Diss ETH No.16375

Influence of Soil Pollution by Heavy Metals on the Water Relations of Young Forest Ecosystems

Dissertation submitted to the

SWISS FEDERAL INSTITUTE OF TECHNOLOGY

For the degree of

DOCTOR OF SCIENCE

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2006

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Influence of Soil Pollution by Heavy Metals on the Water Relations of Young Forest Ecosystems

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www.sierke-verlag.de
ISBN 3-933893-43-7

1. Auflage 2006

Table of Contents

Summary

Among various toxic substances that contaminate the soil, the effects of heavy metals are particularly problematic on different aspects of soil-plant system. Accumulation of heavy metals in the topsoil can adversely affect establishment of seedlings and forest productivity. A number of possible reasons can be implicated, such as: altering catalytic functions of enzymes, damaging cellular membranes, inhibition of root and shoot development, reduction of net carbon dioxide assimilation, and decreasing stomatal conductance and transpiration, which in turn, can affect soil water regime. Knowledge of water regime is the first step to understand other soil-plant relations such as uptake of nutrients and toxic contaminants.

The issue of heavy metal toxicity in a forest ecosystem was being addressed comprehensively by the Swiss Federal Institute for Forest Snow and Landscape Research (WSL) in a project titled "from cell to tree". The first objective of this work was to experimentally investigate and model the water regime of a heavy metal contaminated soil under young forest ecosystems. The second objective was to visualize the root growth in polluted soil using the non-invasive neutron radiography.

The water regime of young forest vegetation consisting of Norway spruce *(Picea abies),* willow *(Salix viminalis), poplar (Populus tremula) and* birch *(Betula pendula)* was monitored from 2001 to 2003 at WSL in 32 lysimeters. The treatments were applied in a latin square factorial design (contaminated vs. uncontaminated topsoil, acidified rain vs. ambient rain) to 16 open-top chambers, with 4 replicates per factorial combination. Each of the open-top chamber contained two lysimeters, one with a calcareous, and the other with acidic subsoil.

In heavy metal contaminated topsoil, water extraction, evapotranspiration and root growth were significantly reduced. Acid rain did not affect water regime of the

young forest ecosystem. The four tree species responded differently with respect to leaf area and biomass to heavy metal pollution and different subsoil types. In the last growing season, leaf area was significantly reduced by heavy metal pollution and acidic subsoil, except for *P. tremula*. Heavy metal contamination significantly reduced above ground biomass of *B. pendula* and *S. viminalis,* however, no such effect was found on *P. tremula* and *P. abies*. Compared to the other species, *P. tremula* also produced highest biomass in acidic subsoil. Heavy metal application did not significantly change the overall water use efficiency of the forest ecosystems. The model MACRO was used to calibrate the water regime of one control and heavy metal contaminated lysimeter.

The root growth imaging studies proved that neutron radiography could be effectively used to study dynamic root growth as well as root responses to heavy metal contamination. Root growth was significantly reduced in boron and zinc contaminated zones. Preliminary experiments with different materials showed that, even though visibility of roots was better with quartz sand than soil, root growth was found to be poor in quartz sand possibly due to higher bulk density.

Zusammenfassung

Neben verschiedenen giftigen Substanzen, welche den Boden verunreinigen, sind die Auswirkungen von Schwermetallen besonders problematisch hinsichtlich verschiedener Aspekte des Boden-Pflanzen-Systems. Die Akkumulation von Schwermetallen im Oberboden kann die Etablierung von Keimlingen und die Produktivität des Waldes nachteilig beeinflussen. Für diese Auswirkungen kommen verschiede Gründe in Frage, wie beispielsweise die Veränderung katalytischer Funktionen von Enzymen, die Beschädigung von Zellmembranen, die Hemmung der Wurzel- und Triebentwicklung, die Verminderung der Netto-Kohlendioxid-Assimilation, sowie die Reduktion der stomatalen Leitfähigkeit und Transpiration, welche ihrerseits das Bodenwasserregime verändern kann. Die Kenntnis des Wasserregimes ist der erste Schritt, um andere Boden-Pflanzen-Beziehungen wie beispielsweise die Aufnahme von Nährstoffen und giftigen Substanzen zu verstehen.

Die Frage nach der Schwermetalltoxizität in einem Waldökosystem wurde umfassend von der Eidgenössischen Forschungsanstalt für Wald, Schnee und Landschaft (WSL) innerhalb eines Projektes mit dem Arbeitstitel „Von der Zelle zum Baum“ behandelt. Ziel der vorliegenden Arbeit war, das Wasserregime eines schwermetallbelasteten Bodens unter einem jungen Waldökosystems experimentell zu untersuchen und zu modellieren. Das zweite Ziel war, das Wurzelwachstum in verschmutztem Boden mittels der nicht-invasiven Neutronenradiographie zu visualisieren.

Das Wasserregime eines jungen Waldbestandes, welcher aus Norwegischer Fichte (*Picea abies*), Weide (*Salix viminalis*), Pappel (*Populus tremula*) und Birke (*Betula pendula*) bestand, wurde von 2001 bis 2003 am WSL in 32 Lysimetern aufgezeichnet. Die Behandlungen wurden in einem faktoriellen Experiment

(lateinische Quadrate) (verschmutzter gegen unverschmutzter Oberboden, saurer Regen gegen Umgebungsregen) auf 16 gegen oben geöffnete Kammern angewandt, mit je vier Wiederholungen pro Faktorkombination. Jede dieser Kammern enthielt zwei Lysimeter, einen mit kalkreichem und einen mit saurem Unterboden.

In schwermetallbelastetem Oberboden wurden die Wasseraufnahme, die Evapotranspiration und das Wurzelwachstum signifikant vermindert. Der saure Regen beeinflusste das Wasserregime des jungen Waldökosystems nicht. Die vier Baumarten reagierten unterschiedlich bezüglich Blattfläche und Biomasse auf die Schwermetallbelastung und die verschiedenen Unterböden. In der letzten Vegetationszeit wurde die Blattfläche signifikant durch die Schwermetallbelastung und den sauren Unterboden vermindert, ausser für *P. tremula*. Die Belastung mit Schwermetallen verminderte die oberirdische Biomasse von *B. pendula* and *S. viminalis* signifikant, hingegen fanden wir diesen Effekt bei *P. tremula* und *P. abies* nicht. Verglichen mit den anderen Pflanzenarten produzierte *P. tremula* ausserdem die höchste Biomasse im sauren Unterboden. Die Zugabe von Schwermetallen veränderte die Wassernutzungseffizienz des Waldökosystems gesamthaft nicht signifikant. Mit dem Modell MACRO kalibrierten wir das Wasserregime einer Kontrolle und eines schwermetallbelasteten Lysimeters.

Die Untersuchungen des Wurzelwachstums mittels Bildanalyseverfahren zeigten, dass die Neutronentomographie für die Untersuchung des dynamischen Wurzelwachstums sowie die Reaktion der Wurzeln auf Schwermetallbelastung geeignet ist. Das Wurzelwachstum wurde in mit Bor- und Zink belasteten Zonen signifikant vermindert. Vorexperimente mit verschiedenen Materialien zeigten dass die Wurzeln, obwohl sie in Quarzsand besser sichtbar waren als in Boden, im Sand schlecht wuchsen, möglicherweise wegen dessen hohen Lagerungsdichte.

Chapter 1. Introduction

The term *heavy metal* refers to metallic chemical elements with a relatively high density above 5 g cm^{-3}. Heavy metals are natural components of soils. Apart from geogenic and pedogenic origin, substantial amounts of these metals have been introduced into some soils from human activities such as industrial waste disposal, traffic, sewage-sludge, fertilizer and pesticide applications, shooting etc. As trace elements, some heavy metals (e.g. copper, zinc) are essential to maintain the metabolism of plants and animals. However, at higher concentrations, all metals can lead to poisoning of the living organisms. Some heavy metals are toxic to plants, animals and humans already at very low concentrations, e.g. Hg, Pb.

The movement of water from soil to atmosphere via plants, i.e. through the soil-plant atmospheric continuum (SPAC), is an important part of soil-plant water regime. This movement of water is governed by a gradient in the water potential, i.e. water moves from higher (soil) to lower (atmosphere) water potential. The water content and water potential control or affects almost all physical, chemical and biological processes in soil. The major factors controlling water regime are: (1) atmospheric (evaporative demand) (2) soil (water potential, hydraulic conductivity and osmotic head) and (3) above and below ground features of the plant (root depth and density). The roots play a critical role in the uptake of water from soil into the plant.

Contaminating heavy metals can adversely affect the functioning of the root system in many ways (detailed review in chapter 2). Heavy metals can have adverse effects on the formation of new laterals and root hairs, spatial distribution of roots, root elongation rate, potential for osmo-regulation, rate of carbon allocation from shoot to root, number and size of xylem elements, hydraulic permeability and conductivity of roots, degree of soil-root contact, root-shoot ratio and integrity of epidermis (Barceló and Poschenrieder, 1990; Punz and Seighardt, 1993; Kahle, 1993). The primary toxicity effects of heavy metals are changes in the catalytic function of enzymes (van Assche and Clijsters, 1988) and damage to cellular membranes (Haug and Caldwell, 1985; Kennedy and Gonsalves, 1987; Ros et al., 1990; DeVos et al., 1991, Ernst, 1998). Due to these primary effects, numerous secondary effects result such as reduced nutrient uptake (Oberlander and Roth, 1978), hormonal imbalance and water stress (Kastori et al., 1992, Prasad, 1995), inhibition of mineralization rate (Aoyama and Kuroyanagi, 1996) and root respiration (Karolewski and Giertych, 1994). Researchers in the past few decades focused on root growth and changes in transpiration or leaf water potentials using small container systems such as pots, hydroponics etc. using selected plant species. The duration of these experiments were usually rather short i.e. in the order of several days or weeks. Such experiments do not represent a composite natural ecosystem, such as a forest stand. For example, metal uptake by plants from a contaminated soil is usually higher in pot experiments than in the field, even if the same soil is used in the pots, due the to fact that roots grown in homogenized soil in a smaller container have no choice to avoid exposure, whereas in the field the roots may extend to uncontaminated zones (Kahle, 1993). For these reasons, there is a need for long-term and large-scale experiments simulating real-world natural conditions.

The hypothesis that was tested in this study was that in the presence of high concentrations of heavy metals the root growth is reduced. As a consequence, plants take up less water and the soil becomes wetter, i.e. less water taken up by plants and more water seeps into the subsurface. It is estimated that in order to produce one kg of fresh weight, approximately 100 kg of water must be transpired (Penman, 1970).

The transpiration is closely linked to photosynthesis and assimilation as water loss through the same stomatal openings though which carbon dioxide diffuses into the leaves. Therefore, a reduction of water uptake will ultimately decrease crop productivity. Over entire forest stands, such changes can have a large impact on the local water cycle as well as forest productivity (Pukacki and Kaminska-Rozek, 2002). In addition, more seepage may also promote heavy metal leaching to deeper layers and eventually into ground water. Until now there have been no experiments to monitor the water regime of heavy metal polluted soils under forest vegetation in realistic ecosystem conditions. Different plants do not only differ in their growth strategies, but also in their responses towards metals. However, little is known on about how such strategies and responses translate into the response of an entire plant community under metal stress in situation of competition and multiple stresses.

Another important issue addressed here is how the root growth and water uptake are affected under partially contaminated situations. The roots of some tree species are know to avoid hotspots of heavy metal contamination (Dickinson et al. 1991; Breckle and Kahle, 1992). It is not known how the water uptake is affected under such situations. If roots invade uncontaminated parts of the profile, there could be a compensatory root water uptake due to root adaptations for uptake of water and nutrients. Modelling approach could be useful to emphasize the effect of contamination on root strategy and modification of the soil structure.

Our understanding of rooting systems is limited because sampling of roots without destructing the surrounding soil and monitoring their activity over time without disturbance is usually very difficult, except for rather artificial experimental conditions. Hence, the development of *in situ* and non-invasive techniques is very important for the investigation of root growth strategy and their dynamics under metal pollution. Some examples of non-invasive and *in situ* techniques are: a) monitoring by means of minirhizotrons (Majdi, 1996), which are transparent plastic tubes inserted into the ground to view the roots using a video camera, b) X-micro tomography (Gregory et al., 2003) or radiography using thin slab systems (Pierret et al., 2003), and c) neutron radiography (e.g., Willat & Struss, 1978; Willat et al.,1978; Couchat et al., 1980; Furukawa et al., 1999) and neutron tomography (Okuni et al., 2001). Although non-destructive, minirhizotrons have an invasive component. Neutron radiography has the potential not only to visualize dynamic root growth but also infiltration and water uptake. A distinct advantage of neutron radiography is its sensitivity to hydrogen (i.e. high attenuation by neutrons) given that the water content of roots often much higher than that of the surrounding unsaturated soil. This allows studying roots very closely with high spatial resolution. In this study, we explored the potential of neutron radiography to visualize root growth strategies in response to heavy metal contaminations.

Summarizing, the objectives of this study were the following:

1. To study water regime of young forest ecosystems on a heavy metal contaminated soil
2. To study the effects of soil metal contamination on individual tree species growing under competition with other trees
3. To evaluate the potential use of neutron radiography as a technique to visualize root growth in the presence of soil pollutants
4. To model water regime of young forest ecosystems in a heavy metal contaminated soil

The experiments with young forest ecosystems were conducted in large lysimeters with juvenile forest ecosystems from 2001 to 2004 at the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland, as part of multi-disciplinary project called “From the cell to tree”. The neutron radiography study was performed at the Paul Scherrer Institute, Villigen, Switzerland.

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Chapter 2. Root Growth and Water Relations in Presence of Heavy Metal Pollution

1. Introduction

Heavy metals are a very heterogeneous group of elements. They greatly differ in their chemical properties and biological functions. Heavy metals do not so much affect plant growth by decreasing the soil osmotic potential. Their influence is because they are essential nutrients on one hand while being toxic on the other hand at relatively low concentrations (10^{-3} M or lower) (Levitt, 1980). An excess of heavy metal ions or soluble metal chelates may induce a series of biochemical and physiological alterations in plants, which present some common characteristics. Membrane damage (Haug and Caldwell, 1985; Kennedy and Gonsalves, 1987; Ros et al., 1990; De Vos et al., 1991, Ernst, 1998), alteration of enzyme activities (Vallee and Ulmer, 1972; van Assche and Clijsters, 1988) and the inhibition of root growth (Punz and Seighardt, 1993; Kahle, 1993) are considered characteristic features of heavy metal stresses. These primary effects lead to a large range of secondary effects, such as a disturbance in hormone balance, deficiency of essential nutrients, inhibition of photosynthesis, changes in photoassimilate translocation, alteration of water relations etc., which further enhance the metal-induced growth reduction. It is extremely difficult to distinguish between primary and secondary effects, which concern practically all-physiological processes. Primary toxicity effects of heavy metals on membrane function, ion balance and enzyme activity may bring about substantial alterations of water relations at both cellular and the whole plant level (detail review by Barcelo and Poschenrieder, 1990).

2. Root growth under the influence of heavy metal stress

In higher plants, roots are the first organ to be exposed to metals in the soil solution and usually accumulate significantly larger metal amounts than the upper plant parts (Breckle, 1989). Metal contamination of soils has been found to reduce root growth (Arduini et al., 1994; Arduini et al., 1995; Ewais, 1997; Helmisaari et al., 1999), to inhibit root respiration (Karolewski and Giertych, 1994), and to interfere with nutrient uptake (Oberlander and Roth, 1978). Under long term exposure, poorly mobile metals such as Pb, Al, Cr, Cu or Ba, and also more mobile Cd, generally reduce the root growth more intensively than shoot growth, decreasing the root/shoot ratio. Both metal uptake and the root growth inhibition may differ between plant species and vary with growth conditions (Breckle, 1989; Matsumoto et al., 1976).

Although the length of roots is widely used to assess the tolerance of plants to metals (Wilkins, 1978), the causes of decreased root elongation (Odjegba and Fasid, 2004, Chen et al., 2003) and their consequences for the whole plant performance are poorly understood. Different metals may inhibit root growth by different mechanisms. Inhibition of root cell division by Al (Taylor, 1988), inhibition of root cell elongation by Zn, Cu and Pb (Wainwright and Woolhouse, 1977; Lane et al., 1978) and the extension of the cell cycle by Zn (Povell et al., 1986) have been found responsible for decreased root growth. Investigation of the primary mechanisms of metal induced root growth inhibition is very difficult. A root does not grow as a unit. Individual tissues can vary in growth to some extent independently of each other (Burström and Svensson, 1972). As metals are distributed unevenly within the different root tissues, there may be different toxicity effects and even different modes of injury within these different tissues (Wierzbicka, 1987; Vazquez et al., 1987; Wagatsuma et al., 1987; Bennet et al., 1985)

Metal toxicity does not only affect the length of primary roots, but also changes the architecture of the entire root system (Breckle, 1989). Toxic levels of Al, Pb, Cu, Zn, Mn, Cd or Cr have been found to enhance lateral root formation, giving rise to more dense and compact root systems. Root hair density is generally decreased under metal toxicity (Breckle, 1989). The morphogenetic changes indicate that metal toxicity may alter the root hormone balance. But the changes of endogenous hormone levels underlying these morphogenetic alterations are virtually unexplored.

3. Water relations under the influence of heavy metal stress

Shankar et al., 2005 reported deleterious Cr effects on the water relations in plants. Leaf gas-exchange rates and water potential were reduced in two varieties of beans (*Phaseolus vulgaris*) at different concentrations of As (Stoeva et al., 2005). Stomatal conductance and water content of cucumber (*Cucumis sativus* L) leaves were decreased by Cu, Cd and Pb (Burzynski and Klobus, 2004). Excess Cd caused a reduction in water and turgor potential, whereas the relative water content was maintained in two varieties of wheat (Milone et al., 2003). The response was different when Ni^{2+} and Cd^{2+} were compared in wheat. Cd^{2+} caused severe reduction in water content and leaf water potential than with Ni^{2+} (Bishoni et al., 1993). Transpiration and relative water content were significantly decreased by excess concentrations of Pb, Cd, Cu and Zn in sunflowers (Kastori et al., 1992).

Studies with solution cultures, in which water availability is not a limiting factor, have shown that water availability alone is not responsible for decreased water content in metal stressed plants. While copper tolerant populations of *Silene cucubalus* maintained their water content when grown in a solution with 40 $\mu g\ L^{-1}$ Cu. A rapid decrease of the water content was found in non-tolerant populations

(Lolkema and Vooijs, 1986). Water contents also decreased in plants grown in solutions containing toxic levels of Zn, Cd, Al or Pb (Paivoke, 1983; Barcelo et al., 1986; Fuhrer, 1982; Gorannson and Eldhuset., 1987; Lane and Martin, 1980).

Impaired spatial distribution and the reduced root hair surface of metal stressed roots lead to bad root-soil contact and lower the capacity of plants to explore the soil for water and nutrients. A decrease in the root size will affect the water relations of plants. The reduced water uptake by metal stressed plants may not only be caused by the small size of the root system, but also by an increased resistance to water flow into and within roots. Several authors reported root browning, as a consequence of metal stress (Breckle, 1989). Browning seems to be caused by enhanced suberisation, which may limit water uptake. Moreover some metals such as Zn and Pb appear to stimulate root lignification (Paivöke, 1983). Structural changes observed in hypodermis, endodermis and the pericycle as well as plasmolysis of cortical cells (Matsumoto et al., 1977; Vazqzuez et al., 1987; Paivöke, 1983) may reduce water uptake in addition to reducing water permeability of membranes. A detailed review on the responses of the tree roots to heavy metals is given by Kahle (1993). Table 1 illustrates the influence of trace metal toxicity on factors influencing water uptake (Barceló and Poschenrieder, 1990).

4. Heavy metal induced changes in transpiration

Low concentrations of potentially toxic metal ions seem to cause a certain amount of osmotic adjustments, due to the accumulation of soluble sugars (Costa and Spitz, 1997). There are several reports on enhanced transpiration after exposure to high metal treatments (Paul and Foresta, 1981; Angelov et al., 1993).

Table 1. Summary of the effects of heavy metals on root growth

Decreased by metals	Increased by metals	Variable or uncertain
1. Formation of new laterals and root hairs 2. Spatial distribution of roots 3. Root elongation rate 4. Potential for osmoregulation 5. Rate of carbon allocation from shoot to root 6. Number and size of xylem elements 7. Hydraulic permeability and conductivity 8. Degree of soil –root contact 9. Root/shoot ratio 10. Integrity of epidermis	1. Degree of root suberization 2. Degree of lignification 3. Rate of root tip dieback	1. Rate of ion transport 2. Root water potential

Enhanced transpiration in plants has been attributed to increased stomatal density (Paul and de Foresta, 1981). It seems that in intoxicated heavy metal stressed plants a premature onset of senescence (van Assche et al., 1980; Vàzquez et al., 1989) may cause a loss of stomatal control, which at the same time cuticle damage may enhance cuticular transpiration (Greger and Jonhansson, 1992).

Other authors, working with growth-reducing concentrations of Cr, Cd or Zn, observed that transpiration decreased in spite of increased stomatal density (van

Assche et al., 1980; Barcelo et al., 1986). Also Barceló and Poschenrieder (1990) report that plants exposed to concentrations clearly above the critical toxicity level exhibit increased stomatal resistance and decreased transpiration rates. Although, the direct effects of toxic metals on stomatal guard cells cannot be excluded, a decrease in transpiratory losses seems to be mainly a consequence of metal-induced water stress. Increased levels of proline (Alia and Sarathi, 1991; Kastori et al., 1992; Bassi and Sharma, 1993a, b; Chen and Kao, 1995; Schat et al., 1997) and abscisic acid (Rauser and Dumbroff, 1981; Barcelo et al., 1986a; Poschenreider et al., 1989), the most frequently used biochemical markers for drought stress (Heuer, 1994; Hartung and Davies, 1994), have been found in plants under metals stress. Exposure to 76 μM Zn caused a 150% increase in proline concentration in the fronds of *Lemna minor*. Copper at the similar concentrations was even more effective: The proline concentration was 3 times higher at similar concentration than in controls (Bassi and Sharma, 1993a). In another study, young wheat plants were exposed to 5 different concentrations of zinc (0, 2, 10, 25, 50 mg L^{-1}) and two different transpiration regimes (Grifferty and Barrington, 2000). Zinc levels did not affect transpiration and at nutrient solution concentration of 50 mg L^{-1} had no effect on plant growth. Vassilev et al. (1998) reported that Cd^{2+} decreased water potential and transpiration rate, whereas relative water content in leaves was not significantly affected in barley (*Hordeum vulgare* L. cv. Hemus).

5. Metal effects on photosynthesis and water use efficiency

Reduced photosynthesis in metal-stressed plants has also been linked to inhibition of photosynthesis (Clijsters and Van Assche, 1983; Prasad, 1997). Vitoria et al., 2003 reported that the main effects observed in radish leaves in response to

Cd were stomatal closure in radish. Some authors suggested that stomatal closure is the main reason for a decrease in photosynthesis observed under metal stress (Carlson et al., 1975). However, others believe that metals act primarily on the metabolic reactions of photosynthesis (Van Assche and Clijsters, 1983). It may be that a metal-induced inhibition of photosynthesis may increase leaf CO_2 concentrations and thus cause stomatal closure as a secondary effect. The water use efficiency (WUE) of metal-stressed plants has been evaluated in different species either in nutrient solution studies or under field conditions. In a comparative study of Pb and Cd-induced effects in *Medicago sativa*, Becerril et al. (1989) found that at similar leaf metal concentrations Pb caused a drastic reduction of water use efficiency, while Cd- inhibited transpiration and CO_2 assimilation to a similar degree and thus did not change WUE. The authors suggested that Cd-induced inhibition of assimilation may be mainly due to stomatal closure. Also, in Cd-stressed *Picea abies* seedlings, decreased CO_2 assimilation was mainly attributed to stomatal closure (Schlegel et al., 1987).

In other studies using Zn (Clijsters and Van Assche., 1983; Schlegel et al., 1987), methyl Hg (Schlegel et al., 1987) and Pb (Bacceril et al., 1989) affected photosynthesis more than transpiration. Wheat plants, when grown on Cu-rich soil, exhibited considerable lower water use efficiency than plants grown on fertile soil (Moustakas et al., 1997). A copper-induced decrease of water use efficiency was also observed in solution-cultured pea plants (Angelov et al., 1993), while Al had similar effects on transpiration and net CO_2 assimilation in *Thiopyrum bessarabicum* (Moustakas et al. 1997). These results indicate that the relative importance of metal-induced stomatal closure for the inhibition of photosynthesis may be different for different metals. However, most of the studies have analysed plant response at only one sample time after relatively long exposure times (several days or weeks). Under these conditions, secondary stress effects such as metal induced changes in mineral nutrition, may contribute to the inhibition of photosynthesis so that it is difficult to evaluate the relative importance of stomatal closure in photosynthesis decline. For this purpose, the time course of metal effects over short intervals has to be investigated (Poschenreider and Barcelo, 1999).

6. Conclusions

In general, high concentration of heavy metals in soil has adverse effects on water uptake and root growth. Heavy metal effects on root growth and water relations are very complex. The influence of plant root growth and water uptake depends on many interconnected factors like concentration of metals, pH, metabolic processes, transpiration conditions, nutrient competition etc. Most of the studies are done in pots and hydroponic cultures. Such investigations are necessary to understand physiological mechanisms and interactions. In order to understand plant responses to metal stress under field conditions, experiments are also necessary in which the effects of metals are studied on plants growing in ecosystem conditions.

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Chapter 3. Root Water Uptake Models

1. Introduction

Plants absorb water from the soil by roots and more than 99% of the water taken up by plants is transpired into the atmosphere. The pathway of water flow from soil to the atmosphere via plants has been termed the "soil-plant-atmospheric continuum" (Philip, 1966). Since most of water that is extracted by plants is lost to the atmosphere, uptake is generally considered to be equal to transpiration.

It is important to know the water uptake under conditions of unlimited as well as limited supply for irrigation scheduling in crop management. Quantification of root water extraction also contributes to an improved understanding of chemical fluxes in the vadose zone in both ecological and hydrological studies (Somma et al., 1998). It is also an important factor in the design of phytoremediation schemes for contaminated sites. Water uptake by root systems can control the timing and the amount of chemical pollutant leaching to ground water, modify the effect of preferential flow on water and chemical movement, and affect the absorption of nutrients and trace elements by plants and thereby reducing their concentration levels in seepage water (Clothier and Green, 1994).

Root water uptake is an important part in water budget models. It is a dynamic process influenced by soil, plant and climate. Soil factors include pressure head, soil hydraulic conductivity, presence of ground water table and osmotic potential. The plant factors are mainly root density, root distribution, root depth, plant resistances, growth stage of plant etc. Evaporative demand is the major climatic factor governing root water uptake.

Being influenced by complex interactions between many factors, modelling of root water uptake is rather difficult. Most soil water models take account of root water uptake by means of rather simple functions (Hammer et al., 1996). A brief summary review on root water uptake models is given below.

2. The concept of root water uptake: introduction of sink a term in the equation of flow

To represent root water uptake, usually a pressure head dependant sink term *S* is introduced into the soil water balance. For one-dimensional vertical flow this results in the equation:

$$\frac{\partial \theta}{\partial t} = \frac{\partial q}{\partial z} - S(h) \qquad (1)$$

where θ is the volumetric water content [L^3L^{-3}], t is the time [T] and S is the water uptake term [$L^3L^{-3}T^{-1}$].

Vertical water flux in soil is described using Darcy's law as:

$$q = -K(h)\frac{\partial(h+z)}{\partial z} \qquad (2)$$

where q is the soil water flux density taken positive upward [LT^{-1}], K is the hydraulic conductivity [LT^{-1}], h is the soil water pressure head [L] and z is the gravitational head as well as the vertical coordinate [L], taken positive upward.

Darcy's law is applied to saturated as well as unsaturated or partly saturated conditions. Combining Eq. 1 and 2 results in Richards' equation (Richards, 1931):

$$\frac{\partial \theta}{\partial t} = C(h)\frac{\partial h}{\partial t} = \frac{-\partial}{\partial z}\left(K(h)\frac{\partial h}{\partial z} + K(h)\right) - S(h) \qquad (3)$$

where C is the differential soil water capacity [L^{-1}]which is equal to the slope of the soil moisture characteristic curve $\theta(h)$.

In terms of water content (θ), the above equation can be written as:

$$\frac{\partial \theta}{\partial t} = \frac{-\partial}{\partial z}\left(D(\theta)\frac{\partial \theta}{\partial z} + K(\theta) \right) - S(\theta) \tag{4}$$

where D is soil water diffusivity [L^2T^{-1}] given by:

$$D = K\frac{dh}{d\theta} \tag{5}$$

The unsaturated soil hydraulic functions in the above equations are the soil water retention curve, the hydraulic conductivity function and soil water diffusivity. Out of several functions available in the literature, the one proposed by van Genuchten (1980) is most widely used in numerical models and is given below.

$$S_e = \frac{\theta - \theta_r}{\theta_s - \theta_r} = \left(1 + \alpha |h|^n\right)^{-m} \tag{6}$$

where θ_r and θ_s are the residual and saturated water contents, respectively. α [L^{-1}] is scaling factor, n and m are shape factors of retention curve and m can be calculated by:

$$m = 1 - \frac{1}{n} \tag{7}$$

3. Root water uptake models

3.1 Early attempts

Earliest attempts to describe water transport into plants date back to the 1920s. Gradmann (1928) was the first to suggest an analogy between steady electric current flow in a series of resistance network and steady water flow from roots, stem and leaves of a plant. Van den Honert (1948) followed this concept and proposed that water flow through a plant part is proportional to the water potential difference across that part and inversely proportional to the water flow resistance (analogous to Ohm's law) as given below:

$$T_a = \frac{h - h_{root}}{R_s} = \frac{h_{root} - h_1}{R_p} \tag{8}$$

where h, h_{root} and h_1 are the pressure heads [L] in the soil , at the root surface and in the leaves, respectively, and R_s and R_p are the flow resistances [T] in soil and plant, respectively, and T_a is the actual transpiration rate [LT^{-1}]. As h_{root} is hardly measurable, the above equation was further simplified to give:

$$T_a = \frac{h - h_1}{R_p + R_s} \tag{9}$$

The relative importance of the resistances in the above equation were studied extensively by Gardner and Ehlig (1962), Cowan (1965), Newman (1969), Feddes (1971), Yang and De Jong (1971), Feddes and Rijtema (1972), Hansen (1974 a,b), Taylor and Klepper (1975), Molz (1975), Jarvis (1975), Herkelrath et al. (1977a,b), Nnyamah et al. (1977), Meyer et al (1978) and Blizzard and Boyer (1980). The majority of these authors believed that R_p is dependent on soil water content and transpiration rate, while some believed that R_p is independent of them. There is evidence that the hydraulic resistance of the root system usually dominates the resistance of soil surrounding roots. If this is true, one may conclude that root water extraction functions expressed in terms of soil hydraulic resistance alone are conceptually wrong. However, there is controversy concerning the role of root resistance.

3.2 Microscopic and macroscopic models

There are mainly two approaches to model the flow of water to roots. The first category deals with the radial flow to a single root and follows a *microscopic approach*. In this category, roots are idealized as an infinitely long hollow cylindrical sink of uniform radius. The second category follows a *macroscopic* approach. Here the root system is treated as a single unit without considering the effect of individual roots realizing the difficulty of measuring the time dependant geometry of the root system.

