Soil chemical and biological changes through the N_2 fixation of black locust (*Robinia pseudoacacia* L.) - A contribution to the research of tree neophytes

Dissertation

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List of Contents

1 General (background hypothesis) 1

2 Impact of cultivating black locust (Robinia pseudoacacia L.) on soils in Hungary 5

2.1 Abstract 5

2.2 Introduction 6

2.3 Materials and Methods 7

2.3.1 Study sites and soils 7

2.3.2 Soil sampling and analysis 8

2.4 Results and Discussion 10

2.4.1 Comparison of soil properties at the landscape level 10

2.4.2 Comparison between selected paired stands 15

2.4.3 Effects of growing black locust for successive generations 19

2.5 Conclusions 21

2.6 References 21

3 Effects of soil inoculation with Rhizobium and of lime application on plant development and nutrient status of black locust (Robinia pseudoacacia L.) seedlings 25

3.1 Abstract 25

3.2 Introduction 26

3.3 Materials and Methods 28

3.3.1 Experimental design 28

3.3.2 Measurements during the observation period 29

3.3.3 Plant growth and analysis 29

3.3.4 Soil analysis 30

3.4 Results and Discussion 30

3.4.1 Plant growth and biomass increment 30

3.4.2 Chemical analysis of plants 35

3.4.3 Soil analysis 40

3.5 Conclusions 52

3.6 References 52

4 Root-induced pH gradients of black locust (Robinia pseudoacacia L.) seedlings grown in soils with different acidity 56

4.1 Abstract 56

4.2 Introduction 58

4.3 Model-Theory 60

4.3.1 Ionic flux equation 60

4.3.2 Equilibrium reactions with CO_2 60

4.3.3 Cation exchange 61

4.3.4 Root mineral nutrient uptake 61

4.3.5 H^+/OH^- excretion 62

4.3.6 Boundary conditions 62

4.4 Materials and Methods 62

4.4.1 Experimental design and measurements 62

4.4.2 Parameter values used for model calculations 65

4.4.3 Comparison of model calculation results and measurements 67

4.5 Results and Discussions 68

4.5.1 Measured parameters 68

4.5.1.1 Plant analysis 68

4.5.1.2 Soil analysis 73

4.5.1.3 Nutrient balances 78

4.5.2 Multispecies rhizosphere model 80

4.5.2.1 Model results 80

4.5.2.2 Modelled pH changes and comparisons with measurements 83

4.6 Conclusions 89

4.7 References 89

5 Effects of soil sterilization on the growth of black locust (Robinia pseudoacacia L.) seedlings 94

5.1 Abstract 94

5.2 Introduction 95

5.3 Materials and Methods 97

5.3.1 Soil origins and treatments 97
5.3.2 Plant material and plant growth 98
5.3.3 Plant and soil analysis 99

5.4 Results and Discussion 99

5.4.1 Plant growth and biomass increment 99
5.4.2 Plant nutrient content 101
5.4.3 Soil N content 105

5.5 Conclusions 107

5.6 References 107

6 Summary 111

7 Zusammenfassung 116

8 Appendix

8.1 List of recent Publications

8.2 Soil degradation by *Robinia pseudoacacia* L. (Black Locust) in Hungary

8.3 Robinie – Fluch oder Segen für den Waldboden?

8.4 Bodenversauerung unter Robinie – Potenzielle Ursache von Wachstumsminderungen?

8.5 Kohlenstoffspeicherung in Böden nach Aufforstung in Abhängigkeit von der Bewirtschaftungsform

List of Tables

Table 2.1: *Climatic and soil conditions of the five forest regions in Hungary.* 9

Table 2.2: *Mean (± standard deviation) dry mass of the organic layers, bulk density of the soils and stocks of C, N, P and K for the non-calcareous and the calcareous sites (n=number of stands).* 10

Table 2.3*: Mean (± standard deviation) cation exchange capacity (CEC_e), exchangeable base (M_b $\sum Na^+$, K^+, Ca^{2+}, Mg^{2+}) and acid cations (M_a $\sum H^+$, Mn^{2+}, Fe^{3+}, Al^{3+}) in the mineral soils under black locust, oak and mixed stands. Significantly different values ($p<0.05$) between stand types within the same depth are superscripted with different letters (^{a,b,c}).* 14

Table 2.4: *Mean (± standard deviation) total element concentrations in the subsoil (30-50cm) for the selected pairs of Robinia and Quercus stands (n=4).* 16

Table 3.1: *Mean (± standard error) absolute biomass increment of Robinia seedlings in soils with different starting pH and treatments (Inoc. = inoculated; Un-Inoc. = uninoculated) from May 2002 to October 2003 and mean number of nodules per plant in October 2003 (n=15).* 33

Table 3.2: *Mean CO_2 production (± standard error) of different sized nodules per mg nodule dry weight (mg CO_2* g^{-1} DW * h^{-}1) and plant (mg CO_2* h^{-1}) from soils with different starting pH and inoculation treatment, measured directly after harvesting the plants in October 2003 (n=15).* 34

