    4.5.1 Effects on NRA and soil solution ............................................................... 74
    4.5.2 Potential as an indicator ............................................................................. 77

5 Rapid $^{15}$N uptake and metabolism in fine roots of Norway spruce

5.1 Abstract ............................................................................................................. 80
5.2 Introduction ........................................................................................................ 82
5.3 Materials and methods ...................................................................................... 84
    5.3.1 Field experiment ....................................................................................... 84
    5.3.2 Laboratory experiment .............................................................................. 87
    5.3.3 Statistics ..................................................................................................... 89
5.4 Results ............................................................................................................... 90
    5.4.1 Field experiment - N uptake ..................................................................... 90
    5.4.2 $^{15}$N distribution within the root system ..................................................... 90
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4.3</td>
<td>Laboratory experiment</td>
<td>93</td>
</tr>
<tr>
<td>5.5</td>
<td>Discussion</td>
<td>96</td>
</tr>
<tr>
<td>5.5.1</td>
<td>$^{15}$N uptake in the field</td>
<td>96</td>
</tr>
<tr>
<td>5.5.2</td>
<td>$N$ in the roots versus $N$ in the soil</td>
<td>97</td>
</tr>
<tr>
<td>5.5.3</td>
<td>$^{15}$N distribution within the root system</td>
<td>99</td>
</tr>
<tr>
<td>5.5.4</td>
<td>Amino acid metabolism</td>
<td>99</td>
</tr>
<tr>
<td>5.5.5</td>
<td>Conclusion</td>
<td>101</td>
</tr>
<tr>
<td>6</td>
<td>Synthesis</td>
<td>103</td>
</tr>
<tr>
<td>6.1</td>
<td>Fine roots as indicators of nutritional imbalances and $N$ status of a forest</td>
<td>103</td>
</tr>
<tr>
<td>6.1.1</td>
<td>Root growth and element concentrations</td>
<td>103</td>
</tr>
<tr>
<td>6.1.2</td>
<td>NRA in fine roots</td>
<td>105</td>
</tr>
<tr>
<td>6.1.3</td>
<td>$N$ uptake</td>
<td>106</td>
</tr>
<tr>
<td>6.2</td>
<td>$N$ deposition and global change</td>
<td>107</td>
</tr>
<tr>
<td>6.3</td>
<td>Sustainability</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td>Acknowledgements</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>Curriculum vitae</td>
<td></td>
</tr>
</tbody>
</table>
Abstract

Forests in large areas of Europe and North America are subjected to high N deposition leading to N saturation and nutritional imbalances. In a field experiment in a spruce forest of the Swiss plateau, the influence of a wood ash recycling (A), an optimal liquid fertilisation (WF), and a water treatment (W) on the fine roots (≤ 2 mm) of Norway spruce has been investigated for two years in comparison to a control (C). Fine root growth and element concentrations have been monitored. The nitrate reductase activity (NRA) of fine roots, assumed to be an indicator of nitrate in the forest soil was measured during the vegetation period. In addition a 15N-tracer experiment was conducted to investigate the N uptake into the fine roots.

The A, WF and also the W treatment resulted in a significant increase of the soil pH. The fine root growth was not affected, although W seemed to reduce the number of root tips and forks, and reduced the root length while the contrary was observed with the A treatment. In addition the roots showed a reduced diameter under the A treatment. Fine root N increased with the WF treatment, while C-concentrations decreased in all three treatments. The Ca and Mg concentrations were significantly increased through A and WF treatments while the K and P concentrations in the fine roots were improved by all three applications. The Al, Fe and Mn concentrations in the fine roots were slightly decreased by the A and WF treatments, and S and Zn showed inconsistent changes over the growing seasons. Heavy metal concentrations of Cu and Cd were not increased by the A treatment. With certain elements as e.g. Mg, Ca, changes were observed in the fine roots, that could not be detected in concentrations of the needles. Therefore, the element concentrations of fine roots are more sensitive in their reaction to changes in element availability of the soil than the element concentrations in the foliage. The NRA of the fine roots in the forest was increased by the WF, but also by the W treatment and most by the A treatment. Nitrate concentrations in the soil solution were enhanced during the WF irrigation. In non-hydroponic laboratory experiments, a correlation between external nitrate concentrations and the NRA in Norway spruce roots could be shown. In the forest the pH of the soil solution was significantly correlated with the NRA in the fine roots.
roots, while a correlation between the nitrate in the soil and the NRA was only significant, when the spatial heterogeneity of the soil nitrate was taken into account. Thus the NRA is assumed to reflect the nitrate conditions in the soil. Nevertheless, the indicator function is limited as shown with the A and W treatments, where the elevated NRA was probably caused by other environmental parameters, which changed the nitrate availability in the soil and/or uptake properties of the roots.

A rapid uptake of the applied $^{15}$N into the fine roots could be observed in the field and in the laboratory experiment. Within one day after application of the tracer to the forest floor 50% of the maximal observed $\delta^{15}$N were detectable in the fine roots, maximum $\delta^{15}$N was reached after one month. The applied $^{15}$N in the laboratory experiment could be retrieved in the major amino acids of Norway spruce seedlings within 4 h to 1 day in root and shoot. The nitrate uptake and incorporation was dependent on the external nitrate concentration applied. The at% $^{15}$N in the amino acids was generally increasing to a maximum within 3 to 7 days and decreasing 10 days after the tracer application again. In the forest the treatments applied in the previous year had no effect on the $\delta^{15}$N in the roots. From 2 months after $^{15}$N application on, the $\delta^{15}$N started to decrease to about 60% of its maximal value within one year, irrespective of what treatment was applied. Nine months after tracer application a detailed investigation of the $\delta^{15}$N distribution of one spruce root system with roots of various sizes and from various soil depths showed significantly higher $\delta^{15}$N values in the roots of the upper soil horizon compared to roots at 30 - 40 cm or 60 - 70 cm depth. Furthermore, the $\delta^{15}$N was higher in fine roots compared to root tissue of roots bigger in diameter than 5 mm. Thus the $^{15}$N was not distributed extensively within the root system, but remained in the fine roots of the topsoil, the sites of nutrient uptake.
Zusammenfassung

Wälder in grossen Teilen Europas und Nord-Amerikas sind durch hohe atmogene Stickstoff (N) Einträge beeinträchtigt, die zu N Sättigung und Nährstoff-Ungleichgewichten führen. In einem Feldexperiment in einem Fichtenwald des Schweizer Mittellandes, wurden über zwei Jahre die Effekte eines Holzasche-Recyclings (A), einer optimalen Flüssig-Düngung (WF) und einer Wasser Variante (W) auf die Feinwurzeln (≤ 2 mm) im Vergleich zu einer Kontrolle (C) untersucht. Der Einfluss dieser Behandlungen auf das Wachstum und die Elementzusammensetzung der Wurzeln wurde beobachtet. Die Aktivität der Nitratreduktase (NRA) in den Feinwurzeln, die als Indikator für Nitrat im Boden fungieren könnte, wurde während der Vegetationszeit gemessen. Darüber hinaus wurde ein $^{15}$N Tracer Experiment durchgeführt, um die N Aufnahme in die Wurzeln zu studieren.


Eine schnelle Aufnahme des $^{15}$N in die Feinwurzeln konnte im Feld wie auch im Laborexperiment gezeigt werden. Innerhalb eines Tages nach Tracer-Ausbringung auf den Waldboden waren bereits 50% des maximal beobachteten $\delta^{15}$N Wertes in den Feinwurzeln erreicht, der maximale $\delta^{15}$N wurde nach ca. einem Monat beobachtet. Das applizierte $^{15}$N im Laborexperiment liess sich innerhalb von 4 h bis zu 1 Tag in den wichtigen Aminosäuren in Wurzeln und Spross von Fichtenkeimlingen wiederfinden. Die Nitrat Aufnahme und Assimilation war dabei von der extern applizierten Nitratkonzentration abhängig. Die at%$^{15}$N in den Aminosäuren erreichte zwischen 3 und 7 Tagen ihr Maximum und begann 10 Tage nach der Tracer-Applikation wieder zu sinken. Im Wald hatten die Behandlungen des vorangegangenen Jahres scheinbar keinen Einfluss auf die $\delta^{15}$N Werte in den Wurzeln. Nach 2 Monaten begannen die $\delta^{15}$N Werte in den Wurzeln wieder zu sinken und erreichten ein Jahr nach der $^{15}$N Applikation ca. 60% des Maximalwertes, unabhängig von der jeweiligen Behandlung. Neun Monate nach Ausbringen des Tracers zeigte eine detaillierte Untersuchung der $\delta^{15}$N Verteilung in einem Wurzelsystem an Hand von Wurzeln verschiedener Grösse und aus verschiedener Bodentiefe signifikant höhere $\delta^{15}$N Werte in Wurzeln des oberen Bodenhorizonts, verglichen mit Wurzeln aus 30 - 40 cm oder 60 - 70 cm Tiefe. Darüber hinaus war der $\delta^{15}$N Wert höher in den Feinwurzeln im Vergleich zu Wurzeln mit einem Durchmesser grösser als 5 mm. So wurde $^{15}$N innerhalb des Wurzelsystems nicht massgeblich verteilt, sondern verblieb in den Feinwurzeln der oberen Bodenschichten, den Orten, wo Nährstoffe vorwiegend aufgenommen werden.
1 Introduction

1.1 N in forests

Rising N emissions through anthropogenic activities (Galloway, 1995) have led to a high N deposition into terrestrial ecosystems (Vitousek et al., 1997; Fowler et al., 1998). In Switzerland for example the emission of N oxides increased from about 20’000 t y\(^{-1}\) in 1920 to more than 100’000 t y\(^{-1}\) in recent years, and a deposition of about 30 kg N ha\(^{-1}\) y\(^{-1}\) into the forest has been documented (Ortloff and Schlaepfer, 1996; SAEFL, 1999). Ecosystems that have usually been N limited, like boreal and temperate forests (Keeney, 1980), are subjected to strong and mainly still unknown changes (Rennenberg and Gessler, 1999). Trees are adapted to N limitation rather than to N excess. The excess availability of N can lead to imbalances in tree nutrition and contributes to an increase of soil acidity (Nihlgard, 1985; Hüttl, 1990; Vitousek et al., 1997; Aber et al., 1998). The high N depositions lead to increasing N saturation in the forest. This N deposition together with the actual N status of the soil affects the nitrate and ammonium levels in the forest soil and forest soil solution (Bredemeier et al., 1998). Although most forests have a remarkable ability to retain N (Johnson, 1992), after a certain time the nitrification rate and the nitrate availability in the forest soil increases (Aber et al., 1998). Nitrate leaching out of the system is described as one symptom of N saturation, although leaching of nitrate can also occur depending on the transport processes in the soil (Hagedorn et al., 2000).
1.2 Forest management

Beside efforts made to minimise the high anthropogenic impact of N on forests, management practices that support or restore the natural forest functioning is desirable. Up to date in Switzerland any fertilisation of a forest is forbidden by law. In other European countries forestry already used application of lime, ashes and composite-fertilisers to improved forest health and/or productivity. In agricultural as well as in a forest ecosystems sustainable fertilisation practices aim at optimised production, while maintaining or improving the soil fertility. The idea is to re-supply nutrient that were exported for example through harvest. The sustainable fertility should be reached with a minimum of environmental negative impact on soil, organisms, water and air (Gisi et al., 1997).

The application of alkalising amendments as lime, peat or wood ash have been tested in various studies (e.g. Persson and Ahlström, 1992; Persson and Ahlström, 1994; Bramryd and Fransman, 1995; Meiwes, 1995; Kahl et al., 1996; Büttner et al., 1998; Bundt et al., 2001b; Nilsson et al., 2001). The idea of bringing wood ash back into the forest is not new as Vance (1996) described in an overview on ash usage. But in recent times technology has been improved, to rise the quality of wood ash deriving from wood chip combustion and to guarantee a minimum contamination with heavy metals or organic pollutants. Thus a responsible recycling into the forest might be possible. The application of wood ash to the forest has the potential to solve two conflicts in one technique. In Switzerland at present more than 25'000 t wood ash deriving from energy production have to be discarded every year and wood as a renewable energy source is underpinned for the future by forest policy in Switzerland (SAEFL, 1999). The discarded wood ash contains relevant basic cations, that have often been recorded to be missing in forest ecosystems on acidic soils. Thus, there is a need to evaluate the possibilities and effects of a wood ash recycling in a Swiss forest.

Another idea of dealing with nutritional imbalances in forest ecosystems is the application of a steady state fertilisation that is adapted to the element 'need' of a certain forest ecosystem (Linder, 1995; Salih and Andersson, 1999). The element
amount applied is calculated according to the results of foliage analyses. An optimal nutrition of the trees aims towards improved forest health and optimum of wood production. Thus, the resistance of the forest ecosystem against many types of stress factors (Hüttl, 1990) is supported.
1.3 Nitrate reductase

In general coniferous forest on acidic soils are characterised by a low nitrification rate and ammonium is the dominant form of inorganic N in the soil solution (Aber et al., 1998). Furthermore, for the nutrition of spruce, nitrate is of minor importance as N source (Kronzucker et al., 1997). But with increasing N deposition the ammonium to nitrate ratio of the soil solution decreases and the change in nitrate reductase activity in the roots might be an indicator of this development.

The nitrate reductase (NR, EC 1.6.6.1-3) is a well characterised enzyme in the N metabolism of plants. It mediates the assimilation of nitrate into nitrite with NADH/NADPH or both as an electron donor. In higher plants the NR is a homodimeric enzyme, each subunit containing a 100-kD polypeptide with a Mocofactor, heme and FAD. The activity, but also the protein degradation is regulated by a phosphorylation/ dephosphorylation mechanism (Kaiser and Huber, 1997). Various factors are known to influence the activity as for example light, anoxia, nitrate supply, ammonium supply, and the concentration of certain amino acids (Tischner, 2000). The NR is mainly located in the cytosol of the plant, but recent research has shown, that there is at least in some species also a plasma-membrane-bound NR (PM-NR). Assumptions concerning the function of the PM-NR are the role as a blue light sensor, a nitrate sensor, or a protection against excess nitrate (Tischner, 2000).

NR is a key enzyme of the N metabolism. Structure and function as described above are well known from studies of model plants like barley, spinach, maize and others (Campbell, 1996; Tischner, 2000). In tissues containing high concentrations of phenolic substances it is difficult to measure the in vitro activity of the NR (Hageman and Reed, 1980; Peuke and Tischner, 1991; own results), especially since this enzyme is rather sensitive and activity is decreasing very fast at temperatures above 0 °C. For the NRA of the roots of spruce trees a reliable in vitro assay has not been established. The in vivo NRA, as measured in the present study, resembles an overall measurement of the nitrate reduction. The results are relative data to the nitrate reduction capacity as it is found in the roots (Bauer, 1997). This method
integrates over influx/efflux, transport and assimilation processes of all NR-isoformes under high nitrate supply (Crawford and Glass, 1998; Tischner, 2000). The enzyme amount, gene expression or the activation state of the NR can not be assessed with this method. But Peuke and Tischner (1991) and Kronzucker et al. (1995) have already shown the correspondences between uptake reactions and in vivo nitrate reduction in the roots of coniferous trees. Furthermore, the in vivo assay has shown to be an elegant tool to gain relative data of the overall nitrate reduction capacity in different tissues (Downs et al., 1993; Cumming and Brown, 1994; Truax et al., 1994; Kronzucker et al., 1995; Muller et al., 1996; Claussen and Lenz, 1999; Thomas and Hilker, 2000) and is also suitable for field studies (Tjoelker et al., 1992; Bauer, 1997; Högberg et al., 1998). Högberg et al. (1986) first showed, that the NRA of Deschampsia might be an indicator towards nitrate availability in the forest soil. Later a higher NRA in the fine roots of Norway spruce on central European sites with higher N deposition, than on northern European sites with low N deposition hinted to similar possibilities of tree roots (Högberg et al., 1998). The contribution of nitrate to the N supply of the tree was estimated to range from 3% in northern Sweden to about 38% in Germany (Bauer, 1997). But an experimental approach in the forest, that changes the nitrate concentrations in the soil to evaluate the suitability of the NRA of fine roots as an indicator, is yet missing.
1.4 Experimental approach

The HARWA project (‘Optimale Ernährung und Holzasche Recycling im Wald’, i.e. 'Optimal nutrition and wood ash recycling in the forest'), which is part of the European COST-action E6 'Forest tree physiology research', aimed to investigate the consequences of a high quality wood ash application to a spruce forest on acidic soil. Furthermore the opportunities of a steady state fertilisation were analysed. The composition of this fertilisation was determined as an 'optimal nutrition' supply according to Ingestad (1979).

1.4.1 Study site

The experiment was conducted in a spruce forest on the Swiss Plateau. The 'Schladwald' (N 47°30'34"/E 08°20'50", 464 m a.s.l.) forest is located about 25 km northwest of Zürich, Switzerland. The 70-year-old stand is classified as a Galio odorati - Fagetum luzuletosum (Ellenberg and Klötzli, 1972) but dominated by Norway spruce (Picea abies (L.) Karst.). The stand of approximately 350 trees has undergone normal silvicultural thinning, resulting in evenly distributed stems. The soil is an acidic brown forest soil classified as Haplumbrept and more detailed information is given by Bundt et al. (2001c). Some characteristics are listed in Table 1.1. The 15-year average of annual precipitation is 1076 mm and air temperature is 9.6° C (SMA, 1998).
Table 1.1: Selected properties of the soil matrix in Unterehrendingen according to Bundt et al. (2001c).

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>pH</th>
<th>Water (%)</th>
<th>C$_{org}$ (g kg$^{-1}$)</th>
<th>N$_{tot}$ (g kg$^{-1}$)</th>
<th>CEC$_{eff}$ (mmol c kg$^{-1}$)</th>
<th>BS$_{eff}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>3.4</td>
<td>34</td>
<td>24.3</td>
<td>1.57</td>
<td>74.6</td>
<td>21</td>
</tr>
<tr>
<td>9-20</td>
<td>3.6</td>
<td>27</td>
<td>12.9</td>
<td>0.88</td>
<td>60.7</td>
<td>18</td>
</tr>
<tr>
<td>20-50</td>
<td>3.8</td>
<td>24</td>
<td>6.4</td>
<td>0.60</td>
<td>58.4</td>
<td>26</td>
</tr>
<tr>
<td>50-100</td>
<td>3.8</td>
<td>24</td>
<td>2.9</td>
<td>0.34</td>
<td>80.4</td>
<td>43</td>
</tr>
</tbody>
</table>

$^a$ effective cation exchange capacity measured in a 1 M NH$_4$NO$_3$ extract

$^b$ effective base saturation (sum Ca, K, Mg, Na percent of CEC$_{eff}$)

1.4.2 Treatments

The experiment was set up as a random block design with four treatments and four repetitions. The plots of each treatment ranged between 200 and 600 m$^2$ in size. The experimental treatments were a control without any treatment (C), irrigation of stream water (W), irrigation of liquid fertiliser (WF, ‘water and fertiliser’), and the application of wood ash (A). The wood ash, deriving from wood chip combustion, was applied by hand on May 25th 1998 and July 23rd 1999, respectively. The WF treatment was based on a nutrient combination according to Ingestad and Lund (1986) related to 70 (1998) or 100 (1999) kg N per ha (Table 1.2). The pH of stream water used for dilution of the fertiliser and for the W treatment was about 8.5. The wood ash and stream water were analysed, and inputs of elements estimated (WF including the contribution of the stream water, Table 1.2). The WF and W treatments were irrigated each night from May 25th 1998 to Sept. 28th 1998 and from May 6th 1999 to Sept. 27th 1999 except for days with intensive rain.
Table 1.2: Element input (kg ha⁻¹ y⁻¹) by the HARWA treatments, A = wood ash, WF = fertiliser, W= water, C= control, n.d. = not determined.