3.2.1 Microscopic approach

By considering the diffusion of water towards a single root, Gardner (1960, 1964) applied the concept of microscopic approach. In this model, the roots were considered to be infinitely long cylinders of uniform radius. For steady state conditions, the soil water flow equation was then solved analytically assuming radial flow. Various water potential distributions surrounding the idealised root were also calculated. The models of Gardner (1964), Nimah and Hanks (1973a, b), Feddes et al. (1974), and Herkelrath et al. (1977a) are in this category.

The major difficulty in solving Eq. 4 numerically or analytically dervies from the unknown form of the *S* term. There has been a tendency to describe the root water uptake analogous to *Darcy*'s equation, assuming that the rate of uptake is proportional to soil hydraulic conductivity and the difference between the total pressure head at the root-soil interface and the corresponding pressure head in the soil. Using radial coordinates, Darcy's flow equation then can be written as:

$$\frac{\partial \theta}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[rK(h)\frac{\partial h}{\partial r} \right] \qquad (10)$$

where r [L] is the radial distance from the centre of the root. Solutions of this equation have been obtained for both steady- and nonsteady-state conditions. For steady state conditions with water flowing from a distance r_2 to a root radius r_1, the solution under the assumption of a constant K is (Feddes, 1981):

$$q_{root} = \frac{2\pi K\left(h - h_{root}\right)}{\ln\left(\frac{r_2}{r_1}\right)} \tag{11}$$

where q_{root} is the rate of water uptake per unit length [L^2T^{-1}]. For a discrete soil layer of thickness Δt and a rooting length per unit volume of soil L [LL^{-3}], the water uptake rate ΔV_{root} [LT^{-1}] is:

$$\Delta V_{root} = L\Delta t q_{root} \tag{12}$$

With the root layer located at depth z, the equation can be written as:

$$\Delta V_{root} = \frac{2\pi}{\ln\left(\frac{r_2}{r_1}\right)} LK(h - h_{root} - z)\Delta t$$

or

$$\Delta V_{root} = BLK(h - h_{root} - z)\Delta t \tag{13}$$

where B is given by:

$$B = \frac{2\pi}{\ln\left(\frac{r_2}{r_1}\right)} \tag{14}$$

and represents a dimensionless geometric and root distribution factor. The soil resistance R_s can be written as:

$$R_s = \frac{1}{BLK} \tag{15}$$

The sink term is described by dividing the root water uptake rate by the depth increment:

$$S = \frac{\Delta V_{root}}{\Delta z} = BLK(h - h_{root} - z) \qquad (16)$$

This approach is useful to understand the root water extraction process. It is difficult to use for the interpretation of field data collected under heterogeneous (non-steady) conditions over a long period of root development (Chang and Coarapcioglu, 1997). The most important limitation of this type of model is the unavailability of the required input parameter values.

3.2.2 Macroscopic approach

In the field, steady state conditions never exist. Moreover the living root system is dynamic and its geometry is time-dependant. Also the water permeability varies with position and time. Furthermore, the detailed geometry of the root system is in practice impossible to measure. Macroscopic models provide reasonable agreement with the field data; hence, they are satisfactory for practical purposes. These models are classified broadly under linear and non-linear models:

3.2.2.1 Different macroscopic models

The macroscopic model of root water uptake was proposed by Molz and Remson, (1970). Their model assumed that water extraction depends only on depth *Z* and actual transpiration rate T_a, i.e.

$$S = -\frac{1.6T_a}{z^2{}_r} z + \frac{1.8T_a}{z_r} \qquad (17)$$

where z_r is the depth of the root zone and z is the depth below soil surface. This model gave better results for higher than for lower contents. As the upper soil layers become drier, more of the transpiration has to come from deeper roots in wetter parts of the soil. Soil water hysteresis is also not taken into account.

In their second attempt, Molz and Remson (1970), described *S* in terms of an "effective root distribution function", *L(z)*, depending on water content, depth, and transpiration rate.

$$S = \left(\frac{D(\theta)R}{\int_0^{z_r} D(\theta)Rdz} \right) T_a \tag{18}$$

where *R* is the effective root distribution defined, i.e. the density of the roots actually effective in soil water uptake at a given time. Later, this model has been modified by Molz (1981), by introducing a third extraction term that depends on transpiration rate, soil water pressure head, xylem water pressure, volumetric water content and length of roots:

$$S = \left(\frac{\theta L(h - h_x)}{\int_0^{z_r} \theta(h - h_x)dz} \right) T_a \tag{19}$$

Here, h_X is the pressure head of the root xylem. Because it is hard to measure, this model never been validated.

The potential water root water extraction rate at certain depth S_p [T^{-1}], for non-homogeneous root distributions be determined by either the root mass or the root length density $L_r(z)$, as fraction of the total root length density over the rooting depth z_r as taken by Nimah and Hanks (1973 a, b):

$$S_p = \frac{L_r(z)}{\int_{z_r}^{0} L_r(z)dz} T_p \qquad (20)$$

where T_p is potential transpiration rate.

Raats (1974) proposed a model that is based on an exponential decrease of water uptake with depth.

$$S = \frac{T_a}{\lambda} \exp\left(-\frac{z}{\lambda}\right) \qquad (21)$$

where λ[L] is an empirical relative root density or activity chosen such that the integral of S over the root zone equals T_a.

Hillel et al. (1976) introduced a model based on hydraulic head measurements (stem and soil) and hydraulic resistance in soil and root.

$$S = (\phi_s - \phi_p)/(R_s + R_r) \qquad (22)$$

In this equation, ϕ_s is the total hydraulic head ($\phi = h + z$) of the soil as a function of depth ; ϕ_p is the hydraulic head in the plants at the base of the stem; R_s is the resistance to water flow in the soil; R_r is the hydraulic resistance of the root.

Rowse et al. (1978) proposed another water uptake term:

$$S = \frac{\Delta z L(h_s - h_p)}{R_s - R_p} \qquad (23)$$

As in the Equation 12, L is the length of roots per unit soil volume; h_s is the bulk soil water potential; h_p is the plant water potential assumed to be constant throughout the root xylem; R_s is the soil resistance to root water uptake per unit length of root and R_p is the resistance to water uptake per unit length of root.

Herkerlrath et al. (1977a, b) proposed a similar model, but taking also explicit account of the influence of soil water content:

$$S = R_p L(h_s - h_r)\theta / \theta_s \tag{24}$$

R_p is the root permeability per unit length of root; *L* is the length of roots per unit volume of soil; h_s is the saturation water potential and h_r is the water potential inside root.

Selim and Iskandar (1978) related transpiration to soil hydraulic conductivity and soil water potential by the following model:

$$S = \frac{T_a L(z) K(h)}{\int_0^v L(z) K(h) dz} \tag{25}$$

T_a is the actual transpiration rate; *L(z)* is the length of roots per unit soil volume; *K(h)* is the unsaturated soil hydraulic conductivity; z is the depth below soil surface; *h* is the soil water potential and *v* is depth of root zone.

Feddes (1976, 1978) introduced a simple *reduction term* to convert potential transpiration to actual transpiration as a function of soil water content or soil water pressure head. The approach is based on the fact that, the extraction term under non-stress condition is simply equal to potential transpiration over the root zone. In his first attempt (Feddes et al., 1976), he proposed a water uptake function depending on water content.

$$
\begin{array}{ll}
S = 0 & 0 \le \theta \le \theta_w \\
S = S_{\max}\left[(\theta - \theta_w)/(\theta_d - \theta_w)\right] & \theta_w < \theta \le \theta_d \\
S = S_{\max} & \theta_d < \theta \le \theta_{an} \\
S = 0 & \theta_{an} < \theta \le \theta_s
\end{array}
\qquad (26)
$$

θ is the water content; θ_w the water content at wilting point; θ_d is the lowest value of θ at which $S = S_{\max}$; θ_{an} is the highest value of θ at which $S = S_{\max}$; θ_s is the saturation water content and $S_{\max}$ is maximum rate of root water extraction.

Later, Feddes et al. (1978), proposed a sink term dependant on soil pressure head.

$$
\begin{array}{ll}
S = 0 & h_{an} \le h \le 0 \\
S = S_{\max}\left[(h - h_w)/(h_d - h_w)\right] & h_w < h \le h_d \\
S = S_{\max} & h_d < h \le h_{an} \\
S = 0 & h \le h_w
\end{array}
\qquad (27)
$$

h is the soil pressure head; h_{an} is the maximum soil pressure head for which $S = S_{\max}$; h_d is the minimum soil pressure head for which $S = S_{\max}$; h_w is the soil pressure head at wilting point and $S_{\max}$ is maximum rate of root water extraction. This approach has become is very popular.

Hillel et al. (1978) introduced the term $\alpha(h)$ to define the pressure head dependent, dimensionless water uptake reduction function:

$$
\alpha(h) = \frac{S(h)}{S_{\max}} \qquad (28)
$$

Integration of $S(h)$ over root depths (z_r) gives actual transpiration rate:

$$T_a = \int_{z=0}^{z=z_r} S dz \tag{29}$$

Prasad (1988) came up with a relatively simple linear equation assuming that the root water uptake at the bottom of root zone equals zero:

$$S_{\max}(z) = \frac{2T_p}{z_r}\left(1 - \frac{z}{z_r}\right) \tag{30}$$

T_p is potential transpiration rate and z_r is the rooting depth.

Hoogland et al. (1981) introduced a model which assumes that root density, and consequently potential root-water uptake, decreases linearly with depth, z

$$S_{\max}(z) = a - bz \tag{31}$$

where *a* and *b* are empirical constants.

Adiku et al. (1996) introduced a stress factor that accounts for the reduction of water uptake as below:

$$S(z,t) = R_L E_f(z,t).q_r \tag{32}$$

where $R_L(z,t)$ is the current root length at depth z; q_r is the maximum uptake per unit length, and $E_f(z,t)$ is water stress factor (environmental modifier)

The model introduced by Chang and Corapcioglu (1997) takes into account daily and diurnal changes of water uptake:

$$S = L_d q_{av}; \tag{33}$$

$$S_w = \frac{q_{\max}}{2} L_d \tag{34}$$

where S is sink term; L_d defined as the length of roots per unit volume of soil; q_{av} is the mean daily root water uptake; $q_{\max}$ is the maximum value of q to be obtained at noon.

Schmidhalter et al. (1994) extended model of Raats (1974) by adding linear term:

$$S = \frac{fT_a}{\lambda}\exp\left(-\frac{z}{\lambda}\right) + (1-f)T_a b \qquad (35)$$

where *f* indicates a fraction of the transpiration rate, and *b* is an empirical parameter representing the non-exponentially distributed part of the transpiration rate, specified for 5 cm increments.

Ojha and Rai (1996) proposed a model describing the dependence of potential soil water extraction $S_{\max}$ on potential transpiration as function of depth and time as:

$$S_{\max} = \left[\frac{T_j}{z_{rj}}(\beta+1)\left(1-\frac{z}{z_{rj}}\right)^{\beta}\right] \qquad 0 \le z \le z_{rj} \qquad (36)$$

where T_j is the potential transpiration on j^{th} day ; z_{rj} is the depth of root zone; β is a model parameter; z is the depth below soil surface.

With β =0 the expansion reduces to the model of Feddes (1978) and for β =1 gives the linear extraction model of Prasad (1988).

4. Conclusions and Outlook

During the past several decades, many root water uptake models have been developed. Macroscopic models are usually used in crop growth models. Despite many different attempts, root water uptake still continues to be weakest and over simplified part of crop growth models (Wang and Smith 2004). In particular, most water uptake model do not explicitly account for stress factors in soil. Only a few model account for water stress (e.g. Li et al. 2001) and salinity stress (e.g. Homaee et al. 2001 a, b, c). No models have been found to describe the effects of metal stresses on the soil water extraction by plant roots.

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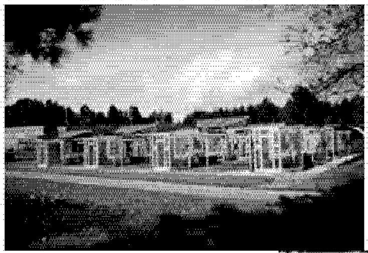

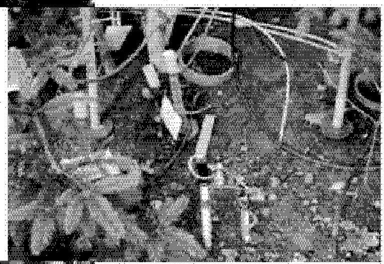
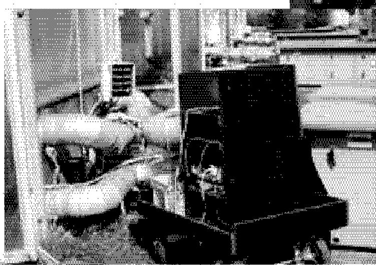
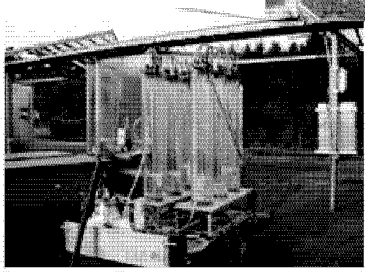

Chapter 4. Water Regime of Metal-Contaminated Soil under Juvenile Forest Vegetation

Water Regime of Metal-Contaminated Soil under Juvenile Forest Vegetation

(*Plant and Soil*, 2005, **271**: 227-241)

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Abstract

In a three-year factorial lysimeter study in Open Top Chambers (OTCs), we investigated the effect of topsoil pollution by the heavy metals Zn, Cu, and Cd on the water regime of newly established forest ecosystems. Furthermore, we studied the influence of two types of uncontaminated subsoils (acidic vs. calcareous) and two types of irrigation water acidity (ambient rainfall chemistry vs. acidified chemistry) on the response of the vegetation. Each of the 8 treatment combinations was replicated 4 times. The contamination (2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu and 10 mg kg^{-1} Cd) was applied by mixing filter dust from a non-ferrous metal smelter into the upper 15 cm of the soil profile, consisting of silty loam (pH 6.5). The same vegetation was established in all 32 lysimeters. The model forest ecosystem consisted of seedlings of Norway spruce (*Picea abies*), willow (*Salix viminalis*), poplar (*Populus tremula*) and

birch (*Betula pendula*) trees and a variety of herbaceous understorey plants. Systematic and significant effects showed up in the second and third growing season after canopies had closed. Evapotranspiration was reduced in metal contaminated treatments, independent of the subsoil type and acidity of the irrigation water. This effect corresponded to an even stronger reduction in root growth in the metal treatments. In the first two growing seasons, evapotranspiration was higher on the calcareous than on the acidic subsoil. In the third year the difference disappeared. Acidification of the irrigation water had no significant effect on water consumption, although a tendency to enhance evapotranspiration became increasingly manifest in the second and third year. Soil water potentials indicated that the increasing water consumption over the years was fed primarily by intensified extraction of water from the topsoil in the lysimeters with acidic subsoil, whereas also lower depths became strongly exploited in the lysimeters with calcareous subsoil. These patterns agreed well with the vertical profiles of fine root density related with the two types of subsoil. Leaf transpiration measurements and biomass samples showed that different plant species in part responded quite differently and occasionally even in opposite ways to the metal treatments and subsoil conditions. They suggest that the year-to-year changes in treatment effects on water consumption and extraction patterns were related to differences in growth dynamics, as well as to shifts in competitiveness of the various species. Results showed that the uncontaminated subsoil offered a possibility to compensate the reduction in root water extraction in the topsoil under drought, as well as metal stress.

1. Introduction

Pollution of forest soils with heavy metals is not perceived to be a major problem because the risk of metals entering human food chain is much less than in polluted agricultural soils. Forest soils may be contaminated by heavy metals in particular due to atmospheric deposition from industrial or traffic (Kahle, 1993), inputs from shooting ranges (Darling and Thomas, 2003), uncontrolled waste disposal or afforestation of contaminated land. Where such pollution occurs, the resulting environmental risks may be even higher than on agricultural lands because of the lower pH values. Whereas agricultural soils are usually maintained close to neutral pH by application of lime and base-rich fertilizers, forest soils are often acidic and poorly buffered. As a result, metal mobility and phytoavailability may be much higher in forest soils than in cultivated soils at comparable total metal contents. This may not only lead to an increased risk of metal leaching into ground and surface waters, but also to higher ecotoxicity and impairment of ecosystem functions, which forests, for example, play in the hydrological and carbon cycle. Since metals cannot be decomposed, even a lowered input of metals still leads to further accumulation in both the upper soil layers and in tree roots (Wittig and Neite,1989).

At landscape scale soil pollution by heavy metals has been found to reduce forest productivity (Pukacki and Kaminska-Rozek, 2002), vitality, biodiversity and stand structure (Chernenkova and Kuperman, 1999). Decreased biomass production is generally associated with reduced transpiration. Any decrease in transpiration results in a corresponding increase in soil water availability and higher regional discharge, which in turn promotes leaching of the metals into the subsurface.

At the plant scale, exposure to metal concentrations above a critical level has often been reported to negatively impact plant water relations and cause water stress

(Barceló and Poschenrieder, 1990; Poschenrieder and Barceló, 1999), resulting in decreased transpiration due to stomatal closure (Kastori et al., 1992; Prasad, 1995; Schlegel et al. 1987). The responses to metal stress can be variable at times and even opposite. Metals induced enhanced transpiration occurs not only at higher metal concentrations (Paul and de Foresta, 1981; Angelov et al., 1993) but also at slightly toxic metal concentrations (Kirkham, 1978; Dueck, 1986). In former case, enhanced transpiration has been attributed to increased stomatal density because of reduction of leaf area (Paul and de Foresta 1981) and in latter case, enhancement of transpiration is due to decreased osmotic potential and turgor maintenance in leaves (Costa and Spitz, 1997).

Due to their direct exposure, roots usually are the plant organs most severely affected by soil-polluting metals. Metal contamination of soils has been found to reduce root growth (Arduini et al., 1994; Arduini et al., 1995; Ewais, 1997; Helmisaari et al., 1999), to inhibit root respiration (Karolewski and Giertych, 1994), and to interfere with nutrient uptake (Oberlander and Roth, 1978). Reduced root growth and functioning compromises the capability of a plant to extract soil water and nutrients. But reduction of transpiration under metal stress must not necessarily be associated with detectable root damage, it may be induced already at metal stresses that are below levels required to cause such effects (Barceló and Poschenrieder, 1990).

Irrespective of the mechanisms leading to toxic effects in the affected organism, the toxicity of metals in soil depends on how available the metals are to the target organism. Soil pH is a key factor for this availability (Kahle, 1993; Krebs et al., 1998). The solubility of metals like zinc and cadmium is particularly sensitive to pH variations in a weakly acidic to near-neutral range, which is the pH range of most soils in temperate humid climates (Fotovat and Naidu, 1998). Levels of pH 3 or 3.5 in the upper soil horizons are quite common in forest stands of Central Europe and North America and under these condition heavy metals are mobilized, pass into soil solution and are taken up by tree roots (Kahle 1998). Because of the continous acid deposition on forest soils, metal-polluted soils that do not have a serious toxicity problem at present due to a sufficiently high pH, may suffer metal toxicity at a future time.

Effects of heavy metals on plant-water relations have mostly been studied in pot experiments with artificial soil substrates under climate chamber or greenhouse conditions or in hydroponic systems. Such studies are useful to understand specific mechanisms and processes, but they can not represent conditions of real ecosystems, where plants grow under competition for above-ground, as well as below-ground resources. Moreover, particularly in hydroponic cultures but also in most pot experiments plants are exposed to homogeneous metal stress in the root zone. In reality, where soils are contaminated with metals from atmospheric deposition or agricultural applications of metal-containing substances, the contamination is usually concentrated in the topsoil, while the subsoil often remains relatively unaffected, because of the slow movement of metals. Root growth has been found to avoid zones of metal stress in soils, where this is possible due to uneven distribution of the pollution (Palazzo et al., 2003, Romney et al. 1981 Schwartz et al. 1999). Thus, the responses of plants on elevated soil metal concentrations may be quite different depending on the spatial distribution of toxic metals in soil in relation to the distribution of required resources, such as water and nutrients. The high spatial heterogeneity of soils, however, makes it in general very difficult to isolate the effects of individual factors, such as metal pollution in field experiments.

The objective was to study the effect of topsoil heavy metal pollution, the influence of uncontaminated subsoil types (acidic and calcareous) and rainfall acidity on plant growth and water regime operating under carefully defined controlled conditions (simulated juvenile forest ecosystem). In order to identify the effects of these factors, a factorial lysimeter study with four replications of each treatment combination was designed. The experiments simulate re-forestation of an agricultural soil, which has been polluted by deposition of metal-containing dust from a nearby non-ferrous metal smelter.

2. Materials and Methods

2.1 Open top chambers and lysimeter compartments

The experiments were performed in the Open Top Chamber (OTC) facility of the Swiss Federal Institute of Forest, Snow and Landscape Research (WSL) at Birmensdorf, Switzerland. The institute is situated close to the city of Zurich at an altitude of 450 m above sea level. The facility consists of 16 hexagonal chambers, which are 3 m high above ground and 3 m wide (internal distance between opposing hexagonal sides). The chambers are equipped with glass walls and roofs. The side glass walls are not completely closed to allow ventilation. The roofs are closed automatically at the onset of rain. Below ground surface, each chamber is divided into two 1.5 m deep concrete-walled soil compartments, each with a surface area of 3 m^2, which are used as non-weighable lysimeters. A cylindrical tube of 1.25 m diameter between the two soil lysimeters allows access to the three bottom outlets collecting the discharge for each compartment (Figure 1).

2.2 Soils and application of heavy metals

The lower 0.5 m of each compartment was filled with a three-layer drainage packing consisting of a fine quartz layer at the bottom, a coarse quartz sand layer at the top and a layer of intermediate texture in between. Before placing the drainage packing into the lysimeters, the sand was thoroughly washed with water to remove fine sediments. On top of the drainage packing, a 0.8 m subsoil layer was packed in the autumn of 1999. In one compartment of each chamber was used calcareous sandy loam originating from a Calcaric Fluvisol (along the river Aare), while in the other compartment was used acidic loamy sand originating from a Haplic Alisol (along the river Rhein). Both sampling sites were under mixed deciduous forests situated in the vicinity of Zurich, Switzerland. For the topsoil in all lysimeters was used a slightly acidic loam that was obtained from an arable field (from Birr), which had been excavated for road construction. The properties of the three soil materials are given in Table 1.

A 0.15 m layer of the topsoil was placed on the top of the subsoil. In half of the chambers, the topsoil was then artificially contaminated with filter dust from a non-ferrous metal smelter. The chemical composition of the dust is given in Table 2. The dust was mixed into the topsoil by hand, after tensiometers and other instrumentation had been installed in the subsoil. The average concentration of HNO_3-extractable metals in the contaminated topsoils after mixing were 2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu, 63 mg kg^{1} Pb and 10 mg kg^{-1} Cd.

Figure 1. View into the Open Top Chambers (OTC) in spring 2002. Each half chamber contained 14 juvenile trees [6 spruce (*P. abies*), 4 poplar (*P. tremula*), 2 birch (*B. pendula*) and 2 willow (*S. viminalis*)] and various understorey plants. In the centre, the access tube to the bottom outlets of the soil compartments is visible.

2.3 Vegetation

The same selection of trees and herbs was planted in each soil compartment. The selected species were birch (*Betula pendula* L.), willow (*Salix viminalis* L.), and poplar (*Populus tremula* L.). The herbaceous plant species, which were chosen to represent different growth strategies, were wood sedge (*Carex sylvatica* Hudson), ramsons (*Allium ursinum* L.) and tansy (*Tanacetum vulgare* L). All plants were planted in spring of the year 2000. The trees were arranged in such a way that different species were mixed in distribution and that the ground was covered as evenly as possible (Figure 1). Deciduous and coniferous trees were planted at fixed positions (without regarding provenance in the case of spruce), but the various understorey plant species were distributed at random in between the trees. Measurements started with the second growing season, i.e. in spring of 2001.

Table 1: Physical and chemical properties of the soil types used in the experiment

Properties	Topsoil	Acidic subsoil	Calc. subsoil
Texture [% sand ; silt ; clay]	36 ; 49 ; 15	87 ; 8 ; 5	74 ; 16 ; 10
pH (0.01M $CaCl_2$)	6.55 ± 0.12	4.2	7.4
C_{inorg} [g/kg]	<1	<1	21
C_{org} [g/kg]	15.1	3.2	11.2
N_{tot} [g/kg]	1.5	<0.3	0.6
C_{org}/N_{tot}	10	n.d.	18
$P_{extractable}$ [mg/kg]	1160	298	296
P_{org} [mg/kg]	862	84	54
P_{avail} [mg/kg]	49 ± 5	18	11
K_{exch} [mg/kg]	283	23	21
CEC [mmolc/kg]	55	12	67
Base saturation [%]	99.9	35.9	99.9

Table 2. Metal contents of the applied heavy metal dust

Element	HNO_3 extractable [g kg^{-1}]	Chemical form [g/kg]			
		Elemental	Oxide	Carbonate	Phosphate
Zn	755.0	-	940.0	-	-
Cu	85.5	48.6	-	71.7	-
Pb	15.2	-	16.4	-	-
Al	6.1	-	8.4	-	7.4
Ca	3.0	-	-	7.6	-
Na	1.8	-	2.4	-	-

2.4 Irrigation

Irrigation was applied by means of sprinklers (6 per soil compartment) with computer-controlled flow meters. The height of the sprinklers was chosen so that splashing of water was avoided. The irrigation was scheduled based on the tensiometer measurements, so as to keep the soil moisture content around field capacity. Irrigation was given during nights with a break of one day prior to soil water potential and water content measurements. In eight OTCs, irrigation water had the chemical composition of ambient rain (pH 5.5). For the irrigation of the other eight OTCs, HCl was added until the pH reached a value of 3.5. Irrigation was discontinued during the non-growing seasons. In 2002, a three-week drought period was imposed purposely from May to June by reducing irrigation. In 2003 irrigation was scheduled to correspond to the long-term rates of monthly average rainfalls, although the summer was exceptionally dry and hot from June to September.

3. Measurements

3.1 Soil Water potential

In each lysimeter 8 tensiometers were installed vertically, two each at 10-15, 35-40, 60-65 and 85-90 cm depths (position of ceramic cups), but the midpoint of each tensiometer cup from above depths is taken (i.e., 12, 36, 62 and 88 cm) to represent each depth. Soil water potentials were recorded manually normally once a week during the growing season, using the method of Marthaler et al. (1983). During the imposed drought period, measurements were taken every 2-3 days.

3.2 Drainage and water content

The drainage water was collected in plastic containers at the bottom outlets of each lysimeter. The volume of the drainage water was determined by volumetric cylinders, into which the containers were emptied weekly by means of a vacuum pump.

Soil water content was measured by Time Domain Reflectometry (TDR), using a Tektronix 1502B instrument. TDR probes of 25 cm length were installed vertically at 0-25, 25-50, 50-75 and 75-100 cm depth. The recorded TDR signals were calibrated and analysed using the procedure of Roth *et al.* (1989). Evapotranspiration was determined from the following water balance expression:

$$ET = I - D - \Delta S \tag{1}$$

where: *ET* is evapotranspiration, *I* is irrigation, *D* is drainage, and ΔS is the change in soil water storage.

3.3 Biomass

Above-ground biomass was harvested at the end of the growing season 2001 except for the very small birch trees and little tree seedlings in the understorey. The harvest included total above-ground biomass of the herbaceous plant species *Tanacetum vulgare* and *Carex sylvatica*. Poplars and willows were cut at 10 cm above ground. In the case of spruce, only one branch was taken per tree from the second whirl from the top and separated into sections grown in 2000 and 2001. The whole tree biomass of spruce was estimated from these samples using allometric relationships between twig aliquots and whole tree biomass, which had been determined in a previous experiment by regression analyses (Egli *et al.* 1998). Dry weights were determined after oven-drying at 65°C till constant weight was attained.

At the end of the growing season 2003, six soil cores of 5 cm diameter and 75 cm depth were sampled from each compartment at the same relative positions using a hollow cylindrical coring device of type HUMAX (brand name). The cores were divided into sections representing 0-12.5cm, 12.5-25 cm, 25-50 cm and 50-75 cm depth. Roots were separated manually by wet-sieving, washed and oven-dried at 65 ^{0}C. Root density was determined as total dry biomass of fine roots (< 2 mm diameter) per unit volume of soil.

3.4 Transpiration

A portable gas-exchange measuring unit (open system; LiCor-6400) was used to measure transpiration rates of single willow and poplar leaves. The measurements were performed in 12 OTCs (3 replicates each per treatment and soil type, 2 trees per species and chamber, 4 fully grown leaves per tree) at the end of June 2001, between 10 a.m. and 4 p.m. at a quantum flux of 100-1000 µmol m^{-2} s^{-1}. Four leaves of each tree were selected representing different positions in the tree with respect to sunlight exposure. Measurements were extrapolated to total tree transpiration by multiplication of mean transpiration with leaf area per tree. To obtain the latter, the area of 10 leaves of each deciduous tree of the experiment was measured in autumn 2001 using a MK2 leaf area meter (Delta-T Devices LTD, Cambridge, England). The total leaf area per tree was then estimated by multiplying the area/weight factor with the total dry foliage weight of the plant.

3.5 Statistical design and analysis

Each of the 4 combinations of topsoil contamination (with/without heavy metal dust) and irrigation water acidity (ambient/acidified) was replicated in 4 chambers using a Latin Square design (Figure 2). Combined with the two types of subsoil in each OTC (acidic and calcareous), this gave a fully balanced factorial design of 2 x 2 x 2 treatments with 4 replications.

Treatment effects were analysed by GLM (General Linear Model) using SYSTAT (version 10.0) and SAS (version 8.2). The following abbreviations are used to denote treatments: HM, heavy metal contaminated topsoil (and ambient rain); AR, acidified “rain” (and no contamination); HMAR, combination of heavy metal contaminated topsoil and acidified rain; CO, control (no contamination, ambient rain). The error bars were calculated by dividing standard deviation by the square root of the number of observations.

4. Results

4.1 Soil water potential

In the first year, the average soil water potentials in each treatment during most of the time varied between -20 and -80 hPa in the topsoils and only occasionally dropped below this range (Figure 3). In the following two years soil water potentials below this range were much more frequent due to the artificially imposed drought period in 2002 and the exceptionally dry summer in 2003. Because the variability between tensiometers increased almost in proportion to the average soil water potential, we chose to display the logarithms of their (negative) values in the figures. A high log value, thus, corresponds to a low soil water potential, which is equivalent to a high capillary water tension or suction or, in other words, to a dry soil. The lowest soil water potentials were observed at the end of the reduced irrigation period in June 2002 (Figure 3). During this period some tensiometers stopped working, as the soil water potentials exceeded the lower limit of their measurement range of around -800 hPa.

Heavy metal contaminated topsoils maintained significantly higher soil water potentials than controls, indicating that less water was extracted from contaminated than from uncontaminated topsoil (Table 3). This effect was particularly pronounced in the dry summer of 2003, i.e. in the third growing season. The acidity of the irrigation water had no significant effect on the recorded soil water potentials. Neither did the type of subsoil significantly influence topsoil soil water potentials, although in metal treated soils topsoil soil water potentials tended to be lower in acidic than in calcareous subsoil in 2003.