<table>
<thead>
<tr>
<th>Elements</th>
<th>C</th>
<th>A</th>
<th>WF 1998</th>
<th>WF 1999</th>
<th>W^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0</td>
<td>n.d.</td>
<td>77</td>
<td>100</td>
<td>7</td>
</tr>
<tr>
<td>S</td>
<td>0</td>
<td>19</td>
<td>17</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td>P</td>
<td>0</td>
<td>66</td>
<td>13</td>
<td>17</td>
<td>0.1</td>
</tr>
<tr>
<td>Ca</td>
<td>0</td>
<td>779</td>
<td>183</td>
<td>184</td>
<td>182</td>
</tr>
<tr>
<td>K</td>
<td>0</td>
<td>96</td>
<td>64</td>
<td>85</td>
<td>3</td>
</tr>
<tr>
<td>Mg</td>
<td>0</td>
<td>101</td>
<td>33</td>
<td>35</td>
<td>25</td>
</tr>
<tr>
<td>Fe</td>
<td>0</td>
<td>22</td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Mn</td>
<td>0</td>
<td>17</td>
<td>0.07</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>0</td>
<td>0.9</td>
<td>&lt;0.01^b</td>
<td>&lt;0.01^b</td>
<td>n.d.</td>
</tr>
<tr>
<td>Al</td>
<td>0</td>
<td>23</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Cu</td>
<td>0</td>
<td>0.6</td>
<td>&lt;0.01^b</td>
<td>&lt;0.01^b</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

^a import of the stream water extrapolated with element concentrations of the water

^b estimated input of stream water negligible
1.5 Objectives

1.5.1 Root growth and elements

The nutritional status and growth of the fine roots were investigated. Root growth was observed according to biomass, root architecture (length, diameter, amount of tips and forks) and growth dynamic by the use of sequential soil coring and ingrowth-cores. Element concentrations in the fine roots have been assessed once a year. The changes due to the treatments applied in the field have been followed for two growing seasons. Based on results of the foliage analyses an evaluation of elements in the fine roots as indicators for the nutritional status of the forest was carried out. The fine roots are probably more sensitive indicators for sustainable forest nutrition than the foliage or soil parameters (Persson et al., 1995; Bakker, 1999).

In chapter 2 the following questions are approached:

- How do the applied elements affect the element concentrations in the fine roots?
  Could the element concentrations be related to changes in soil characteristics?
- Do the treatments affect the root growth or root growth dynamic?
- Are the root element data suitable indicators of forest nutrition? Are the results comparable to the foliage analyses?
- Is forest nutrition improved by the treatments?
1.5.2 NRA in Norway spruce roots

Nitrate is of minor importance as N source for the nutrition of spruce on acid soils (Kronzucker et al., 1997). The soil solution is usually dominated by ammonium, but with increasing N deposition the ammonium to nitrate ratio changes (Aber et al., 1998). The fine roots as organs of nutrient uptake are the first plant parts to be affected by changes in the soil N. A central hypothesis of this project is, that changes in the soil are reflected by the fine roots. In coniferous trees the major part of the nitrate reduction takes place in the roots in contrast to broad-leaved plants, where the main nitrate reduction is located in the leaves. An estimate of the contribution of roots to whole plant level nitrate reduction ranged between 8 and 30% for young broadleaf trees compared to 86% for 140-year-old Norway spruce trees (Gebauer and Schulze, 1997). Thus the NR in the roots of Norway spruce is assumed to be a good indicator to changing nitrate availability in the soil.

The questions, approached in chapter 3 were:

- **How is the in vivo NRA in Norway spruce roots affected by changing nitrate concentrations? How fast does the NRA react, what are the threshold concentrations in the substrate, and what is the influence of ammonium?**
- **In chapter 4 the hypothesis was addressed, that the NRA is suitable as an indicator of soil nitrate in the forest. How is the NRA influenced by other soil factors?**
1.5.3 N uptake

The nitrate and ammonium uptake of higher plants have been characterised to be mediated by several transporters and analysed in detail (Glass et al., 2001). Nitrate uptake has been shown to be on the one hand negatively influenced by the internal nitrate concentrations in the plant and on the other hand is also regulated by the concentrations of certain amino acids (Glass et al., 2001). The N uptake can be measured with the stable isotope $^{15}$N and the pool sizes were estimated by the dilution of the tracer in several studies, observing the ratio of N taken up in relation to N immobilised in the soil (e.g. Buchmann et al., 1996; Bauer et al., 2000). The natural $^{15}$N signature in a forest is mostly within a tight range (Fry, 1991). In tissues the $^{15}$N/$^{14}$N ratio (often described as $\delta^{15}$N, i.e. the ratio of $^{15}$N/$^{14}$N compared to the ratio in air) is determined by the $^{15}$N signature of the N sources and the fractionation processes occurring within the uptake and the eventually participating symbiotic partner (Hobbie et al., 2000). When applying a highly $^{15}$N-enriched N source, the uptake of the ‘tracer’ can be followed into the plant within hours to days.

*In chapter 5 the following questions were studied:*

- How fast is $^{15}$N taken up by the roots and how is it distributed within the root system? How much do the fine roots contribute to N retention in a spruce forest on the Swiss plateau?
- Is the short term $^{15}$N uptake affected by the treatments applied in the field?
- How fast is nitrate assimilated into the free amino acid pool? Is the assimilation dependent on the external nitrate concentration?
1.5.4 Synthesis

In chapter 6 the results are discussed in some general context. The indicator abilities of the fine roots towards the N status of a forest or the N in the soil were investigated. The N metabolism was observed with the changes of N availability in the soil due to the treatments. The question of the suitability and practicability of the treatments to improve nutritional imbalances and thereby improve forest health on the one hand, and the evaluation of the environmental risk of fertilisation treatments in the context of sustainability on the other hand is of interest.
2

Fine root growth and element concentrations of Norway spruce as affected by liquid fertilisation and wood ash

to be submitted to *Plant and Soil*
2.1 Abstract

A field experiment to test various management practices of sustainable forestry has been conducted in a Swiss spruce forest for two growing seasons. Treatments were a control (C), yearly application of 4 t ha\(^{-1}\) wood ash (A), daily irrigation of a steady state fertilisation as ‘optimal nutrition’ (WF) and irrigation of a water control (W). A grid sampling on a 5 m x 5 m distance was conducted once a year with a soil corer to determine fine root biomass (≤ 2 mm) and soil pH of the Ah and B horizon. A subset of the fine root samples was further analysed on its nutrient composition by CN and ICP-AES analyses. The dynamic of root growth was observed with the aid of ingrowth-cores after 10, 17 and 22 months of growth and growth pattern was analysed according to biomass, tips, forks, length and root diameter of the samples. The A, WF and also the W treatment resulted in a significant increase of soil pH in the Ah and B horizon. The fine root density increased over the two growing seasons irrespective of the treatment. The root growth dynamic was only slightly different between the treatments with a initially faster growth under A treatment. The W treatment reduced the number of root tips and forks, and the root length while the A treatment increased the number of root tips, forks and the root length, but reduced the diameter. The differences between the three harvesting times of the ingrowth-cores stressed seasonal differences in root growth and the development of a quasi ‘steady state’ root dynamic. The elements in the fine roots were strongly affected by A and WF and sometimes by W treatment. Fine root N increased with the WF treatment, while C concentrations decreased in A, WF and W treatments. The Ca and Mg concentrations were strongly affected by A but also by the WF treatment. The K and P concentrations in the fine roots were improved by all three applications. Due to the pH increase Al, Fe and Mn concentrations in the fine roots were decreased by the A and WF treatments. S and Zn concentrations showed inconsistent changes over the growing seasons. The results of this study can be placed in a European context and confirm the abilities of the fine roots as indicators of sustainable forest nutrition, to some extent more sensitive than the commonly used foliar analysis.
2.2 Introduction

The consequences of high N depositions and soil acidification are reported as nutritional imbalances, often deficiencies in Mg, P or K (Hüttl, 1990; Schulze and Freer-Smith, 1990; Linder, 1995; Matzner and Murach, 1995; Salih and Andersson, 1999) in relation to N. In Switzerland mainly imbalances of N and P have been reported in spruce forests (Landolt, 1997; Flückiger and Braun, 1998). To compensate nutritional imbalances of trees and acidification of forest soils, several ameliorating methods have been applied (e.g. Rapp, 1992; Persson and Ahlström, 1994; Meiwes, 1995; Vance, 1996; Eriksson et al., 1998; Hahn and Marschner, 1998a; Nilsson et al., 2001). The effects of wood ash applications on forest soils have been studied extensively (e.g. Bramryd and Fransman, 1995; Meiwes, 1995; Kahl et al., 1996; Vance, 1996; Eriksson, 1998), but only few studies were concerned with the effects on the fine roots (e.g. Persson and Ahlström, 1992; Persson and Ahlström, 1994; Clarholm, 1998). Another amelioration technique, which has been applied mainly in Scandinavia, is a steady state or compensatory fertilisation, which supplies the nutrients that are needed. The success of this practice is usually monitored by foliage analyses. Such a steady state fertilisation can result in a long term amelioration of the nutritional status of the trees, improving the resistance of the forest ecosystem against stress factors (Hüttl, 1990). The hypothesis, that fine roots are probably more sensitive indicators for forest sustainability than the foliage or soil parameters (Persson et al., 1995; Bakker, 1999) is investigated in the present study.

The HARWA field experiment was intended to monitor effects of a wood ash recycling and a steady state fertilisation on a forest ecosystem dominated by Norway spruce. The objectives of this study were to investigate the element changes in the fine roots deriving from the ameliorating practices. Furthermore, possible effects on root growth and root growth dynamics should be studied. Together with the soil pH and element changes of the needles an evaluation of the suitability of the management practices and of fine root analyses as indicators of sustainable forest nutrition should be conducted.
2.3 Materials and methods

2.3.1 Field experiment

The experiment was conducted in a spruce forest on the Swiss Plateau, nearby Zürich, Switzerland. The stand was dominated by Norway spruce (Picea abies (L.) Karst.). The soil was an acidic brown forest soil. The treatments applied were a control without any treatment (C), irrigation of water (W), irrigation of liquid fertiliser (WF) and the application of wood ash (A). The wood ash, deriving from wood chip combustion, was applied by hand in May 1998 and July 1999 respectively. WF and W treatments have been irrigated daily during the growing season (May to September) except for days with intensive rain. More detailed information is given in chapter 1 of this thesis.

2.3.2 Sampling design

Sampling of soil and roots was done in April 1998, 1999 and 2000. The samples were taken independent of the plot design in a 5 m to 5 m grid over the whole site (Figure 2.1). At each sampling point 3 soil cores (10 cm depth, diameter 8.5 cm) were collected, divided by horizon A and B and the three samples were pooled. The samples were sieved, the fine roots (≤ 2 mm) of Norway spruce were selected from each bulk sample and washed. The roots were dried at 60°C for at least 3 days and weighted (separated by horizon).

Needles were sampled in winter (December, January) of 1997/98 and 1999/2000 from 136 selected spruce trees within the experimental site. Trees were selected to be located at a minimum distance of 4 m to the neighbour-treatment plot. 100 medial needles were taken of a shoot from the uppermost whorl of the sample tree. Needle samples were dried at 65 °C till constant weight.
2.3.3 Analyses

The analyses of soil pH in a 0.01 M CaCl₂ extract according to Brunner et al. (1999) and fine root biomass (dry weight, DW) was done for the 257 sample points. The fine root material of 110 selected samples of the A horizon (Figure 2.1) and the needle samples of the 136 trees were ground with a mill (Retsch MM2000, Hann, Germany) for further analyses. Total C and N was measured with a CN auto-analyser (N2500, Carlo Erba Instruments, Milano, Italy). Other elements were measured after digestion of the ground material in a high pressure microwave (Milestone MLS Ultraclave) by ICP-AES (Optima 3000, Perkin Elmer).

Figure 2.1: Grid sampling design and assigned treatments, C = control, W = water, WF = liquid fertiliser, A = ash plots, encircled = sampling positions with element analyses of fine roots.
2.3.4 Ingrowth-cores

To study the root growth dynamic 36 sample trees were selected, 9 trees in each treatment. At each sample tree 9 ‘ingrowth-cores’ were installed in May 1998. First, the soil was taken out with a soil corer (diameter 5.5 cm) to about 10 cm depth, divided by horizon and sieved. In the hole a net-cylinder of glass fibre (11 cm height, 5 cm diameter, 5 mm meshes) was installed, before the sieved soil was filled back in the hole again. Minimum distance between two cores was 10 cm in a distance of 1 to 1.5 m of the sample tree.

2.3.5 Harvest ingrowth-cores

The ingrowth-cores were harvested in March 1999, October 1999 and March 2000, i.e. 10, 17 or 22 months after installation. At each sampling event 3 ingrowth-cores per tree were harvested using a big soil corer (diameter 8.5 cm). The soil cores were taken to the lab in plastic bags and stored at 4°C for maximal 3 days until analyses. With a sharp knife the roots and soil were cut off the net-cylinder. The soil depth was measured and the cylinder was opened with the aid of scissors. Horizons were not analysed separately, because at the first harvest it was observed that roots grew as fast in the B horizon as in the A horizon, because of the influence on soil density in the B horizon through the installation of the cores. Roots of Norway spruce were washed out, scanned and the architecture was analyses with the WINRHIZO software (Regent Instruments Inc., Quebec, Canada). Afterwards the roots were dried (60°C, 3 days) and the biomass was determined.
2.3.6 Statistics

Statistical analyses were performed as provided by Statview 5.0 (SAS Inc.). To analyse differences in soil pH, the fine root biomass and fine root elements in grid-samples a repeated-measurement ANOVA and a Fisher’s PLSD post-hoc test were performed within one treatment on the differences between the years. The ingrowth-core data were analysed with a one-way ANOVA on treatment effects, within one harvest. Furthermore, a two-way ANOVA on treatment and time was conducted to reveal differences between the three harvesting events. For the parameters concerning the growth pattern (tips, length, forks, diameter), samples without fine roots were not considered.
2.4 Results

2.4.1 Biomass, growth dynamic and soil pH

The fine root biomass as monitored by the soil coring was not affected by the treatments, when considering Ah and B horizon separately (Table 2.1). If data were related to both horizons, there was a significant difference in fine root density between the harvesting events in samples of C and A treated plots (Table 2.1). In the soil of the A plots a significantly higher root density was observed after two years. The root density in the year 2000 was in the soil of C plots higher than in the year 1998. A general increasing root density could be observed within the two years irrespective of the treatments (Table 2.1).

The ingrowth-cores revealed 10 months after installation a higher proportion of fine root biomass in the cores of the A plots than in the cores of the W plots (Figure 2.2). The biomass of fine roots in the cores at the second or third harvest, in October 1999 and March 2000, were not different between the treatments. The root growth pattern was affected by the treatments. Taken all single differences in account (Figure 2.2), the W treatment seemed to reduce the number of root tips and forks, and reduced the root length. The A treatment increased the number of root tips, forks and the root length, but reduced the diameter of the fine roots (Figure 2.2). Results depended also on the harvest time. A two-way ANOVA revealed a lower fine root biomass in the ingrowth-cores in March 99 than in October 1999. In March 2000 the biomass was comparable to October 99. The diameter of the roots was bigger at the last harvest 2000, than at the harvests in 1999. In October 1999 the roots had a higher density in forks, more tips and a higher length than in both spring harvests.
Table 2.1: Biomass given as fine root density (g dm⁻³) as presented in different horizons and affected by the treatments, different letters indicate a significant difference between the years within one treatment and soil horizon at a 1% probability.

<table>
<thead>
<tr>
<th>Fine root biomass</th>
<th>C</th>
<th>A</th>
<th>WF</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ah-horizon (0-3 cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>1.15 a</td>
<td>2.19 a</td>
<td>1.75 a</td>
<td>1.26 a</td>
</tr>
<tr>
<td>1999</td>
<td>1.34 a</td>
<td>1.86 a</td>
<td>1.61 a</td>
<td>1.26 a</td>
</tr>
<tr>
<td>2000</td>
<td>1.59 a</td>
<td>2.51 a</td>
<td>2.10 a</td>
<td>1.28 a</td>
</tr>
<tr>
<td><strong>B-horizon (3-10 cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>0.19 a</td>
<td>0.27 a</td>
<td>0.28 a</td>
<td>0.22 a</td>
</tr>
<tr>
<td>1999</td>
<td>0.22 a</td>
<td>0.36 a</td>
<td>0.30 a</td>
<td>0.23 a</td>
</tr>
<tr>
<td>2000</td>
<td>0.21 a</td>
<td>0.35 a</td>
<td>0.35 a</td>
<td>0.21 a</td>
</tr>
<tr>
<td><strong>Ah+B horizon (0-10 cm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>0.38 b</td>
<td>0.60 b</td>
<td>0.62 a</td>
<td>0.44 a</td>
</tr>
<tr>
<td>1999</td>
<td>0.47 ab</td>
<td>0.72 b</td>
<td>0.62 a</td>
<td>0.45 a</td>
</tr>
<tr>
<td>2000</td>
<td>0.52 a</td>
<td>0.91 a</td>
<td>0.76 a</td>
<td>0.53 a</td>
</tr>
</tbody>
</table>

When the pH of the soil (Ah horizon) was considered at the same sample points, where the fine roots of the element analysis derived from, an elevated pH in the plots of all three treatments could be observed in both years. While the change of pH in the soil of WF and W treated plots was in a similar range, the pH of the A treated plots made a high shift of about 1.5 units after two years of treatment (Figure 2.3). In
general (results of all sample locations) the pH in the A horizon of the soil was increased by the A, WF and W after the first and even more after the second growing season (Table 2.2). In the mineral soil of the B horizon the pH shift was less pronounced and was significant in the three treatments only after the second year (Table 2.2).

Table 2.2: Development of the soil pH in the soil core samples. Different letters indicate a statistic significant difference between the years within one treatment and soil horizon at a 1% probability.