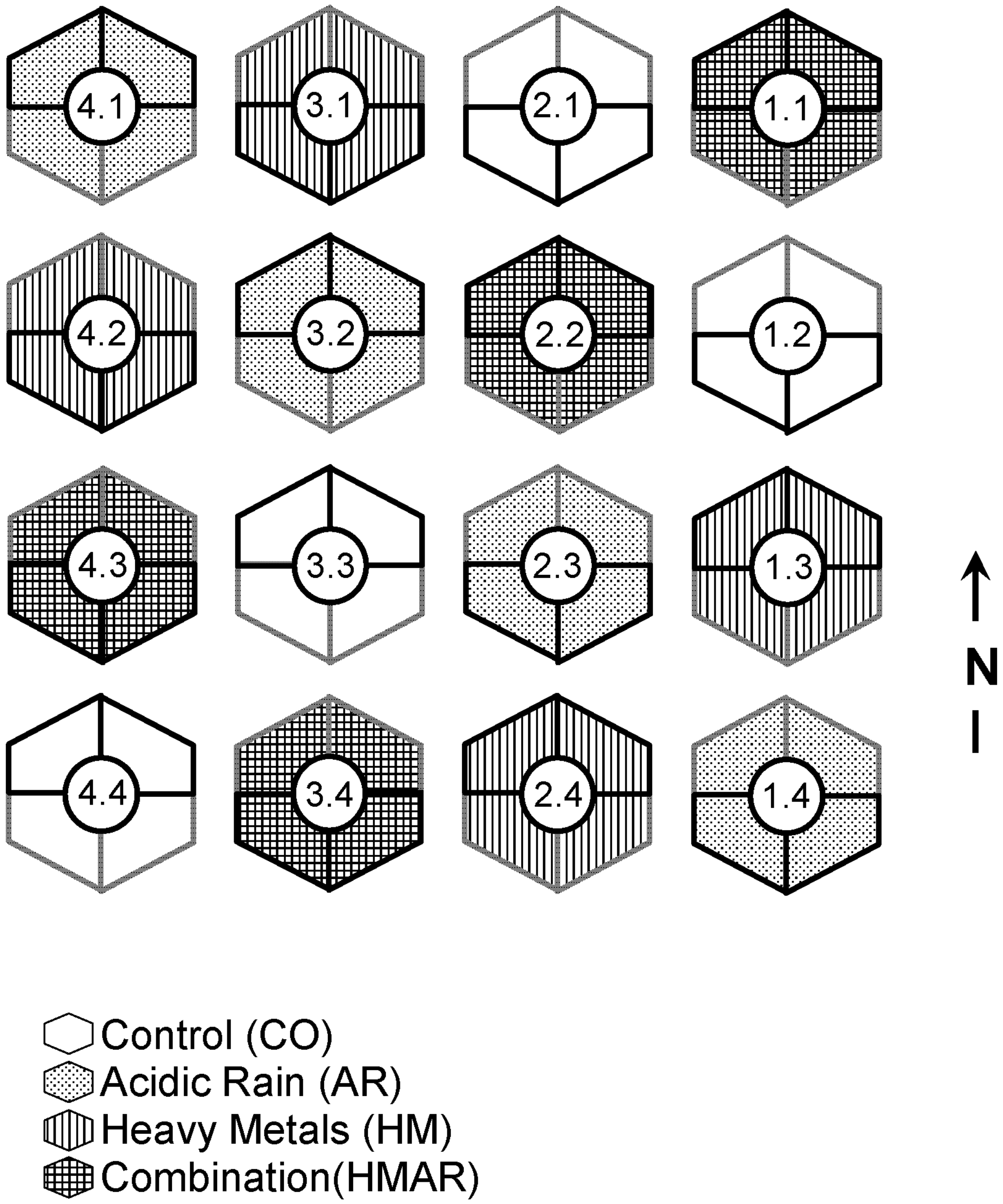

Figure 2. Arrangement of open top chambers (OTCs). Each OTC contains two non-weighing lysimeters (compartments) one with calcareous subsoil (black border), the other with acidic subsoil (grey border).

In the first two seasons, the type of subsoil, however, had a strong influence on soil water potentials in the subsoil. As illustrated in Figure 4, this influence became increasingly pronounced at lower depths and during the imposed drought period and gradually decreased again, when the soil was re-wetted in the weeks thereafter. Soil water potentials decreased faster in the calcareous than in the acidic subsoil. At the lowest two depths of measurement they also reached lower peak values in the calcareous subsoil. In the following season the overall differences in soil water potentials between the two subsoils were no longer significant.

Overall, the metal treatment of the topsoil had no significant effect on the soil water potentials in the subsoil, although Figure 4 suggests that the decrease in soil water potential during the drought period in June 2002 may have been more persistent under uncontaminated than under contaminated topsoil.

A more subtle metal effect on soil water potentials emerges when we compare different depths in Figure 4. During periods of unrestricted water supply (at the start of the imposed drought period and after full re-wetting of the soil profiles) soil water potentials increased with depth in a similar way in all treatments. During the drought in 2002 the vertical soil water potential gradients gradually decreased, indicating a shift of root water extraction activity to lower depth in order to compensate for reduced water availability in the upper parts of the profile. Eventually, the vertical soil water potential gradients even reversed in some treatments, because of the earlier response of soil water potentials in the topsoil and the increasingly delayed responses on drying and re-wetting with increasing depth in the subsoils. This shift in water extraction towards lower depths was pronounced on calcareous than on acidic

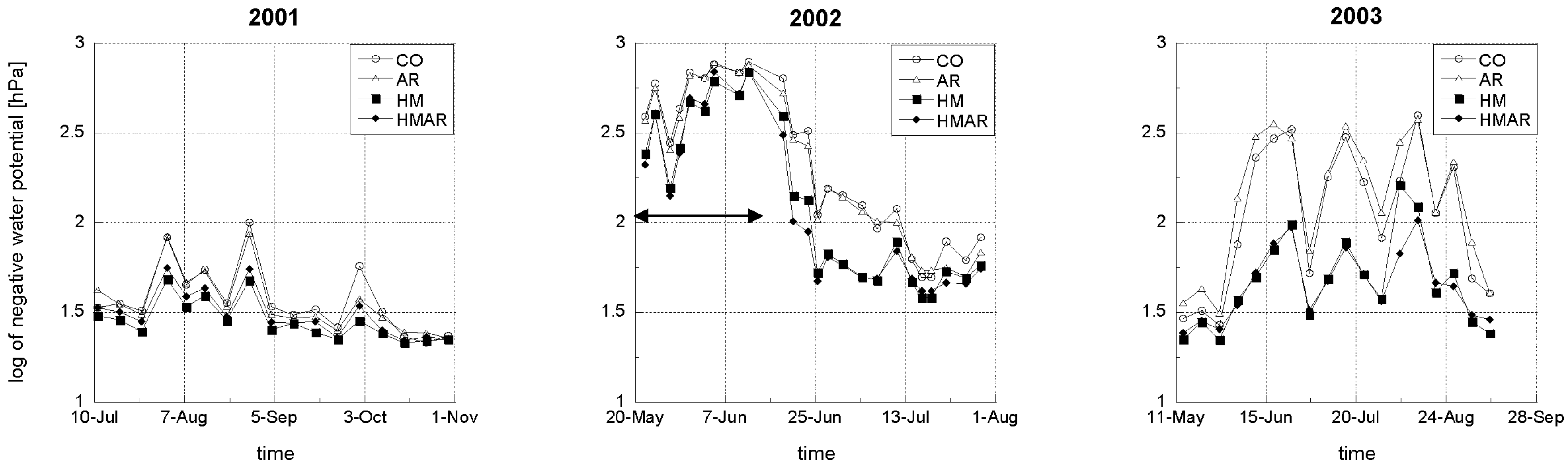

Figure 3. Year-to-year effects of heavy metal and irrigation water acidity on topsoil (12cm depth) water potential (average logarithms of negative potential values). The symbol ↔ denotes the induced drought period.

subsoil. At the end of the reduced irrigation period in June 2002 the average soil water potential in calcareous subsoils even reached lower values under metal-contaminated topsoil than in the controls. It appears that under metal stress in the topsoil roots tried to compensate even more for reduced irrigation water supply by enhanced activity in the subsoil (relative to the topsoil) than in the controls, especially in the calcareous subsoil (Figure 5a and b). In 2003, when dry and hot conditions lasted for several months, while irrigation was continued at pre-scheduled rates, soil water potentials remained higher in the subsoil than in the topsoil. However, as at the end of the artificial drought and in the beginning of the subsequent recovery in 2002, decreases and even reversals of the vertical potential gradients were observed in the calcareous subsoil after peaks of water stress in the topsoil in 2003 (Figure 5c). No such reversals were observed in acidic subsoil (see e.g. profile in Figure 5d). Figure 5 not only shows that soil water potentials increased (i.e.logarithms of negative potentials decreased) much more with depth in the uncontaminated than in the contaminated topsoils, but also that the increase was stronger above acidic than calcareous topsoil for a given treatment.

No such reversals were observed in acidic subsoil (see e.g. profile in Figure 5d). Figure 5 not only shows that soil water potentials increased (i.e.logarithms of negative potentials decreased) much more with depth in the uncontaminated than in the contaminated topsoils, but also that the increase was stronger above acidic than calcareous topsoil for a given treatment.

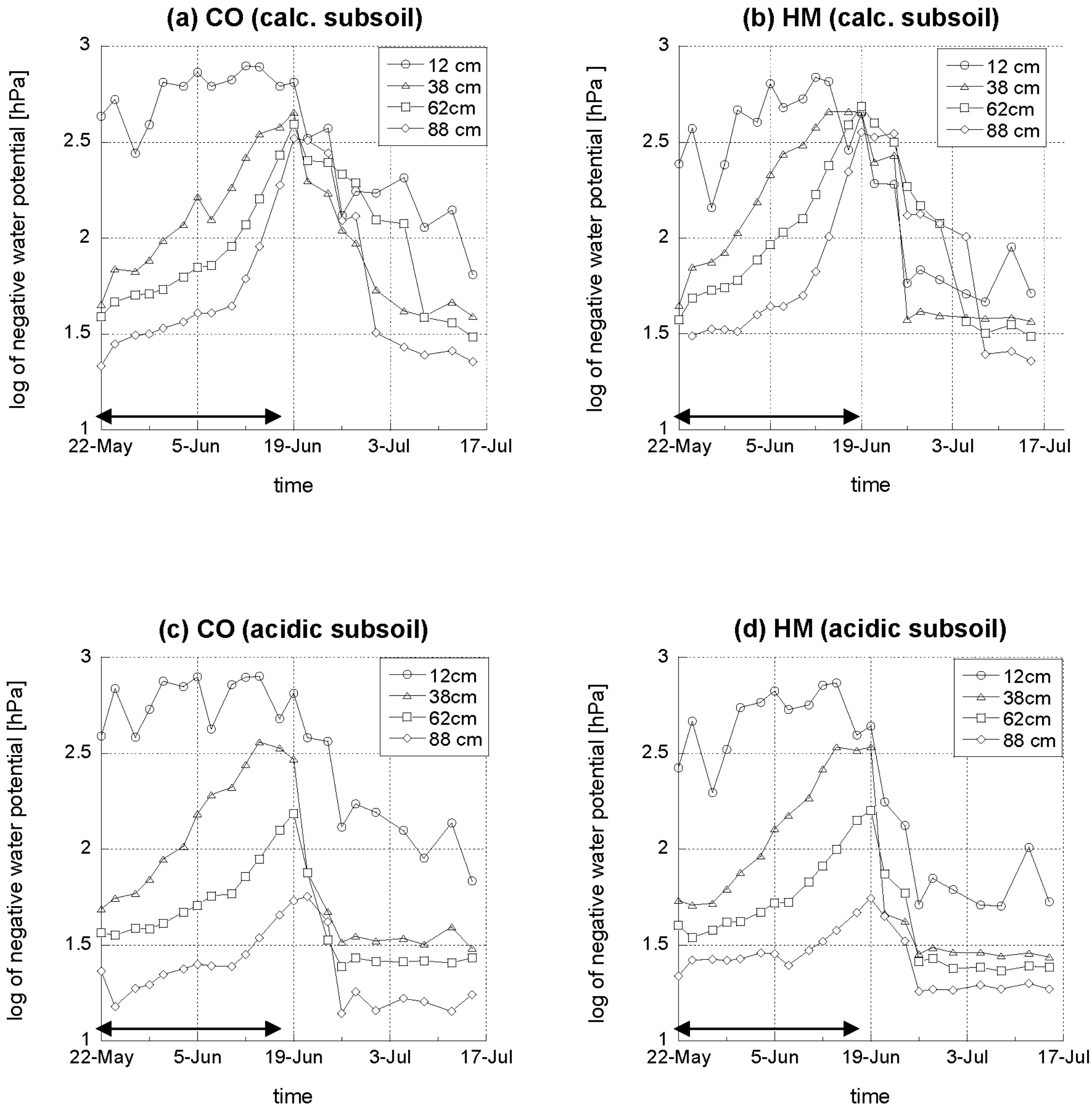

Figure 4. Effect of subsoil type and heavy metals on soil water potential development during the induced period of drought (from 22 May to 19 June) in 2002 at various depths (12 cm represents topsoil). The sub-figures compare control and heavy metal treatments, and between subsoil types. The symbol ↔ denotes the induced drought period.

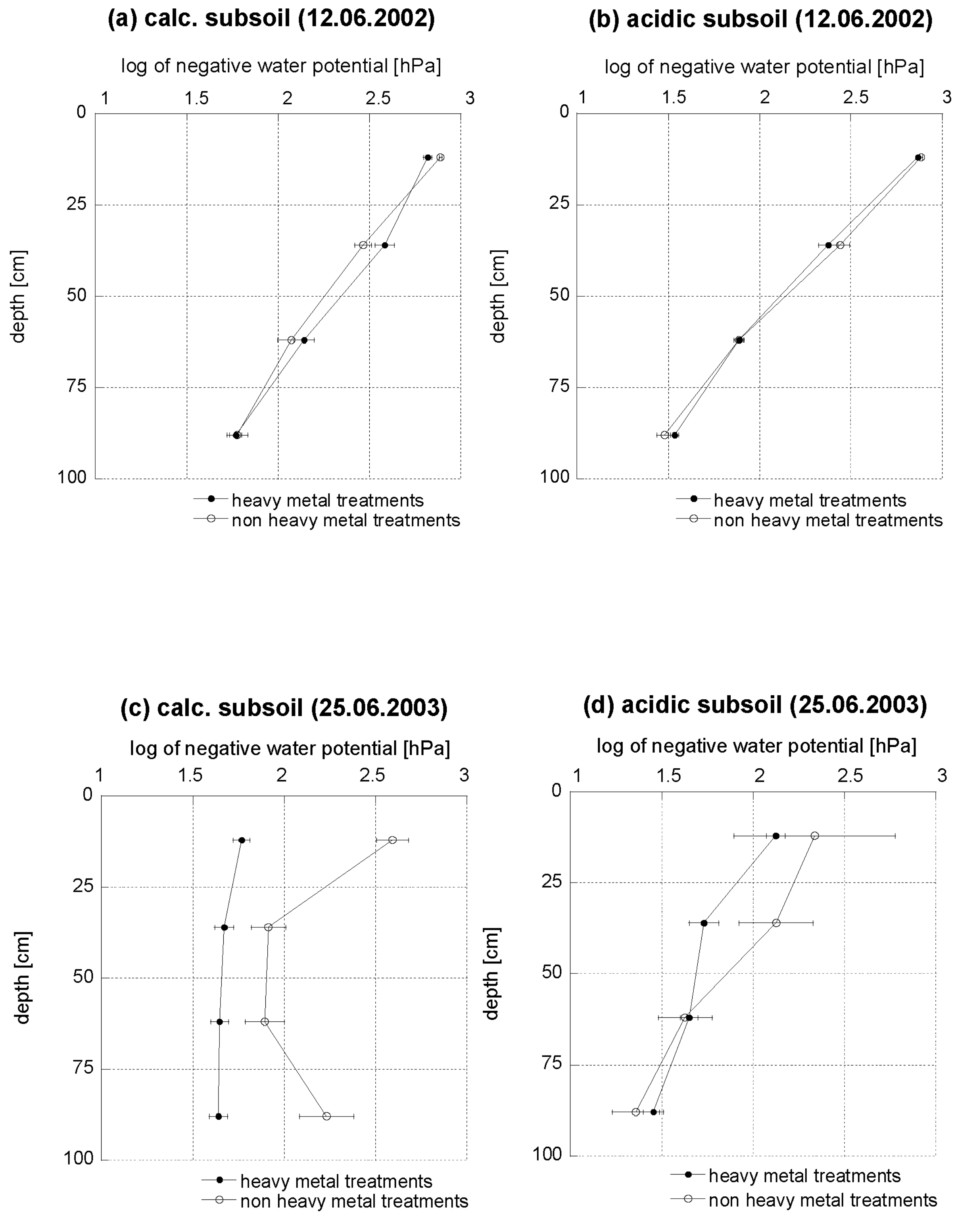

Figure 5. Effect of subsoil types and heavy metals on profile water potential during the peak drought days in 2002 and 2003

4.2 Water balances

Figure 6 shows the treatment effects on the overall water balances of the lysimeters. Metal contamination had a weak, but significant effect (Table 3). The metal effect became more evident from year-to-year, as tree canopies closed and ground area was covered. In contrast, the subsoil had significant effects in the first two years, but not in the third (Table 3). In the growing season of 2001 ET was in average 14.7% and in 2002 - 11.3% higher in the lysimeters with calcareous subsoil than from lysimeters with acidic subsoil. Contrary to our expectation that addition of acidity would enhance the metal effect, acidic rain did not reduce evapotranspiration. Rather there was a tendency over the years that evapotranspiration was larger with acidic than with ambient rain chemistry, although the trend did not become statistically significant.

4.3 Transpiration and biomass

Transpiration rates per tree were not only significantly higher in *P. tremula* (5.18 mmol H_2O s^{-1}) than in *S. viminalis* (3.92 mmol H_2O s^{-1}) at the dates selected for measurements in absence of metal stress, but the two species also responded quite differently to the heavy metal pollution in the topsoil (Figure 7). Heavy metals significantly reduced the transpiration rates in *P. tremula* (P=0.024), whereas they tended to enhance transpiration in *S. viminalis*. The above-ground biomass in Figure 8 show strong fluctuations from year-to- year. In the growing season 2001, trees and herbs together produced much more above-ground biomass than in 2002. Moreover, in 2001 total biomass production was much higher on calcareous than on acidic

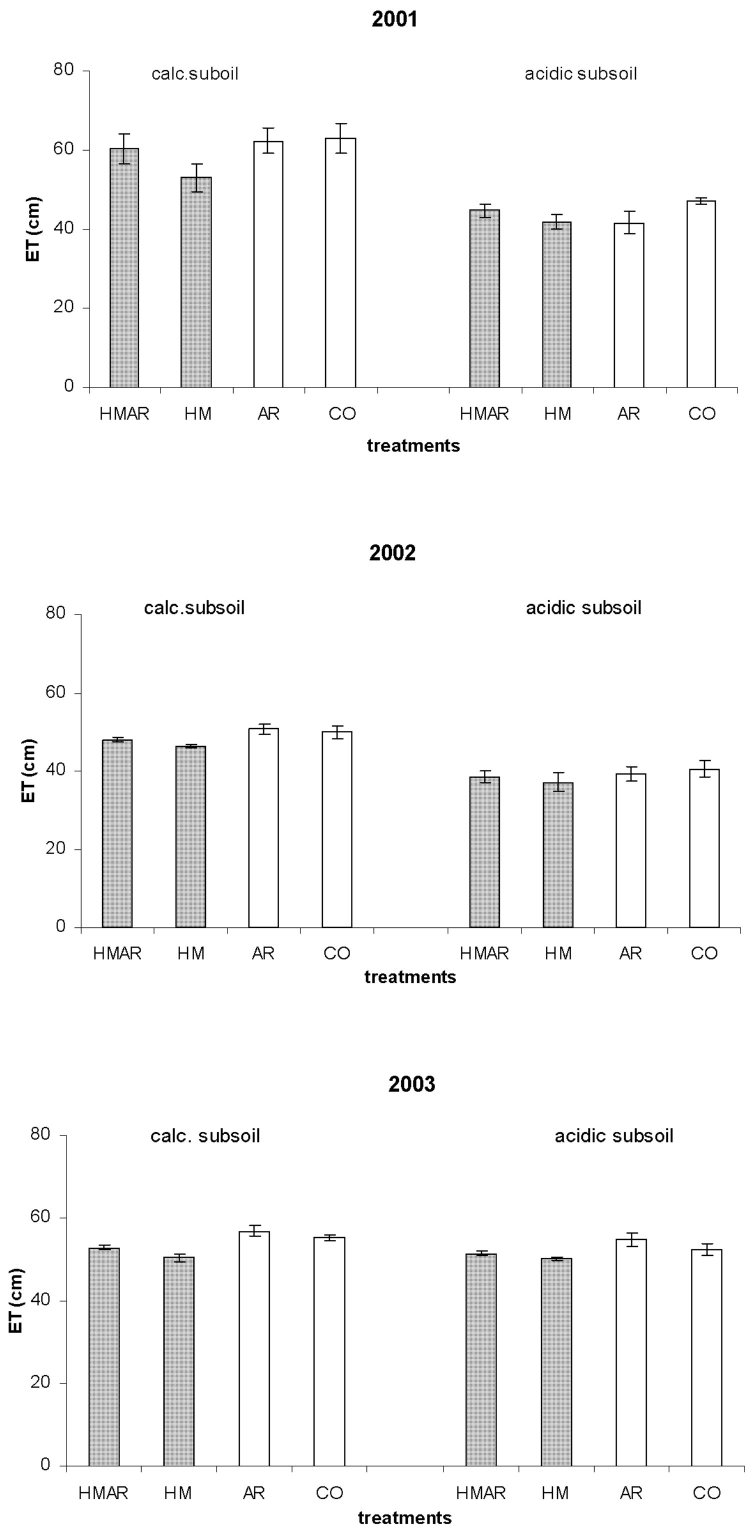

Figure 6: Total evapotranspiration in the three successive growing seasons from 2001 to 2003. The total amount of irrigation during the growing season in year 2001, 2002 and 2003 was 75cm, 57.6 and 59.8cm, respectively.

Table 3. ANOVA results of the different treatments including their significance at 5% level compared to the controls

Measurements	Year	Factors			
		HM*	AR	HM*AR	Subsoil
Evapotranspiration (n=24)	2001	0.032	n.s	0.003	0.000
Evapotranspiration (n=24)	2002	0.001	n.s	n.s	0.002
Evapotranspiration (n=24)	2003	0.001	n.s	n.s	n.s
Topsoil soil water potential (n=64)	2001	0.000	n.s.	n.s.	n.s.
Topsoil soil water potential (n=64)	2002	0.000	n.s.	n.s.	n.s
Topsoil soil water potential (n=64)	2003	0.000	n.s.	n.s.	n.s
Subsoil soil water potential (n=32)	2001	n.s.	n.s.	n.s	0.000
Subsoil soil water potential (n=32)	2002	n.s.	n.s.	n.s	0.000
Subsoil soil water potential (n=32)	2003	n.s	n.s.	n.s.	n.s
Transpiration per whole tree					
S. viminalis	2001	n.s.	n.s.	n.s.	0.002
P. tremula	2001	0.024	0.045	n.s.	0.052
Above ground biomass per half chamber					
Understorey plants	2001	n.s.	0.041	n.s.	0.000
Deciduous trees	2001	0.028	n.s.	n.s.	0.000
P.abies	2001	n.s.	n.s.	n.s.	n.s.
Above ground biomass per half chamber (in total)					
	2000	n.s.	n.s.	n.s.	n.s.
	2001	n.s.	n.s.	n.s.	0.000
	2002	n.s.	n.s.	n.s.	n.s.

* HM= heavy metal contaminated soil, AR= acidic rain, n.s. = not significant

subsoil, whereas no above-ground growth difference was found between the two subsoils in the following year (Figure 8; Table 3). Growth responses in lysimeters where topsoils were contaminated with heavy metal strongly differed between plant species. On the calcareous subsoil, metals distinctly reduced the growth of the deciduous trees, while they enhanced the growth of the herbs and did not affect the biomass of spruce (Figure 9, Table 3). None of these groups showed a metal treatment effect on the biomass production of the acidic subsoils.

Total biomass production was approximately proportional to evapotranspiration, but, in light of the uncertainty in the estimates involved, further analysis of whole-ecosystem water use efficiency (the ratio of aboveground biomass to evapotranspiration) did not reveal any significant insights.

The metal treatment had a strong effect on the fine root density (Figure 10). In the presence of metals fine root growth was reduced by roughly 50 % in the topsoil above both types of subsoil. This metal effect decreased with depth and little or no differences between metal treatments and controls were found below 25 cm depth. Also the subsoil influenced the vertical profiles of fine root density distribution. Calcareous subsoil favoured deeper rooting, whereas acidic subsoil resulted in higher fine root densities in the topsoil.

5. Discussion and Conclusions

The metal contamination in the topsoil produced weak, but nonetheless significant effects on the soil water relations of the investigated juvenile forest ecosystem., Evapotranspiration was reduced under metal stress. The effect became clearer from year- to-year with progressing development of the young stands. At the same time the subsoil effect on evapotranspiration decreased, while there appeared to be a slight tendency of higher ET in the lysimeters, receiving acidified irrigation.

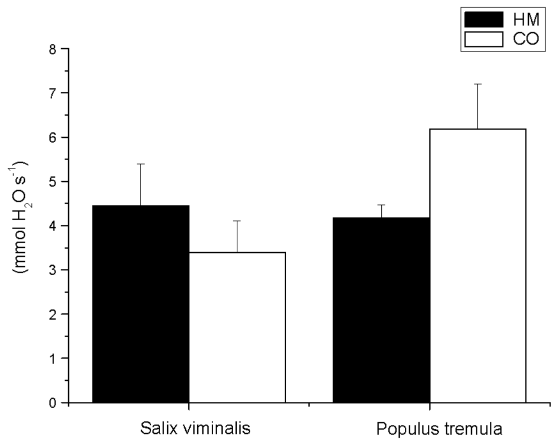

Figure 7: Transpiration rate per tree in 2001 for the heavy metal compared to the control treatment (mean + SE; N= 12); comparison of willow and poplar.

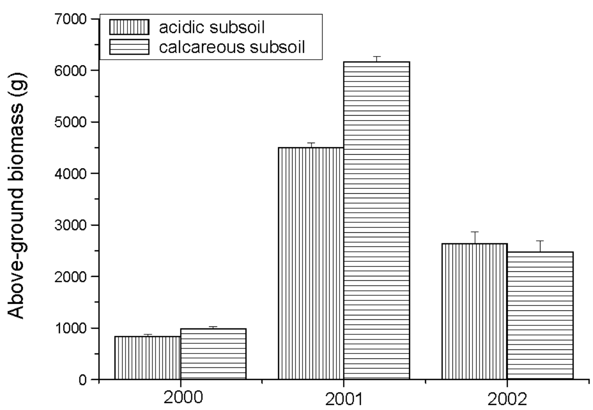

Figure 8: Annual total above-ground biomass production of trees and understorey plants on acidic and calcareous subsoil type (means +SE; 2000: N= 18; 2001, 2002: N=16).

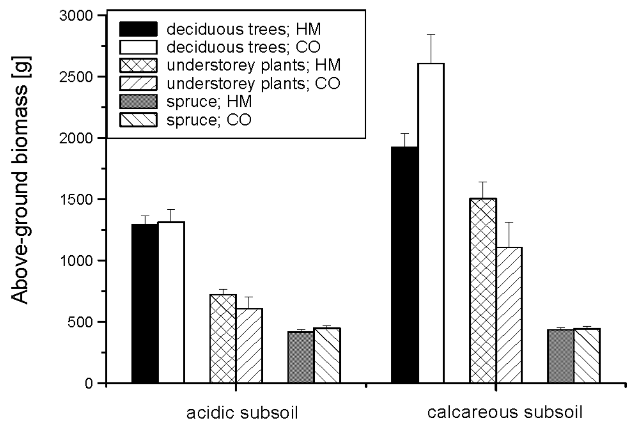

Figure 9. Influence of topsoil heavy metal contamination and subsoil type on above-ground biomass production in 2001, for different groups of species (mean + SE; N=8).

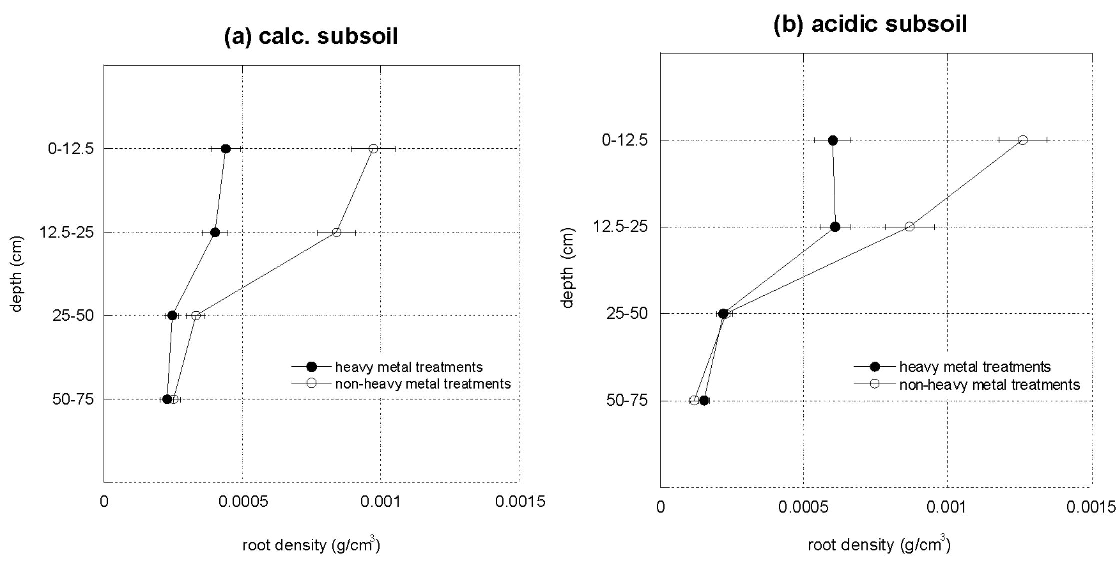

Figure 10. Vertical profiles of the fine root density distribution at the end of the third growing season in the lysimeters (a) with calcareous subsoil and (b) with acidic subsoil

The latter two trends were not expected. On the contrary, addition of acidity to the metal-contaminated soils was expected to increase the availability of the metals and, thus, to amplify the heavy metal-induced stress effects. The responses of different plant species or groups, as well as the soil water potentials at different depths, revealed the complexity of the system, while providing some hints on possible explanations. The measured soil water potentials can be interpreted in terms of the vigour, with which the roots extracted water from the respective soil depths. Under unrestricted water supply, root water extraction activity was by far the highest in the topsoil, as would be expected under such conditions (Figure 3, year 2001). Under water stress (induced drought conditions), the root activity gradually shifted into the subsoil, in particular in the calcareous subsoil, which provided more favourable growth conditions, especially for deciduous trees than the less fertile acidic subsoil (Table 1). According to measurements of metal concentrations in soil solution extracts, significant metal leaching into either of the two subsoils can be ruled out (Rais, 2004), thus the subsoil represents a zone for root activity not directly affected by the heavy metal stress. This explains why, relative to the root activity in the topsoil, the compensatory water extraction from the subsoil observed during periods of water shortage was more pronounced in the metal treatments than in the controls particularly evident in calcareous subsoil (Figure 4). The observed shifts in root water extraction activity must not necessarily reflect responses of individual plant species, but may equally well result from a shift in the relative contributions of plants with different root systems to water extraction. The water balances give total soil water losses due to evaporation and transpiration, but can not differentiate between contributions of the various system components. The transpiration measurements on the other hand give no information on the water balance of the system, but provide insight into the variation of responses between different tree species. The leaf measurements at selected dates showed that willow trees transpired rather more water under metal stress, while the opposite was found for poplars. Willows are known for their high tolerance to soil metal pollution (Pulford and Watson, 2003). As we do not know how the two species in monoculture would have reacted to the treatments, we can not say whether this observation only reflects the different tolerances to metal stress of the two species or whether competition effects played an additional role. Nonetheless, the latter seems likely if we consider also the understorey growth, which profited from reduced tree growth in the metal treatments, at least for calcareous subsoil (Figure 9).