<table>
<thead>
<tr>
<th>pH</th>
<th>C</th>
<th>A</th>
<th>WF</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ah–horizon (0–3 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>3.28 a</td>
<td>3.26 c</td>
<td>3.25 c</td>
<td>3.34 b</td>
</tr>
<tr>
<td>1999</td>
<td>3.30 a</td>
<td>3.70 b</td>
<td>3.38 b</td>
<td>3.45 b</td>
</tr>
<tr>
<td>2000</td>
<td>3.35 a</td>
<td>4.63 a</td>
<td>3.50 a</td>
<td>3.55 a</td>
</tr>
<tr>
<td>B-horizon (3-10 cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>3.46 a</td>
<td>3.47 b</td>
<td>3.45 b</td>
<td>3.50 b</td>
</tr>
<tr>
<td>1999</td>
<td>3.49 a</td>
<td>3.51 b</td>
<td>3.46 ab</td>
<td>3.51 b</td>
</tr>
<tr>
<td>2000</td>
<td>3.50 a</td>
<td>3.67 a</td>
<td>3.51 a</td>
<td>3.55 a</td>
</tr>
</tbody>
</table>
Figure 2.2: Biomass given as fine root density (g dry weight dm$^{-3}$), amount of forks, tips, length (cm) per cm$^3$ soil and fine root diameter (mm) of the ingrowth-cores samples, columns represent means ± SE of the respective parameter (n=27). Different letters indicate a significant difference at 5 % probability according to a one-way ANOVA on the factor treatment within one harvest.
2.4.2 Elements in the fine roots

No differences (one-way ANOVA probability level 1%) in the fine root elements were detected between the designated plots in the samples of 1998, before the start of the treatments (data not shown). Within the two years of treatment Mg, Ca, P and K concentrations in the fine roots of Norway spruce were improved by the A as well as by the WF treatment (Figure 2.3 and 2.4). The W treatment had also a significant effect on K, P and, after two years of treatment, on Ca and the Ca/Al molar ratio in the fine roots and a tendency ($p < 0.1$) towards higher Mg concentrations after two years. The Al and Fe concentrations were higher in 1999 than in 1998 in the C and W treated samples and lower in 2000 than in 1998 in the A and WF treated roots. The Ca/Al was increased after two years in the roots of A and WF plots (Figure 2.3). In the roots sampled 1999 the S and Zn concentrations were increased compared to 1998, notwithstanding of the treatment applied, while in the samples of 2000 all S and Zn concentrations were in the range of 1998 again (Figure 2.4). The N concentration of the roots was only significantly changed in the WF treatment after one year. The N concentration after two years was not significantly changed in any treatment (Figure 2.4). The C concentration in the root was negatively influenced by the A, LF and W treatment after one year but in the second year this negative effect could only be observed in the root of the A treatment. The C/N ratio in the fine roots was decreased by the WF and the W treatment, while the A treatment seemed to have no effect compared to the control (Figure 2.4). The Mn concentration was significantly reduced by the A and WF treatments, in contrast to a significant increase in the roots of C plots that was observed after two years (Figure 2.4).

Mean Cu and Cd concentrations in the fine root were in the range of 9.0 - 10.4 and 1.7 - 2.2 mg kg$^{-1}$, respectively. An accumulation, that means increased concentrations in the fine roots due to the A treatment, of heavy metals like Cu, Cd (data not shown) and Zn (Figure 2.4) were not observed in the roots after two years with a total wood ash input of 8 t ha$^{-1}$. 
Figure 2.3: Changes of elements in the fine roots and the soil pH after one and after two years of treatments, columns represent a mean ± SE (n=25 to 31), C = control, W = water, WF = liquid fertiliser, A = ash. Significant differences to the element concentrations of the analyses before treatments are indicated: *p < 0.05, **p < 0.01, ***p < 0.001.
Figure 2.4: Changes of elements in the fine roots after one and after two years of treatments, columns represent a mean ± SE (n=25 to 31), C = control, W = water, WF = liquid fertiliser, A = ash. Significant differences to the element concentrations of the analyses before treatments indicated: $p < 0.05 \ast$, $p < 0.01 \ast\ast$, $p < 0.001 \ast\ast\ast$. 
2.4.3 Element change in current needles

Within two years an increase of the N and P concentrations in the current needles could be observed with the WF treatment. But in general the N concentrations in the needles were higher in the 2000 samples than in the sampling before treatments (Table 2.3). Mg, Ca, S and Al concentrations were not significantly changed in the current foliage. K concentrations were increased with the A and WF treatment but also in the control plots. The Mn concentrations decreased in the C and W treatments, while Zn was increased in the foliage of the A and WF treated trees. Fe concentrations were increased irrespective of the treatment (Table 2.3).
Table 2.3: Element concentrations (mg g^{-1} dw) in the current needles before the treatments and after two years of treatment (harvest in autumn of the previous year), ns = not significantly different, *, **, *** difference with p<0.05, p<0.01, p<0.001, respectively.

<table>
<thead>
<tr>
<th>Needles</th>
<th>C</th>
<th>A</th>
<th>WF</th>
<th>W</th>
<th>suff. Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15.1</td>
<td>15.9*</td>
<td>14.9</td>
<td>15.7*</td>
<td>14.4</td>
</tr>
<tr>
<td>S</td>
<td>0.93</td>
<td>0.88^{ns}</td>
<td>0.92</td>
<td>0.96^{ns}</td>
<td>0.93</td>
</tr>
<tr>
<td>P</td>
<td>1.40</td>
<td>1.32^{ns}</td>
<td>1.38</td>
<td>1.43^{ns}</td>
<td>1.31</td>
</tr>
<tr>
<td>Ca</td>
<td>4.60</td>
<td>4.14^{ns}</td>
<td>4.54</td>
<td>4.47^{ns}</td>
<td>5.33</td>
</tr>
<tr>
<td>K</td>
<td>3.37</td>
<td>4.00**</td>
<td>3.68</td>
<td>5.10***</td>
<td>3.37</td>
</tr>
<tr>
<td>Mg</td>
<td>0.92</td>
<td>0.94^{ns}</td>
<td>0.94</td>
<td>1.05^{ns}</td>
<td>1.02</td>
</tr>
<tr>
<td>Fe</td>
<td>0.038</td>
<td>0.042**</td>
<td>0.039</td>
<td>0.046***</td>
<td>0.038</td>
</tr>
<tr>
<td>Mn</td>
<td>1.96</td>
<td>1.66***</td>
<td>1.85</td>
<td>1.72^{ns}</td>
<td>2.21</td>
</tr>
<tr>
<td>Zn</td>
<td>0.016</td>
<td>0.018^{ns}</td>
<td>0.017</td>
<td>0.024**</td>
<td>0.021</td>
</tr>
<tr>
<td>Al</td>
<td>0.08</td>
<td>0.07^{ns}</td>
<td>0.08</td>
<td>0.08^{ns}</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* a) range of concentration described as sufficient for the respective element by Bergmann (1993).
2.5 Discussion

2.5.1 Root growth

In general a high variation in ingrowth-cores data could be observed. This variation was also reported by comparable studies earlier (e.g. Persson et al., 1998). The variation was not dependent on the distance from the tree, in which the ingrowth-core was installed (data not shown). Laboratory experiments have shown that high N supply could lead to reduced root growth (George et al., 1999) and also to an increased root growth (George and Marschner, 1996), while the majority of studies revealed a change of the root to shoot ratio that favours shoot growth (Marschner, 1995). The WF treatment contained 70 to 100 kg N ha\(^{-1}\) y\(^{-1}\), but a change in relative root growth was not observed.

The fine root density measured in the ingrowth-cores was higher than the comparable measurements of the grid samples with the soil corer. This observation was confirmed in former studies (Stober et al., 2000), and can be contributed to a change of soil density through the installation of the cores. According to the ingrowth-cores results of the present study, a trend after the first 10 months towards faster root growth with the A and WF treatments was observed. The WF treatment enhanced the nutrient input into the soil, while the A treatment improved the soil supply with cations. But the higher dynamic did not lead to an overall increased fine root density at the WF or A treated plots, as was confirmed by biomass data from the grid sampling. Magill et al. (2000) did not observe an influence on fine root biomass after several years of nitrogen addition in a pine or a hardwood stand. Nevertheless, a change in storage N due to enhanced N concentrations in the fine roots was reported (Magill et al., 2000), what is similar to the results of the present study in a spruce forest.

In general there might be a time lag, till differences concerning the growth in a forest could be observed, since two growing seasons are a short time in the time scale of trees. For example within a fertilisation experiment in Austria, changes of tree growth were observed only after 5 years, but not after two years of treatment.
Growth and elements

(Jandl et al., 2001). The relevance of the time period was also reported by Stober et al. (2000), who observed significant differences in fertilised against unfertilised ingrowth cores within two growing seasons in various spruce forests, while after one growing season no significant differences were observed (Stober et al., 2000). Similarly, Raich et al. (1994) used the ingrowth-cores filled with a fertilised substrate to detect nutrient limitations in a forest. After a period of 6 months they observed increased growth in the cores with additional N compared to the remaining cores (Raich et al., 1994). In the present study the higher proportion of roots grown in the cores after 10 months with the WF and A treatment could be contributed to a foraging of the fine roots. But the later harvests showed that the ingrowth-cores, that were subjected to other treatments, were colonised to the same extent with time, i.e. the final fine root density was not affected by the treatments, but only the initial growth dynamic has been influenced by A and WF treatment.

Increasing root densities in the topsoil could be observed irrespective of the treatment with the grid sampling. Only in the C and the A plots, a significantly higher root density was observed after two years of treatments. The fine root density was already different among the plots designated to the treatments before the start of the experiment in spring 1998. In Sweden wood ash application lead to a decrease in fine root biomass in the topsoil (Clemensson-Lindell and Persson, 1995). With a high input of Mg in an experiment in Germany the fine root biomass was strongly enhanced in a spruce forest within 3 growing seasons. But after a combined K and Mg fertilisation no growth effects were observed (Raspe et al., 1994).

The A treatment reduced the root diameter and C concentration of the fine roots. The smaller the roots are the higher the concentrations of most nutrients (e.g. N, Mg and P) and the lower the concentration of C (Gordon and Jackson, 2000). Consequently, in the present study the observed change in root size might have contributed to the change in element concentrations, although this contribution can be estimated to be very small. An enhanced root length with higher Ca/Al molar ratios was reported earlier as reviewed by Cronan and Grigal (1995). But considering the critical Ca/Al ratio for Al stress in the fine roots of 0.2, the roots of the present study, which had a Ca/Al molar ratio of about 1.5 (data not shown) before the
treatments, were far off being in Al stress. This is also due to the remarkable high Ca concentrations in the fine roots and needles, and therefore an assumedly good Ca supply, in Swiss forests (Table 2.3 and 2.4, Landolt, 1997; Brunner et al., 2001).

The influence of the A treatment on the root growth was probably a result of mitigated soil acidification. Persson et al. (1998) observed a stabilisation of growth with exclusion of N and S, the main contributors to soil acidification, by a roof. The roots grew thicker and shorter, what the authors concluded to be a sign of higher root vitality and enhanced mycorrhization. In contrast, in the present study the A treatment resulted in more branched, longer and thinner root growth pattern and nutrient data confirmed, that these roots had a nutritional balance that was comparable to roots of other European forests. An experiment in a Norway spruce forest in Sweden also reported increased length of fine roots caused by wood ash application (Clemensson-Lindell and Persson, 1995). Thus the change to a higher branching intensity and length in root growth could have been caused by an improved soil pH and nutrient availability.

A considerable part of the nutrients needed for growth might derive from remobilization within the plant (Millard, 1996). Millard (1996) reported that 18% to 93% of the N required for leaf growth might come from remobilisation. Remobilisation is also important for other nutrients and it contributed up to 82% and 52% of K or Mg, respectively, to the spring growth of young Pinus sylvestris, when external supply of these nutrients was low (Proe et al., 2000). Thus tree growth could be decoupled from nutritional supply over a period of time, what might explain the lack of effects on the root biomass in the present study.

### 2.5.2 Elements

The elements in the fine roots of the present study were in the range of reported values of other European study-sites (Table 2.4). The N concentration was moderate compared to high values reported from Germany and France and low values in Sweden. The Ca, Mg and K concentrations in the roots were high compared to German, French and Swedish results. N is enhanced in the fine roots only by the WF
treatment. As observed before the fine roots could be a significant sink for added N (Magill et al., 1997). Magill et al. (1997) calculated that about 15% of the over six years applied N was retained by a change in root storage N of red pine. The observation that N addition led to higher N concentrations in spruce fine roots was among others reported by Stober et al. (2000). Hahn and Marschner (1998b) investigated the element concentrations of fine roots of Norway spruce under acid irrigation and liming. On the acid irrigated plot Ca and Mg in the roots decreased, but 2 years after the end of irrigation no difference could be found. Liming increased root concentrations of Ca and Mg, and reduced Mn and Al. The results of liming were similar to the wood ash effects observed in the present study. In a field experiment on the effect of alkalising compounds wood ash and lime on the fine roots of spruce, the effects on the fine root chemistry were comparable to that observed in the present study: Ca, Ca/Al ratio, Mg, P and K were enhanced in the roots (Persson and Ahlström, 1994). In contrast to the results presented here, the concentrations of Mn increased two years after the ash application, while we observed decreasing concentrations in the roots.

Hüttl (1990) described the long lasting improvement through a balanced fertilisation, correcting deficiency in Mg and K supply. In contrast Ohno and Erich (1990) observed a decreasing relative availability of Mg and K through wood ash additions. The result that Ca was the most available nutrient after wood ash application (Ohno and Erich, 1990) concludes with the present results. The changes observed with the A treatment in the fine roots were, furthermore, conclusive with the results observed in the upper soil after wood ash application in the USA (Kahl et al., 1996), where the availability of Mn and Al was decreased, while the Ca, K and Mg availability in the topsoil increased with increasing pH.

The effects on soil and roots as observed in the present study were certainly dependent on the soil and site characteristics as has been shown in similar experiments before (Raspe et al., 1994; Berger and Glatzel, 2001).
Table 2.4: Element concentrations (mg g\(^{-1}\)) in fine roots of Norway spruce of Unterehrendingen compared to results from other Swiss and European spruce forests (LWF\(^*\), NIPHYS/CANIF\(^**\)). n. d. = not determined.

<table>
<thead>
<tr>
<th></th>
<th>Unterehrendingen</th>
<th>Alptal *</th>
<th>Beatenberg *</th>
<th>Chironico *</th>
<th>Waldstein **</th>
<th>Aubure **</th>
<th>Skogaby **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Switzerland</td>
<td>Germany</td>
<td>France</td>
<td>Sweden</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>70</td>
<td>200</td>
<td>200</td>
<td>160</td>
<td>80</td>
<td>100</td>
<td>30</td>
</tr>
<tr>
<td>Elevation (m asl)</td>
<td>450</td>
<td>1160</td>
<td>1510</td>
<td>1360</td>
<td>700</td>
<td>1050</td>
<td>100</td>
</tr>
<tr>
<td>Soil pH (0-10cm)</td>
<td>3.3</td>
<td>5.4</td>
<td>2.9</td>
<td>3.7</td>
<td>3.5</td>
<td>3.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Element (mg g(^{-1}))</td>
<td>N</td>
<td>13.9</td>
<td>10.1</td>
<td>11.6</td>
<td>13.9</td>
<td>16.9</td>
<td>16.2</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.98</td>
<td>0.78</td>
<td>n.d.</td>
<td>1.16</td>
<td>1.28</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>1.18</td>
<td>0.65</td>
<td>n.d.</td>
<td>0.88</td>
<td>1.05</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>Ca</td>
<td>4.13</td>
<td>7.96</td>
<td>4.31</td>
<td>7.13</td>
<td>3.40</td>
<td>2.94</td>
</tr>
<tr>
<td></td>
<td>K</td>
<td>3.13</td>
<td>3.30</td>
<td>2.21</td>
<td>3.69</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>0.76</td>
<td>1.05</td>
<td>0.78</td>
<td>1.33</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td></td>
<td>Fe</td>
<td>1.59</td>
<td>2.33</td>
<td>0.12</td>
<td>4.19</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Mn</td>
<td>0.80</td>
<td>0.29</td>
<td>0.19</td>
<td>0.32</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Zn</td>
<td>0.08</td>
<td>0.10</td>
<td>0.13</td>
<td>0.38</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Al</td>
<td>2.03</td>
<td>2.81</td>
<td>0.20</td>
<td>5.78</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\(^*\) data Alptal, Beatenberg and Chironico according to Brunner et al. (2001)

\(^**\) data for Aubure, Waldstein, Skogaby according to Bauer et al. (2000).
2.5.3 **Foliage elements versus fine root elements**

All elements measured in the needles were within a 'typical' range of Norway spruce needle elements in Switzerland (Landolt et al., 1989). In comparison to a European survey (Stefan et al., 1997), N and Mn concentrations were in the upper third, while K, S, Mg and Zn concentrations were in the lower third of the European range (Stefan et al., 1997). This observation is confirmed by the comparison with general plant nutrition knowledge (Bergmann, 1993): P, Mg, Zn and K were close to the lower limit whereas other nutrients were basically in the range of 'sufficient' supply (Bergmann, 1993). Mn was measured in high concentrations compared to the range defined as 'sufficient' (Table 2.3). But the Mn concentrations in the shoot are quite variable, and especially with a low soil pH, high Mn concentrations have been reported (Marschner, 1995).

With a biogeochemical model it was calculated that the fine roots (assuming one turnover a year) contribute 33% of global annual net primary production (Jackson et al., 1997) and represent an important part, not only from the physiological point of view as sites of nutrient uptake, but furthermore from the quantitative contribution to the nutrient budget of a tree. Foliage analyses have been used for a long time to estimated the nutritional status of a tree or a whole forest. But in some aspects the element concentration of the needles might be misleading. For example heavy metals are immobilised very fast and mostly enriched in the roots and not transported to the shoots as known from laboratory evidences (e.g. Stienen and Bauch, 1988). Also less mobile nutritional elements such as Ca might be underestimated, if they were analysed only in the foliage. Changes in the soil will be reflected earlier in the roots than in the shoots as has been confirmed by the present study. While a significant influence of the applied wood ash on the Ca concentrations in the fine roots was observed, no such effect was evident in the current needles (Table 2.3). Ca is known to be rather immobile in a plant and is located mainly in the apoplast and vacuoles (Marschner, 1995). But in contrast to its low concentration in the cytosol, Ca has elementary functions in the plant metabolism (Reddy, 2001). In addition to Ca concentrations, the concentrations of Mg were also changed in the roots, but not in
the foliage. In contrast to Ca the Mg concentrations have been shown to be more critical in forest nutrition (Hüttl, 1990). Low amounts of Ca are sufficient to avoid deficiency and a surplus of Ca can be detoxified in the apoplast or as Ca-oxalate in the needles (Gülpen et al., 1995). Comparable to the Ca most of the Al supplied to a Norway spruce root is immobilised in the root apoplast (Heim et al., 1999), therefore Ca/Al ratios to estimate the influence of a potential Al stress might be more suitable in roots than in the shoots.