Shifts in the relative root system development between the different plant species or groups of plant species also provide a likely and plausible explanation for the observed changes in the patterns of treatment effects on water balances and soil water potentials between successive seasons. In particular, this could explain why water consumption increased much more on the acidic than on the calcareous subsoil from 2002 to 2003, making the subsoil effect on evapotranspiration disappear in 2003. Judging from the soil water potential measurements, the increased evapotranspiration on acidic subsoil was primarily due to a stronger exploitation of water in the upper subsoil, while in the lysimeters with calcareous subsoil the lower part of the subsoil contributed most to the slight increase in water consumption. This suggests that from the second to the third year of the experiment, root systems developed more in topsoil with acidic subsoil, while in compartments with calcareous subsoil more root growth occurred in deeper soil layers.

The root density data are in good agreement with this interpretation. Root growth may have been reduced in the acidic subsoil, because of nutrient scarcity. It may also have been inhibited due to aluminium toxicity in the acidic subsoil (Table 1). Single-species root growth allocation and toxicity alone, do not explain why the difference in evapotranspiration between lysimeters with different subsoils disappeared in the third year. All these observations can be reconciled, however, if we consider that they are the combined effects resulting from the growth and activity of a mixed vegetation, in which different species with different growth dynamics and responsiveness to site conditions were competing with each other.

It appears that biomass production was affected by metal stress, where growth conditions were favourable (in calcareous subsoil), while in the acidic soils, the heavy metal effect was probably masked by the unfavourable growing conditions. The biomass data suggest that the deciduous trees suffered on the acidic subsoils and that in comparison with spruce trees developed much better. Spruce is known to develop rather shallow root systems and be more tolerant to acidic soil conditions, and to grow slower than willows and poplars. A shift to a stronger development of spruce relative to the deciduous trees with time on the acidic subsoils, would agree well with the differences in fine root distribution profiles found at the end of the experiment, as well as with the observed shifts in water extraction. It would also provide a plausible explanation for the observation that evapotranspiration tended to be larger under acidified than under ambient rainfall chemistry, including the fact that this tendency became obvious only in the last experimental year.

As acidic rain had no effect or even slight enhancing effect on water consumption, we conclude that the acidity of the irrigation water did not significantly increase soil metal toxicity in the system. As heavy metal treatment caused a significant reduction in evapotranspiration, any mobilisation of metals due to the addition of acidity into the topsoil, should have further reduced evapotranspiration.

So, it seems likely that some additional effect other than increasing metal solubility and thereby toxicity was exerted by the acidic rain treatment, compensating for such mobilisation. It would be in agreement with the hypothesis of shifts in competitive strength between different species, if the increase in evapotranspiration was due to an increased growth of the spruce, favoured against the competition of the deciduous trees by more acidic soil conditions.

Metals have been reported to reduce transpiration in various ways, ranging from reduction of root growth and metabolism, inhibition of root water uptake by blocking water channels, blockage of xylem vessels, and decrease of stomatal aperture (e.g. Robb et al. 1980; Schlegel et al., 1987; Poschenrieder and Barceló, 1999). The soil water potential measurements suggest that the primary metal effect occurred locally in the topsoils, where roots were exposed to metal stress. This does not exclude an active secondary response of the plants at the leaf level down-regulating stomatal water loss in order to avoid excessive water stress, as discussed by Schlegel et al. (1987). The observed shifts of water extraction from the topsoil into the subsoil may in fact indicate that inhibition of water uptake in the topsoil induced compensatory root activity at lower depths. The local reduction of root water extraction activity also does not exclude direct effects of accumulated heavy metals on stomatal regulation. Zn in particular was highly accumulated in the leaves of the deciduous trees (Günthardt-Goerg and Vollenweider, 2003; Hermle, 2004).

In conclusion, the results support the hypothesis that the uncontaminated subsoil offered a possibility to compensate the reduction in root water extraction in the topsoil under drought, as well as metal stress. This was more pronounced in the case of the calcareous subsoil, which is more favourable for root activity than the acidic subsoil. The results also show that heavy metal effects on vegetation of a given composition may vary considerably over time and also between different soils due to differences in growth dynamics of the various plant species, as well as due to shifts in competitiveness of the plants.

Acknowledgements

The authors are grateful to Werner Attinger, Peter Bleuler, Michael Lautenschläger and Martin Keller for their technical support throughout the experiment. The collaboration of Ueli Bühlmann, Terry Menard, Véronique Michellod, Daniele Pezzotta and Susanne Scheid is highly acknowledged. The project was funded by Swiss National Science Foundation and Swiss Federal Office for Education and Science, COST-Action 837.

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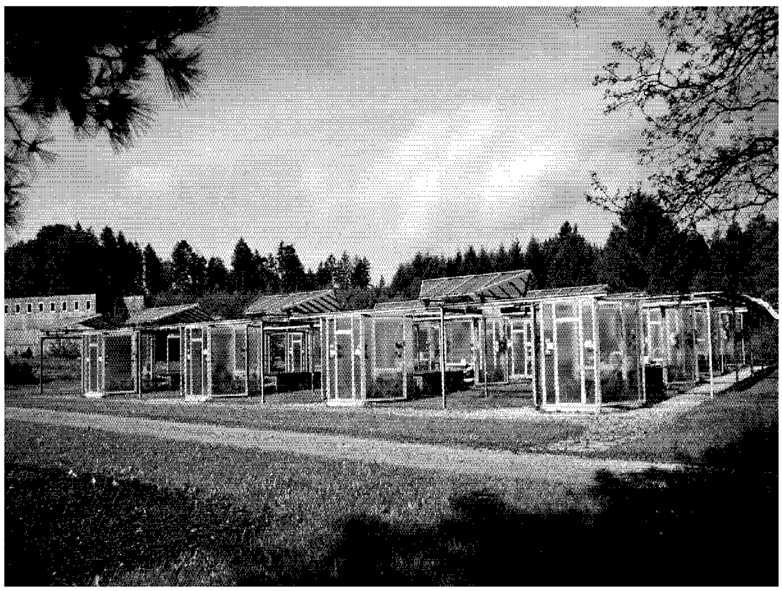

Chapter 5. Effects of Heavy Metal Pollution and Acid Rain on Growth and Water Use Efficiency of a Young Model Forest Ecosystem

Effects of heavy metal pollution and acid rain on growth and water use efficiency of a young model forest ecosystem

(Prepared for *Plant and Soil)*

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Abstract

In a 4-year lysimeter experiment, we investigated the effects of top soil heavy metal pollution (2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu and 10 mg kg^{-1} Cd) and simulated acid rain (pH 3.5) on growth and water use efficiency of young forest ecosystem consisting of Norway spruce (*Picea abies*), willow (*Salix viminalis*), poplar (*Populus tremula*) and birch (*Betula pendula*) trees and a variety of herbaceous understorey plants. The treatments were applied in a Latin-square factorial design (contaminated vs. uncontaminated topsoil, acidified rain vs. ambient rain) to 16 open-top chambers, with 4 replicates each. Each open-top chamber contained two lysimeters, one with a calcareous, and the other with an acidic subsoil. The four tree species responded quite differently to heavy metal pollution and type of subsoil. The fine root biomass of *P. tremula* was strongly reduced by metal pollution, while the above-ground biomass was not significantly affected. Shoot and root growth of *P. tremula* tended to be higher on the acidic than on the calcareous subsoil, whereas *S. viminalis* produced higher biomass above and below ground on the calcareous than the acidic subsoil. In contrast to the previous growing seasons, the growth of *S. viminalis* was significantly reduced in the metal treatment on calcareous subsoil in 2003. Also in *P. abies* and *B. pendula*, growth was generally less on the acidic subsoil. In contrast to S. viminalis, *P. abies* showed a clear metal effect on growth only on the acidic subsoil, while in *B. pendula* metals reduced growth of roots and shoots on both types of subsoil. Acid rain produced few significant effects. In the absence of metal contamination acid rain generally tended to increase growth on calcareous subsoil, while clear inhibitive effects were observed only on root growth of *P. tremula* and *P. abies* in lysimeters with acidic subsoil. Treatment effects on water consumption were similar as those on total tree biomass, but weaker in proportion to the respective controls. As a result, water use efficiency tended to be larger on calcareous than acidic subsoil and was slightly increased by acidic rain in the absence of metal contamination and was decreased by metal treatment. None of these trends proved to be significant, however.

Keywords: forest ecosystem, heavy metals, lysimeters, acid rain, soil pollution, water use efficiency, tree growth, acidic subsoil, calcareous subsoil

1. Introduction

In addition to large areas of agricultural soils, there are also many sites at which forest soils have been polluted by various heavy metals, in particular through atmospheric deposition from industrial and traffic emissions (Hesske et al., 1998; Kahle, 1993), shooting activities (Darling and Thomas, 2003) or waste disposal. Heavy metal pollution has been found to decrease forest productivity (Puckacki and Kaminska-Rosek, 2002), vitality, biodiversity and stand structure (Chernenkova and Kuperman, 1999). The risks from heavy metal pollution depend on the type(s) and concentration of metal (s), soil properties such as pH, CEC, redox potential, soil texture, clay and organic matter content etc (Prasad, 1997). Soil pH is a key factor, which determines metal availability and toxicity to plants (Krebs et al., 1998). The upper horizons of many forest soils are acidic. Under these conditions the risks of metal leaching and uptake by plants are generally higher than under basic or neutral pH conditions (Kahle, 1993). Decrease of soil pH due to acid rain (Walker and Mclaughlin, 1993) has increased forest soil acidity in many parts of Europe and North America in the past and further aggravated the problem of metal leaching and toxicity (Guo et al., 2005).

Many previous investigations showed adverse effects of heavy metals on root growth and root functions (Arduini et al., 1994; Arduini et al., 1995; Ewais, 1997; Helmisaari et al., 1999; Karolewski and Giertych, 1994; Oberlander and Roth, 1978). This in turn changes the water relations of affected plants as reviewed by Poschenrieder and Barceló (1999). Most of these studies were plant and metal-specific. They were usually performed in pots or in hydroponics and provided valuable information about physiological responses of individual plants to metal stress under well-defined conditions. However, they do not represent field conditions under which roots are not usually confined to a small volume of homogenized soil substrate and also, where they may have to compete for above and below-ground resources. The duration of these experiments are very short (several days or months), it will not give an opportunity to study the response of plants under long term exposure. On the other hand, there are also many studies, which compared the responses of plants grown under field conditions on metal-contaminated soils with plants grown on uncontaminated or less-contaminated “reference sites”. The problem here is the lack of control of factors, in particular soil conditions, so that the effect of the metal contamination can be clearly separated from the influence of other factors.

In order to avoid the problems in these two approaches, a factorially designed lysimeter study was performed at Swiss Federal Institute of Forest, Snow and Landscape (WSL), Birmensdorf, Switzerland, in which young forest ecosystem were replicated in lysimeters with reconstructed soil and exposed to different combination of well-defined treatments. The goal of this multi-disciplinary project was to study the effects of a mixed metal contamination of the topsoil, originating from the deposition of dust from metal smelter, under the influence of different types of rain water acidity and uncontaminated subsoil on various physical, chemical and biological processes and functions from the sub-cellular to the ecosystem scale.

In a previous publication, we focused on water regime of metal-contaminated soil (Menon et al., 2005). The heavy metal treatment significantly reduced root growth and evapotranspiration (ET) on one hand and increased soil wetness and drainage discharge on the other hand. However, the type of subsoil had initially an even stronger effect. In the first two growing seasons, ET was higher on calcareous than acidic subsoil, but in the third year, this effect disappeared. Under contaminated topsoil, root growth was shifted to deeper depths, particularly in the calcareous subsoil. On the other hand, there was almost no influence of acidified irrigation on the water regime, although it increased soluble Zn and Cd concentrations in the topsoil (Rais, 2005). Despite this increase in HM concentration, the overall water consumption actually increased rather than decreasing, although not with statistical significance.

All these findings led to the hypothesis that competition between different species and shifts in relative competitiveness occurring with the development of the young forest stands played an important role in the response of the vegetation to the metal contamination of the topsoil. After the experiment in 2003, all trees were harvested and analyzed biometrically, allowing us to compare growth responses between the various tree species and to relate them to the overall water use efficiency of the stands. Our hypothesis was that the responses to the applied treatments would vary strongly among the different tree species. In particular, we expected to find that metal stress would reduce root growth in some species and favour it in others.

2. Materials and Methods

2.1 Open Top Chambers with Lysimeters

The experiment was carried out in the open-top chamber (OTC) facility of the Swiss Federal Institute of Forest, Snow and Landscape Research (WSL) Research, Birmensdorf, Switzerland. The glass walls of the 16 hexagonal 3 m high OTC were partially opened when it did not rain in order to increase ventilation and closed when it rained. Also the glass roofs of the OTC were set to close automatically upon the onset of rain. Below ground, each OTC was divided into two lysimeters of 1.5 m depth and 3 m^2 surface area.

2.2 Soils

Each lysimeter was filled from bottom to top with 0.5 m thick quartz sand packing, 0.85 m subsoil and 0.15 cm topsoil. The quartz sand drainage packing consisted of three layers with increasingly coarse grain sizes towards the concrete bottom. In each OTC, the subsoil of one of the lysimeters was a calcareous (pH 7.4) and the other one an acidic loamy sand (pH 4.2). The loamy topsoil was non-calcareous (pH 6.4) and consisted of the same material in all lysimeters. The physico-chemical properties of the soil materials and the experimental design have been described in more detail by Menon et al. (2005).

2.3 Forest Vegetation

The same selection of trees and understorey plants was planted in each lysimeter. The selected species were birch (*Betula pendula* L.), willow (*Salix viminalis* L.), poplar (*Populus tremula* L.) and three provenances of spruce (*P. abies*). The understorey was composed of wood sedge (*Carex sylvatica* Hudson), ramsons (*Allium ursinum* L.) tansy (*Tanacetum vulgare* L) and different small tree seedlings. All plants were planted in spring of the year 2000. The plants were arranged in such a way that a mixed distribution was obtained and that the ground was covered as evenly as possible (Figure 1). Positions were fixed with respect to groups of plants, i.e. for deciduous trees, conifers or understorey plants, but random within each group.

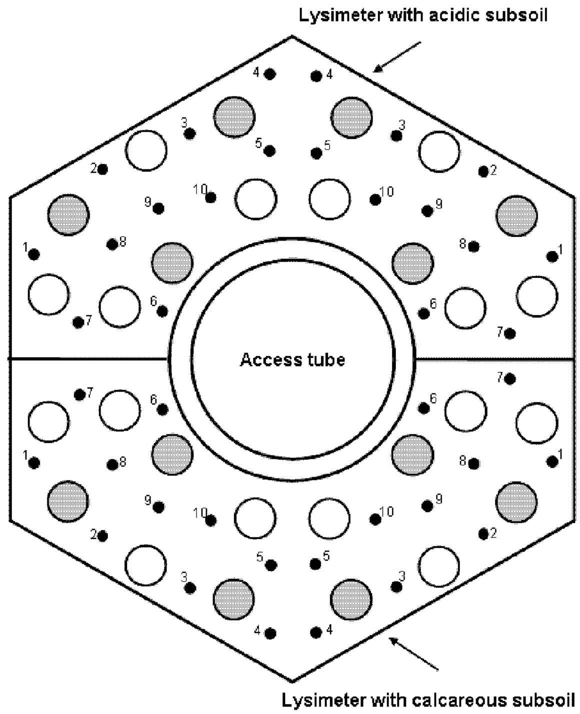


Figure 1. Ground view of OTC. White circles indicate positions of decidous trees, dark circles those of conifers (i.e. *P. abies*) and black dots those of understorey plants.

2.4 Treatments

In 8 chambers, the topsoil was artificially contaminated with filter dust from a non-ferrous metal smelter. The dust was mixed into the topsoil by hand, after tensiometers and other instrumentation had been installed in the subsoil. The average concentrations of HNO^{3-} extractable metals in the contaminated topsoils after mixing were 2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu and 10 mg kg^{-1} Cd, respectively.

Irrigation was applied by means of sprinklers (6 per soil compartment) with computer-controlled flow meters. The irrigation was scheduled based on tensiometer measurements so as to keep the soil moisture content around field capacity and added as ambient rain composition (30 years mean values of the site) with the pH adjusted in eight OTC to 5.5, in the other eight OTCs to 3.5 by addition of HCl.

The metal and acid rain treatments were assigned to the 16 OTC according to a 2 x 2 Latin-square design with four replicates of each combination. Combined with the two types of subsoil in each OTC (acidic and calcareous), this gave a fully balanced factorial design of 2 x 2 x 2 treatments with 4 replications (Menon et al., 2005). The following abbreviations are used to denote the treatments: HM, heavy metal contaminated topsoil (and ambient rain); AR, acidified "rain" (and no contamination); HMAR, combination of heavy metal contaminated topsoil and acidified rain; CO, control (no contamination, ambient rain).

3. Measurements, sampling and data analysis

3.1 Water regime

Evapotranspiration (ET) was calculated during the growing season from the water balance equation:

$$ET = I - D - \Delta S \qquad (1)$$

where *ET* is evapotranspiration, *I* is amount of irrigation, *D* is amount of drainage, and ΔS is the change in soil water storage. Drainage water was collected in plastic containers at the bottom of an access tube in the middle between the two lysimeters. The containers were emptied at weekly or biweekly intervals by means of a pump. In order to obtain ΔS, profile soil water contents was measured periodically by Time Domain Reflectometry (TDR), using a Tektronix 1502B instrument. TDR probes of 25 cm length were installed vertically at 0-25, 25-50, 50-75 and 75-100 cm depth. The recorded TDR signals were calibrated and analysed using the procedure of Roth *et al.* (1989). WUE was calculated by dividing the aboveground biomass produced by ET.

3.2 Above- and below-ground biomass

The total duration of the experiment was 4 years (2000-2003). The aboveground biomass of the deciduous trees and understorey plants was harvested annually at the end of the first three growing seasons by coppicing. In autumn 2003, all trees were harvested, and foliage, wood and root biomass (<2 mm diameter) were determined separately for each species.

3.3 Specific leaf area (SLA)

Ten leaves were selected over each tree from the bottom to the top. Single leaf area (using a leaf area meter) and projected needle area of the rhomboid *P. abies* needles (an aliquot of 20 current year needles) and dry mass was determined. SLA was then calculated by dividing leaf area by leaf dry weight. Total leaf area was obtained by multiplying the SLA for each tree with its foliage dry biomass. Leaf area of understorey plants was not taken into account.

All statistical analysis was performed using SYSTAT (version 11.0) and error bars in figures represent standard error.

4. Results

4.1 Fine root biomass

At the final harvest fine root biomass was in general significantly reduced in metal treatments, except in the case of *S. viminalis*, which showed large variation within the treatments (Figure 2). On the other hand, *S. viminalis* showed the strongest subsoil effect on fine root mass. The root mass of *S. viminalis* was much less in the lysimeters with acidic than in those with calcareous subsoil. The effect was highly significant despite the large variability found in the lysimeters with calcareous subsoils.

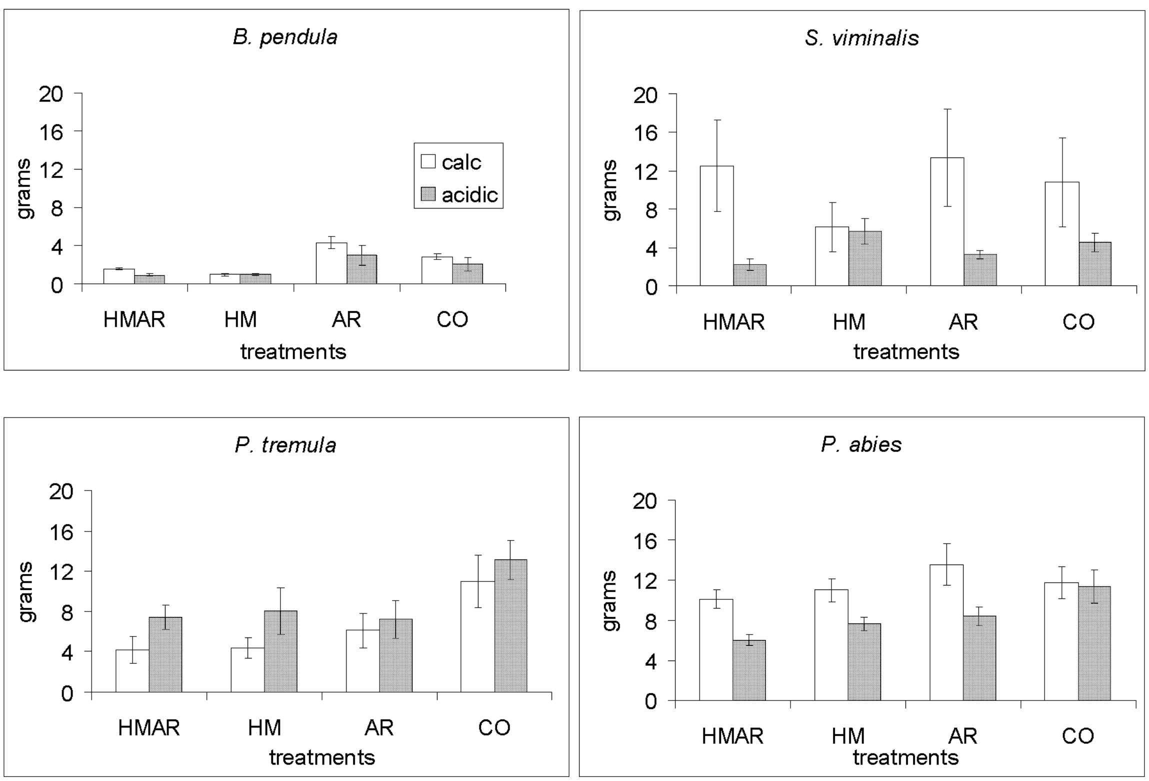


Figure 2. Average fine root biomass per tree after the harvest in 2003.

A significantly reduced fine root biomass in lysimeters with acidic subsoil was also found in *P. abies*, while poplar showed a tendency to the opposite. *B. pendula* grew more fine root biomass in lysimeters with calcareous subsoil than on acidic subsoil, like *S. viminalis* and *P. abies*; however, the difference was not significant.

Acid rain had no significant effect on fine root growth, except for *P. tremula*, where root growth was reduced under acid rain. However, acid irrigation tended to increase rather than decrease root growth in the other three tree species in the lysimeters with calcareous subsoil, in birch also in the lysimeters with acidic subsoil. In contrast, root growth of willows and spruce tended to be decreased in lysimeters with acidic subsoil. Poplar was the only tree species in which root growth was decreased also in the treatments with calcareous subsoil.

4.2 Above ground biomass

Overall, the picture of treatment effects on above-ground biomass obtained from the final harvest (Figure 3) was quite similar to that observed below ground (Figure 2, see 4.1). In 2003, the above-ground biomass production of *B. pendula* and *S. viminalis* was significantly less in the metal treatments than in the controls, while in *P. abies* a similar tendency was observed only on acidic subsoil. Subsoil effects on above-ground biomass were significant, except for *P. tremula*, which differed from the other species by a tendency to higher growth on acidic than on calcareous subsoil, as in root growth (see 4.1). None of the trees responded significantly in above-ground growth to the acidic rain treatment. But there were similar tendencies in willows, spruce and birch to respond positively to acid irrigation on calcareous subsoil, and in willow again also a slight trend to respond negatively on acidic subsoil.

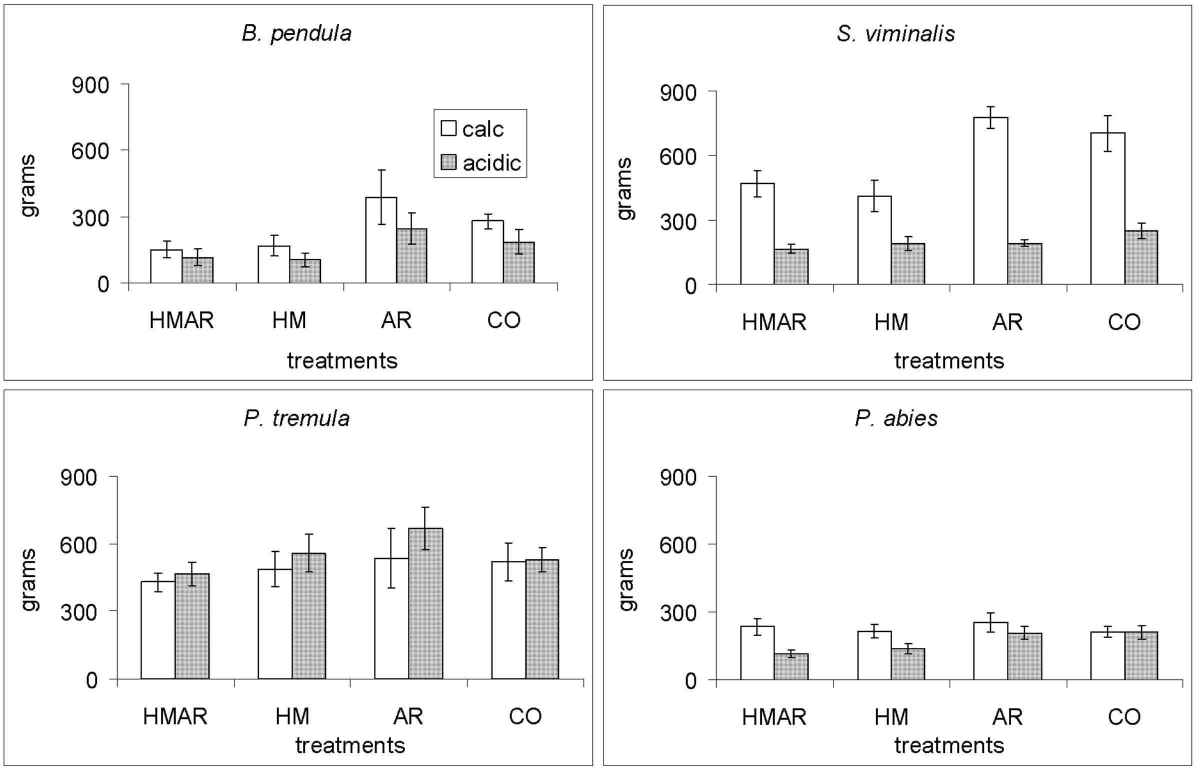


Figure 3. Above-ground biomass produced in the experimental year 2003.

Comparing the data on above-ground biomass production in 2003 with the respective data on leaf and needle production from 2002 (Figure 4), it can be observed that the general patterns of treatment effects are quite similar, but with some interesting deviations:

- The metal effect on poplar was much clearer in 2002 than in 2003, while there was no tendency yet of a subsoil effect on this species (cf. Hermle (2004) for more detail).

- Willow and spruce showed similar metal and subsoil effects in both years, but the metal effect on willow was much weaker and not significant in 2002.

- There was no indication of any positive acid rain effect on growth in 2002.

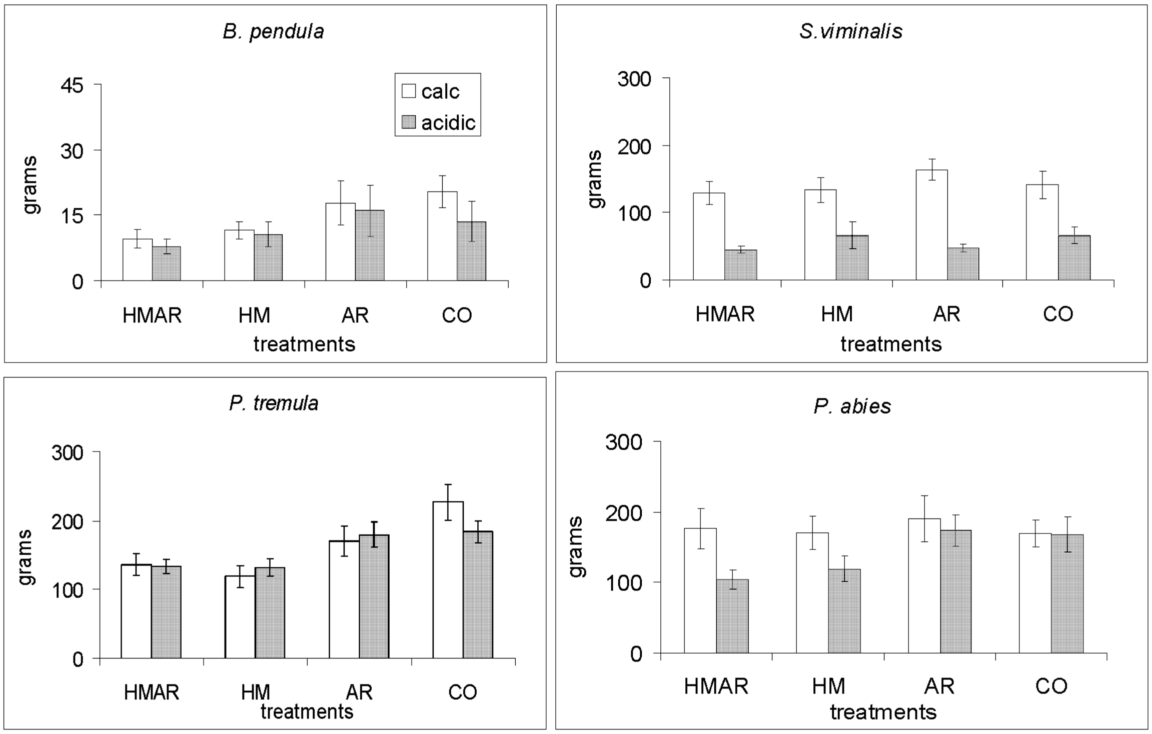


Figure 4. Above-ground biomass produced in the experimental year 2002.

4.3 Specific Leaf Area (SLA)

Specific leaf area was very similar in the three deciduous tree species and showed very little response to the treatments (Figure 5). There was no subsoil and no acid rain effect, while heavy metal stress reduced SLA in *S. viminalis* and in tendency also in *P. tremula*. Acid rain appeared to counteract the metal effect. The interaction between acid rain and heavy metal treatment was statistically significant not only for *S. viminalis, but also* for *P. tremula* (Table 1). Little dependence of SLA on metal stress has been reported also by Sebastiani at al. (2004) for hybrid poplars.