The change of soil pH had a direct influence on the nutrient availability in the soil. For example the increase in Ca, Mg was most probably influenced by the pH change and the decrease in Mn concentration in the fine roots were a consequence of a decreased availability of Mn to the roots because of an increased pH with the A and WF treatment (Marschner, 1995). Thus the results of the present study support the evaluation of Bakker (1999) that fine root nutrients are suitable indicators of forest nutrition, but should be supported by certain soil parameters.

2.6 Conclusions

The application of wood ash or of a fertilisation, as observed in this study on the fine roots of Norway spruce, was appropriate to mitigate acidification and its consequences, like the deficiency in nutrients, e.g. K, P, and Mg. Thus, the recycling of wood ash to the forest is confirmed as a suitable method in the short term view, always baring in mind certain prerequisites to the quality and amount of the wood ash (Kahl et al., 1996). Although no accumulation of heavy metals in the fine roots could be observed in the present study, in the long term view mobilisation of heavy metals could occur (Zhan et al., 1996). Therefore, heavy metal concentrations in the wood ash have to be kept as low as technical possible.

Analogue to their physiological position, the fine roots as indicators of the nutritional status of a forest soil can be assumed to be, in a temporal point of view, in between the soil, which reflects a rather actual status on the one hand and the foliage, which integrates over a long period of time on the other hand. Thus concerning the sustainability of the fertilisations a long term evaluation must include all aspects from the effects on the soil biology to the effects on the tree growth.
Reactions of the nitrate reductase activity in the roots of Norway spruce seedlings to nitrate
3.1 Abstract

In an experimental system using vermiculite as a substrate the reactions of the nitrate reductase activity (NRA) in the roots of Norway spruce seedlings to elevated ammonium and nitrate concentrations were investigated under controlled climatic conditions. A frequently applied fertilisation with 15 mM nitrate resulted in a 40% increase in NRA compared to an ion-equivalent control treatment, whereas a fertilization with 15 mM ammonium led to a 40% decrease in NRA. When a 4:1 mixture of ammonium and nitrate was applied to the plants, no significant difference in the NRA of the roots to the control could be observed. Measurements of the nitrate concentrations in the substrate solution after harvesting showed an enrichment to a concentration of 24 mM nitrate in the pure nitrate treatment, while in the ammonium/nitrate treatment the concentration measured in the substrate solution was 3 mM.

After applying a single nitrate fertilisation, no change in NRA was determined with a 2 mM nitrate treatment within 10 days. But after application of 10 mM or 20 mM nitrate, a tendency towards enhanced activity on day 3 and 7 and a significant increase on day 10 were measured. When the 40 mM nitrate solution was applied to the spruce seedlings, the NRA of the roots was already significantly higher on the 3rd day after treatment. Thus, 2 - 7 mM nitrate in the substrate induced the NRA of Norway spruce roots after 10 days, while 8 - 11 mM nitrate in the substrate induced it within only 3 days. The nitrate concentrations measured in the solution 1 to 10 days after the N fertilisation were significantly correlated with the NRA of the young roots measured at the same time.
3.2 Introduction

The nitrate reductase is an inducible enzyme which holds a key position in the N metabolism of plants (Oaks, 1992; Campbell, 1996; Kaiser et al., 1999). It mediates the reduction of nitrate to nitrite, which is further metabolised to ammonium and incorporated into the amino acids. The nitrate reductase activity (NRA) is influenced by internal and environmental factors such as light, pH, nitrate, nitrite or ammonium (Beevers and Hageman, 1983; Campbell, 1996; Kaiser et al., 1999; Tischner, 2000). In this regard foliar NRA showed to be a valuable marker for assimilation of atmospheric N oxides (e.g. Norby, 1989; von Ballmoos et al., 1998).

The N deposition together with the actual N status of the soil affects the nitrate and ammonium levels in the forest soil (Bredemeier et al., 1998). One signal of N saturation in the forest on acidic soils is the increasing nitrification and consequently rising availability of nitrate in the soil. Furthermore, Högberg et al. (1998) reported that the NRA in roots of spruce and beech increased along a North-to-South N deposition gradient across Europe. Thus the NRA in the fine roots might be an indicator of the N saturation in forest.

The time period needed to induce the NRA also differs depending on the plant species and organ. Whereas the induction in spinach leaves was observed within 30 min upon light activation (Huber et al., 1992), NRA in Norway spruce needles needed 4 h to react to N oxide fumigation (von Ballmoos et al., 1998). Concerning the situation in roots, the NRA induction in coniferous trees took several days compared to a few hours in broadleaf trees (Yandow and Klein, 1986; Min et al., 1998), when high nitrate concentrations were applied to the experimental system. Most studies dealing with NRA of tree roots have used hydroponic (Peuke and Tischner, 1991; Sarjala, 1991; Kronzucker et al., 1995; Kronzucker et al., 1997; Min et al., 1998; Thomas and Hilker, 2000) or sand systems (Cumming and Brown, 1994). Few studies have taken a closer look on the kinetics of NRA induction in tree roots (Yandow and Klein, 1986; Min et al., 1998).

The present study aims to assess the nitrate concentrations, which lead to an observable increase in the NRA of Norway spruce roots. The time period needed to
provoke such increases should also be estimated. In addition the influence of ammonium on the NRA in a laboratory experiment should be investigated under non-hydroponic conditions. The observations are discussed in respect to relevant nitrate concentrations in a forest soil.
3.3 Materials and Methods

3.3.1 Plant material

Ten boxes (34 x 95 x 8.5 cm) were sealed at the bottom with a plastic foil, with several holes for drainage. Vermiculite was sieved (> 2 mm) and autoclaved 2 times for 60 min before it was put in the prepared boxes. *Picea abies* (L.) Karst. seeds (WSL-Nr. 846 Tägerwilen, 520 m above sea-level) were surface sterilised with 30% H₂O₂ for 35 min and rinsed with sterile water before sowing. About 600 - 700 seeds were sown in each box (containing 3.5 - 4 L vermiculite), wetted with demineralised water and covered with transparent foil to ensure moist conditions for germination. Then the boxes were placed in a growth chamber at 20 °C and 70 % humidity (16 hours light per day, 100 µE m⁻² s⁻¹). Once the seedlings had germinated the cover was removed.

3.3.2 N source experiment

Three weeks after sowing the seeds, when the primary needles had developed, the treatment with various nutritive solutions began. Seedlings were treated with a 1:5 diluted modified Melin Nokrans (MMN) solution (Marx and Bryan, 1975) without vitamins, glucose and malt which contained either nitrate (15 mM KNO₃), ammonium (7.5 mM (NH₄)₂SO₄), nitrate and ammonium (6 mM (NH₄)₂SO₄ + 3 mM KNO₃) or potassium-sulphate (7.5 mM K₂SO₄) as ion-equivalent control. The pH of the fertiliser solutions ranged between 4.6 and 4.7. Each treatment was applied to two boxes with 500 ml per box. Another two boxes were kept with the same amount of demineralised water (pH 6) as an additional control. The solutions were applied frequently (10 times in total) for five weeks. Care was taken to wet only the substrate in order to avoid uptake effects deriving from the shoots. After eight weeks of treatment, plants were harvested.
3.3.3 Time course experiment

Three weeks after sowing, the germinated seedlings were irrigated regularly (1-2 times a week) with demineralised water. In the fifth week a 1:5 diluted MMN solution as described above was added (500 ml per box) instead of the demineralised water. Eight weeks after sowing, the plants were treated with 4 different nitrate concentrations at 2 mM, 10 mM, 20 mM and 40 mM, added as a potassium nitrate solution. Each nitrate solution was applied once to two boxes (500 ml per box). Two boxes were irrigated with equal amounts of water as controls. Only the substrate was wetted in order to avoid uptake effects deriving from the shoots. Shortly before the application 50 - 70 plants from each treatment were harvested. Further samples were taken 1, 3, 7 and 10 days after nitrate fertilisation.

3.3.4 Harvest

At each harvest the plants were carefully taken out of the substrate. Seedlings were divided into root and shoot, washed with demineralised water and immediately frozen in liquid N. All the samples were stored at −80 °C until enzyme assay. At each harvest a sample of substrate solution was extracted by centrifugation (4 °C, 20 min, 12,000 g) of approximately 250 ml of substrate to determine the nitrate concentration in this solution. In the time course experiment only 50 - 70 plants per treatment were harvested at one time. Also in this experiment the amount of water was determined by drying an aliquot of the substrate. At each harvesting all the roots were non-mycorrhizal and no contaminating fungi were visible on the root surface.

3.3.5 Nitrate reductase activity

To determine the NRA in the roots of seedlings a modified in vivo-assay (Yandow and Klein, 1986) was applied. Frozen samples were broken into small pieces with a Retsch mill (MM2000, amplitude 40, time 10 s). About 100 mg fresh weight of the root material were incubated in 1.5 ml K-phosphate-buffer (0.1 mM, pH=7.5),
containing 12 mM KNO₃, 1% Isopropanol and 30 µg ml⁻¹ ampicillin. Incubation was carried out at 25 °C under vacuum for 3 hours in the dark.

After incubation the sample-buffer was cleared by centrifugation (20 min, 20,000 g, 4 °C) and with the aid of activated charcoal (about 15 mg/sample). The charcoal was afterwards removed by centrifugation (20 min, 20,000 g). No charcoal was applied in the N sources experiment. Half a millilitre of the supernatant was used for nitrite detection with 0.25 ml sulphanilamide (0.1 % in 2 N HCl) and 0.25 ml 0.02 % N-Naphtyl ethylenediamine. In blanks N-Naphtyl-ethylenediamine was replaced by distilled water. The colour reaction was measured after 30 min at 546 nm with a Photometer (Shimadzu UV-160). All the tubes used in the assay were pre-weighed after 24 hours drying at 105 °C. The incubated root samples were dried for 24 hours at 105 °C and weighed to calculate the NRA on a dry weight basis.

3.3.6 Nitrate concentration

Nitrate was analysed by capillary electrophoresis (BioFocus 3000, BioRad) using a 3 mM pyromellitic acid buffer (pH 8.0 adjusted with TEMED, Fluka) and a 40 cm x 50 µm fused silica capillary. The separation voltage was 20 kV at a temperature of 20 °C. The nitrate was detected by indirect UV absorption at 230 nm (Göttlein and Blasek, 1996). Measurements were conducted 2-5 times for each harvest and concentration.

3.3.7 Statistics

One and two factorial ANOVA in combination with Fisher's PLSD Test and simple regression analysis were performed on the data-set as provided by Statview 4.0 (SAS, Cary, NC). All statistics are at 1 % level of confidence.
3.4 Results

3.4.1 Effects of inorganic N sources

NRA in the roots of Norway spruce seedlings was low with the pure water treatment (Table 3.1). The roots of ion-equivalent control treatment (potassium-sulphate) without any N but with essential nutrient solution, showed significantly higher NRA than water treated samples. NRA of the nitrate treatment was about 5-fold higher than in the pure water treatment and about 1.4-fold higher than in the potassium-sulphate treated roots (Table 3.1). The ammonium fertilised roots showed a 60% lower NRA than the nitrate fertilised roots and still a 40% lower NRA than in the ion-equivalent controls. No significant difference was observed between the enzyme activities of the roots of the ion-equivalent controls and the roots fertilised with both N sources. The latter performed 3.5-fold higher activity than the water treated roots and a significantly higher activity (50%) than the roots, treated with ammonium only (Table 3.1).

The measurement of nitrate concentration in the substrate solution revealed an enrichment in the nitrate treatment to 24 mM nitrate. The combined nitrate and ammonium fertilisation resulted in a final concentration of 3 mM nitrate. In the solutions of the other treatments, nitrate was below the detection limit (Table 3.1).
**Table 3.1:** Mean NRA in the roots of Norway spruce seedlings after 2 months treatment and corresponding nitrate concentrations measured in the substrate solution. Different letters indicate significant differences at 1% level of confidence.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>NRA</th>
<th>Nitrate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nmol NO₂ h⁻¹ g⁻¹ dw</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>20</td>
<td>58 d</td>
<td>n. d.</td>
<td></td>
</tr>
<tr>
<td>Potassium-sulphate</td>
<td>23</td>
<td>225 b</td>
<td>n. d.</td>
<td></td>
</tr>
<tr>
<td>Ammonium</td>
<td>20</td>
<td>135 c</td>
<td>n. d.</td>
<td></td>
</tr>
<tr>
<td>Ammonium + Nitrate</td>
<td>18</td>
<td>206 b</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Nitrate</td>
<td>20</td>
<td>307 a</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

* n. d. = not detected, below detection limit of 30 μmol L⁻¹

### 3.4.2 Time course of nitrate reductase induction

Within 10 days after single application of pure water or 2 mM nitrate solution, no significant change in the NRA in the roots of Norway spruce seedlings compared to the unfertilised controls was detected (Figure 3.1). Ten days after the application of 10 mM nitrate a significant increase in NRA was observed. A slight increase in NRA compared to controls was observed after 3 and 7 days (Figure 3.1). In the treatment supplying 20 mM nitrate solution a strikingly low NRA was measured before N application that was significantly different from measurements of the later harvesting. After the 20 mM nitrate solution was applied the roots showed comparable reactions in the NRA like the roots supplied with a 10 mM nitrate solution (Figure 3.1).
Figure 3.1: Time course of NRA (±SE) in roots of Norway spruce seedlings in dependence on various nitrate concentrations. Letters indicate significant differences with time at 1% level for the respective nitrate treatment.
Low activities of nitrate reductase were measured before the application of 40 mM nitrate as already observed in the 20 mM treatment. Three days after the application a significant increase in the NRA of roots was observed (Figure 3.1). On day 7 and day 10 after treatment the effect of the fertilisation on NRA was even more obvious. Compared to the 3rd day NRA was significantly higher at the 10th day (Figure 3.1). The difference represented a 80% increase in activity compared to the unfertilised control.

**Figure 3.2**: Correlation between the means (±SE) of nitrate reductase activity (NRA) in roots of Norway spruce seedlings and the measured nitrate concentrations (mM) in the substrate solution (±SE).
Nitrate concentrations and water content that have been measured in the time course experiment are shown in Table 3.2. Nitrate was below the detection limit in the substrate solution of the control. The applied nitrate concentrations were found to be about 4 to 5-fold diluted in the substrate solution. The application of 10 mM nitrate for example led to a nitrate concentration in the substrate solution of around 2 mM except on the 10th day when a nearly doubled nitrate concentration of 4 mM was measured (Table 3.2). The application of 40 mM nitrate resulted in the highest nitrate concentration in the substrate solution. At the last harvest a concentration of 17 mM nitrate could be observed. A significant ($p = 0.0003$) correlation between the NRA and the nitrate concentrations remaining in the solution within the first 10 days after N application was observed (Figure 3.2). The vermiculite was saturated with water after the application and about 25 - 30% of the water were lost from the boxes by evapotranspiration during the experiment (Table 3.2).
Table 3.2: Measured means of the nitrate concentrations in the substrate solution (± SE) and the water content of the substrate.

<table>
<thead>
<tr>
<th>Applied Nitrate</th>
<th>Harvest</th>
<th>Nitrate</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>mM</td>
<td>days after application</td>
<td>mM</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>n. d. *</td>
<td>49.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>n. d.</td>
<td>40.7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>n. d.</td>
<td>39.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>n. d.</td>
<td>33.6</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>0.63 ± 0.03</td>
<td>44.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.53 ± 0.08</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.16 ± 0.01</td>
<td>39.7</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.28 ± 0.04</td>
<td>34.9</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>2.29 ± 0.13</td>
<td>47.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.03 ± 0.32</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>2.65 ± 0.00</td>
<td>39.3</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4.05 ± 0.18</td>
<td>34.2</td>
</tr>
<tr>
<td>20</td>
<td>1</td>
<td>5.96 ± 0.56</td>
<td>44.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5.64 ± 0.04</td>
<td>40.8</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.06 ± 0.57</td>
<td>39.6</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>7.25 ± 0.28</td>
<td>33.5</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
<td>10.56 ± 0.10</td>
<td>44.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8.41 ± 0.09</td>
<td>39.7</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>12.05 ± 0.94</td>
<td>37.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>17.25 ± 0.62</td>
<td>33.5</td>
</tr>
</tbody>
</table>

* n. d. = not detected, below detection limit of 30 μmol L⁻¹
3.5 Discussion

The reaction time of the NRA of Norway spruce roots to a change in the nitrate concentration is slow compared to crop plants (Yandow and Klein, 1986; Kaiser et al., 1999). In the present study the first significant changes could be observed with the highest nitrate concentrations in the substrate solution (8-11 mM) after three days. After 10 days the NRA was significantly higher with a nitrate concentration in the solution of about 2 - 7 mM. A comparable long induction time of 4 - 12 days observed Min et al. (1998) with Pinus contorta trees whereas the NRA of Populus roots was induced within 12 hours (Min et al., 1998).

Peuke and Tischner (1991) observed an induction of the NRA of Norway spruce at very low nitrate concentrations of 0.07 mM in a hydroponic system. In the present experiment an induction of the NRA could not be observed at such low nitrate concentrations in the substrate solution. Yandow and Klein (1986) observed with Picea rubens, grown in perlite, a doubling of NRA after one week with nitrate or with a nitrate-ammonium mixed fertilisation and even a 4-fold higher NRA after two weeks of fertilisation. Cumming and Brown, (1994), using Picea rubens in sand cultures, reported a significant induction of the NRA in the roots with 0.8 and 1.4 mM applied nitrate concentration after 10 weeks.

Sarjala (1991) could not measure a significant change in NRA of Pinus silvestris roots after 20 hours in a nitrate solution (12 mM, 30 mM). Similar Thomas and Hilker (2000) found no reaction of the NRA of Quercus robur after 3 days of nitrate solution. Nevertheless, both studies could observe significant differences of the NRA when measuring with an 'internal' enzyme assay, that means without addition of nitrate to the incubation buffer.
Table 3.3: Examples of nitrate concentrations in the soil solution of the topsoil (max 30 cm depth) in forests dominated by conifers natural occurring and after fertilisation

<table>
<thead>
<tr>
<th>Forest</th>
<th>Country</th>
<th>Nitrate (mM)</th>
<th>Fertilization (N ha(^{-1}) y(^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce</td>
<td>CH</td>
<td>0 - 1.5</td>
<td>0.2 - 4.5</td>
<td>Zimmermann, (pers. communication.)</td>
</tr>
<tr>
<td>Spruce</td>
<td>CH</td>
<td>0.01 - 0.1</td>
<td>0.01 - 0.2</td>
<td>Hagedorn et al., 2001</td>
</tr>
<tr>
<td>Spruce</td>
<td>D</td>
<td>0.16 - 0.8</td>
<td>-</td>
<td>Sandhage-Hofmann, 1993</td>
</tr>
<tr>
<td>Spruce</td>
<td>D</td>
<td>0.1 - 1.2</td>
<td>-</td>
<td>Göttlein and Stanjek, 1996</td>
</tr>
<tr>
<td>Spruce</td>
<td>D</td>
<td>0.14 - 0.5</td>
<td>-</td>
<td>Bredemeier et al., 1995, 1998</td>
</tr>
<tr>
<td>Spruce</td>
<td>D</td>
<td>0.4 - 1.1</td>
<td>-</td>
<td>Gessler et al., 1998</td>
</tr>
<tr>
<td>Spruce</td>
<td>D</td>
<td>0.003 - 0.02</td>
<td>0.03 - 0.8</td>
<td>Armbruster and Feger, 1998</td>
</tr>
<tr>
<td>Spruce</td>
<td>DK</td>
<td>0.1 - 0.5</td>
<td>0.1 - 1.2</td>
<td>Gundersen, 1998</td>
</tr>
<tr>
<td>Spruce</td>
<td>F</td>
<td>0.1 - 1.2</td>
<td>-</td>
<td>Daldoum and Ranger, 1994</td>
</tr>
<tr>
<td>Spruce</td>
<td>UK</td>
<td>0 - 0.4</td>
<td>0 - 0.8</td>
<td>Emmett et al., 1995</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1 - 1.2</td>
<td>Currie et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1 - 2.2</td>
<td>Currie et al., 1996</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1 - 1.4</td>
<td>Currie et al., 1996</td>
</tr>
</tbody>
</table>

\(^{a)} \text{AN = Ammoniumnitrate} \quad \(^{b)} \text{AS = Ammoniumsulfat} \quad \(^{c)} \text{SN = Sodiumnitrate}
Under natural conditions nitrate concentrations in the soil solution of forests can be in the range of about 0 - 1.5 mM. Under fertilised conditions, however, nitrate concentrations are enhanced to 2.2 - 4.5 mM (Table 3.3). These values are comparable to the nitrate concentrations at which we observed an increased NRA. The correlation between nitrate concentration of the substrate solution and the enzyme activities in the roots (Figure 3.2) proves the ability of this enzyme to be a marker of N impacts although under field conditions additional environmental factors can influence the enzyme reactions. It is generally assumed that the nitrate reducing enzymes are bypassed when ammonium is fed to the plant (Oaks, 1992) which would lead to a lower activity of the nitrate reductase. Peuke and Tischner (1991) observed in their experiment with Norway spruce seedlings a negative effect of ammonium on the NRA in roots. With only 10% ammonium-N of the total applied N the NRA showed a reduction to nearly half of the activity compared to roots supplied solely with nitrate. Other studies confirmed this result, by showing that with 10% ammonium-N a reduction in the nitrate uptake to about 60% of the amount taken up with nitrate as a sole N source occurred (Gessler et al., 1998). In the present study a treatment with a mixture of both inorganic N source containing 80% as ammonium resulted in a NRA that was comparable to the ion-equivalent control without nitrate. In studies with Pinus and Pseudotsuga the same effects of nitrate, ammonium, or a mixture fertilisation on the root NRA were described (Bigg and Daniel, 1978; Adams and Attiwill, 1982). Yandow and Klein (1986) and Margolis et al. (1988) reported only a slight reduction of the NRA through 50% ammonium compared to the sole nitrate treatment with jack pine and red spruce.

In the present experiment an activity of the nitrate reductase was still observed without any N fertilisation in the potassium-sulphate control as well as in the pure ammonium treatment. This constitutive NRA was also reported in several former studies in the roots of various tree species (e.g. Peuke and Tischner, 1991; Downs et al., 1993; Truax et al., 1994). The very low NRA with the water treatment in the present study is most likely the result of a deficiency effect deriving from the lack of nutrient due to the pure water treatment.
The NRA in the fine roots of trees might be an indicator to observe N saturation in a forest. In Norway spruce roots, stimulation of the NRA appears to require an increased nitrate availability for at least one week and the increase must be large enough to compensate a certain inhibition deriving from the presence of ammonium. Thus, the root *in vivo* NRA integrates over the temporal (and spatial) variability in the field and one could expect to observe reactions of the NRA in the roots to changes in the soil N in the range of months. As a results of our study the NRA in roots of Norway spruce could serve as an additional indicator beside the nitrate leaching for high N loads of forest soils (Gebauer *et al.*, 1988; Högberg *et al.*, 1998). Further field studies are needed to evaluate the *in vivo* NRA in roots as an indicator to determine the N status of forest ecosystems.
The effects of fertiliser or wood ash on nitrate reductase activity in Norway spruce fine roots

4.1 Abstract

In a field experiment, lasting two years, an intensive monitoring of the nitrate reductase activity (NRA) of Norway spruce fine roots was used to investigate the question of whether the NRA is suitable as an indicator for N availability in the soil. The treatments were a liquid complete fertilisation containing 70 - 100 kg N (64% as nitrate), composed as an optimal nutrition, a non-treated control, and a water treatment as an additional control. Another treatment was the application of wood ash, which recycled mainly basic cations back into the forest soil (Ca, Mg, K). This treatment caused a mineralisation pulse leading to a nitrate peak in the soil solution.

The NRA of the fine roots was increased by the liquid fertilisation (averages 34% compared to control), but also by the water treatment (averages 28 % more than control) and most by the wood ash treatment (averages 82 % more than control). Nitrate concentrations in the soil solution were enhanced during the irrigation with the fertiliser. The pH was distinctly elevated in the soil solution by the ash, but also by the liquid fertiliser treatment. The soil solution applied to the water treated plots was not monitored, but results from an investigation of soil extracts revealed also an elevated pH in the soil of the water treated plots. The pH of the soil solution was significantly correlated with the NRA in the fine roots, while a correlation between the nitrate concentration in the soil solution and the NRA was not significant. When taking the spatial heterogeneity of the soil nitrate into account, a correlation between the nitrate concentration of the soil extract and the root NRA was found. Although only a weak correlation between the NRA and the actual soil nitrate was observed, the NRA is assumed to reflect the nitrate conditions in the soil, possibly only in a time scale of months. As shown with the wood ash and water treatments an elevated NRA can also be caused from other environmental parameters which can change the nitrate availability in the soil and uptake properties of the roots. Concluding, the NRA of fine roots as a marker for nitrate concentrations in the soil cannot be applied on a regional and short term scale in the field if other environmental parameters are subjected to pronounced changes.
4.2 Introduction

The nitrate reductase is an inducible central enzyme in N metabolism (Tischner, 2000). In laboratory and greenhouse studies, the nitrate reductase activity (NRA) of tree roots already showed a potential as an indicator of N conditions by reflecting various N regimes applied (e.g. Margolis et al., 1988; Kronzucker et al., 1995; Thomas and Hilker, 2000). So far, however, there is little information available on the NRA of fine roots of trees in the field, although the knowledge on N cycling in forests has grown considerably through such projects as NITREX (Wright and Rasmussen, 1998) or ARINUS (Rennenberg, 1998). In Norway spruce the main nitrate reduction takes place in the roots in contrast to other trees (Gebauer and Schulze, 1997), and a connection between the in vivo NRA of tree roots and an N deposition gradient across Europe was assumed (Högberg et al., 1998). But an experimental approach in the forest on the ability of the NRA in fine roots as indicator on soil nitrate has not yet been reported. Wood ash amendments recycles many relevant nutrient cations (such as K, Ca, Mg etc.) to the forest floor. Through its high acid neutralising capacity wood ash initiates a mineralisation and nitrification leading to a nitrate peak in the soil solution (Meiwes, 1995; Büttner et al., 1998).

In what is known as the HARWA project the effects of a wood ash and a liquid fertilisation on a forest dominated by spruce are being studied. In the present study we report on the results of the fine root investigations, in particular the effects of the wood ash and liquid fertilisation on the NRA of the fine roots of Norway spruce. Furthermore, this study aims at detecting correlations between the NRA of the roots and nitrate and pH in the soil under experimental conditions in the field. Part of the question approached in this study was, whether the assumed nitrate peak caused by the ash treatment is reflected in the NRA in the roots.
4.3 Materials and methods

4.3.1 Experiment

The study was conducted in a spruce forest located about 25 km northwest of Zürich, Switzerland. The 70-year-old stand is dominated by Norway spruce (Picea abies (L.) Karst.) mixed with beech (Fagus silvatica L.), Douglas fir (Pseudotsuga menziesii (Mirb.) Franco), silver fir (Abies alba Mill.), and pine (Pinus silvestris L.). The soil is an acidic brown forest soil.

The experimental design comprised four different treatments with four replication plots ranging between 200 and 600 m² in size (Figure 4.1). On four plots 4 t ha⁻¹ y⁻¹ of wood ash (A), deriving from wood chip combustion, were applied by hand on May 25th 1998 and July 23rd 1999 respectively. In the corresponding control plots (C) no treatment was applied, four plots were irrigated solely with stream water (W). The pH of stream water was about 8.5. Another treatment was the application of a complete fertilisation (WF). The WF and W treatments were irrigated each night from May 25th 1998 to Sept. 28th 1998 and from May 6th 1999 to Sept. 27th 1999. For further details confer the experimental description in chapter 1 of this thesis.

4.3.2 Sample trees

On three plots of each treatment and the controls, three spruce trees were selected for the sampling of fine roots. If possible the trees were chosen standing in the vicinity of the soil chemistry measurement stations (Figure 4.1). An imaginary ring around each sample tree, reaching from about 50 to 100 cm distance from the stem, was divided into four equal parts. Three fine root samples were taken in the quarters 1, 2, and 3, respectively, from each selected spruce tree at every sample collection. The fine roots (diameter ≤ 2mm) were gently removed by hand from the topsoil (0 – 10 cm) from a 20 x 20 cm² area. After each harvest a marker was set on the edge of the collecting place and at the following sampling roots from the clockwise adjacent area were sampled. Then the fine roots were washed with demineralised water in the field and
frozen immediately in liquid N. During washing brittle or disintegrating roots were considered as dead and excluded from the sample. The samples were stored at −80 °C until enzyme analyses.

**Figure 4.1:** Illustration of the experimental design, C = control, W = water, WF = fertiliser, A = ash plots with four repetitions, triangles = spruce trees for fine root sampling, squares = soil solution measurement stations on the plots.

### 4.3.3 Sampling

The first samples of fine roots were taken from the 36 trees (4 treatments, 3 replicates, 3 trees per replicate) in May 1998 before any treatment had started, and
then in June, August, and October, i.e. one sample before, two during, and one after the irrigation period. In 1999 fine roots of the sample trees were taken analogue to the previous year in April, June, August, and October.

In August 1999 soil samples were taken at the same sites, from which the fine roots were collected (3 samples at each sample tree). After homogenisation, 25 g (fresh weight) of soil were shaken for 1 h with 1 M KCl at a 1:4 soil/solution ratio. Filtrates (Schleicher & Schuell 598/3) were used for photometrical nitrate detection at 210 nm according to Norman and Stucki (1981). In addition, the pH of the extract was measured with a pH-meter (Orion 520A).

4.3.4 Soil solution

In each plots from which the sample trees were chosen, a monitoring station for the soil solution was installed (Figure 4.1). The soil solution was collected from below the organic litter layer of about 3 cm, by the aid of gravitation-lysimeters (referred to as ‘0 cm’). In addition suction-cups were installed at 10 cm (gathering solution at 10 - 15 cm) depth, where a vacuum of −600 mbar was renewed every 10 hours (maximum rising to −400 mbar). The solution was collected regularly, in the growing season every week and in winter every 2 - 4 weeks. The collected solutions were filtered (0.45 µm, Schleicher & Schuell, ME 25) and if not measured at once stored at −20 °C. All samples were analysed for nitrate by ion-chromatography (Dionex DX-120). The pH was measured with a PHM-250 (Radiometer-Copenhagen) equipped with a phC-8000-4 electrode. Data represent the mean of three plots for one treatment. For technical reasons, the soil solution on plots with the W treatment was not sampled.

4.3.5 Nitrate reductase activity

To determine the NRA in fine roots, a modified in vivo assay (Yandow and Klein, 1986) was applied. Frozen root samples were broken into small pieces with a Retsch
mill (MM2000, low amplitude). The root material was incubated in a 0.1 mM K-
phosphate-buffer (pH 7.5), containing 12 mM KNO₃, 1% isopropanol and 30 µg ml⁻¹
ampicillin for 3 h at 25 °C in the dark. After incubation the sample-buffer was
cleared with the aid of activated charcoal and the supernatant was used for nitrite
detection with 0.1% sulphanilamide and 0.02 % N-naphthyl-ethylenediamine. In blanks
N-naphthyl-ethylenediamine was replaced by distilled water. The colour reaction was
measured after 30 min at 546 nm with a photometer (Shimadzu UV-160) and
incubated root samples were dried and weighed to calculate the NRA on a dry weight
(DW) basis. For further details confer to chapter 3.

4.3.6 Statistics

Differences in the NRA between the treatments and between the sample collections
from each treatment were calculated with a one-way ANOVA and LSD post-hoc test
as provided by Statview 5.0 (SAS Inc.). Data were averaged for each tree, because
samples from one tree were not regarded to be independent. With the soil solution
data a one-way ANOVA on the differences in pH and nitrate between treatments was
also conducted. To combine the NRA and the soil solution data-sets, the root NRA of
each collection of fine roots was averaged for each plot and combined with the soil
solution data recorded for the last soil solution sampling (within 0 - 8 days) before
each root sampling. Correlation analysis with Fisher’s r to z test between the soil
solution data (pH and nitrate) and NRA of fine roots was calculated. For the data of
the detailed study in August 1999, the correlation coefficients between NRA of the
roots and nitrate and pH of the soil extract were also computed. For each analysis,
data were divided into two subsets: data from plots with WF, W, and C treatments
and data from A and C plots. In this way the ‘wet’ (or irrigated) treatments together
with the control were analysed on the one hand and the ‘dry’ applications on the
other.
4.4 Results

4.4.1 Nitrate reductase activity

Time course

In 1998 the NRA of the fine roots sampled from the C plots showed no differences between the samplings, but in 1999 there was a significant seasonal increase in the summer (Figure 4.2, C). In the A, W, and the WF treated roots, the NRA was enhanced in October 1998 compared to the earlier samplings. In 1999 a maximum NRA of fine roots was observed in August for all treatments. The NRA of fine roots was lower at the next sampling in October with the WF and C treatment while with the W and A treatment, the NRA of the fine roots remained high in the October samples as well. The NRA in the fine roots of August and October samples of the W and WF plots were significantly higher than the April data (Figure 4.2).

Treatment effects

NRA data were analysed by one-way ANOVA in two subsets: WF, W, and C data, and the A and C treatment data. The W and WF treatments resulted in a higher NRA in the end of the first year, 1998 (Table 4.1). In the second year, 1999, the NRA of fine roots was enhanced by the WF treatment at all samplings \((p < 0.05)\) compared to the control, but by the W treatment only in October \((p < 0.01)\). Comparing the WF to the W treatment, significant differences in the NRA were not detected, although in spring and summer 1999 there was a tendency to a higher NRA in the roots that were sampled on the fertilised plots (WF, Table 4.1).

In total, the effects on the NRA of the fine roots of the A treatment were higher (averages 82% increase compared to roots from C plots) than the effects deriving from WF (34% increase) and W (28% increase) treatment.
Figure 4.2: Soil solution pH, nitrate concentrations (mM) at 0 cm and 10-15 cm depth and mean NRA (nmol NO\textsubscript{2} h\textsuperscript{-1} g\textsuperscript{-1} DW) ± SE of Norway spruce fine roots over two years: C = control, W = water, WF = fertiliser, A = ash, letters above columns indicate a significant (p<0.05) difference between samplings in one treatment.
The first A treatment in May 1998 was followed by an increase in the NRA of fine roots, that showed up only a few weeks after ash application (Table 4.2). The difference to the control was observed as a trend ($p=0.17$) from a few weeks after the first application onwards. In autumn of the same year the differences in the NRA of the fine roots were significant ($p<0.01$, Table 4.2). In the second year, the NRA of the fine roots of the A treatment was higher than the NRA of roots of the C plots at all samplings (Table 4.2).

**Table 4.1: Statistical analyses of treatment effects on NRA in fine roots, differences ($\Delta$) between control (C), fertilisation (WF) and water (W) - irrigated from May to September: mean differences (nmol NO$_2^-$ h$^{-1}$ g$^{-1}$ dry wt) in the NRA of the fine roots and probability values (not significant n.s., $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***), as provided by a one-way ANOVA on the treatment effect on the NRA within data of one sampling.**

<table>
<thead>
<tr>
<th>Time</th>
<th>$\Delta_{WF-C}$</th>
<th>$\Delta_{W-C}$</th>
<th>$\Delta_{WF-W}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\Delta$</td>
<td>$p$</td>
<td>$\Delta$</td>
</tr>
<tr>
<td>1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>-0.9</td>
<td>0.86 n.s.</td>
<td>4.7</td>
</tr>
<tr>
<td>June</td>
<td>0.7</td>
<td>0.91 n.s.</td>
<td>2.9</td>
</tr>
<tr>
<td>August</td>
<td>0.3</td>
<td>0.97 n.s.</td>
<td>8.9</td>
</tr>
<tr>
<td>October</td>
<td>21.5</td>
<td>0.005 **</td>
<td>21.1</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>14.1</td>
<td>0.019 *</td>
<td>4.6</td>
</tr>
<tr>
<td>June</td>
<td>23.0</td>
<td>0.028 *</td>
<td>9.9</td>
</tr>
<tr>
<td>August</td>
<td>45.6</td>
<td>0.006 **</td>
<td>17.2</td>
</tr>
<tr>
<td>October</td>
<td>40.3</td>
<td>0.004 **</td>
<td>44.7</td>
</tr>
</tbody>
</table>
Table 4.2: Statistical analyses of treatment effects on NRA in fine roots, differences (Δ) between wood ash (A) application (May 1998 and July 1999) and control (C): mean differences (nmol NO$_2$ h$^{-1}$ g$^{-1}$DW) in the NRA of the fine roots and probability values (not significant n.s., p<0.05 *, p<0.01 **, p<0.001 ***) as provided by a one-way ANOVA on the treatment effect on the NRA within data of one sampling.