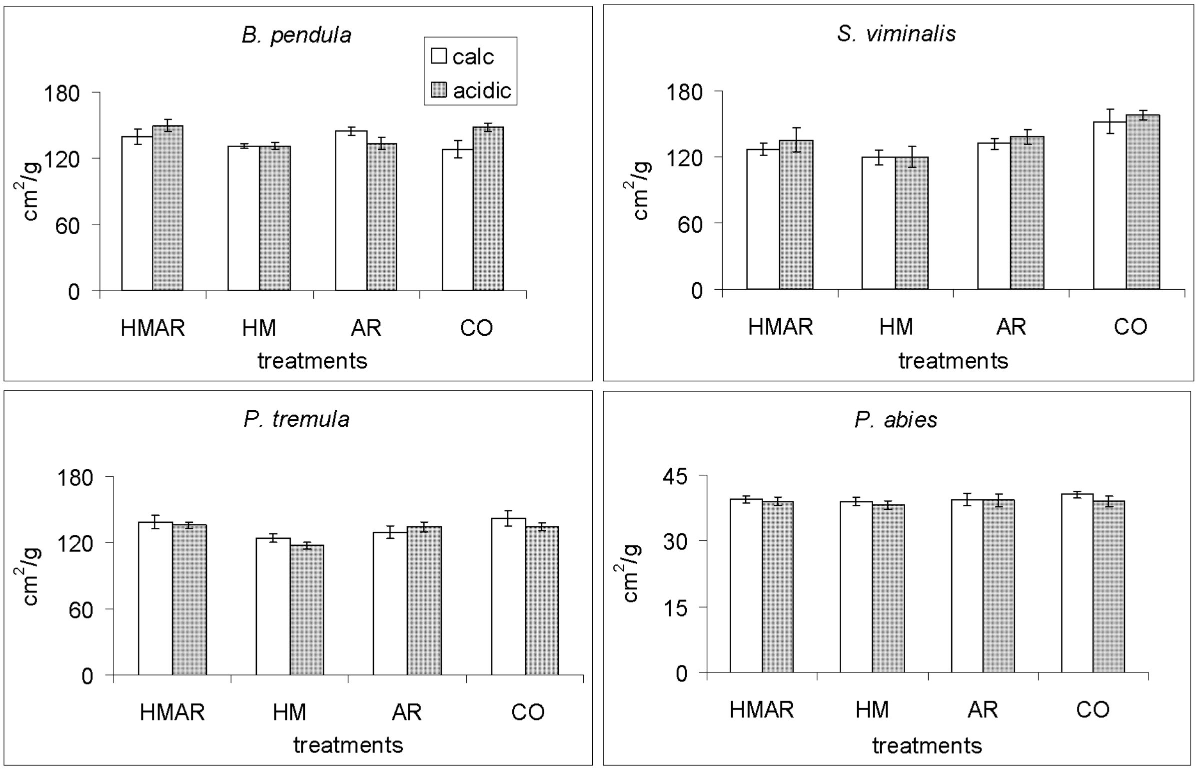


Figure 5. Specific leaf area (SLA) of trees in the year 2003.

4.5 Leaf Area

Given that specific leaf area varied only very little within each species between treatments (including subsoil conditions), the total leaf area per tree showed very similar patterns of treatment responses as above-ground biomass (Figure 6). For the same reason also the treatment effects on leaf area show the same changes between 2002 and 2003 as the treatment effects on above-ground biomass. The leaf area of *S. viminalis* was two to three times as large on calcareous than on acidic subsoil. In addition it showed a significant reduction in the metal treatments on calcareous subsoil, but only a small reduction on acidic subsoil in 2003 and no effect in 2002

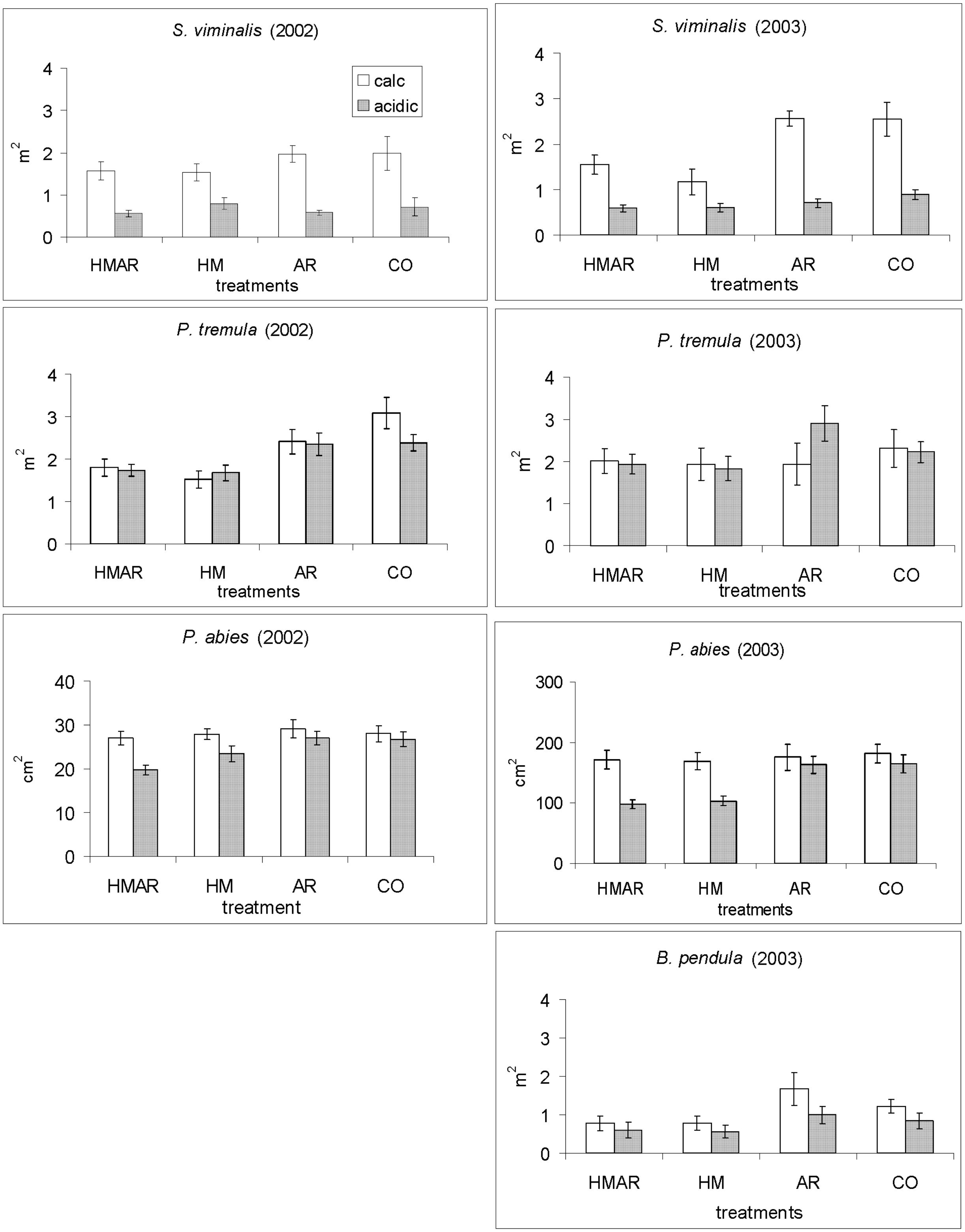


Figure 6. Leaf area per tree in 2002 (left) and 2003 (right). Note differences in scales. No birch leaves were collected in 2002.

B. pendula showed a similar metal effect on both subsoils, but only a tendency of larger leaf areas on calcareous than acidic subsoil (No leaves were collected in 2002). In the absence of metals, the leaf area differences between subsoils were even smaller in *P. abies* than in *B. pendula*, and in contrast to the other tree species, *P. abies* showed no metal effect at all on calcareous subsoil, while its leaf area was significantly reduced on acidic subsoil. This latter effect became even much stronger in 2003 than it was already in 2002. Similar as in *B. pendula* in 2003, metals also reduced the leaf area of *P. tremula in 2002* on both subsoils*, but* this metal effect disappeared in 2003. There was no tendency of a larger leaf area on acidic subsoil, except perhaps in the acid rain treatment in 2003. In none of the four tree species there was clear tendency neither towards a positive, nor a negative acid rain effect on leaf area, except perhaps for a slightly negative influence on *S. viminalis* on acidic subsoil in 2002.

4.6 Evapotranspiration and water use efficiency

Figure 7 shows the treatment effects on the overall water balances of the lysimeters in the year 2002 and 2003. Metal contamination had a weak, but significant effect in both years. In contrast, the subsoil had significant effects in 2002, but not in the 2003. Contrary to our expectation that addition of acidity would enhance the metal effect, acidic rain did not reduce evapotranspiration. Rather there was a tendency over the years that evapotranspiration was larger with acidic than with ambient rain chemistry, although the trend did not become statistically significant (Menon et al., 2005).

Water use efficiency (WUE) i.e. biomass produced per unit amount of ET, was higher on calcareous subsoil than acidic subsoil, and it decreased in HM treatments

(Figure 8). Acid rain chemistry tended to increase WUE on calcareous subsoil in controls and to decrease it in metal treatments and also on acidic subsoil in control treatments. Decrease in WUE was strongest in metal-contaminated soil on acidic subsoil. The above-ground biomass of 2003 showed stronger correlation with ET (Figure 9a) on calcareous subsoil (R^2=0.8989) than on acidic subsoil (R^2=0.6677).

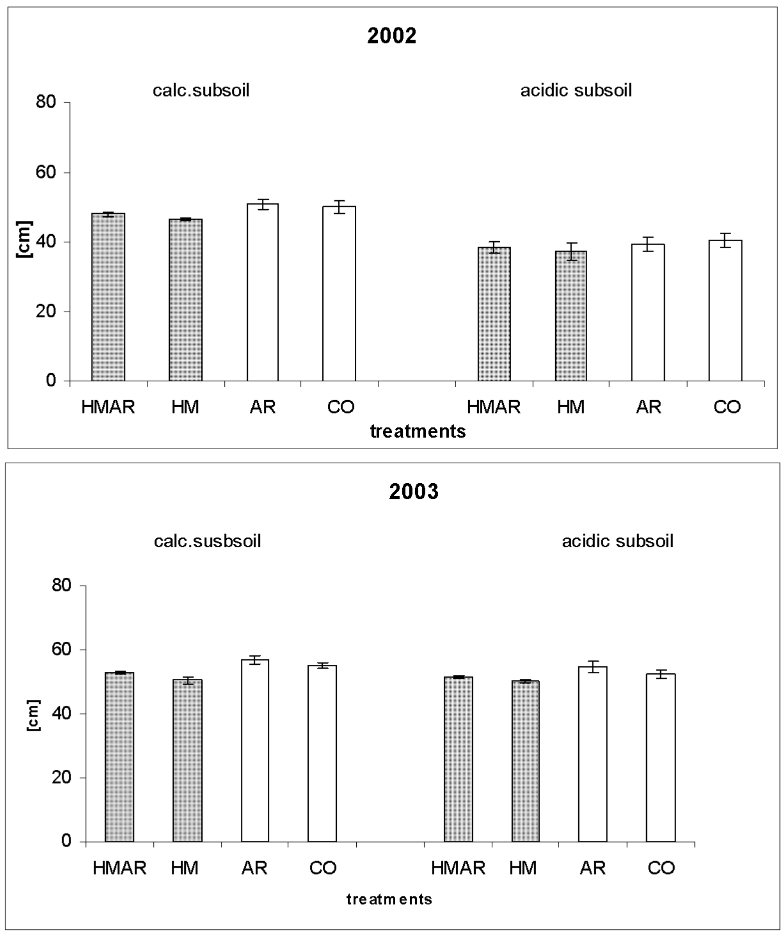


Figure 7. Total evapotranspiration in the year 2002 and 2003 (Menon et al., 2005).

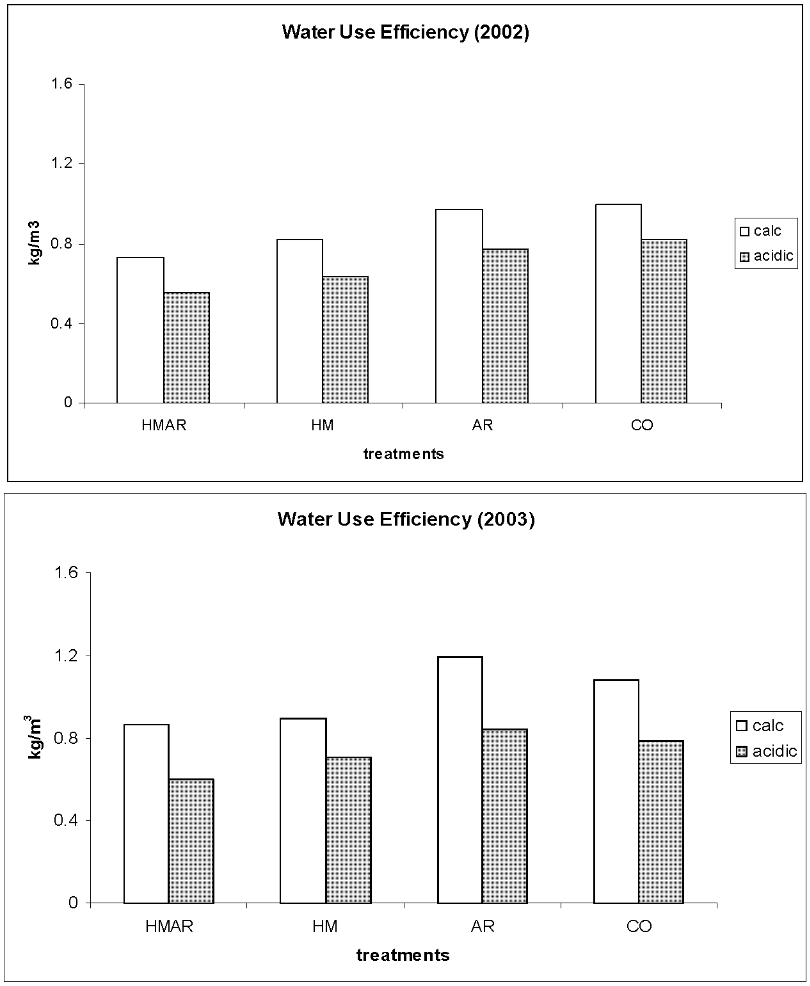
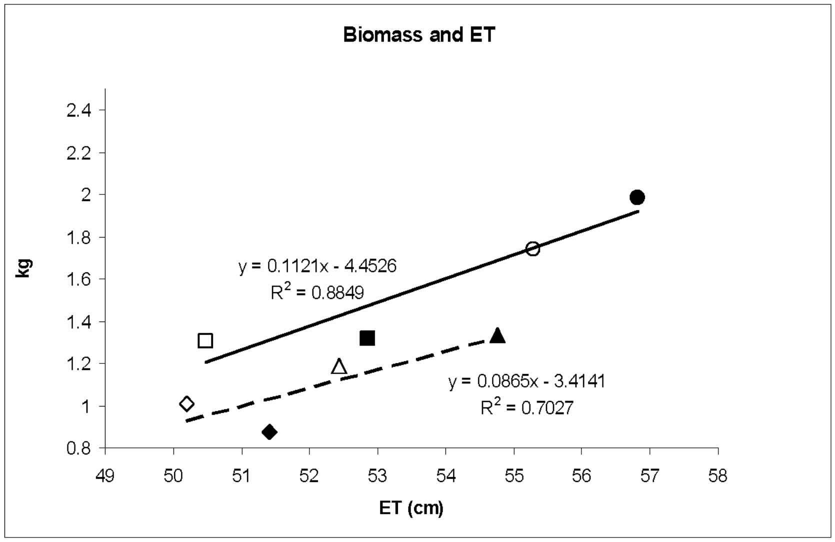


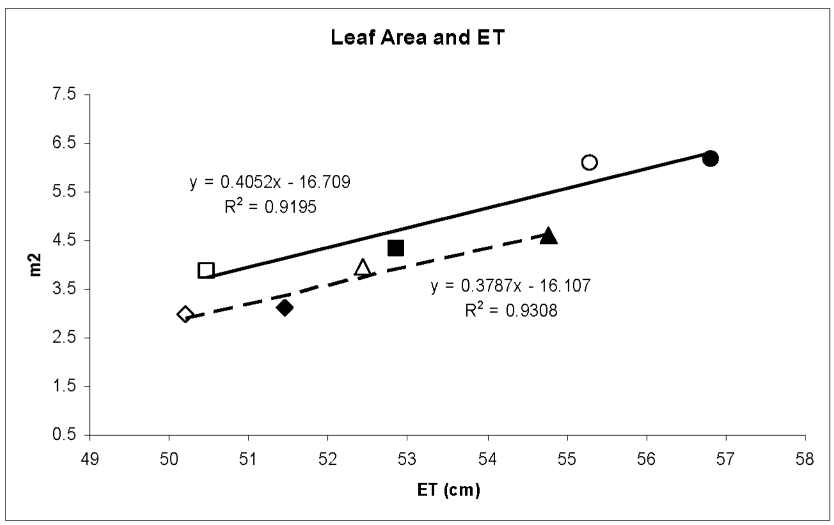
Figure 8. Water Use Efficiency of young forest ecosystem in the year 2002 and 2003.

ET showed a close correlation with total leaf area in 2003 for both subsoil types (Figure 9b). The acid rain treatment led to a clear parallel shift of this relationship in the case of the calcareous subsoil.

(a)



b)



○ CO(calc) ● AR(calc) □ HM(calc) ■ HMAR(calc)

△ CO(acidic) ▲ AR(acidic) ◇ HM(acidic) ◆ HMAR(acidic);

Calcareous subsoil (——) Acidic subsoil (------)

Figure 9. Relationship between above ground biomass and ET (a) and leaf area and ET (b) of the year 2003.

5. Discussions and conclusions

The results show that each of the four tree species responded differently and partially even in opposite directions to the applied treatments including the variation of subsoil type. To some extent the effects also varied between the two years of observation. Effects on root growth above and below ground were in general closely related to each other, but there were nonetheless also interesting differences.

S. viminalis did not show a metal effect on final root biomass and in 2002 also no clear metal effect on above-ground biomass, but in 2003 the above-ground biomass was significantly reduced on calcareous subsoil. The latter response was rather surprising in light of the fact that *S. viminalis* grew much better on the calcareous subsoil, while the metal stress was limited to the topsoil and was the same as above acidic subsoil. If there was any metal leaching, it should have led to higher soluble metal concentrations in the acidic than in the calcareous subsoil. If different responses to the metal treatments should have been expected on the two subsoils, the effects observed in *P. abies* thus were the more plausible ones. This species showed no or at best a very slight tendency of reduced growth in the metal treatments on calcareous subsoil, but clear metal-induced growth reduction above and below ground on the acidic subsoil. In the absence of metal stress, *P. abies* was less dependent on the type of subsoil than *S. viminalis*, but still showed a tendency towards better growth on the calcareous subsoil, which fits well to the higher metal tolerance on this type of subsoil.

Spruce was surprisingly metal-resistant also on the acidic subsoil in our study, compared to other studies in which this species was found to be rather metal-sensitive. For example Godbold et al. (1987) found that the elongation of *P. abies* roots was significantly reduced compared to controls if they were grown in culture solutions with 30 and 60 µM Zn or Cd. In another solution culture study, application of 65 µM Zn or 15 µM Cd reduced root biomass production of spruce seedlings to 68% and 59% of the control treatments, respectively (Godbold et al.,1985).

Also *S. viminalis* has not been portrayed as a particularly metal-resistant species in the literature, at least in comparison to other species of the genus *Salix*. For example, Vandecasteele et al., (2005) compared the metal tolerance of *Salix fragilis* 'Belgisch Rood' and *Salix viminalis* 'Aage' in a greenhouse pot experiment with six sediment-derived field-contaminated soils with Cd levels ranging from 0.9 to 41.4 $mg.kg^{-1}$ and found that at all levels dry weight root biomass and total shoot length were significantly lower in *S. viminalis* than in *S. fragilis* for all treatments. Also, in greenhouse and field studies (Watson et al., 2003) *S. viminalis* clones and *S. triandra* produced less biomass at elevated metal concentrations in soil than e.g. *S. burjatica* 'Germany,' *S.x dasyclados*, *S. candida* and *S. spaethii, al*though the latter species accumulated higher concentrations of Cu and Ni in the bark.

In agreement with the literature cited before, Hermle et al. (*in press*) found that root biomass of *S. viminalis* was significantly reduced and that *P. abies* failed to grow altogether under these conditions when seedlings (of the same clones or provenances) of these two species were grown in pots filled with the same contaminated topsoil as used in our lysimeter study. This indicates that the apparently high metal tolerances observed in our lysimeter study may have been due to the fact that roots had the possibility to evade the highly contaminated topsoil by growing into the uncontaminated subsoil. Rais (2005) showed that the contamination of the topsoil had very little effect on the soluble metal concentrations in the subsoils of our lysimeters. Soluble zinc concentrations remained below the detection limit at a depth of 40 cm in the calcareous subsoil and were about doubled in the respective depth of the acidic subsoil from around 0.1 μM in the controls to around 0.2 μM below contaminated topsoil, in the treatments with ambient rain chemistry. Total copper concentrations in solution on the other hand showed no effect under these conditions in the acidic subsoil and were even reduced in the calcareous subsoil (0.1 - 0.15 μM as compared to around 0.2 μM in the controls) below contaminated topsoil, due to a decrease in dissolved organic carbon. For comparison, the respective concentrations varied around 1.5 μM Cu and 20 μM Zn in the contaminated topsoil and around 0.3 μM Cu and below 0.05 μM Zn in the control topsoil.

A further difference between the pot experiment of Hermle (2004) and the lysimeter experiment here was that the contamination of the topsoil was much more heterogeneously distributed on a small scale, i.e. in the range of cm (Luster et al., in prep.), because for practical reasons it was not possible to achieve the same fine mixing in the lysimeters than in the preparation of the pots, as utmost care had been taken to avoid contamination of the subsoils during the preparation, planting and instrumentation of the lysimeter soils. As a result, roots would have had the possibility also to reduce metal stress by growing into local niches of lower metal concentrations.

The differences in treatment effects between the two years of study may to some extent just reflect the development of the root systems, in particular in the subsoil. In addition, differences in climate conditions likely played some role. The summer of 2003 was exceptionally hot and dry. Irrigation, was, however, purposely applied at similar rates as in the year before so that some moisture stress evolved in late summer as documented by Menon et al. (2005). It is possible that willows were less able to cope with this situation than the other tree species under metal stress on the calcareous subsoil, as soil water availability here probably became the limiting factor. The absence of such a similar year-to-year change in metal sensitivity in spruce is in line with a higher drought tolerance of this species. The higher metal sensitivity on acidic subsoil in this species was similar in both years and in line with the before-mentioned hypothetically stronger restriction of root development to the contaminated topsoil.

Birch showed the highest degree of metal sensitivity in our study. Biomass was likewise negatively affected above and below ground and independent of subsoil type, despite a general tendency of slightly stronger growth on the calcareous subsoil. We found no data in the literature which are directly comparable to our results. Utriainen et al. (1998) found that different clones of *B. pendula* varied considerably in their responses to Zn and Cu. Osteras et al. (2000) observed a high Cd tolerance of birch roots. The root weight of *B. pendula* was significantly reduced only at Cd levels of 100 ppm. In terms of shoot sensitivity, on the other hand, birch was the most sensitive species

A rather unexpected result of our study was that the growth response of *P. tremula* to the two subsoils, which only emerged in 2003 after no such effect was still apparent in the year before, went into the opposite rather than into the same direction as in the other three species. Apparently *P. tremula* has a higher tolerance to acidic soil conditions than the other three species. This tolerance became more relevant over time with progressive development of the root system into the depth of the soil profile. This shift of root activity to lower depths would also explain the tendency of decreasing metal sensitivity of *P. tremula* over the two years of this study. To some degree the complementary effects of the two subsoils on poplar growth on one side and on the growth of the other tree species on the other side may also be the expression of interspecies competition that became increasingly severe as metal stress restricted the development and activity of foraging roots in the contaminated topsoil.

Interestingly, there was no significant acidic rain effect on growth and water consumption. Such an effect may have been expected as it is known that increased proton activity solubilizes metal cations. In fact, in at least qualitative agreement with theory, Rais (2005) found that Zn and Cd concentrations were increased by roughly a factor of two in the solutions of contaminated topsoils during acidic irrigation. The fact that this increase did not translate into negative growth effects, but rather the opposite on the calcareous subsoil, means that the input of acidity had effects that were overriding any adverse metal effects. Most likely this compensation was due to a concomitant mobilization of nutrients, in particular phosphate. *P. tremula* was again the only tree species that showed a tendency that was opposite to the other three, which nicely agrees with the competition hypothesis. On the nutrient-poor acidic subsoil (Hermle, 2004) the compensatory effect of nutrient mobilization was much less, if there was any, explaining why increased metal solubility under acidic irrigation could have caused some growth inhibition, although the observed trends were not significant.

The observed treatment effects on evapotranspiration can be interpreted as composite effects on the individual tree species, although it is not possible to quantify the contribution of each species on the basis of the available data. The disappearance of the subsoil effect on ET in 2003 can be attributed to an enhanced development of the poplars, in particular of subsoil roots, in the lysimeters with acidic subsoils towards the end of the study. The slight tendency to increased ET under acid rain agrees well with the respected trends observed in biomass production.

In conclusion, this study showed how differently trees can respond to heavy metal soil contamination in a near-natural ecosystem situation. In some species, these responses depended significantly on the type of subsoil. The calcareous subsoil favoured the growth of *S. viminalis*, *B. pendula* and *P. abies*, while *P. tremula* produced more biomass, especially below ground, in the lysimeters with acidic subsoil.

Table 1. ANOVA results for the different treatments (level of significance: $P<0.05$ n.s = not significant; * only leaf biomass).

Parameters (2002)	Tree	Treatments			
		HM	AR	HM*AR	Subsoil
Above ground biomass	**B. pendula* (n=63)	0.01	n.s.	n.s.	n.s.
	S. viminalis (n=64)	n.s.	n.s.	n.s.	n.s.
	P. tremula (n=122)	0.001	n.s.	n.s.	n.s.
	P. abies (n=176)	n.s.	n.s.	n.s.	0.000
Leaf Area	*S. viminalis* (n=64)	n.s.	n.s.	n.s.	n.s.
	P. tremula (n=120)	0.001	n.s.	n.s.	n.s.
	P. abies (n=176)	n.s.	n.s.	n.s.	0.000

(Continued on next page)

(2003)					
Fine root mass	*B. pendula* (n=63)	0.000	n.s.	n.s.	n.s.
	S. viminalis (n=64)	n.s.	n.s.	n.s.	0.004
	P. tremula (n=117)	0.011	0.026	n.s	n.s
	P. abies (n=186)	0.006	n.s.	n.s.	0.001
Above ground biomass	*B. pendula* (n=63)	0.001	n.s.	n.s.	0.048
	S. viminalis (n=64)	0.000	n.s.	n.s.	0.000
	P. tremula (n=84)	n.s.	n.s.	n.s.	n.s.
	P. abies (n=182)	n.s.	n.s.	n.s.	0.002
Specific Leaf Area	*S. viminalis*(n=64)	0.000	n.s.	0.025	n.s.
	P. tremula (n=81)	n.s.	n.s.	0.001	n.s.
	P. abies (n=243)	n.s.	n.s.	n.s.	n.s.
Leaf Area	*B. pendula* (n=63)	0.003	n.s.	n.s.	0.028
	S. viminalis(n=64)	0.000	n.s.	n.s.	0.000
	P. tremula (n=85)	n.s.	n.s.	n.s.	n.s.
	P. abies (n=177)	0.000	n.s.	n.s.	0.000

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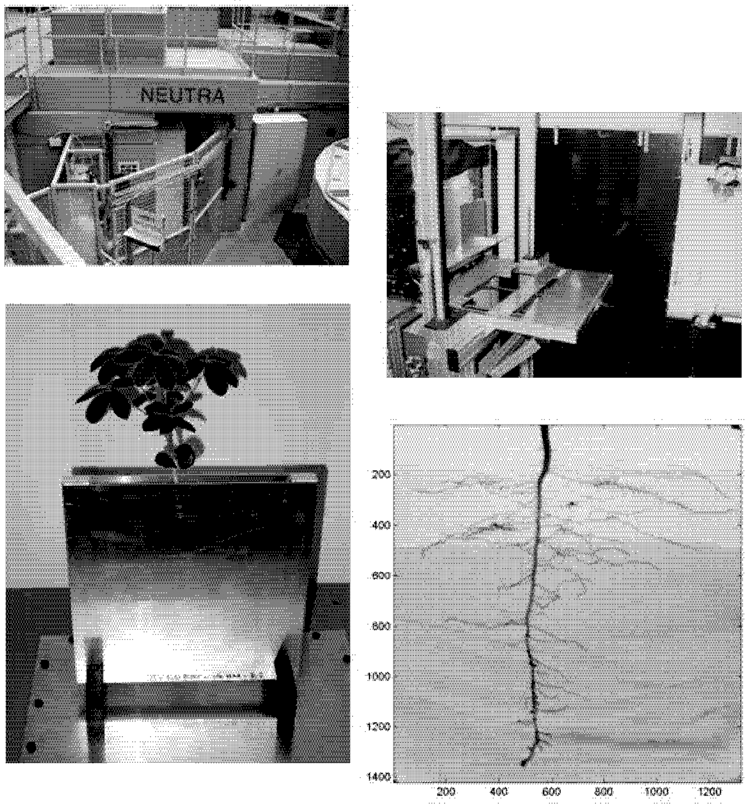

6. Visualisation of Root Growth in Heterogeneously Contaminated Soil using Neutron Radiography

Visualisation of root growth in heterogeneously contaminated soil using neutron radiography

(Submitted to *European Journal of Soil Science*)

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Summary

We used Neutron Radiography (NR), a non-invasive and *in situ* technique to study living plant roots in soil. Plant roots have higher water content than their unsaturated surrounding media. As water strongly attenuates a neutron-beam, NR can identify root structures in great detail. We investigated the use of NR to visualise the root growth of lupin in quartz sand and a loamy sand field soil. Further experiments revealed the root growth of lupin in the loamy sand that was heterogeneously contaminated with 10 and 20 mg kg^{-1} boron (B) and 100 mg kg^{-1} zinc (Zn). We obtained high quality images of root growth dynamics in both media with, a resolution range of 110-270 µm. The images with quartz sand revealed fine structures such as proteoid roots that are difficult to locate *in situ* by other methods without destruction of the soil. Though quartz sand provided excellent visibility of roots, it proved to be a poor medium to grow plants, probably because of its high density (1.8 Mg m^{-3}). The images with field soil showed normal root growth with slightly less contrast than the

quartz sand. The lower contrast was due to the higher neutron interaction with soil water and organic matter in the soil. In the heterogeneously contaminated soil, root growth was significantly reduced in contaminated part of the soil in all treatments of B and Zn. This study shows that NR has potential as a non-invasive method to investigate root growth over time as well as the response of roots to various abiotic stress factors.

1. Introduction

Roots are the hidden half of terrestrial plants. Our understanding of root systems is limited by the difficulty of observing root growth and activity without destruction of the surrounding soil. The development of *in situ* and non-invasive techniques for root measurement would alleviate these difficulties. At present, the non-destructive techniques available for measuring root growth are: a) monitoring by means of minirhizotrons, which are transparent plastic tubes inserted into the ground to view the roots using a video camera, b) X-radiography (Gregory *et al.*, 2003; Pierret *et al.*, 2003), and c) neutron radiography (Willatt & Struss, 1978; Willat *et al.*,1978; Couchat *et al.*, 1980; Furukawa *et al.*, 1999). Although non-destructive, minirhizotrons have an invasive component (Majdi, 1996), while neutron radiography (NR) and X-radiography (X-ray) are complementary non-invasive imaging techniques. X-rays interact with the electronic shells of atoms whereas neutrons interact with the nuclei of atoms. Therefore, neutron radiographs and X-radiographs show different characteristics of the imaged object. Both methods can provide two-dimensional images in transmission, such as in traditional medical X-ray imaging, or be used to perform three-dimensional tomography, as in the case of X-ray computer tomography (CT).

Radiography techniques (X-ray or NR) are based on the exponential law (similar to the Lambert-Beer law) of attenuation of radiation passing through matter (Kasperl and Vontobel, 2005):

$$I = I_0 e^{-\Sigma d} \qquad [1]$$

where, I is the attenuated neutron flux [$L^{-2}T^{-1}$] after an incident neutron flux I_0 passes through a material of thickness d [L] with an attenuating constant Σ [L^{-1}], which is a characteristic of the material.