<table>
<thead>
<tr>
<th>Time</th>
<th>ΔA-C</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>0.4</td>
<td>0.96 n.s.</td>
</tr>
<tr>
<td>June</td>
<td>14.5</td>
<td>0.17 n.s.</td>
</tr>
<tr>
<td>August</td>
<td>18.3</td>
<td>0.09 n.s.</td>
</tr>
<tr>
<td>October</td>
<td>55.1</td>
<td>0.005 **</td>
</tr>
<tr>
<td>1999</td>
<td></td>
<td></td>
</tr>
<tr>
<td>April</td>
<td>46.2</td>
<td>0.006 **</td>
</tr>
<tr>
<td>June</td>
<td>56.4</td>
<td>&lt;0.001 ***</td>
</tr>
<tr>
<td>August</td>
<td>61.6</td>
<td>0.008 **</td>
</tr>
<tr>
<td>October</td>
<td>65.8</td>
<td>0.002 **</td>
</tr>
</tbody>
</table>

4.4.2 Soil

The nitrate concentrations in the soil solution were strongly affected by the WF treatment. Directly below the litter layer the nitrate concentration was enhanced up to nearly ten-fold, and at 10-15 cm depth the maximum concentrations measured were about six times higher than before the treatment (Figure 4.2, WF). This increase was highly dependent on the irrigation time. After the irrigation period in late summer the nitrate concentration dropped back to the original level, at 10-15 cm more slowly than at the surface. There was a nitrate peak in the surface soil solution after each ash
application, which could be observed after a lag time at 10 - 15 cm depth (Figure 4.2, A). In the control plots a seasonal aspect of the nitrate in the soil solution was observed. Throughout the growing season, higher nitrate concentrations in the soil solution than in the winter were detected (Figure 4.2, C). Compared to the A and C treatment, the nitrate concentrations in the WF treatment were significantly higher ($p<0.001$) at both soil depths measured. With the WF treatment the pH in the surface soil solution was elevated on average by more than 1 unit compared to C, and with the A treatment it was more than 0.5 above the pH with the WF treatment ($p<0.0001$). The effect on the pH was renewed with the second ash application in July 1999 (Figure 4.2, A). In the deeper soil solution of 10 - 15 cm the pH was not affected (Figure 4.2).

In August 1999 soil samples were taken together with the root samples from identical sites. The soil extract of these samples revealed the highest pH in the A plots and slightly enhanced pH ($p<0.05$) in the soil extracts from the irrigated (WF and W) plots (Figure 4.3). In August 1999 the NRA in the fine roots was highest with the A treatment and lowest in the C plots (Figure 4.3). The KCl extractable nitrate in the soil of the WF plots was significantly higher than with all other treatments, while nitrate concentrations in the soil of the W, A and C treatments were low and did not differ from each other (Figure 4.3).
Figure 4.3: Means ± SE of pH and nitrate concentration (ug g⁻¹) of soil samples and mean NRA (nmol NO₂⁻ h⁻¹ g⁻¹ dw) of Norway spruce fine roots from identical sites, C = control, W = water, WF = fertiliser, A = ash, letters indicate a significant (p<0.05) between the treatments.
4.4.3 Correlation NRA to soil data

The NRA of the fine roots was significantly correlated to the pH of the soil solution from below the litter layer in both data subsets (Table 4.3). The nitrate concentration did not show a significant correlation to the NRA of either fine roots of the WF and C subset, or of the A and C subset. In the WF and C subset, however, a trend ($p=0.14$) between nitrate concentration of the soil solution and NRA of the fine roots was observed (Table 4.3).

Table 4.3: Correlation coefficients ($r$) between the NRA of fine roots and pH and nitrate concentration in the soil solution (0 cm); NRA and nitrate data were log transformed for the analysis, subset WF and C treatments ($n=46$), subset A and C treatments ($n=45$), (not significant n.s., $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***) as provided by a simple correlation and a Fisher’s $r$ to $z$ test.

<table>
<thead>
<tr>
<th>Soil solution</th>
<th>WF and C</th>
<th>A and C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.240</td>
<td>0.14 n.s.</td>
</tr>
<tr>
<td>pH</td>
<td>0.505</td>
<td>0.0004 ***</td>
</tr>
</tbody>
</table>
The pH of the soil extracts of August 1999 was significantly correlated with the NRA of fine roots in both subsets (Table 4.4). In addition a significant ($p<0.05$) correlation between the nitrate concentration in the soil extract and the NRA of the fine roots was observed in the data subset of WF, W, and C plots (Table 4.4). Considering only data from the A and C plots, the correlation between nitrate of the soil and NRA of the fine roots was not significant (Table 4.4).

**Table 4.4:** Correlation coefficients ($r$) between the NRA of fine roots and pH and nitrate concentration in the soil (KCl extract); NRA and nitrate data were log transformed for the analysis, subset WF, W, and C treatments ($n=78$), subset A and C treatments ($n=53$), (not significant n.s., $p<0.05$ *, $p<0.01$ **, $p<0.001$ ***, $p<0.0001$ ****) as provided by a simple correlation and a Fisher’s $r$ to $z$ test.

<table>
<thead>
<tr>
<th>Soil extract</th>
<th>Fine root NRA</th>
<th>WF, W, and C</th>
<th>A and C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$r$</td>
<td>$p$</td>
</tr>
<tr>
<td>Nitrate</td>
<td></td>
<td>0.229</td>
<td>0.0439 *</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td>0.514</td>
<td>$&lt;0.0001$ ****</td>
</tr>
</tbody>
</table>
4.5 Discussion

4.5.1 Effects on NRA and soil solution

In the present study the NRA of Norway spruce fine roots, nitrate and soil pH were monitored for two years in a field experiment. The NRA of the fine roots of Norway spruce reacted positively to the W, WF, and A treatments. The nitrate concentration in the soil solution was influenced by both fertilising treatments. The nitrate concentrations were enhanced in the WF plots during the irrigation period at both soil depths and in the A plots after the ash applications as expected from earlier investigations (e.g. Kahl et al., 1996). The seasonal pattern in the soil solution of the mineral soil solution (10 - 15 cm depth) as observed for the A and C plots was similar to that reported for spruce forests in Denmark (Gundersen, 1998). The differences in the soil solution nitrate concentrations (on the non-irrigated A and C plots) between the two years may have been caused by a better water supply in the second year: 1998 was a mostly dry and warm year compared to the long term average (SMA, 1998), while in 1999 23% more precipitation fell than the average for 1984 - 1998 (SMA, 1999). Through the moist conditions in 1999, the uptake properties of the plants were improved, which might have been a reason for lower nitrate concentrations in the soil solution on the one hand and a higher influence of the treatments on the NRA on the A, W, and WF plots in the second year on the other. On the irrigated plots the differences in precipitation between the two years were of minor importance, because 1.2 mm water per day were added throughout the irrigation period. This approximated 30 - 45% of the natural monthly precipitation (compared to the long-time mean, SMA 1999). It is therefore concluded that the results of the second year showed, at least for the WF treatment, a cumulative effect of the fertilisation.

The main nitrate reduction in mature spruce trees takes place in the roots (Gebauer and Schulze, 1997) and, therefore, a correlation between the soil nitrate and the NRA of the needles is not obvious (Bauer, 1997; Gebauer et al., 1998). Although the soil
solution in the present study was collected from the centre of the plots, and the sample trees for the fine root samples were nearby, the nitrate in the soil solution was not correlated with the *in vivo* NRA. However, a significant correlation between NRA in the fine roots and extracted nitrate of the soil was observed, when soil was investigated at the identical sites that the root samples originated from. Thus the spatial heterogeneity of nitrate in the soil as described for example by Göttlein and Stanjek (1996) was taken into account.

The *in vivo* NRA, as measured in the present study, approximates the nitrate reduction capacity of plant tissues (Högberg *et al.*, 1986; Bauer, 1997). Furthermore, greenhouse experiments have shown that the NRA in the roots of conifers reacts slowly to changes in the nitrate concentration compared to broadleaf trees (Min *et al.*, 1998; Genenger, unpublished). This being so, another explanation of the lack of correlation between the current nitrate concentration (i.e. in the range of one week) in the soil solution and the NRA of the Norway spruce fine roots, could be that the NRA of the fine roots is only increased with an elevated nitrate concentration over a period of months.

The wood ash treatment resulted in a significant increase in the NRA of fine roots. This increase was obviously not connected to the nitrate mobilisation peaks in the soil solution. One possible explanation for the enhanced NRA might be an effect of the basic cations supplied by the ash treatment. In general cations often improve the uptake of anions, Ca at a low soil pH in particular is known to cause synergism in the uptake of different ions and can have a positive feedback on the nitrate uptake (Marschner, 1995).

In August 1999 the pH in the soil extracts from the A plots was significantly enhanced by more than 1 unit compared to the other treatments. Comparable drastic effects on the pH of the topsoil with wood ash application have been described earlier (Bramryd and Fransman, 1995; Eriksson, 1998) and were in our study also observed in the soil solution. The elevated NRA of the fine roots could thus be interpreted as a stress reaction provoked through the drastic changes of the soil conditions. The pH change through the irrigation (W and WF) treatments was
presumably caused by a relatively high pH of the water from the stream that was used. In the present study the pH of the soil is significantly correlated to the NRA of the fine roots. A direct influence of pH on the NRA of maize roots has been shown in the laboratory (Mengel et al., 1983): at a pH 7 of the medium the NRA was significantly increased compared to pH values of 4 and 5. An influence of the elevated pH can be suspected as well from the laboratory findings that at an optimal pH of 5.5 in the substrate the roots of Norway spruce took up twice as much nitrate as at a pH of 4.0 (Peuke and Tischner, 1991). A higher uptake subsequently leads to a higher NRA in the fine roots.

Another possible explanation for the higher NRA with the A treatment is based on the spatial variation of soil properties: Grogan et al. (2000) concluded from a field experiment that ash (from a wildfire) led to a higher spatial heterogeneity in the soil N availability to plants in a burnt pine forest in California. In our experiment this heterogeneity might have caused the NRA of some root samples to react strongly while others showed a non-induced NRA (as reflected in the high standard errors compared to the other treatments). The N availability and the plant uptake of N is enhanced through ash from a wildfire (Grogan et al., 2000), an effect which is important with the A treatment in our study. However, Grogan et al. (2000) concluded from $^{15}$N data, that the N readily taken up after the fire is mainly ammonium and not nitrate, which would not tally with the enhanced NRA measured in the present study.

In general the in vivo NRA of the fine roots in this field study was lower than the NRA measured in the roots of Norway spruce seedlings in laboratory experiments (Peuke and Tischner, 1991; Genenger, unpublished). The lower NRA in the field might have a number of explanations. First, in the root system of seedlings the proportion of root tips per unit root material is higher than in the fine roots harvested in the field, and root tips have a generally higher NRA than other fine root material (Marschner et al., 1991; Schmidt et al., 1991). Secondly, the roots in the laboratory experiments were non-mycorrhizal. Mycorrhization leads to a reduction of in vivo NRA in the roots as is known from laboratory experiments (Vézina et al., 1989;
Sarjala, 1991; Brunner et al., 2000). Nevertheless, the nitrate reducing capacity of the whole plant root, at least in seedlings in a laboratory experiment, was enhanced through mycorrhization, because of higher production of root biomass (Constable et al., 2001).

4.5.2 Potential as an indicator

The potential of NRA as an indicator has been shown in various studies. For example Norby (1989) proved the suitability of the foliar NRA from red spruce (Picea rubens Sarg.) as a marker for atmospheric NOx concentrations. Gebauer et al. (1988) detected a close correlation between the NRA of the leaves of various plant species to their N figures (i.e. the corresponding N status of the soil the species usually growth on) as determined by Ellenberg (1979) and stressed the suitability as an indicator of the NRA ‘for the degree of nitrate supply of the plants’. However, in later studies a correlation between nitrate availability in the soil and the NRA in the leaflets of Fraxinus excelsior L. (Stadler and Gebauer, 1992) or the roots of Picea abies (Gebauer et al., 1998) was not verified. Nevertheless, Högberg et al. (1998) confirmed the ability of the in vivo NRA of Norway spruce fine roots to determine the different N saturation states of the soil in another study along an N-deposition gradient in Europe. In a review on the nutrient uptake kinetics of roots, Bassirirad (2000) also stressed that in the context of global change investigations, root N uptake kinetics, and hence also nitrate reductase kinetics, might be an accurate biological indicator of the ecosystem capacity to retain N. The results of the present study confirmed that the NRA of spruce fine roots reacts in the field to changes deriving from higher N input. Nevertheless, other environmental factors, as in our study the pH, could be of great importance, either indirectly by influencing the nitrate availability for the plant or directly (which we cannot determine from the results of the present study). Therefore the NRA in fine roots might not be useful as an indicator of nitrate availability if other environmental parameters (for example the
pH) were subjected to remarkable changes at the same time. In addition the order of magnitude might be of importance: On a regional scale and in a short term view (in the context of a forest ecosystem) the indicator properties might not come into play, while in a range of decades and in comparing different forest sites the integrating indicator qualities are probably improved.
Rapid $^{15}\text{N}$ uptake and metabolism in fine roots of Norway spruce
to be submitted to *Tree Physiology*
5.1 Abstract
An elegant tool to investigate the N cycle in an ecosystem is the use of stable isotopes as tracers, either in natural abundance or through enrichment of a certain N pool with subsequent tracking the changed $^{15}$N signature into other N pools. In a field experiment the short-term N uptake into the roots of Norway spruce was investigated after $^{15}$NH$_4$$^{15}$NO$_3$ tracer application. The influence of wood ash or liquid fertiliser treatments of the previous year on the N uptake was studied. A laboratory experiment aimed to describe short-term $^{15}$N-nitrate uptake and assimilation into free amino acids with two different nitrate concentrations (2 mM or 20 mM). In the forest a rapid uptake of the applied N into the fine roots was observed. About 50 % of the maximum observed $\delta^{15}$N value could be measured within 1 day after application and within 1 week even 70-90% of the maximal measured $\delta^{15}$N. The $^{15}$N signature in fine roots was not affected by the treatments applied in the previous year. $\delta^{15}$N increased until about two months after $^{15}$N application and decreased to 60% of its maximal value within one year, irrespective of what treatment was applied. Nine months after tracer application, a detailed analysis of $\delta^{15}$N distribution of one root system was carried out. Significantly higher $\delta^{15}$N values in the roots of the upper soil horizon compared to roots at 30 - 40 cm or 60 - 70 cm depth were observed. Furthermore, the $\delta^{15}$N was higher in fine roots compared to root tissue of roots that were bigger in diameter than 5 mm. The $^{15}$N was not translocated through the root system, but remained mainly in the fine roots of the topsoil, the site of nutrient uptake.

In the laboratory the applied $^{15}$N enriched nitrate could be retrieved in the major amino acids of Norway spruce seedlings within four hours to one day in roots and shoots. Further observations tally with the amino acid metabolism as it is known from literature. N was incorporated into Gln, Glu, Asp, Ala, Ser and Asn. The nitrate uptake and incorporation was dependent on the external nitrate concentration applied. The at% $^{15}$N in the amino acids was generally increasing to a maximum within 3 to 7 days and decreasing 10 days after the tracer application again, depending on the place of the respective amino acid in the N metabolism of Norway
N uptake spruce. The results of the study contribute to a better understanding of the short-term N uptake processes in Norway spruce forests.
5.2 Introduction

The high atmospheric deposition of N due to anthropogenic activities (Galloway, 1995) has consequences on N limited ecosystems like forests that are still not fully understood (Rennenberg and Gessler, 1999). To study parts of the N cycle and possible changes of it, the stable N isotope $^{15}$N provides an elegant tool to trace sources to sink relations. The relative amounts of the isotopes $^{15}$N and $^{14}$N compared to N$_2$ in air are expressed in the $\delta^{15}$N or at% enrichment values. The natural abundance of $\delta^{15}$N in plant tissue is generally within a small range of -5% to 8% (Fry, 1991), and therefore, with addition of a small amount of highly enriched $^{15}$N the soil, the signal can easily be followed into different N pools (Nadelhoffer and Fry, 1994). Plants are integrators of $\delta^{15}$N of available N sources, and especially fine roots of trees might give reliable information on the $\delta^{15}$N of available N in the forest soil (Högberg, 1997).

The amino acid pool is subjected to a complex control by the enzymes of the amino acid biosynthesis and is closely connected to the carbon metabolism in plants (Lea and Ireland, 1999). The main metabolic pathways can also be followed, if $^{15}$N is used as N source. The free amino acids are central within the N metabolism of a plant. The assimilation of N into the amino acid pool is for example dependent on the N source. If ammonium is taken up the $^{15}$N can earlier be detected in the amino acids than nitrate as reviewed by Lea and Ireland (1999).

After the $\delta^{15}$N of plant available N in the soil was experimentally changed, we investigated the time span, in which the N in the fine roots was affected. The $^{15}$N fertilisation experiment was conducted within the scope of a forest management project, investigating the influence of wood ash and optimal fertilisation on a Norway spruce forest. Thus the possible influences of the fertilisation treatments on $^{15}$N uptake were another topic to study. In addition we investigated the influence of the soil depth, root size and root tissue on its $\delta^{15}$N signature within the root system of a tree. The field results were supported by a laboratory study on the metabolism of
Norway spruce seedlings. We investigated the effects of different $^{15}$N-nitrate regimes on free amino acids in roots and shoots over a period of 10 days.
5.3 Materials and methods

5.3.1 Field experiment

The experiment was conducted in a 70-year old spruce (Picea abies (L.) Karst.) forest, called 'Schladwald' (464 m a.s.l.), which is located about 25 km northwest of Zürich, Switzerland. The soil is an acidic brown forest soil and the experimental treatments were a control without any treatment (C), irrigated plots (W), water and fertiliser plots (WF) and plots supplied with wood ash (A). The WF (basically NPK full nutrient fertilisation containing 100 kg N ha\(^{-1}\) y\(^{-1}\)) and W treatments were irrigated daily during the vegetation period 1998, 1999 and 2000. The A treatment was applied by hand in Mai 1998 and July 1999 with 4t ha\(^{-1}\) of dry wood ash. For more details confer chapter 1.

As illustrated in Figure 5.1, mature spruce trees were selected for sampling in this experiment which ranged in age between 43 and 62 years. A heavy storm ‘Lothar’ in December 1999 caused a considerable damage to the forest site and reduced the number of experimental trees from 16 at the first sampling to 12 for the last sampling.

\(^{15}\text{N} \text{labelling and sample analysis}

In April 1999, the 16 spruce trees were supplied with N solutions of 58.4 mg N m\(^{-2}\) in the form of the double-labelled \(^{15}\text{NH}_4^{15}\text{NO}_3\) tracer (98% enriched, Cambridge Isotope Laboratories, Inc., USA). This tracer amount was estimated to ensure a visible \(^{15}\text{N} \text{signal in the roots (and in the needles), yet small enough not to cause any unwanted physiological effects. The tracer was evenly distributed by encircling an area of 30 m}^2 \text{ with strings around each experimental tree and applying the tracer solution in subdivisions by hand. Fine roots (diameter} \leq 2\, \text{mm}) \text{ were sampled before the tracer experiment in April 1999 and then 1, 3, 7, 28 days, and 2, 6 and 12 months after the application. Three root samples per tree were collected in a distance of 1 m from the trunk and maximum 5 cm below ground by hand, rinsed with demineralised}
water, frozen in liquid N and then stored at -80°C. Finally, the fine roots were freeze-dried for 48 h.

Figure 5.1: Illustration of the experimental design, C = control, W = water, WF = liquid fertiliser, A = ash plots, localisation of spruce trees marked with $^{15}$N (triangles), trees affected by the storm (horizontal triangles) and fallen tree investigated for $^{15}$N distribution (encircled).
Single tree study

From one of the labelled spruce trees, which was uprooted by the heavy storm in December 1999 (Figure 5.1, tree 499), root samples were taken from various depths and root sizes. Size classes were fine roots (FR ≤ 2 mm), petite roots (PR ≤ 5 mm), small roots (SR ≤ 10 mm) and coarse roots (CR ≤ 20 mm). Depth classes were 0 - 10 cm (surface), 30 - 40 cm and 60 - 70 cm soil depth. The roots have been lyophilised for 48 h. FR and PR have been analysed in total. The bark of the SR was separated with a knife from the woody tissue and analysed separate. The bark of the CR was also separated and, in addition, the centre of the woody tissue was cut out of the remaining wood by a little core (diameter 4 mm).

$^{15}$N analysis by Mass Spectrometry

The different root samples were ground with a mill (Retsch MM2000, Hann, Germany). For analyses of total N and isotopic signature 4.5 to 5 mg dry material of the regular fine root samples was needed. The same amount was taken to analyse the FR and PR from the fallen tree. Furthermore, 7.5 to 8 mg of the bark of SR and CR, 15 to 16 mg of the SR wood, 35 to 36 mg of the central wood of CR and 30 to 31 of the remaining wood of CR was used.

The total N concentration and the isotopic signature of each sample were measured by combustion in an Elemental Analyser (EA-1110, Carlo Erba, Italy). The elemental analyser was connected to a continuous flow mass spectrometer (DELTA-S Finnigan MAT, Germany). The isotopic signatures are expressed in the delta notation $\delta^{15}N = [(R_{\text{sample}}/R_{\text{standard}})-1]*1000$ (%), relative to the international standards ($N_2$ in air). To analyse the wood samples properly an ‘empty measurement’ (without any sample material) had to be conducted after each sample, because of a memory effect with the high amount of material.
5.3.2 Laboratory experiment

Ten boxes (34 x 95 x 8.5 cm) were sealed at the bottom with a plastic foil (sterilised with Ethanol), with several holes for drainage. Vermiculite was sieved (> 2mm) and autoclaved 2 times for 60 min before it was filled into the prepared boxes. *Picea abies* seeds (WSL-Nr. 846) were surface sterilised with 30% H₂O₂ for 35 min, rinsed with sterile water and 600-700 seeds were sown in each box (containing 3.5 - 4 L vermiculite). The boxes were covered with transparent foil to ensure moist conditions for germination and placed in a growth chamber at 20 °C and 70 % humidity (16 h light per day, 100 μE m⁻² s⁻¹). Once the seedlings had germinated the cover was removed.

Three weeks after sowing, the germinated seedlings were irrigated regularly (1 - 2 times a week) with demineralised water. In the fifth week a 1:5 diluted modified Melin Nokrans (MMN) solution (Marx and Bryan, 1975) without vitamins, glucose and malt was added (500 ml per box) instead of the demineralised water. Eight weeks after sowing, the plants were treated with either 2 mM or 20 mM nitrate, added as a potassium nitrate (98 % ¹⁵N enriched, Sigma-Aldrich) solution. Each nitrate solution was applied once to two boxes (500 ml per box). Two boxes were irrigated with equal amounts of water as controls. Only the substrate was wetted in order to avoid ¹⁵N contamination of the shoots. Samples of about 50 - 70 plants were taken 4 hours, 1, 3, 7 and 10 days after nitrate fertilisation.

*Sampling and amino acid extraction*

At each harvest the plants were carefully taken out of the substrate. Seedlings were divided into root and shoot, washed with demineralised water and immediately frozen in liquid N. All the samples were stored at −80 °C until extraction. At each harvesting all the roots were non-mycorrhizal and no contaminating fungi were visible on the root surface.

Extraction of amino compounds from the roots based on the method described by Stoermer *et al.* (1997). The root material was freeze-dried for 48 h and ground 3 times 1 min in liquid N with a mill (Retsch MM2000). 15 mg of the material was
homogenised in 200 μl of a 0.02 M HEPES buffer containing 5 mM EGTA and 10 mM NaF. As a standard, 50 μl of 10 mM norleucin (Fluka) were added to each sample. 1.6 ml methanol:chloroform (3.5:1.5, v/v) were added and incubated for 20 min on ice. The water-soluble compounds were extracted twice with 2.4 ml dest. Water. The aqueous phases were combined and freeze dried in a centrifugal evaporator (RC10, Jouan Inc., Winchester, USA) overnight. The dried extracts were dissolved in 400 μl bidest (and if necessary centrifuged). The extracts were cleaned after Chalot et al. (1994) over a Dowex 50W 8X-200 (Sigma-Aldrich) column, and eluded with 5 ml of 4.5 M ammonium hydroxide.

**Derivatisation and measuring**

The purified samples were derivatised and measured according to Chalot et al. (1995). Therefore, the purified samples were freeze dried in a centrifugal evaporator and then resolved in 10 μl DMF and 50 μl MTBSTFA (Pierce). Samples were mixed and heated in a water-bath at 80 °C for 25 min, afterwards samples were allowed to cool down for about 90 min at room temperature. The GC-MS was a Hewlett Packard (Palo-Alto, USA) 5989A MS engine interfaced to a Model 5890 GC and a Model 7673 auto-sampler. The capillary column (30 m x 0.25 mm, HP5-MS, Hewlett Packard) was initially held for 3 min at 110 °C, then temperature was increased by 8 °C per min to 250 °C, the injector temperature and the max. detector temperature was 260 °C. The MS peak [M-57]+ was monitored and used for calculation of at% 15N of all amino acids (Mawhinney et al., 1986). Ile and Leu could not be separated and the applied procedures were not suitable to measure Arg properly (double peak, partly overlaid with His peak, Mawhinney et al., 1986). 15N enrichment of each amino acid is expressed in at% excess compared to the control samples. Data are presented as a mean of two extractions and measurements.
5.3.3 Statistics

Statistics were performed as provided by Statview 5.0 (SAS Inc.) for Macintosh. The differences in the distribution was of $^{15}\text{N}$ or $\%\text{N}$ in the root system was analysed by a one-way ANOVA with Fisher’s PLSD test on depth or tissue effects at a 5% probability level.
5.4 Results

5.4.1 Field experiment - N uptake

A very rapid uptake of the labelled N into the fine roots of Norway spruce could be observed. Within one day after application about 50 % of the maximum observed $\delta^{15}$N value could be measured in the roots. Within the first week after tracer application 70 - 90% of the maximal measured $\delta^{15}$N was detected (Figure 5.2). The maximal $\delta^{15}$N values were reached within one to two months. One year after the tracer application the enrichment in the fine roots of Norway spruce was about 60% of the maximum measured $\delta^{15}$N values (Figure 5.2, inset). Beside the tendency towards a faster $^{15}$N uptake in the irrigated plots (WF and W) within the first week, significant effects of the treatments applied in the year prior to the $^{15}$N experiment were not observed.

5.4.2 $^{15}$N distribution within the root system

The root system of a wind-thrown tree was analysed on the $^{15}$N enrichment nine months after tracer application. The roots showed a significant difference depending on the depth they were growing in the soil, and on the root size and tissue. Concerning the effects of the various tissues there was a significantly higher $\delta^{15}$N in the fine and petite roots of the samples from the topsoil (0 - 10 cm depth) compared to all the other root sizes and tissues measured in that horizon. A continuous trend towards lower $\delta^{15}$N was detected the bigger the roots were in general and the more woody tissue was analysed. This trend could also be made out in the root samples of 30 - 40 cm soil depth. But the differences among the root sizes were not statistically significant. The root samples at a soil depth of 60 - 70 cm did not show any significant differences between the root sizes and tissues either (Table 5.1).
Figure 5.2: $\delta^{15}N$ ($\%\delta$) in Norway spruce fine roots over time (days), split by different treatments: $C$ = control, $W$ = water, $WF$ = liquid fertiliser, $A$ = ash plots, means ($n=12$) ± SE, inset: long term development.

Comparing the $\delta^{15}N$ values between the three depth classes all root samples from the upper soil were significantly higher $^{15}N$ enriched after nine months than the samples from the 60 - 70 cm soil horizon. Furthermore, all tissues of the topsoil root samples had higher $\delta^{15}N$ values than the root samples of the 30 - 40 cm depth, except for bark and wood of the small roots. Bark and wood of the roots, that were bigger than 1 cm in diameter at 30 - 40 cm were significantly higher enriched with the tracer than the respective tissues of the root samples at 60 - 70 cm. All other tissues were not significantly different in their $^{15}N$ to $^{14}N$ relations at 30 - 40 cm compared to 60 - 70 cm depth (Table 5.1, upper part).
The N content was higher in the FR than in all tissue of roots bigger than 0.5 cm. The N content of PR was in between. In FR, PR, in the wood of SR and the inner wood of CR there was a significantly higher N content in the roots of samples from 0 - 10 cm than in roots deriving from deeper soil layers (Table 5.1, lower part).

**Table 5.1**: Stable N enrichment values ($\delta^{15}$N) and N content (%N) in root samples of a fallen tree of different root sizes or tissues and from 3 different rooting depths. Different letters indicate significant differences between the depths at a 5% probability level (one-way ANOVA).

<table>
<thead>
<tr>
<th>Soil depth</th>
<th>Fine 0–2 mm</th>
<th>Petite 2–5 mm</th>
<th>Small 5–10 mm</th>
<th>Coarse 10–20 mm</th>
<th>Ø root</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total</td>
<td>total</td>
<td>bark</td>
<td>wood</td>
<td>bark</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-10 cm</td>
<td>227.1a</td>
<td>163.8a</td>
<td>51.6a</td>
<td>58.1a</td>
<td>36.8a</td>
</tr>
<tr>
<td>30-40 cm</td>
<td>33.9b</td>
<td>37.3b</td>
<td>28.6ab</td>
<td>31.7ab</td>
<td>9.8b</td>
</tr>
<tr>
<td>60-70 cm</td>
<td>33.6b</td>
<td>-4.0b</td>
<td>-2.7b</td>
<td>-2.6b</td>
<td>-4.2c</td>
</tr>
</tbody>
</table>

| % N | | | | | | |
| 0-10 cm | 1.32a | 0.87a | 0.69a | 0.39a | 0.66a | 0.33a | 0.28a |
| 30-40 cm | 0.73b | 0.71b | 0.68a | 0.25b | 0.70a | 0.15a | 0.17b |
| 60-70 cm | 0.82b | 0.75ab | 0.72a | 0.21b | 0.69a | 0.15a | 0.13b |
5.4.3 Laboratory experiment

In Norway spruce seedlings changes in the free amino acids of roots and shoots were detected after nitrate fertilisation. The natural enrichment with $^{15}\text{N}$ of the seedlings was in roots comparable to the data in shoots between $-10$ to $+10$ at% (Figure 5.3). The enrichment of the amino acids in the shoots was generally lower than in the roots with both tracer concentrations at all harvests. Within 4 hours after the tracer application the signature of $^{15}\text{N}$ was enhanced in most amino acids of the roots compared to the shoots. The differences between root and shoot were most distinct with the three amino acids Glu, Gln and Asp after the 20 mM nitrate treatment. The signature of $^{15}\text{N}$ in all amino acids was increasing up to the 3rd day after the 2 mM nitrate treatment and up to the 7th day after the 20 mM nitrate application. Thereafter the at% $^{15}\text{N}$ of the measured amino acids decreased again.

Effects on the concentrations of amino acid were not as consistent as the changes of the N signature (Figure 5.4). Within 4h after the nitrate application no differences in the amino acid concentrations could be observed. After 1 and 3 days an increase of the amino acid concentrations in the shoots of the spruce seedlings was observed that was most pronounced in Gln, Glu and Asp, while in Ala, Ser and Asn the effects were faint. After 7 days there was a decrease of at% $^{15}\text{N}$ again and 10 days after the nitrate application the amino acid concentrations measured were in the range of the control. In general the application of 20 mM nitrate resulted in higher at% $^{15}\text{N}$ values and amino acid concentrations than the 2 mM nitrate treatment.
Figure 5.3: Enrichment of $^{15}$N (at% $^{15}$N) in free amino acids of Norway spruce roots (filled symbols) and needles (open symbols) as affected by different nitrate regimes with time (days): □ - control, ○ - 2 mM nitrate, Δ - 20 mM nitrate.
Figure 5.4: Concentrations (μmol g⁻¹) of free amino acids of Norway spruce roots (filled symbols) and needles (open symbols) as affected by different nitrate regimes with time (days): □ - control, ○ - 2 mM nitrate, Δ - 20 mM nitrate.
5.5 Discussion

5.5.1 $^{15}$N uptake in the field

The natural enrichment before tracer application was in the range of comparable European forests (Emmett et al., 1998). The $^{15}$N application in the forest lead to high $\delta^{15}$N values in the fine roots. Forest management treatments that had been applied in the year before the $^{15}$N experiment, had obviously little effect on the short-term uptake of the tracer. Only slightly faster $^{15}$N increase within the first week in the roots of the WF and W treatment were observed. These treatments had increased the N input (WF added 100 kg N ha$^{-1}$ y$^{-1}$; W about 5 - 8 kg N ha$^{-1}$ y$^{-1}$) to the forest and thus the uptake capacity in the year before the experiment was improved. In the present study a saturation effect on the uptake deriving from the high N inputs with the WF treatment could not be observed. This confirmed the results of Nadelhoffer et al. (1995), who investigated the influence of low and high nitrate input rates on the recovery in various tree species in the US.

The inorganic N added in the field is assumed to be immobilised very fast mainly in the upper organic soil (Buchmann et al., 1996) and especially in coniferous forests the release into new plant available N compounds is obviously slow (Gebauer et al., 2000). Gebauer et al. (2000) estimated the period of time of immobilisation in the soil to be less than two weeks, the time period in which we observed a very high increase of $\delta^{15}$N in the fine roots in the field. Later other immobilised N could serve as N source as already showed for amino acids that were taken up by Norway spruce in the laboratory (Johnsson et al., 1999) as well as in the field (Näsholm et al., 1998), probably mediated by the abilities of the external hyphae of mycorrhizas.

Bundt et al. (2001a) observed maximal $\delta^{15}$N enrichment in total fine roots of the topsoil after one month (the first sampling time), which was about the same temporal range as in the present study for the fine roots of Norway spruce. This experiment was conducted within the same forest the presented results were from. The maximal enrichment that was measured in the present study was higher than in a comparable experiment of Buchmann et al. (1996). In the latter root material up to 10 mm
diameter was analysed, while we used only the finest root up to 2 mm in diameter for the general analyses. Thus the different root sizes analysed were one reason of the differing results. This conclusion is supported by the results of the $\delta^{15}$N distribution in the root system nine months after tracer application. Probably minor contribution to the differences were deriving from the use of a single N-source, respectively, and differences due to the fact that Buchmann et al. (1996) used mixed roots of various species.

When calculating a recovery rate (with biomass results, Genenger, unpublished) for $^{15}$N according to Buchmann et al. (1996), we found the recovery of $^{15}$N in the finest roots of the topsoil to be in the range of about 1 mg $^{15}$N m$^{-2}$, i.e. 0 - 3% recovery after 6 - 12 months. These results are comparable to the results of Buchmann et al. (1996), who applied similar amounts of $^{15}$N m$^{-2}$. They described the recovery of $^{15}$N with ammonium to account for about 0.6 mg $^{15}$N m$^{-2}$ (1.0%) and with nitrate about 2 mg $^{15}$N m$^{-2}$ (3.5%). Therefore, the major part of the tracer was assumed to be immobilised in the soil and litter (Buchmann et al., 1996; Schleppi et al., 1999).

5.5.2 N in the roots versus N in the soil

In an undisturbed forest the $\delta^{15}$N values of the roots increase with increasing soil depth (Mariotti et al., 1980; Gebauer and Schulze, 1991; Högberg, 1997; Bundt et al., 2001a) while the N content decreases with increasing soil depths. This reflects the situation in the soil. By applying a highly $^{15}$N enriched tracer on the forest floor the $\delta^{15}$N values in the present study were increased to be highest in the roots of the topsoil and decreasing with depths as observed nine months after application. At 60 - 70 cm depth most $\delta^{15}$N values of roots were in the range of natural $^{15}$N ratios again. Some high variation in the $\delta^{15}$N values measured in the roots might (especially in the mineral soil) originate from the phenomenon of preferential flow. Bundt et al., (2001a) discovered differences in the $^{15}$N dynamic between flow path and soil matrix in $^{15}$N distribution in a tracer application experiment, leading to a higher rise and a stronger decrease of $\delta^{15}$N in a flow path compared to the matrix. A close correlation
between changes of $\delta^{15}N$ in the soil with the changes in the fine roots in a spruce forest was confirmed (Bundt et al., 2001a). But the phenomenon of preferential flow is of minor importance in the upper organic soil horizon compared to the mineral soil, where the flow paths remain stable at least over decades (Bundt et al., 2000).

The amount of N in the tracer of the present experiment was with estimated 2% of the annual N deposition vanishing compared to the deposition and N pool in the soil. Coarse roots are storages for N, as Dyckmans and Flessa (2001) concluded from an enhanced translocation of N to coarse roots in N depleted trees. In the present experiment in small and coarse roots, differences in the N depending on the soil depth were observed only in woody tissue. This relation could be due to a storage function of the wood of roots in contrast to the bark with stable N concentrations, although the main N storage tissue in the whole evergreen trees is assumed in their needles (Millard, 1996).

5.5.3 $^{15}N$ distribution within the root system

In our study we applied double labelled ammonium-nitrate. The analyses of various root material nine months after tracer application revealed $\delta^{15}N$ values in the roots of Norway spruce ranging from about 50 %o (in roots of 5 - 10 mm diameter) to more than 200 %o in the finest roots (diameter smaller than 2 mm), which confirmed the results of Buchmann et al. (1996). The authors described $\delta^{15}N$ enrichments of bulk samples of roots of several species with an diameter smaller than 10 mm from the topsoil rising from −4.4%o to about 55%o with labelled ammonium and to about 137 %o with labelled nitrate addition. In the present study after nine months the $\delta^{15}N$ values in the root system were highest in the fine roots in the upper soil horizon, which confirmed the results of other studies e.g. Gebauer and Schulze (1991), that the bulk of N is taken up from the organic soil layers. The $^{15}N$ mainly stayed in the tissue, where it has been taken up, and is not distributed in the root system to a noticeable extent. But a transportation in the shoot certainly takes place rather fast. At the first sampling of needles after one month the $^{15}N$ was highly enriched in the needles within the sample trees of this experiment (Jaeggi et al., unpublished data).
In a comparable experiment Buchmann et al. (1995) detected as early as 11 days (first sampling) after tracer application a significant increase of $\delta^{15}$N in the current foliage of 15-year old spruce trees. In our laboratory experiment, the major amino acids were enriched in the shoots after four hours to one day.