Since materials differ in their attenuating behaviour, a neutron beam passing through a sample provides information about the composition and structure of the sample. Neutron radiography is a powerful tool for imaging water distribution in a sample, since hydrogen has a relatively high Σ value. The volumetric water content of plant roots generally ranges between 70 and 95%, while that of soils at field capacity usually ranges between 5 and 30% (Kramer, 1969). This difference in water content provides sufficient contrast to distinguish roots from surrounding soil using NR. The neutron radiograph of a sample is a result of all the neutron attenuation processes occurring while the neutron beam penetrates a sample. Therefore, the visibility of roots depends on the thickness, water content or organic matter of the porous media. For example, dry quartz sand has higher neutron transmission than a dry soil of same thickness and moisture content. Some workers used neutron radiography in the 1970s and 1980s to study early germination and root growth of corn (Couchat *et al.*, 1980) and soybean (Willatt & Struss, 1978; Willatt *et al.*, 1978). At that time low-resolution images were acquired using imaging plates or films and required much longer exposure times than modern CCD (Charge Coupled Device) image capture methods.

Recently, NR has improved due to fast digital imaging and increased resolution. Digital imaging techniques using a CCD camera combined with image processing tools yield quantifiable images with a resolution of about 100 μm. The required exposure time for such images is in the order of seconds. Images acquired using imaging plates or films required several minutes of exposure to the potentially damaging neutron beam. These advances open up the possibility of using this technique to study root system development in soils and simultaneously monitor soil moisture distribution.

Here we use neutron radiography to measure root growth in response to elevated concentrations of B and Zn in soil. Several investigations have shown, at high concentrations, these essential elements inhibit plant root growth (Breckle, 1989; Fargasova, 2001; Helmisaari *et al.*, 1999; Ebbs & Kochian, 1997; Ewais, 1997; Arduini *et al.*, 1995). Both these elements are more soluble under acidic conditions (McLaren and Cameron, 1996). In plants, B and Zn concentrations are usually in the range of 10-50 mg kg^{-1} and 20-100 mg kg^{-1} respectively (McLaren and Cameron, 1996).

The spatial distribution of soil contaminants is usually heterogeneous, not only over scales of meters and beyond, but also at smaller scales. The heterogeneous distribution of soil contaminants affects plant root-growth. The roots of some tree species avoid hotspots contaminated with heavy metals (Dickinson *et al.* 1991; Breckle and Kahle, 1992). Conversely, roots of the Zn hyperaccumulator *Thlaspi caerulescens* actively forage Zn-rich hotspots in soils (Schwartz *et al.*, 1999; Whiting *et al.*, 2000).

The response of roots to heterogeneously distributed soil contaminants may profoundly affect their uptake by plants as well as their leaching into receiving waters. The underlying principles of how heterogeneously distributed trace elements influence plant root growth is not well understood. In order to reveal such mechanisms, it is important to visualize root growth and activity with minimum disturbance.

The aim of this study was to elucidate the potential of NR to monitor root growth in heterogeneously contaminated media. For this purpose, we chose to investigate the growth of lupin (*Lupinus albus* L.) in soil that was heterogeneously contaminated with B and Zn.

2. Materials and Methods

2.1 Neutron radiography system

The experiment was performed at the NEUTron RAdiography facility (NEUTRA) at the Paul Scherrer Institute (PSI), Villigen, Switzerland (Lehmann *et al.*, 1999). The Neutron Radiography (NR) facility consists of neutron source, collimator, sample, and image detector. The neutron generating source can be a reactor, the target of an accelerator, or a neutron emitting isotope. The NEUTRA facility at PSI uses neutrons emitted from a lead (Pb) target that is bombarded with high energy protons (Pleinert *et al.*, 1997). High energy neutrons are then converted to thermal neutrons using a heavy-water moderator.

The collimator is a beam-forming assembly that determines the geometric properties of the beam and contains filters that reduce the intensity of gamma rays. The image resolution depends on the collimator geometry and is expressed by the collimation ratio L/D, where L is the collimator length and D is the diameter of the inlet aperture of the collimator on the side facing the source. In our study, a neutron flux of 3×10^{10} m^{-2} s^{-1} was used with a mean energy of 25 meV and a collimation ratio of L/D = 550. The beam was transmitted through the sample and a plane position sensitive detector recorded the resulting image. This detector records a two-dimensional image that is a projection of the object on the detector plane. We used a CCD camera detector with an array of 1024 x 1024 pixels, giving a resolution of 110-270 µm in the digital images.

2.2 Material testing

We grew the plants quartz sand and a natural soil (loamy sand). Sand and soil samples were prepared in slab-shaped aluminium containers because of the transparency (low attenuation) of aluminium to neutrons. We used slabs with inner dimensions of 0.17 x 0.15 x 0.013 m. Preliminary tests revealed that a slab thickness of 0.013 m gave good contrast in the images, while providing adequate space for root development.

2.2.1 Experiment 1

Quartz sand

Two slabs were filled with acid-washed dry quartz sand, with grain size of 100 to 500 μm. The average bulk density of packed sand was 1.8 Mg m^{-3}. Seeds were sown directly onto the surface of the slabs, a single seed in the centre of each slab. The plants were grown in a controlled environment chamber at temperature of 21/16 ^{o}C (day/night), daily photoperiod of 16 h, using fluorescent lighting (9000-10,000 Lux) for 10 days before first imaging. The sand slabs were irrigated with Hoagland's nutrient solution (Hoagland and Arnon, 1938) with a pH of 6.

Loamy sand field soil

Three slabs were prepared with an acidic loamy sand soil (Haplic Alisol), which was obtained from a site along the river Rhein under mixed deciduous forests situated in the vicinity of Zurich, Switzerland (texture: 87% sand, 8% silt, 5% clay, pH = 4.2, organic matter = 3.2 g kg^{-1}, bulk density = 1.4 Mg m^{-3}). The plants were grown in the controlled environment chamber for 2 weeks before the first imaging. Soil slabs were irrigated with tap water.

During the imaging period, planted slabs were not removed from the NEUTRA facility for safety reasons. Fluorescent lighting and air conditioning maintained a day/night temperature of 21/16^{o}C and a daily photoperiod of 16 h. Images of the sand slabs were taken after weekly for a period of 4 weeks. In the soil slabs, we stopped imaging after the 3rd week due to a faster root growth, which had filled the entire soil volume of the slabs.

2.2.2 Experiment 2

Based on the results of Experiment 1, which proved soil to be a better growing medium, we selected the loamy sand for further experiments. In order to determine a suitable level of contamination to be applied in the imaging experiment, we performed two pot experiments using soils spiked with B and Zn to study the growth of lupin (*Lupinus albus* L*)*, maize (*Zea mays*), poplar (*Populus tremula*), willow (*Salix viminalis*) and mustard (*Brassica juncea*). Potting mixture was mixed with 0,.6, 12, 25, 50 and100 mg kg^{-1} of B and 0, 125, 250, 500, 1000 and 2000 mg kg^{-1} Zn. We found that concentrations between 6 and 25 mg kg^{-1} B and 125 mg kg^{-1} Zn were sufficient to induce toxicity symptoms in leaves of lupin, yet not result in plant death.

2.2.3 Experiment 3

Based on the results of Experiment 2, we chose 3 treatments: two levels of B (10 and 20 mg kg^{-1}) and one level of Zn (100 mg kg^{-1}) for further investigation. We prepared three replicates of slabs containing control soil, homogeneously contaminated soil, and heterogeneously contaminated soil. The treatment levels were achieved by mixing H_3BO_3 or ZnCl into a loamy sand soil.

The heterogeneously contaminated slabs consisted of two vertical bands; A 0.05 m contaminated zone, and a 0.10 m uncontaminated zone (Figure 1). Controls were prepared with only uncontaminated soil. Three replicates were prepared for each treatment. The final bulk density of the soil was 1.4 Mg m^{-3}. The slabs were initially saturated with water to allow settling of soil. In each slab, one seed was sown in the centre, i.e. in the uncontaminated zone. Plants were irrigated every second day to maintain a water content at around 25%. After two weeks in the above-mentioned growth chamber, the slabs were transferred to the NR facility for imaging. At the conclusion of the experiment, the slabs were opened and the root mass was measured directly. For the heterogeneously contaminated soil, the slabs were opened at one side and then soil was cut into to 6 segments (0.075 x 0.05 m) and the individual segments were carefully removed. Roots were separated from soil using a 2 mm sieve. After washing and drying, the biomass of the roots was obtained for each individual segment.

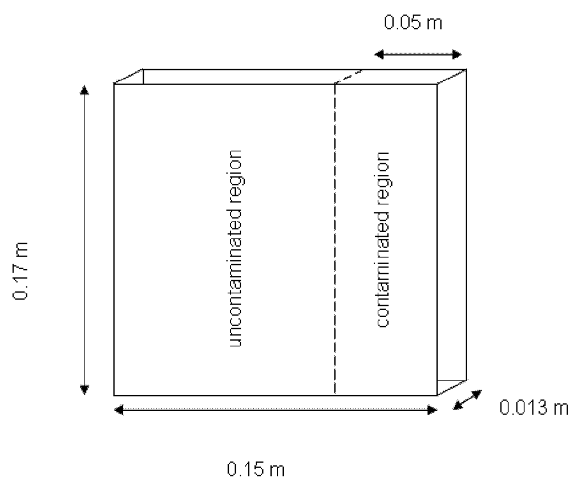


Figure 1. Experimental design of the heterogeneously contaminated slabs

2.2.4 Imaging procedure

The images were taken at least two days after irrigation to decrease the heterogeneity of the water distribution in the slab. This optimized the root-soil contrast at the time when the images were taken, without stressing the plants by severe dryness. Samples were moved to the NEUTRA imaging system and fixed in the same position each time using a 3-dimensional positioning system (Figure 2). This consistent placement was important for the comparison of subsequent images.

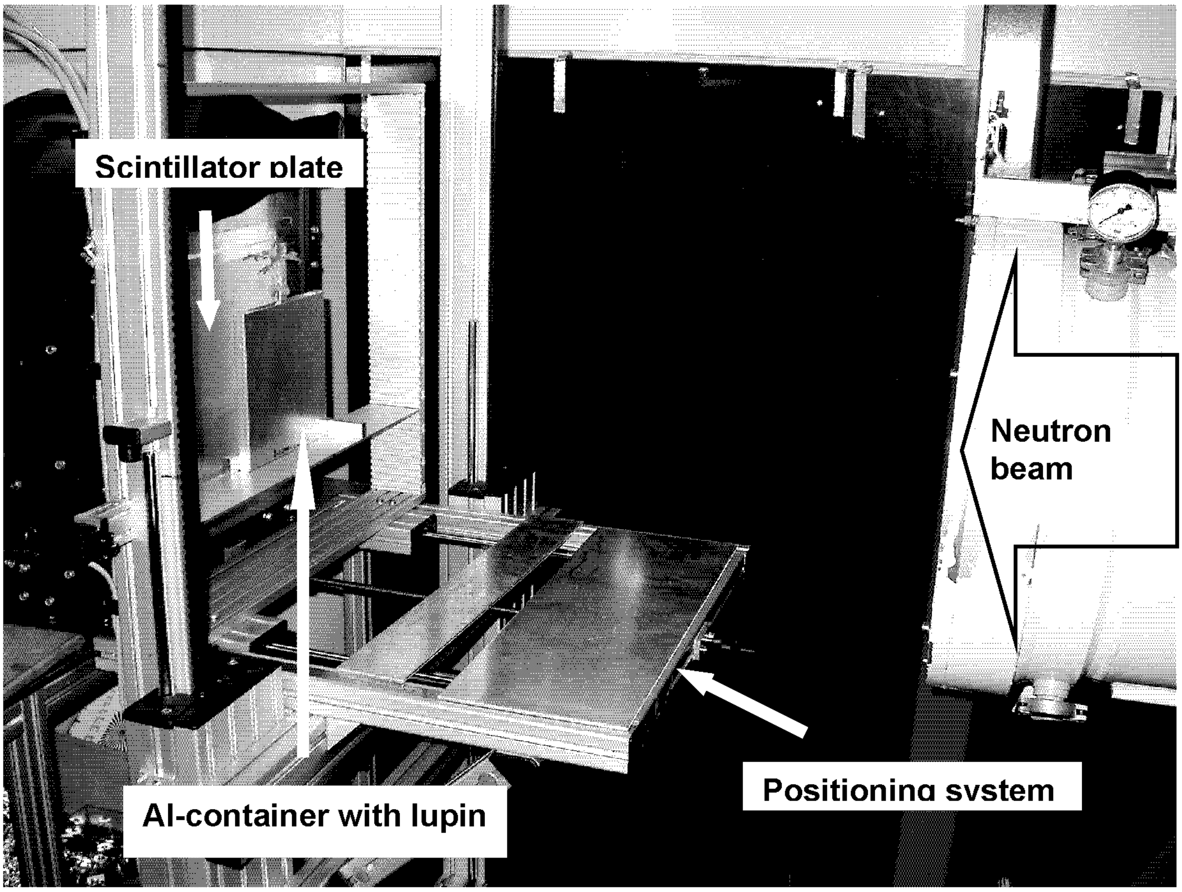


Figure 2. The neutron imaging set up during the experiment. The position of the sample was adjusted using a calibrated positioning system.

We used exposure times of 60 s and 20 s per image for the sand slabs and soil slabs, respectively. These exposure times gave the best signal-to-noise ratio and allowed the differentiation of the main roots from the porous medium. To correct variations of the beam intensity over the image area, we collected open beam images without a sample as well as background images, with neither sample nor beam. The neutron radiation dose received by plants was 0.003 mSv h^{-1}, some two orders of magnitude lower than the minimum value of 0.2 mSv h^{-1} required to affect plant growth (Real *et al.*, 2004).

2.2.5 Image analysis

The radiography images that show the neutron shadow of the sample were first corrected for beam variations and camera noise using a flat field correction:

$$I' = \frac{I_{raw} - I_{dark}}{I_{openbeam} - I_{dark}} \qquad [2]$$

This operation is done pixel-wise, were I_{raw} is the image as registered by the camera, I_{dark} is dark noise image without beam, and $I_{openbeam}$ contains the spatial field variation of the beam without object. The logarithm of the corrected image was computed since the attenuation exponential (Eq. 1)

The image was analysed further to differentiate roots from surrounding media. This was achieved by segmentation the root network

The background grey-level intensity within each image varied so much that a single global threshold could not segment the roots satisfactorily, i.e. the grey-level of roots in one part of the image were the same as the background in another part. Hoover *et al.* (2000) developed an algorithm for segmenting blood vessels in retinal images that had similar characteristics to ours. Hoover’s segmentation method consists of several steps as schematically described in Figure 3. The first step is to enhance the root contrast using a matched filter response (MFR) image (Chaudhuri *et al.*, 1989). The MFR image is computed pixel-wise selecting the maximum response from a set of images filtered by a bank of oriented Gaussian convolution kernels. The intensity variations in the MFR image are flattened. Next, a threshold of between 80% and 97.5% of the pixels in the MFR image are labelled as background. The percentage depends on the size of the root network. This pre-segmented image is then skeletonised using a thinning algorithm described by Lam *et al.*, (1992). The final initialization step before the region growing iterations is to remove branch points

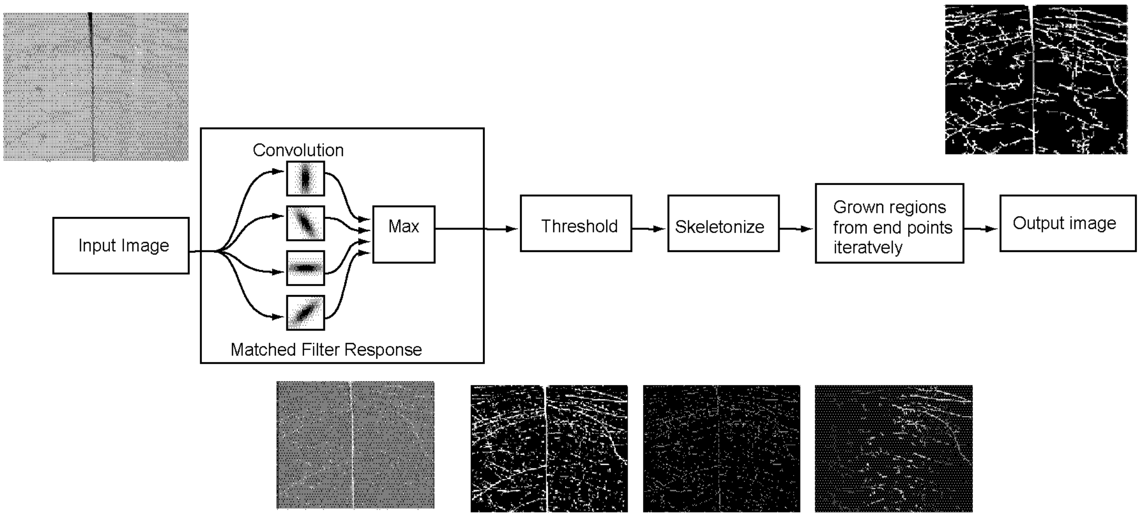


Figure 3. Schematic description of Hoover's blood vessel segmentation method.

in the skeleton and to identify the end-points. The skeleton end-points are used as seeds for region growing. The region growing acts locally on pieces connected to the end points, were all pixels with a grey-level higher than the end point are added to the piece. When all pixels for a given level are collected, a number of shape tests are made. When all tests are positive, the threshold level is decremented and a new iteration is started. If, on the other hand, one of the structure tests fails the threshold probing, then the iteration will be terminated. In order to be labelled as a root segment the piece must not exceed given size criteria. The skeleton endpoints of accepted pieces are added to the list of end-points and the main iteration loop is repeated. The endpoints of the accepted pieces are added to the list of end-points. The iteration procedure is repeated until there are no end-points left to segment.

We calculated root volumes using the Euclidean distance map (D_e) and the skeleton of the segmented roots. We assumed that a large number of cylinder segments with the radius taken as the distance for each skeleton pixel would approximate the root volume. The cylinder height was defined as the pixel resolution. The total volume can thus be described as:

$$V = \pi k^3 \sum_{p \in g} D_e(g(p))^2 g_{SK}(p) \qquad [3]$$

where g is the segmented image and g_{SK} is the skeleton of it, the constant k is the side length of a pixel, and p represents the pixel coordinates. In our investigation we did not compute the volume for the whole image but rather the six segments of the image as shown in Figure 4.

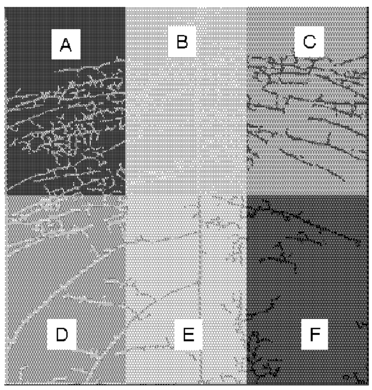


Figure 4. Image regions for volume estimation corresponding to the segments from heterogeneously contaminated soil slabs.

3. Results and discussion

3.1 Comparison of root growth in quartz sand and soil

Figure 5 shows the NR images of root development in the quartz sand after 17 days and soil slabs after 14 days. The contrast and resolution were excellent, particularly in the sand slabs. The sand slabs showed thin horizontal bands of higher moisture content, resulting from the capillary barrier effect of thin beds of coarser sand particles underlying beds of finer sand. The roots can be clearly distinguished from these bands by continuity and orientation (Figure 5a and b).

The root-soil contrast was less in the slabs filled with soil than in the quartz sand. Nevertheless the roots were sufficiently visible to determine their growth pattern (Figure 5c and d). Roots grew much faster in the soil slabs than in the quartz sand. After 14 days, the tap root and the laterals had reached the edges of the containers filled with soil, whereas in sand this was achieved only after 24 days of growth. Furthermore, tap roots were straighter and longer in the soil than in the sand slabs, where roots were more twisted and shorter. In the soil, the laterals branched off in a more regular pattern, grew thicker and followed a rather straight, slightly oblique direction downwards, while in the quartz sand they were more fibrous and spread out. The bulk density of the quartz sand slabs was much higher than of soil slabs. This was the likely reason for the reduced root penetration resulting in a more branched root system (Baver *et al.*, 1972). Therefore, we conclude that although quartz sand provided better quality images than field soil media, its use for studying root growth patterns is limited because it impedes root growth.

An additional problem with using quartz sand was the separation of the size-fractions when filling. The roots grew preferentially along the bands of fine sand that

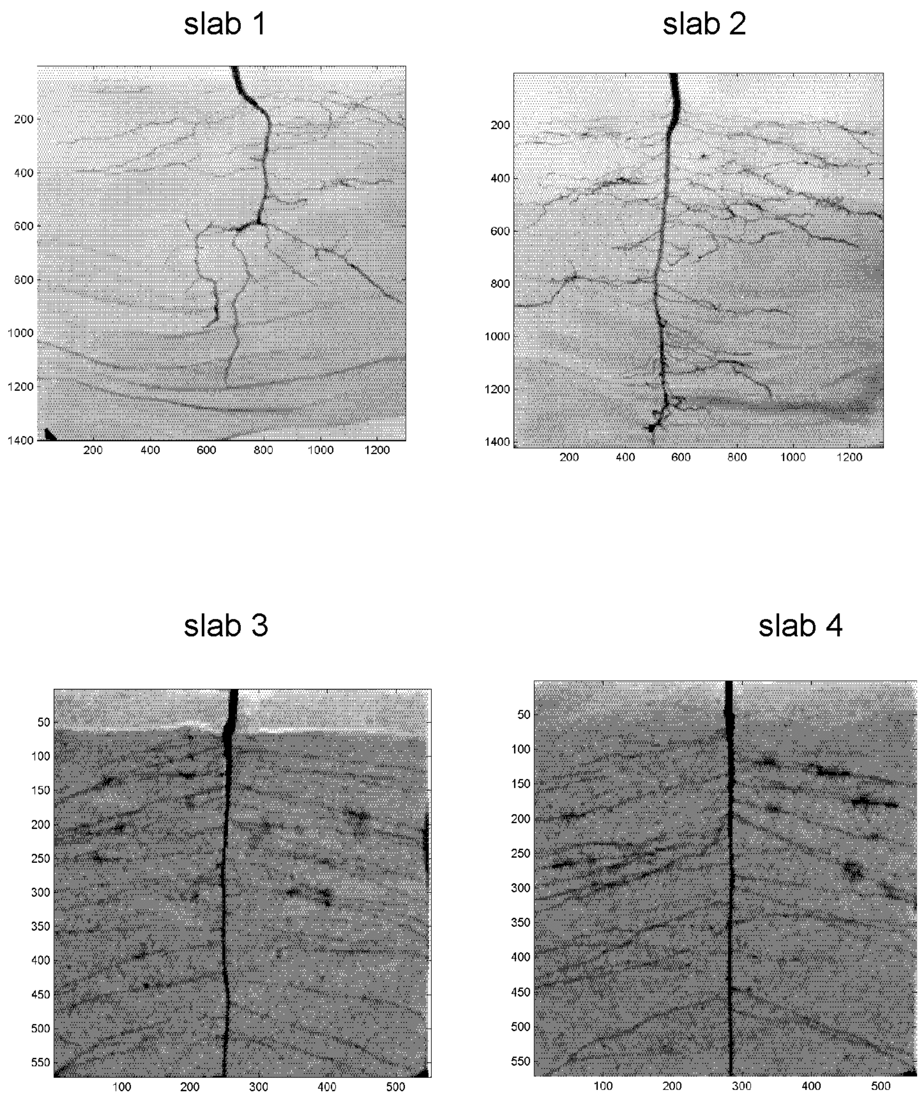


Figure 5. Comparison of root growth in quartz sand and field soil media. Sand (slab 1 and 2) and soil (slab 3 and 4)

had relatively high moisture content (Figure 6a). This shows the potential of NR to monitor variations in the distribution of soil moisture simultaneously with root growth, but creates an extra complication when investigating other phenomena.

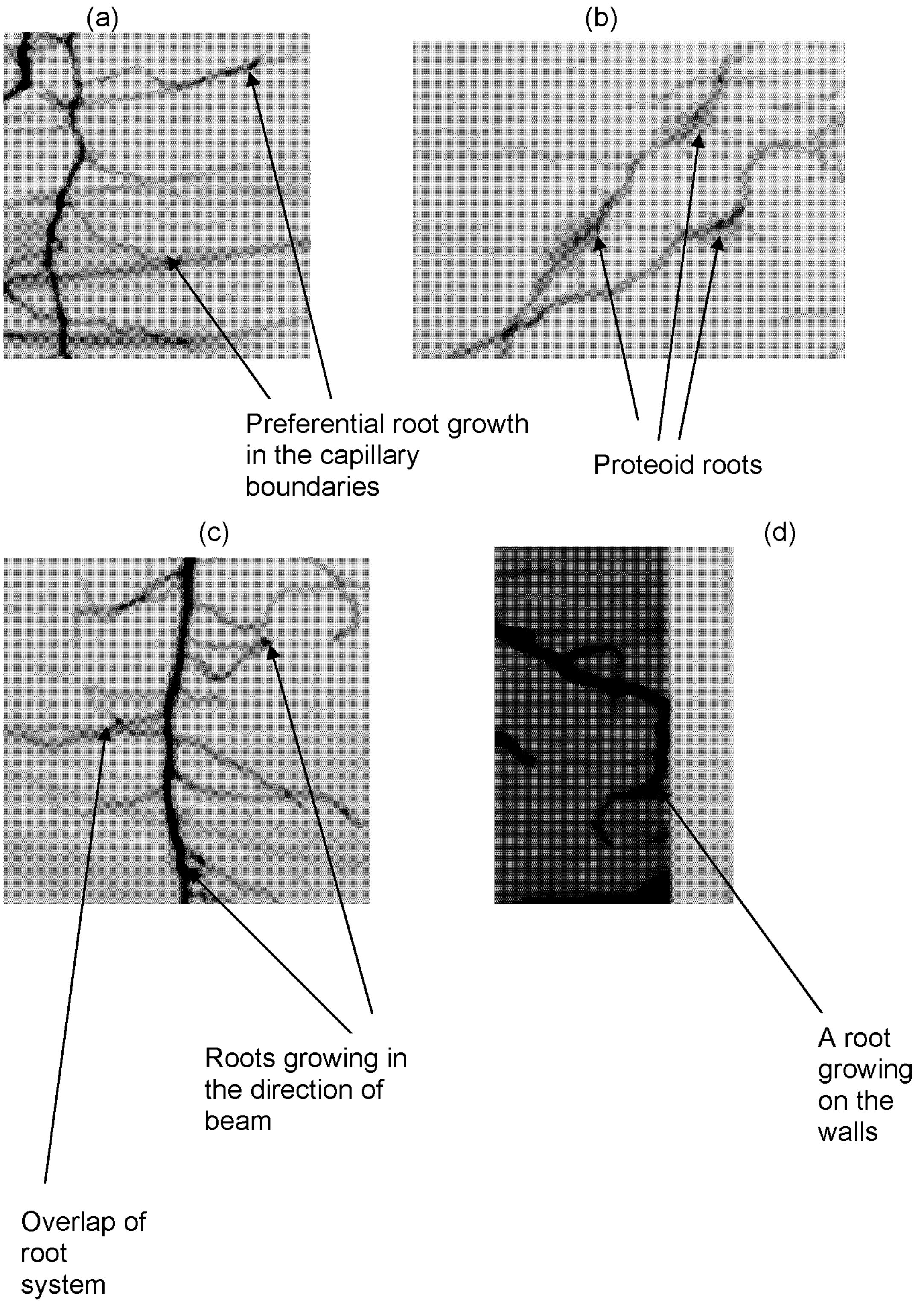


Figure 6. Additional features observed from the images. a) preferential root growth in the capillary barriers; b) proteoid roots; c) dark spots, which results from root growth in the direction of beam or overlap of roots; d) wall effects.

The development of proteoid roots was another feature recognisable in many images (enlarged in Figure 6b). This was observed in both quartz sand and soil slabs, but with lower resolution in the latter. Proteoid roots, also called cluster roots, are stout bottle-brush-like clusters of rootlets. They arise from the pericycle opposite the protoxylem poles along the lateral roots in many plant families (Neumann and Martinoia, 2002).

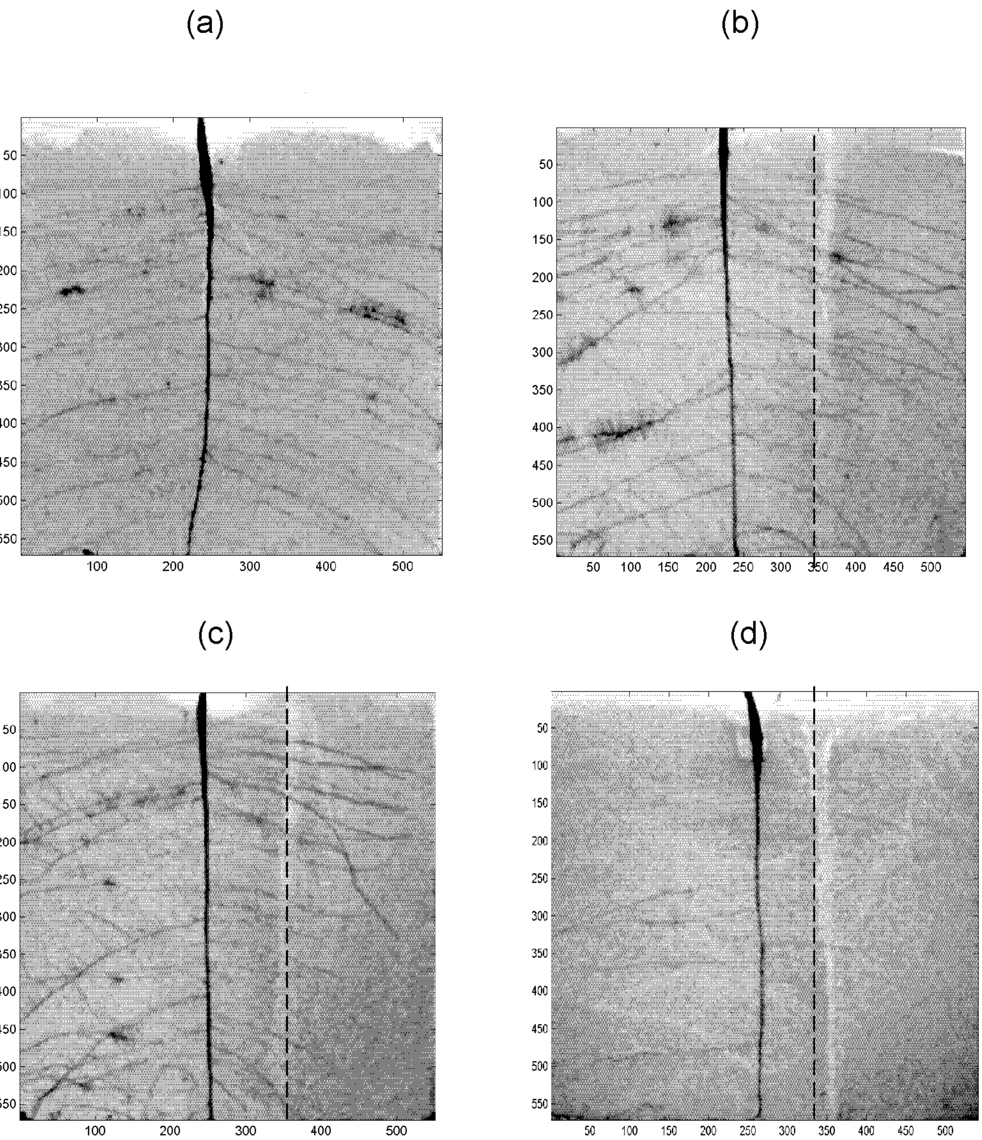
The visibility of roots in NR images depends on their orientation. Roots that grow in the direction of the beam only appear as darker pixels (Figure 6c), because the beam in transmission has a higher amount of water to penetrate and is thus attenuated more strongly. Similarly, darker spots occur where two roots overlap or cross each other. The images demonstrate that the thickness of the porous medium was sufficient to allow growth in all directions. There was enough space for lateral roots to cross each other.

The images further show how roots react when they reach container walls and at about what time this occurred. After 4 weeks only a few roots had just reached the walls, while a few days later, the development of root system was strongly influenced by this space limitation. (Figure 6d). This limitation is a principle problem in pot experiments, as roots start to coil around the walls of the containers within a short time. NR allows to monitor the distance of root tips to their wall and to determine when they reach the walls. This may provide valuable information for planning and interpretation of pot experiments.

3.2 *Root growth under B and Zn contamination*

The spatial distribution of B and Zn affected lupin root growth (Figure 7). In all treatments (Figure 7e, f, g) significantly fewer roots were produced in homogeneously contaminated slabs compared to the control slabs (Figure 7a). In the heterogeneously contaminated slabs, lateral roots initially grew into the contaminated zone. However, as the plants developed root growth was inhibited in the contaminated zone (Figure 7b, c, d). This trend occurred in all heterogeneously-contaminated slabs.

A significant reduction in shoot and root dry biomass was observed in all homogeneously-contaminated slabs compared to both control and heterogeneously-contaminated soils for both B and Zn treatments. In the Zn treatments, shoot and root weights were significantly reduced in the heterogeneously contaminated slabs (Figure 8). The percentage of roots (ratio of roots weight in each segment to the total root weight) was significantly reduced in all contaminated portions of these slabs (Table 1). The lowest percentage of roots was observed in the lower part of the contaminated zones (“F” zone in Table 1). Computed root volumes from the heterogeneously contaminated slabs also showed a linear relationship with measured percentage of roots (Figure 9).


(a)
(b)
(c)
(d)

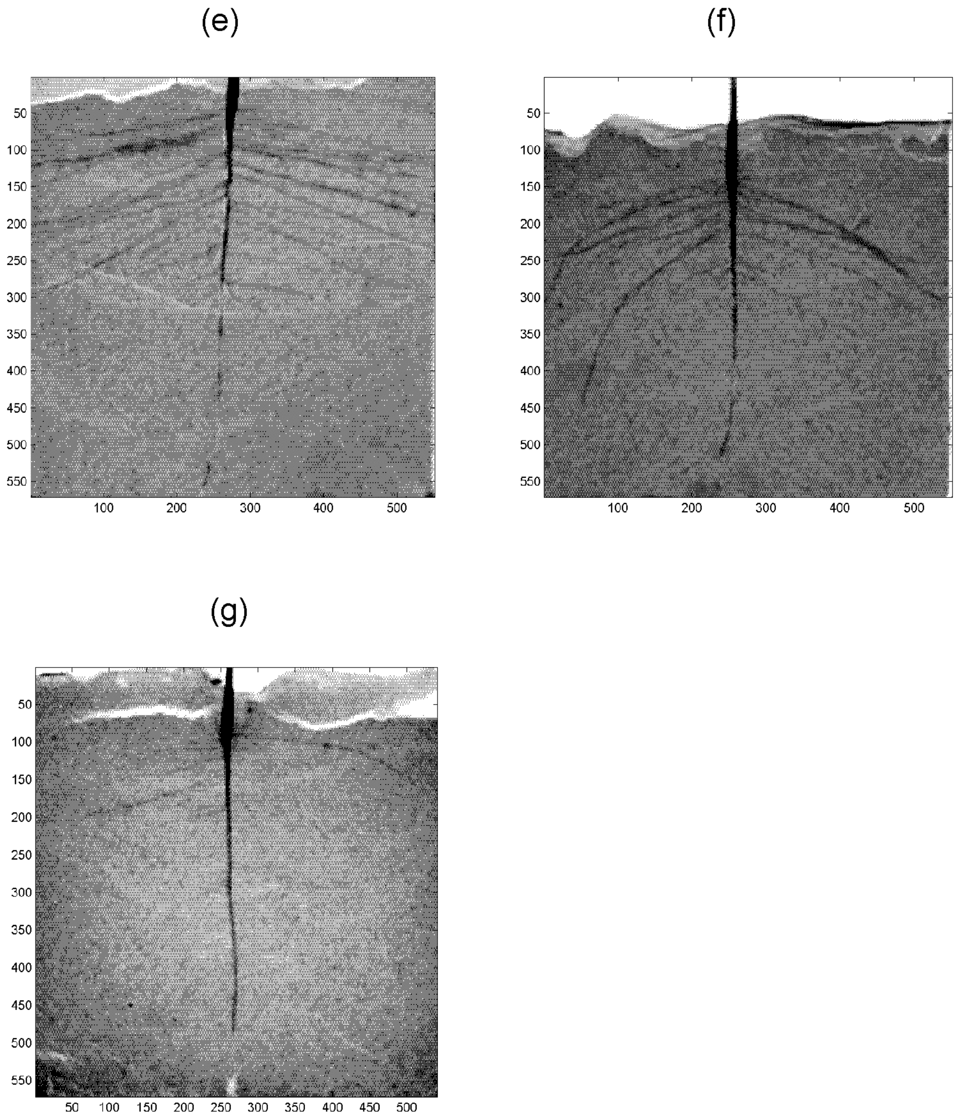


Figure 7. Root growth patterns in slabs with control (a), heterogeneously contaminated with 10 mg kg-1 B (b), 20 mg kg-1 B (c), 100 mg kg-1 Zn (d), fully contaminated with 10 mg kg-1 B (e), 20 mg kg-1 B (f), 100 mg kg-1 Zn (g).

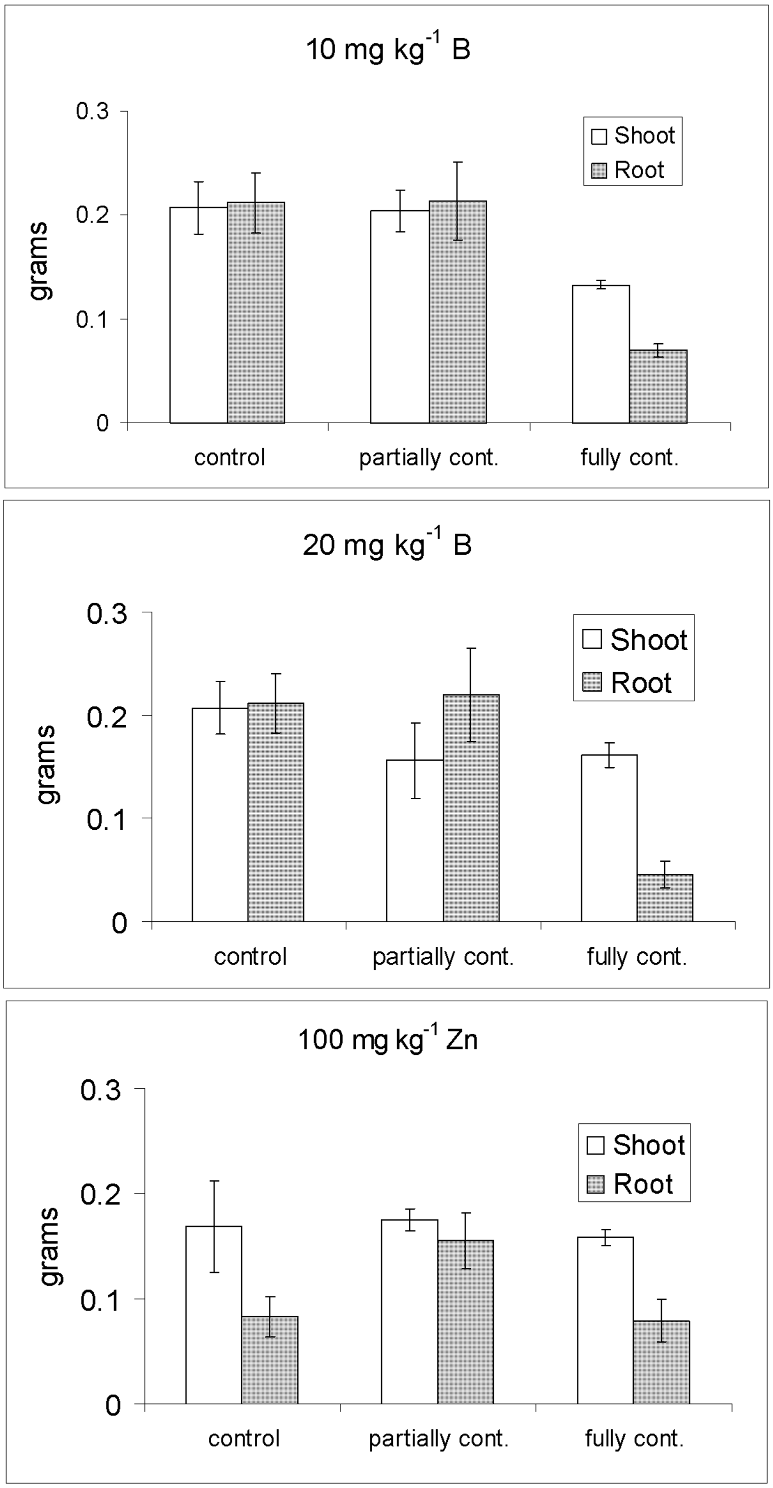


Figure 8. Average shoot and root biomass in control, heterogeneously (partially cont.) and fully contaminated (fully cont.) slabs.

Table 1. Each heterogeneously contaminated slab was divided into six segments of 0.075 x 0.05 m size named from A to F as demonstrated in Figure 4. The table shows the average percentage of roots in each segment of heterogeneously contaminated slabs. Sections A, B, C and D were uncontaminated, while C and F were contaminated

	Concentration of B and Zn in the contaminated segments zone		
Segments	10 mg kg^{-1}B	20 mg kg^{-1}B	100 mg kg^{-1}Zn
A	12.84 ±1.72	10.96 ± 3.58	15.67 ± 13.56
B	52.48 ± 11.57	48.78 ± 1.46	41.18 ± 15.13
C	7.43 ± 1.96	10.74 ± 1.89	6.88 ± 5.45
D	15.03 ± 6.63	17.90 ± 5.58	20.24 ± 4.22
E	9.93 ± 4.15	10.36 ± 1.46	15.50 ± 7.88
F	1.85 ± 0.43	1.15 ± 0.54	0.50 ± 0.50

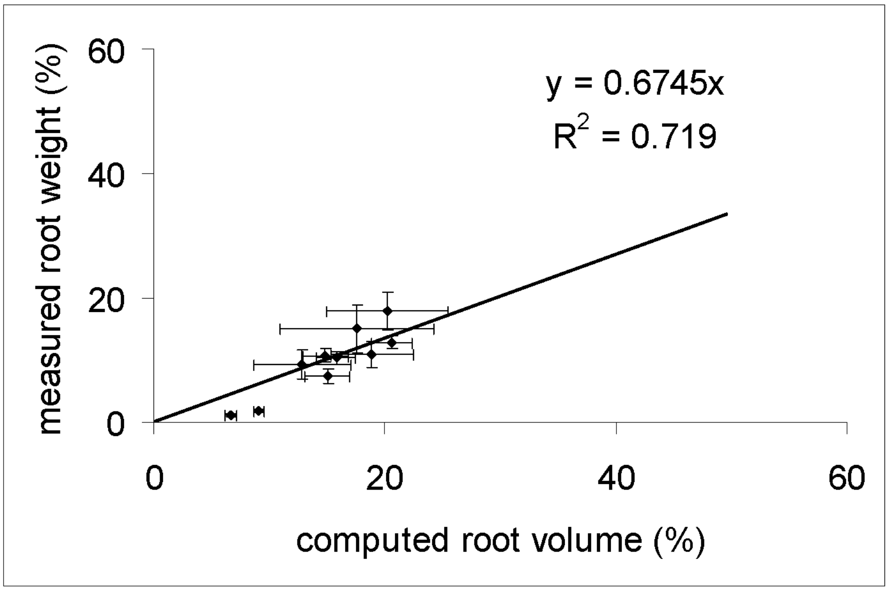


Figure 9. The relationship between the averages (n=3) of percentage of computed root volume and measured root weight of segments in the heterogeneously contaminated slabs. Error bars represent standard error.

4. Conclusions

Neutron Radiography is an effective tool for the non-destructive investigation of root growth. Although sand provided better contrast of roots than real soil, the root growth was significantly perturbed. The root growth in soil gave significant contrast to elucidate the decreased growth in the zones contaminated with B or Zn. Testing new materials under different conditions combined with new image processing techniques will doubtless lead to improved image contrast.

Acknowledgments

This study was funded by Swiss National Science Foundation. We highly appreciate the technical support from Mr. H. Laeser from Institute of Terrestrial Ecology, ETH, Mr. P. Vontobel & Mr. S. Hartmann from Paul Scherrer Institute.

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Chapter 7. Modelling the Water Regime of Heavy Metal Polluted Soil with Young Forest Vegetation

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1. Introduction

Heavy metal contamination of soils has been found to reduce root growth of forest tree species (Arduini et al., 1994; Arduini et al., 1995; Ewais, 1997; Helmisaari et al., 1999). Plant roots develop different strategies in response to contamination in order to compensate water and nutrient uptake. It is for example often the topsoil that is contaminated and there is a strong influence of uncontaminated subsoil, which serves as "safe" region for roots to proliferate and compensate water uptake. With the exception of hyperaccumulator plants, roots have been found to avoid zones of metal stress in soils, in which pollution was distributed unevenly (Palazzo et al., 2003; Romney et al., 1981; Schwartz et al., 1999). It has been also been found that plants compensate water uptake in presence of heavy metal pollution by increasing root density in the uncontaminated part of the profile (Menon et al., 2005). Due to this difference in the root growth either in contaminated or uncontaminated part of the profile, the soil structure and macroporosity might change, which in turn might change soil hydraulic parameters.

Water uptake by plants is usually studied with the help of models as it is difficult to measure directly under in situ conditions. Until now, the water regime of contaminated soil under forest vegetation is not modelled so far. MACRO (Larsbo et al., 2005) is a popular model used for simulating water flow and solute transport in structured soils. MACRO considers macropore flow, which is very common under the field conditions.

The aim was to quantify the effects of topsoil contamination by heavy metal on water regime of a forest model ecosystem in order to identify the role of roots responses to the metals in these effects.

2. Experimental setup and data collection

The study was performed in hexagonal Open Top Chambers (OTCs), which were 3 m high above ground and 3 m wide (internal distance between opposing hexagonal sides). The chambers are equipped with glass walls and roofs. The side glass walls were not completely closed to allow ventilation and the roofs are closed automatically at the onset of rain. Below ground surface, each chamber was divided into two 1.5 m deep concrete-walled soil compartments, each with a surface area of 3 m^2, which are used as non-weighing lysimeters. These compartments were filled with 15 cm of acidic loamy topsoil (36% sand, 49% silt and 15% clay), overlying 80 cm of loamy sand subsoil (87% sand, 8% silt and 5% clay), and 50 cm of a quartz sand drainage layer at the bottom. Two lysimeters were studied in this investigation: in the first the topsoil was contaminated with heavy metals, in the second the topsoil was free from contamination (control). The average heavy metal concentrations in the artificially contaminated topsoil were 2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu and 10 mg kg^{-1} Cd. In 2000, a young forest ecosystem consisting of seedlings of Norway spruce (*Picea abies*), willow (*Salix viminalis*), poplar (*Populus tremula*) and birch (*Betula pendula*) trees and a variety of herbaceous understorey plants were established. The experiment lasted for 4 years. During the growing seasons, the lysimeters were watered artificially. Irrigation water (ambient rain chemistry) was applied using sprinklers between midnight and 5 a.m. The amount of irrigation water and the timing of application varied between 10 to 14 mm per application, every one to four days.

We chose the period from June 1 to July 15, 2003 for investigation, because of the fine time-resolution of the measurements for this period. Water contents and soil water potentials were recorded hourly during the summer of 2003 using a data logger system. Water contents were measured by means of Time Domain Reflectometry using double probes (TDR, model Tektronix 1502B), which were installed vertically in the soil profile at 0-25, 25-50, 50-75 and 75-100 cm. Tensiometers set at depths 10-15, 35-40, 60-65 and 85-90 cm depths (position of ceramic cups), were used to measure the water potentials at mid point of at 25 cm depth intervals covered by the TDR probes. Free drainage water was collected using canisters located at the bottom part of the lysimeters. A vacuum pump was used to empty the canisters weekly or fortnightly and to determine the volume of drainage water. In December 2003, soil samples were collected from the lysimeters using a 75 cm core sampler with a diameter of 5 cm. From each chamber, 6 core samples were drawn and subsequently divided into 0-12.5, 12.5-25, 25-50 and 50-75 cm sections. Root mass was collected and dried and root density was calculated for all depths of lysimeters.

3. MACRO model

The MACRO model was used to simulate and analyze the experiments. MACRO (Larsbo et al., 2005) is a one-dimensional dual-permeability model simulating transient fluxes of water, heat, and solute in the unsaturated zone. The total porosity is partitioned into two separate flow regions (micropores and macropores), each characterised by a degree of saturation, conductivity, water flow rate, solute concentration and solute flux density. The division between the two pore domains is defined by a given boundary water potential ψ_b (m) corresponding to a saturated micropore water content θ_b ($m^3 m^{-3}$) and a saturated micropore haydraulic conductivity K_b ($m s^{-1}$). Vertical water and solute fluxes are first calculated in the micropores and updated values of water storage are used to determine the excess amount of water routed to the macropores. Water fluxes originating in the macropores are then calculated and the solute concentrations in both domains which solve the solute balance are derived. Richards' equation is used to calculate vertical fluxes in the micropores:

$$\frac{\partial \theta_{mi}}{\partial t} = \frac{\partial}{\partial_z}\left[K_{mi}\left(\frac{\partial \psi}{\partial z}+1\right)\right] \pm S_w \tag{1}$$

where *mi* refers to micropores, θ is the water content, *t* is the time[T], *z* is the depth [L] and *K* is the hydraulic conductivity [MT^{-1}], ψ is the soil water potential [L], and S_w is a source-sink term for lateral water exchange between micro- and macroporosity [T^{-1}]. In the micropores, the water retention characteristic $\psi(\theta_{mi})$ is given by the modified van Genuchten's (1980) function, as follows:.

$$\theta(h) = \begin{cases} \theta_r + \dfrac{\theta_s - \theta_r}{\left[1+|\alpha h|^n\right]^{1-\frac{1}{n}}} \\ \theta_s \end{cases} \tag{2}$$

in which θ_r and θ_s denote the residual and saturated water content [$L^3 L^{-3}$], respectively, α [L] is the inverse of the air entry value, and *n* is a pore size distribution index. The hydraulic conductivity of the micropore domain is described by Mualem's Model (1976)

Water flow in the macropores is modelled with a modified kinematic wave approach (Germann and Beven, 1985), where macropores are assumed to drain by gravity. The macropore hydraulic conductivity K_{ma} is expressed as a power law function of the degree of saturation S_{ma}:

$$K_{ma} = (K_s - K_b)S_{ma}^{n^*} \tag{3}$$

where K_s is the saturated hydraulic conductivity of the total pore system, and n^* is an emperical "kinematic" exponent accounting for pore size distribution and tortuosity in the macropores.

Water exchange from macropores to micropores is treated as first order approximation to the water "diffusion" equation that results from Richards' equation when the influence of gravity is neglected:

$$S_w = \left(\frac{3D_w\gamma_w}{d^2}\right)(\theta_b - \theta_{mi}) \tag{4}$$

where D_w is an effective water diffusivity [m^2s^{-1}], γ_w is a scaling factor introduced to match the approximate and exact solutions to the diffusion problem (set to 0.8, see Jarvis, 1994), and d is an effective diffusion pathlength (m). Water flow in the reverse direction (micropores to macropores) occurs instantly if the micropores are saturated.

Root water uptake is described by a simple empirical function, which Jarvis (1989) adapted from Feddes (1978). It is thus assumed that the ratio between actual and potential root water uptake (E_a/E_p) varies as a function of a dimensionless water stress index ϖ^*. The stress index is calculated by combining two functions describing the distribution of roots and water content in the profile:

$$\varpi^* = \Sigma_{i=l}^{i=k} r_i \varpi_i \tag{5}$$

where k is the number of soil layers in the profile containing roots and r_i and ϖ_i are the proportion of the total root length and water stress 'reduction factor' in layer *i,* respectively. Total stress must exceed a critical value of the water stress index ϖ^* (root adaptability factor) before transpiration is reduced. This means plants can adjust to stress in one part of the root system by increasing uptake from other parts where conditions may be more favourable (Jarvis, 1989). Again, a threshold type of response is assumed for the reduction factor, ϖ (Feddes et al., 1974), accounting for soil conditions that are either too wet or dry.

Root length is assumed decrease experimentally with depth (Feddes et al., 1974, Gerwitz and Page, 1974):

$$r_i = \zeta\left(\frac{\Delta z_i}{z_r}\right)e^{-\zeta\left(\frac{z_{m(i)}}{z_r}\right)} \qquad (6)$$

where Δz_i and $z_{m(i)}$ are the thickness and mid-point depth below the soil surface of layer *i*, z_r is the root depth and ζ is an empirical root distribution parameter. The root water uptake is reduced at each depth according to the stress in the layer. The water uptake sink is given as:

$$S = \left(\frac{E_a}{\Delta z_i}\right)\left(\frac{r_i \varpi_i}{\varpi^*}\right) \qquad (7)$$

In MACRO, water is preferentially extracted from in the macropores. Any excess demand is then satisfied from water stored in the micropores.

4. Modelling strategy

The total soil profile was divided into 3 layers: 1) topsoil (15cm), 2) subsoil (80cm) and 3) quartz sand (50cm). The simulations were carried out for the period from May 9 to July 15, 2003 to allow a warming up period of 3 weeks. The initial condition was given by the measured profile of water content at the starting date. Free drainage was set as the lower boundary condition. Since no measurements of evapotranspiration (ETP) were available, ETP was calculated by the model using the Penmann-Monteith equation (Monteith, 1965) using measured weather data. Global radiation and wind speed data were obtained from neighbouring *MeteoSwiss* metrological station, whereas temperature and humidity data were measured within the OTC.

4.1 Model parameterization

MACRO was parameterised using either measured values (crop characteristics), or indirectly, through inverse modelling procedures (hydraulic parameters and root adaptability factor). The inverse modelling software SUFI (Sequential Uncertainty Fitting-Abbaspour et al; 1999) was used the latter latest purpose. SUFI performs a combined optimization and uncertainty analysis using a global search procedure: the procedure starts with a given uncertainty of the parameters to be estimated, which is then with a reduced iteratively. At each step of the procedure, a user-defined goal function is calculated, by comparing simulation results and observed variables. The multiplicative form of Root Mean Squared error (RMSE) was chosen as the goal function in the present study. It is defined as:

$$g(x) = \prod_{i=1}^{n} \sum_{j=1}^{S_i} \sum_{k=1}^{t_i} \left(x_{ijk}^{m} - x_{ijk}^{p}\right)^2 \tag{8}$$

where x^m is a measured variable, x^p is a simulated variable, w_i is the weight for variable i, t_i is the number of measurements over time for the variable i, s_i is the number of measurements over space for the variable i and n is the number of variables used in the objective function.

SUFI was applied in two ways: (1) The parameters of the Van Genuchten equation (Eq 2) were first calibrated on the retention data for the first layer (0-15 cm), for which a the data on water potential and water content covered a large range of the retention curve. (2) SUFI was used together with MACRO for the calibration of the parameters of the Van Genuchten equation for the second layer (15-90 cm) as well as the root adaptability factor, and the saturated volumetric water contents of the micropores for the two soil layers. The target data were the water potentials and the water contents at 4 depths and the cumulative outflows.

We assumed a perennial crop of height 1.5m; a LAI of 8, a rooting depth = 0.75 m (from Menon et al., 2005) and a boundary water tension head of 0.1 m; The parameters for the drainage layer estimated as Ks = 0.250 m hr^{-1}, N = 1.192 and α = 0.054 m.

4.2 Model evaluation

The model efficiency EF was calculated for each variable over the calibration period in order to compare the performance of the model, following (Loague and Green, 1991):

$$EF = \frac{\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2 - \sum_{i=1}^{n}\left(P_i - O_i\right)^2}{\sum_{i=1}^{n}\left(O_i - \bar{O}\right)^2} \qquad (9)$$

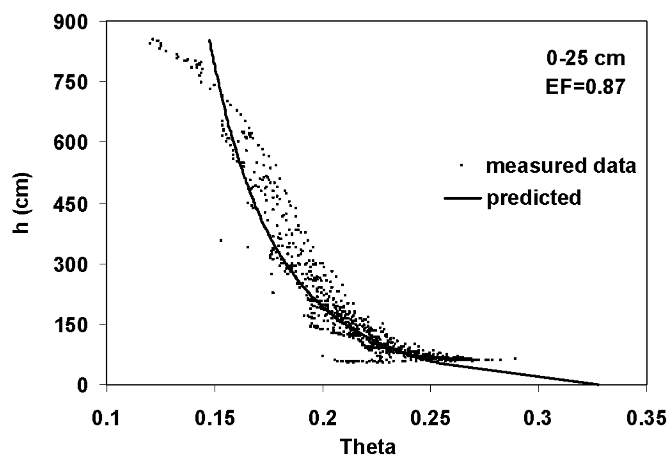
where n is the number of observations; O_i is i^{th} measurement of the target variable, $\bar{O}$ is the average of respective and P_i is predicated values by model prediction of the i^{th} measurement. The best value EF is 1 and a negative value of EF means that the model performs worse than if just the average value of the measurements, would be taken as the prediction at individual point.

5. Results

5.1 Calibration of topsoil hydraulic parameters

Figure 1 shows calibrated retention curves for the contaminated and uncontaminated topsoil. The van Genuchten parameters are given in Table 1.

a) Control



b) Heavy metal

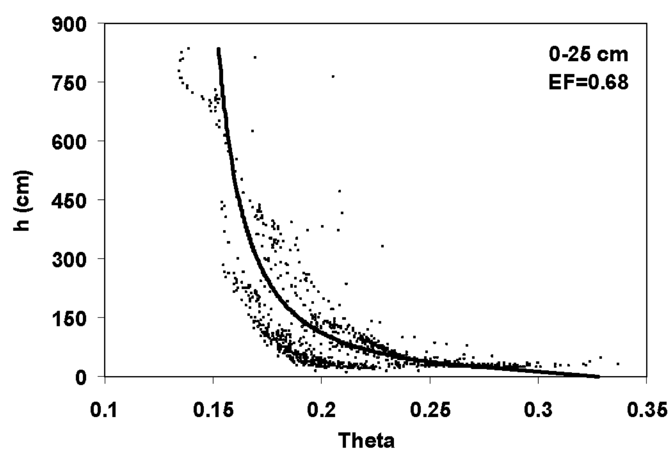
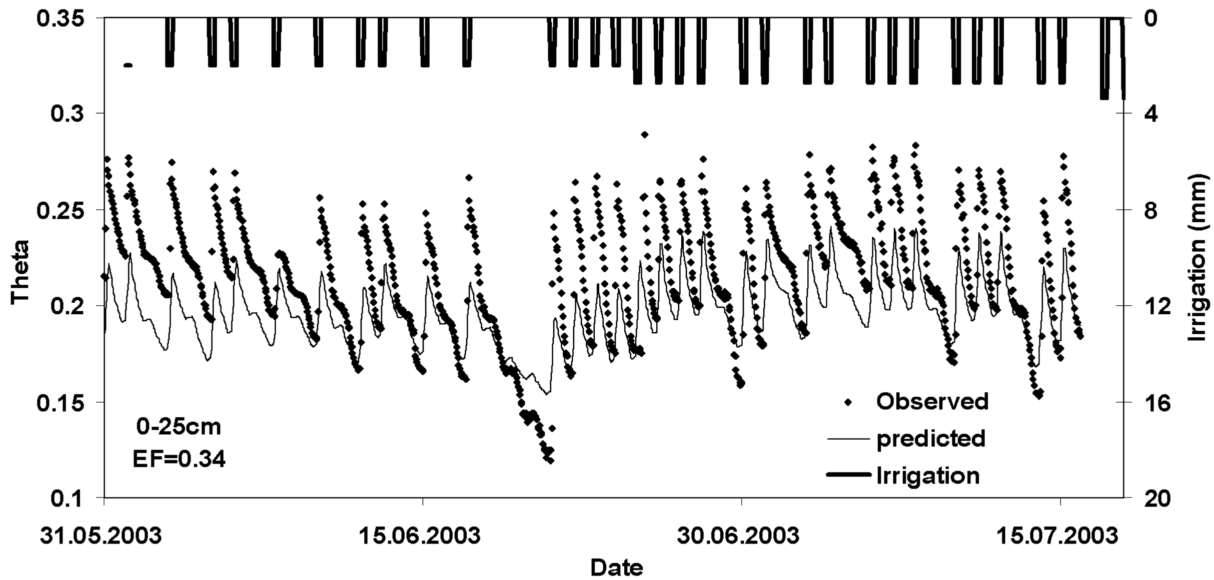


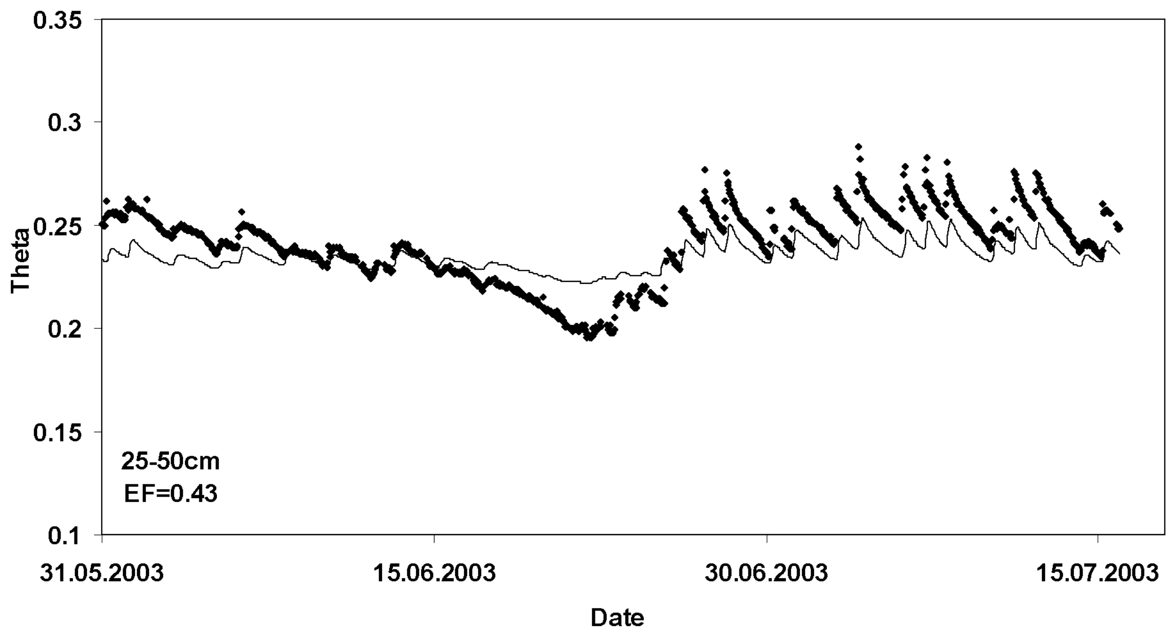
Figure 1. Retention curves of topsoil of control and heavy metal contaminated soil

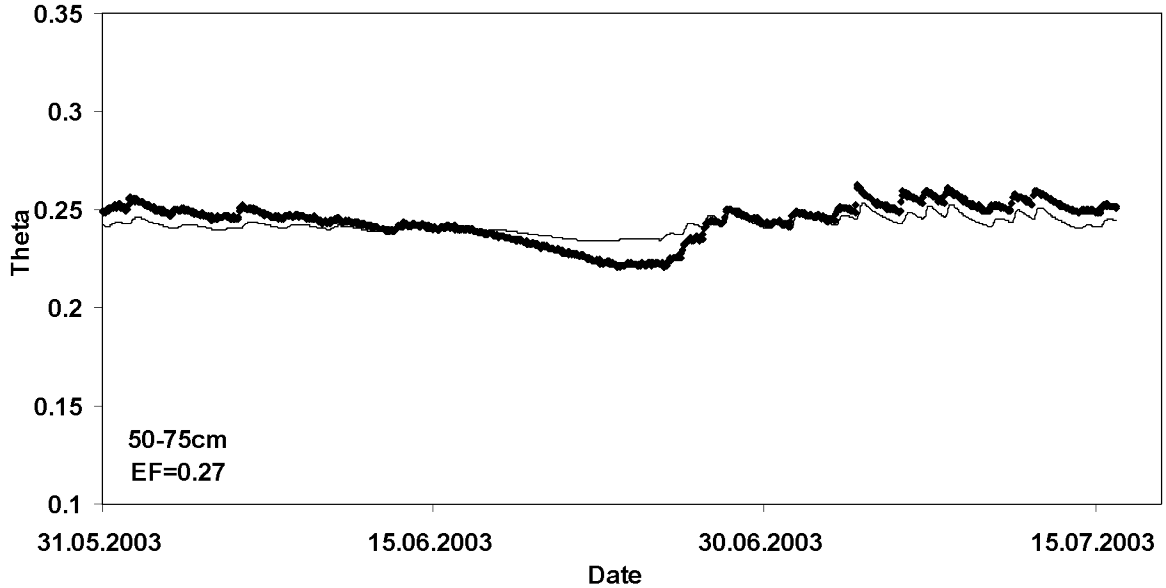
5.2 Model output - control lysimeter

The water contents and water potentials calculated for the control lysimeter are given in Figure 2. Water contents were well described by the model and closely followed the dynamics of the observed data. The model efficiencies were positive in all cases, except for the water potential at 62 cm depth. The water potentials for all layers were under-estimated throughout the simulated period for all layers apart from peak during the drought period that occurred around the June 21, 2003.