### 5.5.4 Amino acid metabolism

The time course as it was observed in the amino acid concentration fits well to the results from laboratory experiments with *Picea glauca* Moench (Kronzucker et al., 1995) that described the induction of nitrate uptake and reduction. The maximum influx rates were observed 3 days after exposure to elevated nitrate and declined afterwards again (Kronzucker et al., 1995).

Concerning the biochemical pathways in plants in general, the amino acids can be grouped into biogenetic families (Fowden, 2001): e.g. Glu is among other amino acids the precursor of Gin, Pro and Arg; Asp is the precursor of Asn, Thr, Meth and Lys; Ala is precursor of Ser, Gly, Val and Ile. The temporal resolution of this study is not sufficient to follow the $^{15}$N signal metabolising in detail from one amino acid to the other. But considering the groups Gin and Glu, Asp and Asn, Ala and Ser a time shift was observed in the enrichment of these amino acids from the first to the latter.

Previous reports of short-term ammonium $^{15}$N labelling of mycorrhizal *Eucalyptus* (Turnbull et al., 1995) or spruce (Chalot et al., 1991; Aarnes et al., 1995) seedlings reported the greatest flow of $^{15}$N enters via the amide group of Gin, with label also in the amino group of Gin, Glu Ala and $\gamma$-aminobutyric acid. While the latter was not measured in the present study, the high enrichment patterns after four hours to one day of Gin, Glu and Ala were confirmed with non-mycorrhizal spruce seedlings. The isotope $^{15}$N could also be followed fast into Asp, while in the Ser and Asn pool the signal was obviously enhanced slightly later, i.e. 3 days after tracer application. Compared to incorporation of ammonium in amino acids of *Daucus* and *Picea glauca* (Lea and Ireland, 1999) the uptake and incorporation of nitrate is slower. While the former report times of minutes to hours until observation of the $^{15}$N signal in the amino acids, here times were in the range of days. In general spruce
is known to prefer ammonium over nitrate (Kronzucker et al., 1997). But the different kinetic could also be due to species dependent differences in the N uptake and metabolism kinetics (Min et al., 1998).

The main N storage tissue in evergreen trees is assumed in their needles (Millard, 1996) and up to 90% of the total leaf N of a tree can be mobilised for tree growth. Therefore it is not astonishing that the amino acid concentrations reacted to the nitrate fertilisation mainly in the shoot of the Norway spruce seedlings, while concentrations in the roots stayed at the same level. That means the surplus of N has been transported to the main pools of storage. The free amino acids contribute for less than 1% of the total N in needles (Bauer, 1997). So changes in the concentrations of that pool are interpreted as primary physiological signal of an nutritional improvement rather than being quantitatively relevant for N storage (Bauer et al., 2000). In a laboratory experiment with *Pinus* Flaig and Mohr (1992) reported, that a distinct accumulation of free amino acids did not occur with intensive nitrate fertilisation. This observation is partly confirmed in our study. The free amino acid pool seemed (especially in the roots) to be in a homeostasis. In the needles an increase (depending on the fertilisation amount) of Glu and Asp could be observed, that lasted only for about a week and returned to a control level afterwards as observed before with *Pinus* (Flaig and Mohr, 1992). Considering these observations it can be concluded that a surplus of N is metabolised in a steady state to storage pools as arginine or storage proteins (which were not measured here).

In the present study, Gln and Asp were measured in comparable low concentrations in the roots, in contrast to Glu, while other studies (Chalot et al., 1991; Aarnes et al., 1995; Gessler et al., 1998) described Gln as one of the main amino acid in the roots of Norway spruce seedlings. Maybe this is a hint to N depletion in our study, as far as no N was applied before the tracer application. Comparable values in needles of pine were observed on sites, where the N supply is low in general (Raitio and Sarjala, 2000).

The differences between the at% $^{15}$N values in roots and shoots derived from a dilution effect, as already described by Aarnes et al. (1995) with white spruce.
An enrichment of $^{15}\text{N}$ in amino acids could not be measured in samples deriving from the field experiment (data not shown), because of the dilution of applied $^{15}\text{N}$ in the huge N pool of the soil.

5.5.5 Conclusion

The results of this study confirmed a fast uptake of N in the laboratory as well as in the field. In the field the potential influence of the soil immobilisation properties on the kinetic of uptake of N, that is deposited on the forest floor, was observed. The N that is taken up is not distributed within the root system to a noteworthy extent in the range of several months. Nevertheless, a transport to the shoot could be observed fast in laboratory as well as in the field experiment. No significant effects of an intensive fertilisation on the N uptake were observed in the field. Therefore, the uptake capacities of trees might not be suitable indicators of N saturation in the forest soil in the short-term range.
6 Synthesis

In the present study the effects of an optimal nutrition and wood ash recycling on the fine roots in a Norway spruce stand on the Swiss plateau were investigated. Root growth was observed by sequential soil coring and with ingrowth-cores. Effects on the element composition of the fine roots were observed. Further insight into the N-metabolism of the forest was gained by monitoring the NRA of the spruce fine roots together with soil nitrate and pH. In addition, in laboratory experiments, the reaction of the NRA towards elevated inorganic N supply was analysed. Finally N uptake was assessed under laboratory conditions and in the forest using $^{15}$N labelling with nitrate or ammonium-nitrate applications, respectively.

6.1 Fine roots as indicators of nutritional imbalances and N status of a forest

6.1.1 Root growth and element concentrations

The root growth in general is variable and dependent on the plant growth, the microclimate and soil conditions. Therefore, root growth is not easy to access and different methods can lead to different results (Stober et al., 2000). Although the different treatments studied in this field experiment induced changes in the element concentrations of the fine roots, no significant effect on the fine root biomass has been observed within two years. Root biomass either reacted slowly or in the range of the spatial and temporal variability and therefore it is not suitable as short term indicator. Under certain conditions the root growth can be affected exceptionally fast and strongly as was shown e.g. in the NITREX experiment (Bredemeier et al., 1995), where root growth was greatly improved when excluding S and N depositions. In the present study a significant change in root growth pattern was observed with the wood ash (A) treatment, leading to a more branched root system. Nevertheless, the enhanced root length observed under the A treatment could be due to a change in the Ca/Al molar ratio (Cronan and Grigal, 1995). The change in growth pattern went along with an increase of the NRA of the fine roots.
In the HARWA project an increase in needle weight and an influence on the phenology of beech trees in autumn due to the liquid fertiliser (WF) treatment could be detected (Hallenbarter, unpublished). Furthermore, the needle fall of Norway spruce deriving from abscission (in contrast to mechanically induced needle fall), was reduced under the A and the WF treatment (Hallenbarter, unpublished). These effects might result in a feedback on the root growth as well in the long run. A support for this hypothesis would be the increased root growth dynamic with the A treated roots as investigated with the ingrowth-cores in the first 10 months. On sensitive soils a negative influence of N input (as sodium- or ammonium-nitrate) on the tree growth in general has also been reported (Emmett, 1999). A reduced root growth could not be observed in the present study, although the WF treatment added a reasonable amount of N. The effect of N fertilisation on forest growth does not only depend on the amount of N and the N source applied, but also on the N saturation status of the forest system (Aber et al., 1998) and on the nutritional balance with other nutrients.

Foliage analyses have been used for years to evaluate the nutritional status of trees and forests, and thereby, with the definition on what is a ‘deficient’, ‘sufficient’ or ‘surplus’ range, a conclusion on the forest 'health' could be drawn. An improvement of simply considering the element concentrations might be the use of relations of the nutrients to each other (Linder, 1995). For foliage analyses the biomass or leaf area is sometimes related to the element concentrations to estimated the element content of the foliage (Linder, 1995; Salih and Andersson, 1999) and to describe 'net changes' in the element budget.

The N concentrations in the fine root and in the needles were changed by the WF treatment, which resembled a high input of N to the forest. But the N concentrations in the needles were also changed with the control (C) and A treatments, although to a minor extent. These results are consistent with the observation, that N fertilisation often led to an enhanced biomass while an increase of N concentrations in the needles might be transient (Aber et al., 1985). The C concentrations of the fine roots decreased in A, WF and water (W) treatments and the Ca and Mg concentrations were increased by A, but also by the WF treatment. The K and P concentrations in
the fine roots were improved by all three applications. The decrease of Al, Fe and Mn concentrations in the fine roots by the A and WF treatments was most probably caused by a pH increase and the subsequent change in the availability of these elements in the soil.

The heavy metal input of the wood ash applications is rather high compared e.g. to the yearly need of Zn and Cu according to Ingestad (1979). This leads to an concentration of these elements in the soil, which will possible gain mobility when the pH is decreasing after some seasons (Zhan et al., 1996). Thus heavy metal concentrations in trees must be judged in relation to the soil pH. After two growing seasons an accumulation of heavy metals in the fine roots of the topsoil could not be observed in the HARWA experiment, but the question of heavy metal impact has to be addressed in the long term range.

6.1.2 NRA in fine roots

The N cycle of a forest on acidic soil is usually dominated by ammonium. When the state of the forest increased towards N saturation the nitrate gains importance by nitrification. The definition of what is ‘healthy’ in regard to the N status of a forest is difficult and has been linked to the 'vitality' and productivity of a forest (Linder, 1995). A major export of N by nitrate leaching and gaseous losses, which equals or exceeds the import, nevertheless, is a symptom of an N saturated forest.

The NRA was enhanced by the optimal WF fertilisation, by the A treatment, and in autumn by the W treatment. Possible reasons for this increase were detected as (i) a change in soil nitrate (especially with WF treatment) and so an increase in substrate availability, (ii) a pH change, which is a multifunctional property, that could have effects on a series of other factors (e.g. nutrient availability, uptake processes, internal pH, electrochemical balance), and (iii) a possible influence by the impact of other nutrients as e. g. Mo, Ca, K (Marschner, 1995).

Although the NRA of the A treated roots was increased, the N concentrations in these roots were not enhanced. This might partly be contributed to a translocation of the assimilated N to the shoots where increases in growth (needle weight, tree-ring-
growth, Hallenbarter, personal communication) were observed. The N concentration in the needles was increased in comparison to the pre-fertilisation situation, but in the other treatments and in the control the N concentrations were enhanced as well. In the roots the N concentrations were only enhanced with the WF treatment, which matches with the enhanced NRA in the fine roots.

In the laboratory experiment the NRA could be directly correlated to the nitrate concentrations in the substrate. High ammonium concentrations inhibited the NRA in the roots. In the forest, the NRA of the fine roots is an integrator on the spatial and temporal heterogeneity of nitrate in the soil. The ability of the NRA in the fine roots to indicate changes in the nitrate content in the forest soil could be shown. Nevertheless, on the one hand, the soil pH and probably the availability of nutrients beside nitrate could have a strong influence on the NRA and on the other hand the temporal range of the indicator function of the NRA is difficult to estimate.

The in vivo NRA of white spruce, *Picea glauca* (Moench) Voss., has been shown to be down-regulated after several days of exposition to elevated nitrate (Kronzucker *et al.*, 1995). Thus, the NRA might adapt to elevated nitrate in the soil and switch back to a 'constitutive' activity after some time. If so, the NRA of the roots is only suitable to detect recent changes in the nitrate availability and the N saturation status of the forest.

6.1.3 N uptake

Although in this study the NRA in the fine roots showed increases in the roots of A, WF and W treated plot, the total N uptake by the roots as assessed by the use of $^{15}\text{N}$ was not affected by the treatments. This is partly due to the minor quantitative role of nitrate as N source of spruce (Kronzucker *et al.*, 1997). In addition, the increase in nitrate assimilation might have gone along with a decrease in ammonium assimilation. Thus the N uptake capacity did not seem to be a good indicator of changes in the soil N, at least on the observed time scale.

The free amino acid pool is a rather small N pool in the plant. In needles of spruce it contributes for example only for 1% of the total N (Bauer *et al.*, 2000).
Nevertheless, the free amino acid pool has a signalling function, for example also regulating the nitrate assimilation (Oaks, 1992; Tischner, 2000). Furthermore, high concentrations of free arginine were assumed to be a good indicator towards nutritional imbalances (especially of N/P ratios) of a forest (Ericsson et al., 1993; Boxman et al., 1998).

The $^{15}$N labelled amino acid pool in the laboratory experiment of the present study did react to an increased nitrate input within hours. But this effect was subjected to adaptation with time, after 10 days the concentrations of the main amino acids had been regulated back to an amount, close to that before experimental fertilisation. The changes of the free amino acid pool can therefore be interpreted as a primary physiological signal towards changing N supply rather than as a permanent indicator of elevated N deposition.

### 6.2 N deposition and global change

The C and N assimilation are the two most energy-requiring processes in plants (Gebauer and Schulze, 1997). The C metabolism is closely connected to the N metabolism. For example Dyckmans and Flessa (2001) showed that N depleted beech trees reduced their C assimilation. Furthermore the N depletion had no direct influence on N uptake as long as internal N stores could be used for translocation (Dyckmans and Flessa, 2001).

In the context of rising CO$_2$ concentrations in the atmosphere it is assumed, that N saturation of forests is counteracted by a rising N retention capacity of the soil, depending on the soil characteristics (Hagedorn et al., 2000). The assimilation of nitrate requires more energy than the assimilation of ammonium. Bassirirad et al. (1996) observed a higher nitrate uptake of the roots, that was possibly induced by a higher carbohydrate content of the roots (Bassirirad et al., 1996) under elevated CO$_2$. In a laboratory experiment different responses concerning the N uptake and reduction under elevated CO$_2$ between birch and pine was observed: For example pine took up more N but did not change the growth, while birch increased root length but did not take up more N (Bauer and Berntson, 2001). The NRA was only reduced in the birch
leaves while in other tissues and in pine it was not affected by the elevated CO$_2$
(Bauer and Berntson, 2001). Thus, interactions between enhanced N deposition and
elevated CO$_2$ are complicated by species dependent reactions. However, overall the
elevated N deposition is unlikely to be a major contributor to a CO$_2$ sink in northern
temperate forest (Nadelhoffer et al., 1999).

6.3 Sustainability
The term 'sustainable' already implies a long term view on the effects of a treatment.
Thus, the sustainability of the applied HARWA treatments cannot be judged from the
results of the project up to now, although the fine roots did not reveal a negative
influence of the treatments.

Productivity is an important indicator of forest sustainability, but a different
possible perspectives and emphasis concerning a 'plantation forest' and a 'native
forest' has to be considered (Richardson et al., 1999). While in the first case the
productivity is one of the key criteria, in a virgin forest more emphasis is given to
conservation of biodiversity and socio-economic benefits (Richardson et al., 1999).
The soil fertility has to be maintained by the aid of balanced nutrient budgets
(Ranger and Turpault, 1999). The supply with basic cations and the elements in the
roots have been judged as good indicators towards soil fertility, and with that of
sustainability of an applied forest management (Smith et al., 1999). Sustainable
management of a system includes the preservation of the biodiversity. The vegetation
within the HARWA project showed no significant reactions towards the treatments
within two seasons (Thürig and Kienast, unpublished results). Similarly with a N
fertilisation experiment in a montane forest (Schleppi et al., 1999) could not observe
a change in the botanical composition. By excluding N and S depositions in a pine
forest in the Netherlands, effects on the undergrowth was observed only after a lag
phase of 3-4 years (Boxman et al., 1998). In general eutrophication and acidification
of a forest ecosystem have been shown to be most important factors contributing to a
change in the species of the ground vegetation in France within 20 years (Thimonier
et al., 1994). In a microcosm experiment the application of wood ash resulted in a
change of the microbial community structure (Fritze et al., 2000). In the HARWA project only effect of the A treatment were investigated and small changes in the microbial community structure were observed, with a tendency towards higher functional diversity (Shannon index) with the ash application (Zimmermann and Frey, unpublished). The optimal nutrition of the forest was focussed on the nutrition of the spruce trees. The effects of a management that ensures the optimal nutrition of spruce trees on other organisms within the forest have to be considered as well. Certain species of animals, plants, fungi as well as microbes will be favoured while other will be discriminated. This shift in species composition will certainly also depend on the site, where the spruce grow. If the forest is a natural spruce forest there might be minor shifts compared to a forest, where spruce has been planted and where usually other trees would be dominating.

The species composition of ectomycorrhizal fungi in a forest can change upon N fertilisation and liming (Kuyper, 1989; Peter et al., 2001). The contribution of the ectomycorrhizal fungi to N (and element) uptake and nitrate assimilation is only partly known (Smith and Read, 1997; Botton and Chalot, 1999), especially as it probably differs between various species or even strains. To investigate the contribution of both partners and especially of the fungal extramatrical mycelium, that might have an essential role in N nutrition (Brandes et al., 1998), is a possible direction of future research. Furthermore a recent review on the topic of N saturation suggest an important role of the ectomycorrhizal fungi to N retention capacity in a forest (Aber et al., 1998; Berntson and Aber, 2000; Hobbie et al., 2000). Mycorrhizas were not investigated within the HARWA project.

In a sustainable forest management recirculation of nutrients through the application of ashes from wood chip combustion can be an essential way to guarantee healthy and vital forests. Beside recirculating elements, the application of wood ash counteracts soil acidification and therefore, is especially suitable at sites, which exceed the critical load of acidity and, thus, are in danger of acidification. Nearly one third of Switzerland, about 1.2 Mio ha, is covered by forest. A calculation with deposition data of 1990 concluded that about 63% of these areas were subjected to an exceeded critical load of acidity (Rihm, 1994). In recent years the acid
depositions were decreased and so one could assume that about 50% of the forests in Switzerland might be suitable for a wood ash recycling. Noger et al. (1996) suggested a threshold of 8 t ha\(^{-1}\) in three years. This would equal a wood ash amount of about 1.6 Mio t per year. But the amount of 8 t in three years is still a high limit. In a more modest calculation on the basis of a forest productivity of 10 m\(^3\) ha\(^{-1}\) y\(^{-1}\), a wood density of 0.8 t m\(^3\) and ash content of about 2%, the amount of wood ash to be recycled may not exceed 0.5 t ha\(^{-1}\) within 3 years (Zimmermann, personal communication). Thus the estimated amount of wood ash totals about 100'000 t that could potentially be recycled to the forest in Switzerland every year. In 1995 more than 23'000 t wood ash were produced. Only the high quality ash part would be suitable for forest recycling. To our estimate the amount of suitable wood ash might then be recycled totally to the forest, with a capacity for increasing the energy production with wood. The risk of heavy metals and organic contaminants that might be relevant in the wood ash has to be considered (Bundt et al., 2001b). Therefore, an effective quality control of the wood chip combustion and the wood ash is absolute essential in the context of wood ash recycling. Along with this problem goes the acceptability of the Swiss society of wood ash in the forest. The wood ash is derived from pure wood and contains only the elements that were taken out of the forest. Thus the acceptability might increase especially since energy production with wood is one of the CO\(_2\) neutral alternative energy sources, that must be promoted to tackle climatic change.
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