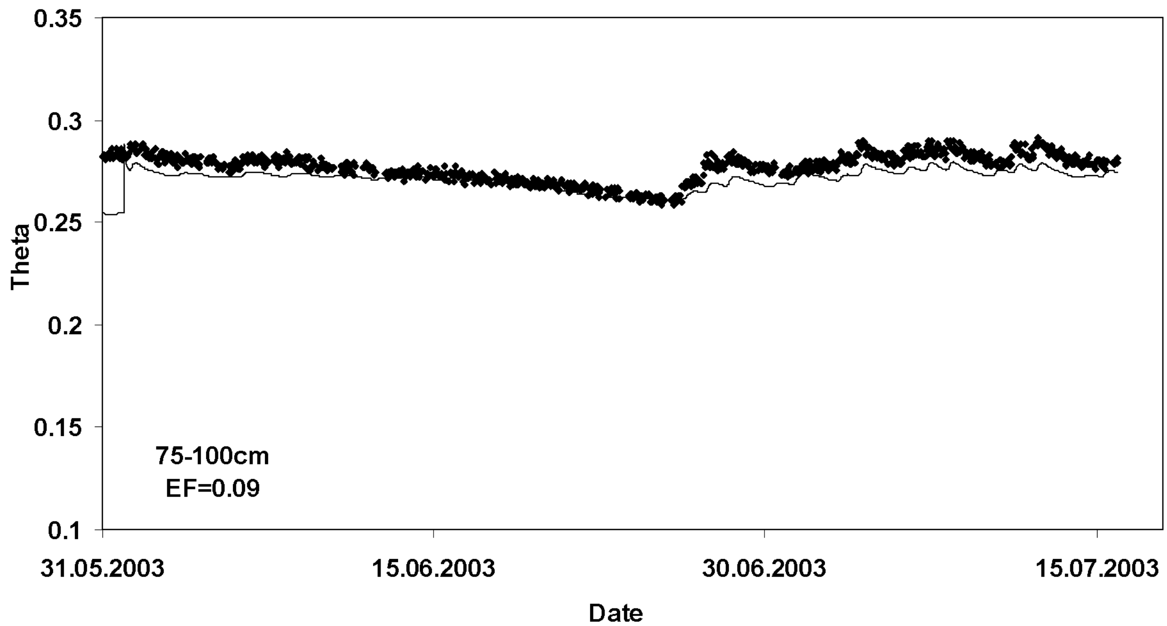
Water content





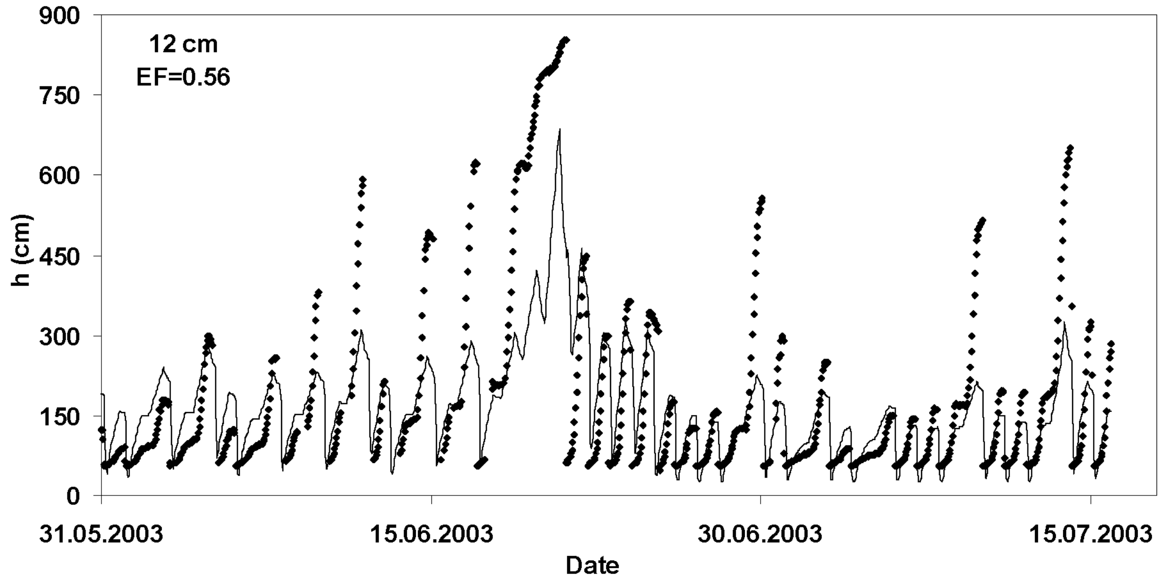


0.35
0.3
0.25
0.2
0.15
0.1
Theta
50-75cm
EF=0.27
31.05.2003
15.06.2003
30.06.2003
15.07.2003
Date



0.35
0.3
0.25
0.2
0.15
0.1
Theta
75-100cm
EF=0.09
31.05.2003
15.06.2003
30.06.2003
15.07.2003
Date

Water potential



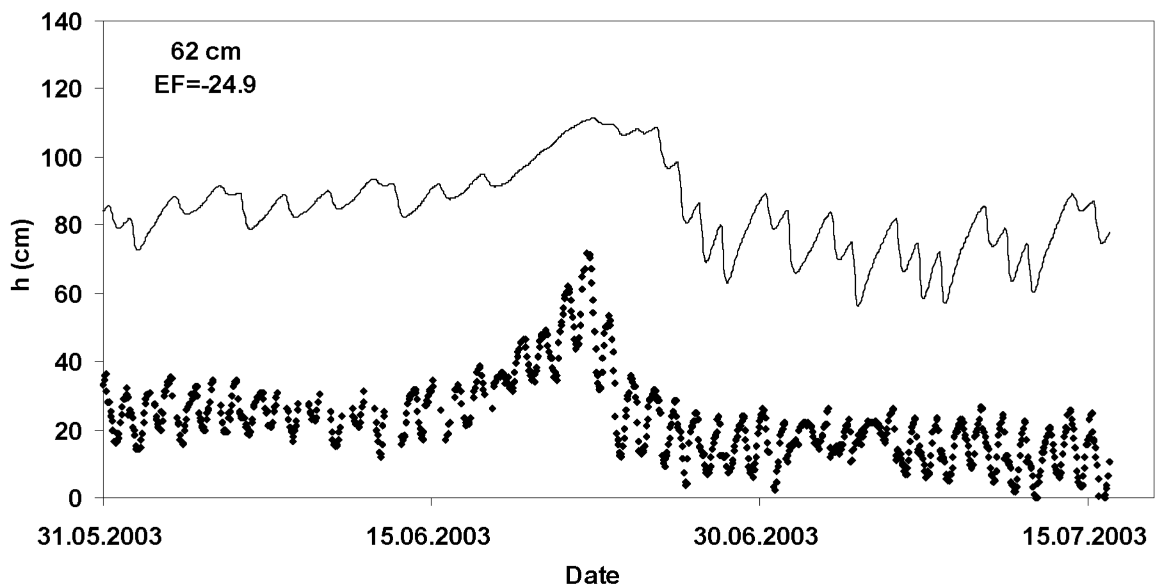


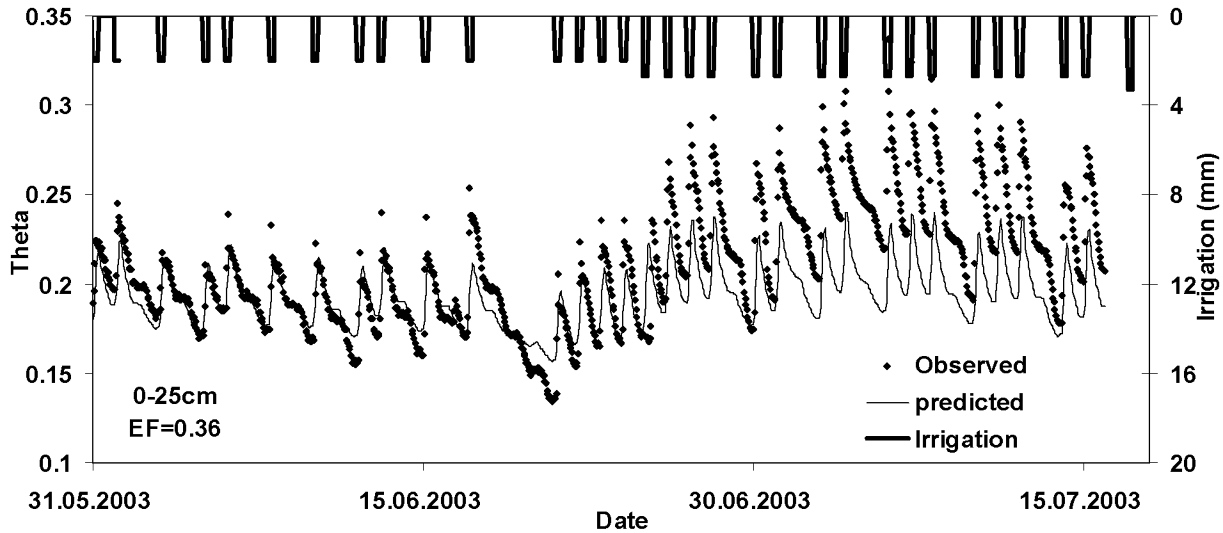
Figure 2. Simulated and observed water contents and water potentials in the control

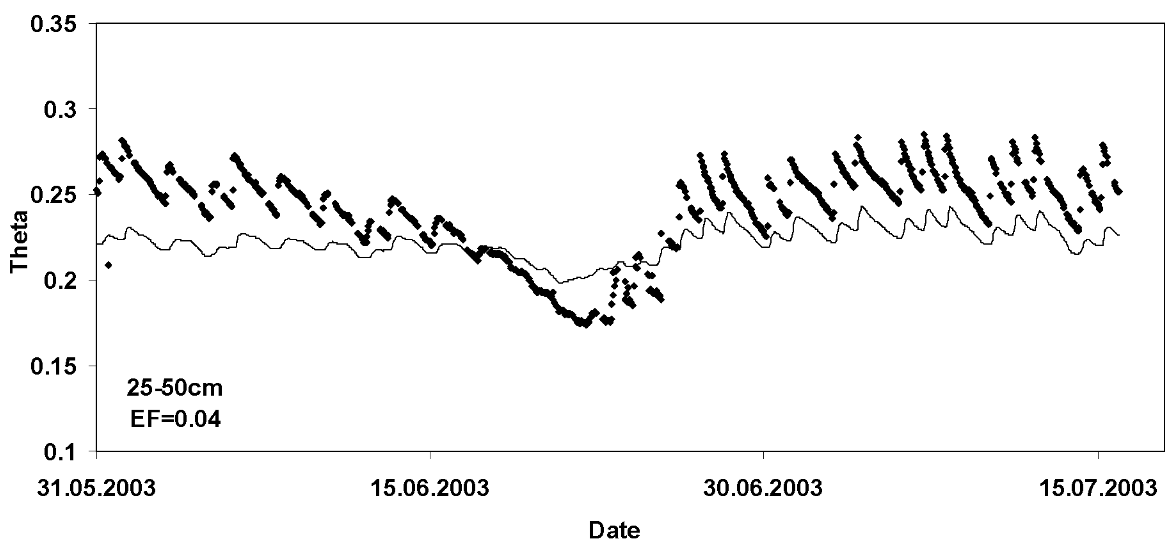
5.3 Model output-heavy metal contaminated lysimeter

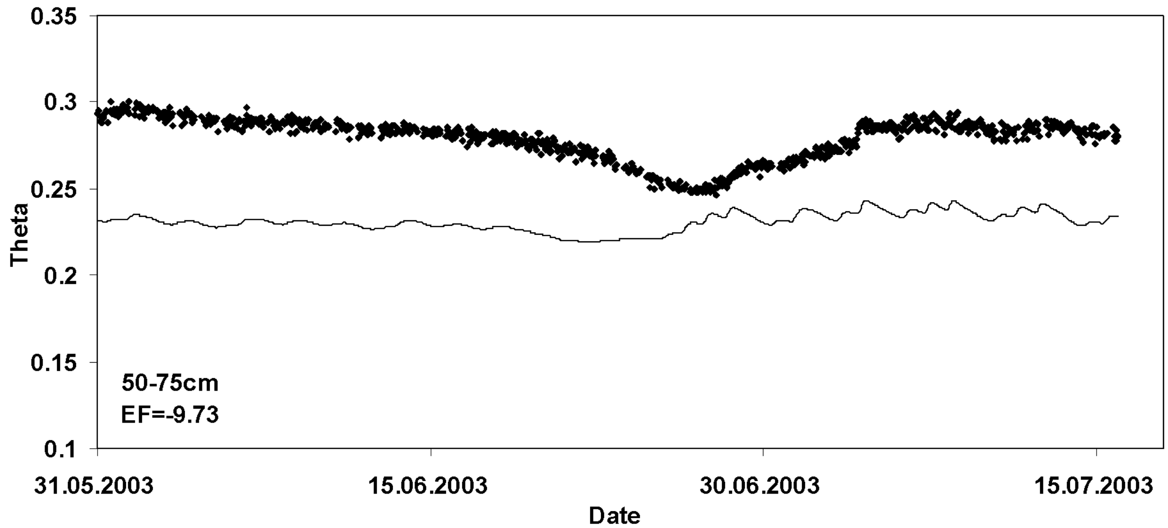
The water content in the contaminated topsoil was not uniformly well described by the model. Prior to the dry period occurring around the June 21, the measured water contents were well reproduced. However, during the second part of simulated period the measurements were underestimated. For the deeper layers, the driest period was simulated approximately three days too early. Moreover, the model underestimated the measured water contents by 20% in average throughout the simulated period, resulting in negative model efficiencies.

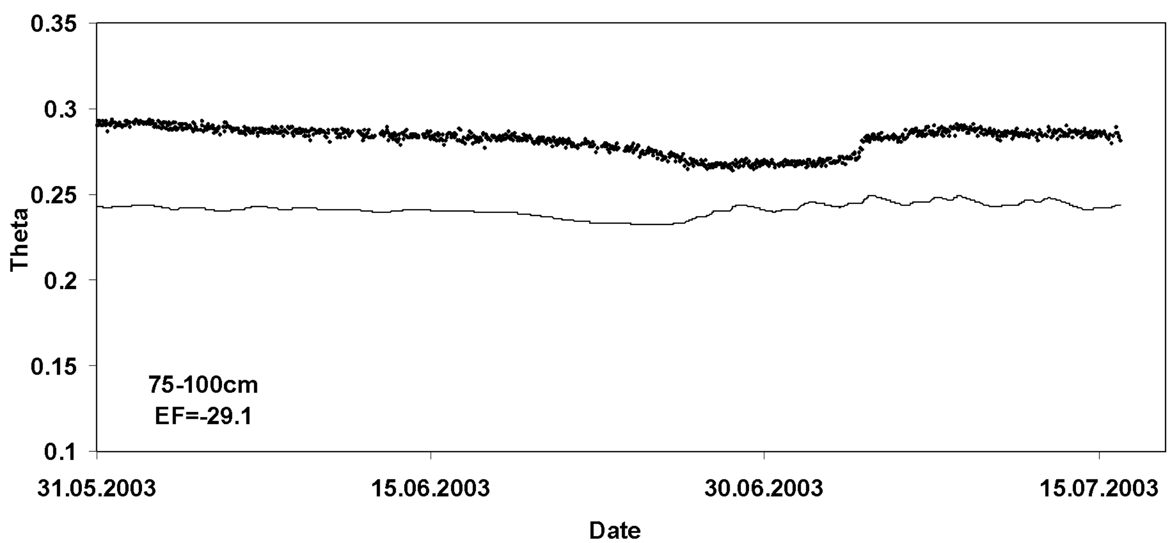
Even though the global pattern of the topsoil water potential was well simulated, the model failed to reproduce the driest period. The model overestimated the water potential at 62 cm, but to a lesser extent after dry period.

Water content

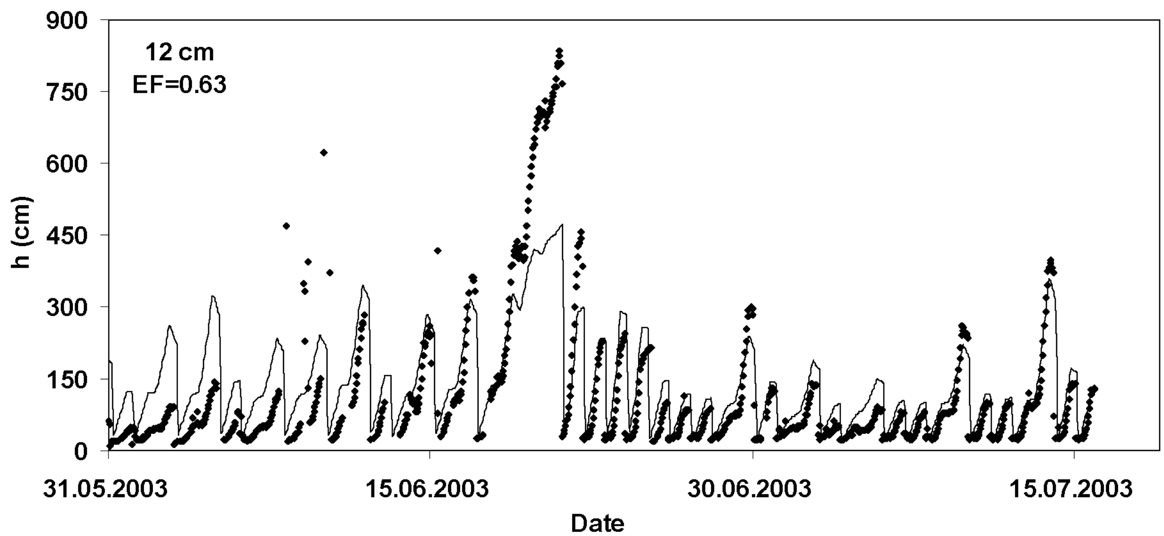





0.35
0.3
0.25
0.2
0.15
0.1
Theta
50-75cm
EF=-9.73
31.05.2003
15.06.2003
30.06.2003
15.07.2003
Date


0.35
0.3
0.25
0.2
0.15
0.1
Theta
75-100cm
EF=-29.1
31.05.2003
15.06.2003
30.06.2003
15.07.2003
Date

Water potential



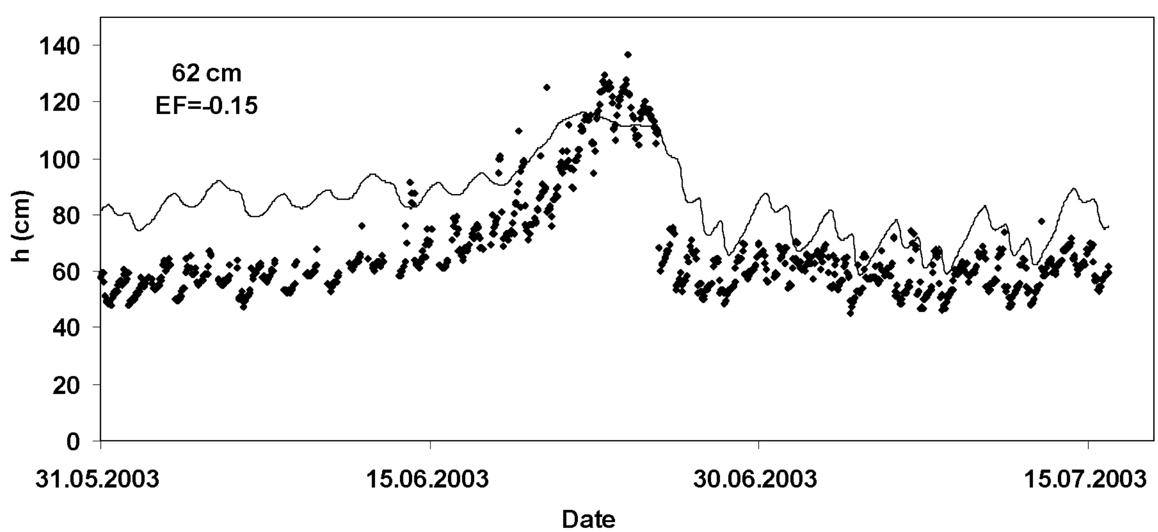


Figure 3. Simulated and observed water contents and water potentials in the heavy metal contaminated lysimeters

Table 1. The hydraulic parameters of the MACRO model estimated using SUFI for the two lysimeter soils.

Topsoil Parameters (SUFI)	Control lysimeters	Metal contaminated lysimeter
θ_r	0.0150	0.110
θ_s	0.328	0.328
n	1.23	1.575
α	0.0485	0.0525
Boundary water content (0-15cm)	0.23	0.24
Subsoil Parameters SUFI-MACRO		
θ_r	0.079	0.100
θ_s	0.325	0.330
n	1.25	1.35
α	0.0450	0.0400
Boundary water content (0-15cm)	0.29	0.3
Root adaptability factor, ω*	0.65	0.1

6. Discussion and future work to be done

The simulated of water contents of the top layer differed between control and metal treatment. The model predicted a drier profile throughout the simulation period in the case of control whereas in the contaminated lysimeter, this underestimation occurred only after dry conditions. This might be because of the subsoil compensation of root water uptake under contaminated topsoil during water stress.

Many of the observed water potential peaks could not be well simulated by the model. Tensiometers measurements are point measurements, and the proximity of roots around the suction cups can lead to large deviation from the potentiald further away the roots.

The simulated forest ecosystem can be considered partially an annual crop (above ground biomass is harvested every season except for spruce) and partially as perennial crop (permanent root system). The model input requirements are different for both situations. The scenario is even more complicated due to the mixed vegetation. The model has now been calibrated for 'perennial' crop with LAI of 8 obtained from literature, which is much higher than the LAI measured for 2003. Since latter value was obtained only recently, there is a need to check above calibration against measured crop characteristics obtained after the experiments such as LAI and crop height. After calibration, the model has to be tested with other replicates.

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Chapter 8. Conclusions

The aim of the project was to experimentally investigate the influence of heavy metal pollution on the root growth and water uptake. The experimental investigations on water regime and growth of trees were carried out at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. The root growth imaging experiments were carried out at Paul Scherrer Institute, Switzerland. The results are given in the following subheads:

1. Water regime of metal-contaminated soil under juvenile forest vegetation

This study was performed as part of a comprehensive project "From cell to tree" at Swiss Federal Institute for Forest, Snow and Landscape Research (WSL), Birmensdorf, Switzerland. In a three-year factorial lysimeter study in Open Top Chambers (OTCs), we investigated the effect of topsoil pollution by the heavy metals Zn, Cu, and Cd on the water regime of newly established forest ecosystems. Furthermore, we studied the influence of two types of uncontaminated subsoils (acidic vs. calcareous) and two types of irrigation water acidity (ambient rainfall chemistry vs. acidified chemistry) on the response of the vegetation. Each of the 8 treatment combinations was replicated 4 times. The contamination (2700 mg kg^{-1} Zn, 385 mg kg^{-1} Cu and 10 mg kg^{-1} Cd) was applied by mixing filter dust from a non-ferrous metal smelter into the upper 15 cm of the soil profile, consisting of silty loam (pH 6.5). The same vegetation was established in all 32 lysimeters. The model forest ecosystem consisted of seedlings of Norway spruce (*Picea abies*), willow (*Salix viminalis*), poplar (*Populus tremula*) and birch (*Betula pendula*) trees and a variety of herbaceous understorey plants. Systematic and significant effects showed up in the second and third growing season after canopies had closed. Evapotranspiration was reduced in metal contaminated treatments, independent of the subsoil type and acidity of the irrigation water. This effect corresponded to an even stronger reduction in root growth in the metal treatments. In the first two growing seasons, evapotranspiration was higher on the calcareous than on the acidic subsoil. In the third year the difference disappeared. Acidification of the irrigation water had no significant effect on water consumption, although a tendency to enhance evapotranspiration became increasingly manifest in the second and third year. Soil water potentials indicated that the increasing water consumption over the years was fed primarily by intensified extraction of water from the topsoil in the lysimeters with acidic subsoil, whereas also lower depths became strongly exploited in the lysimeters with calcareous subsoil. These patterns agreed well with the vertical profiles of fine root density related with the two types of subsoil. Leaf transpiration measurements and biomass samples showed that different plant species in part responded quite differently and occasionally even in opposite ways to the metal treatments and subsoil conditions. They suggest that the year-to-year changes in treatment effects on water consumption and extraction patterns were related to differences in growth dynamics, as well as to shifts in competitiveness of the various species. Results showed that the uncontaminated subsoil offered a possibility to compensate the reduction in root water extraction in the topsoil under metal stress, particularly under drought conditions.

Using the measurements on water regime, we used MACRO to model the water uptake under contaminated lysimeters. The model has been calibrated and it has to be tested with other replicates.

2. Effects of heavy metal pollution and acid rain on growth and water use efficiency of a young forest ecosystem

The four tree species responded quite differently to heavy metal pollution and type of subsoil. The fine root biomass of *P. tremula* was strongly reduced by metal pollution, while the above-ground biomass was not significantly affected. Shoot and root growth of *P. tremula* tended to be higher on the acidic than on the calcareous subsoil, whereas *S. viminalis* produced higher biomass above and below ground on the calcareous than the acidic subsoil. In contrast to the previous growing seasons, the growth of *S. viminalis* was significantly reduced in the metal treatment on calcareous subsoil in 2003. Also in *P. abies* and *B. pendula*, growth was generally less on the acidic subsoil. In contrast to S. viminalis, *P. abies* showed a clear metal effect on growth only on the acidic subsoil, while in B. pendula metals reduced growth of roots and shoots on both types of subsoil. Acid rain produced few significant effects. In the absence of metal contamination it generally tended to increase growth on calcareous subsoil, while clear inhibitive effects were observed only on root growth of *P. tremula* and *P. abies* in lysimeters with acidic subsoil. Treatment effects on water consumption were similar as those on total tree biomass, but weaker in proportion to the respective controls. As a result, water use efficiency tended to larger on calcareous than acidic subsoil, to be slightly enhanced by acidic rain in absence of metal contamination and to be reduced by the metal treatment. None of these trends proved to be significant, however.

3. Visualisation of root growth in heterogeneously contaminated soil using neutron radiography

We used Neutron Radiography (NR), a non-invasive and non-destructive technique, to study living plant roots in soil. Plant roots have higher water content than their unsaturated surrounding media. As water strongly attenuates a neutron-beam, NR can identify root structures in great detail. We investigated the use of NR to visualise the root growth of lupin in quartz sand and a loamy sand field soil. Further experiments were conducted to determine the root growth of lupin in the loamy sand heterogeneously contaminated with 10 and 20 mg kg^{-1} boron (B) and 100 mg kg^{-1} zinc (Zn). We obtained high quality images of root growth dynamics in both media with a resolution range of 0.0109-0.027 cm. The images with quartz sand revealed fine structures such as proteoid roots that are difficult to locate in situ by any other available method without destruction of the soil. Though quartz sand provided excellent visibility of roots, it proved to be a poor medium to grow plants. possibly because of its high density (1.8 Mg m^{-3}). The images with field soil, showed normal root growth with slightly less contrast than the quartz sand. This was because of the higher neutron interaction with soil water and organic matter in the soil. In the heterogeneously contaminated soil, root growth was significantly reduced in contaminated part of the soil in all treatments of B and Zn. This study shows that NR has potential as a non-invasive method to investigate root growth over time as well as the response of roots to various abiotic stress factors.

4. Outlook

This study showed root growth and water uptake under two different spatial scales and the results showed root growth is inhibited under soil contamination and roots behave differently under partially contaminated situations. Also, in future, we need to understand the mechanisms behind roots adaptations partially contaminated using modern techniques in plant physiology and biotechnology. Role of competition between the plants in an ecosystem is to be further investigated.

Acknowledgements

The successful completion of a study, which is the prime objective of a Ph.D. thesis, is rarely achieved singularly. This dissertation, by no means, is an exception. I consider myself fortunate in having been guided over the course of my research by Prof. Dr. Rainer Schulin. His encouragement, support and enthusiasm for the project are the foremost reason for its expeditious completion.

I am grateful to Dr. Eberhard Lehmann for his generous support to do neutron radiography imaging at Paul Scherrer Institute. I would also like to thank Dr. Brett Robinson and Dr. Anders Kaestner for their great help and support to do imaging studies. I am particularly indebted to Dr. Karim Abbaspour and Dr. Stephanie Roulier for helping in the modelling. I also would like to thank Dr. Sascha Oswald for his supervision during his tenure in our group.

It would be impossible for to do experiment without technical support. I got excellent support from Mr. Werner Attinger, Mr. Martin Keller, Mr. Hanspieter Laser, Ms. Anna Grunwald, Mr. Rene Saladin, Mr. Peter Vontobel and Mr. Stephan Hartmann. I am grateful to them.

There is never enough space for the acknowledgement of friends. I am grateful to many of them for their support during my stay in Zurich. I also thank my family, friends and relatives in India for their mental support and prayers.

I am also grateful for the financial support provided by Swiss National Science Foundation for the successful completion of this study.

And finally, without *His* grace and support, nothing would probably work…I thank *Him* for giving me courage and patience in the toughest times.

Yours Sincerely,

Manoj Menon

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