Table 3.3: *Concentrations (mg/g dry weight) and accumulation (mg/plant) of P, S, K, Ca, Mg, Mn, Fe and Al in black locust seedlings from soils with different initial pH values and treatments (inoculated, uninoculated). Data presented as mean values ± standard error (n=15).* 39

Table 3.4: *Mean (± standard error) pH (KCl) values, total element concentrations, exchangeable cation concentrations, cation exchange capacity (CEC_e) and base saturation (BS) in the soils with different lime applications [(a) = unlimed, (b) = moderately limed, (c) = strongly limed] at the beginning of the experiment in May 2002 (n=5).* 40

Table 3.5: *Mean (± standard error) stocks ($kmol_c$/ha) of exchangeable cations in the soils with different starting pH values (pH 3.4, 4.8 and 6.2) and treatments (Inoculated, Uninoculated and unplanted Controls); planted variants n=15, controls n=5.* 43

Table 3.6: *Mean PO_4-P, K, Ca, Mg, Mn, Fe, Al and H concentrations (± standard error) in the percolate under the pots with different initial soil pH and inoculation treatment for the whole observation period (n=16 months).* 48

Table 3.7: *Mean (± standard error) concentrations (mg/L) in and absolute leaching losses (mg) of the different N fractions with the percolate under the soils with different initial pH values and inoculation treatments over the complete observation period (n=16 months).* 51

Table 4.1: *Ion-concentrations in the solution of bulks soils with different starting pH values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 66

Table 4.2: *Mean dimension (± standard deviation), number (nodules) and biomass (dry weight) of plant compartments at the end of the observation period (October 2003); n=10.* 68

Table 4.3: *Mean (± standard deviation) cumulative root length, surface area and volume of fine roots ($D \leq 2.0mm$), medium and coarse roots ($D>2.0mm$) per plant after cultivation of black locust seedlings in soils with different initial pH values [(a), (b), (c)] for one growing season (n=10).* 70

Table 4.4: *Mean (± standard deviation) element concentrations (mg/g) and content (mg) of black locust seedlings at the beginning of the experiment (reference; n=5) and after cultivation under different soil environments [(a), (b), (c)] for six month (n=10).* 71

Table 4.5: *Mean (± standard deviation) accumulation of N_{org}, anions ($\sum H_2PO_4^-$, SO_4^{2-}) and cations ($\sum Na^+$, K^+, Ca^{2+}, Mg^{2+}) in the whole plant, as well as calculated excretion of H^+ or OH^- ions from May to October 2003 depending on the N species taken up by plants (n=10).* 72

Table 4.6: *Mean (± standard deviation) exchangeable cation concentrations, cation exchange capacity (CEC_e) and base saturation (BS) in soils with different initial soil pH values (a, b, c) at the beginning (Start; n=5) and end (End; n=10) of the experiment.* 74

Table 4.7: *Mean (± standard deviation) cumulative leaching losses (mmol per pot) of the mineral N fractions under soils with different initial soil pH values (a b, c) from May to October 2003 (n=10).* 76

Table 4.8: *Mean (± standard deviation) cumulative leaching losses of anions (SO_4^{2-}, PO_4^{3-}) and cations (H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}) per pot under the different soil treatments from May to October 2003 (n=10).* 76

Table 4.9: *Measured mean element content in soils at the beginning (May 03) and at the end (Oct 03) of the experiment, element losses through leaching and plant uptake over the whole study period as well as the calculated element content of soils in October 2003 (n=10).* 79

Table 4.10: *Calculated average daily nutrient uptake rates of black locust seedlings cultivated in soils with different starting pH values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 82

Table 5.1: *Chemical soil characteristics of the Hungarian Robinia sites. Values are arithmetic means with standard deviation for the whole soil profile (0-50cm), n=5.* 98

Table 5.2: *Mean and standard deviation of biomass increment (dry weight) during the observation period (June-October 2003) and number and dry weight of nodules in October 2003 for the soils of different origin and treatment (n=5).* 101

Table 5.3: *N-status of seedlings at the beginning of the experiment mean and standard deviation, n=4).* 102

Table 5.4: *Total concentration of P, K, Mn and Fe (mg/g dry weight) in the separated plant compartments from soils of different sites and treatments. Data presented as mean values with standard deviation (n=5).* ____ *104*

Table 5.5: *Changes of N-status in soils after sterilization with Basamid before replanting soils with black locust seedlings (May 2003); data presented as mean values (n=5) with standard deviation.* ____ *105*

Table 5.6: *Concentrations of nitrate and ammonium at the end of the experiment (October 2003) and absolute change of concentrations during the observation period from May to October 2003, data presented as means with standard deviation (n=4).* ____ *106*

List of Figures

Figure 2.1*: Map of Hungary with the five experimental sites distributed over the whole country.* ____ *8*

Figure 2.2: *Mean C/N ratios for the whole profile and C/P ratios for the organic layer at all non-calcareous sites in Hungary; values are presented as median (for C/N with quartiles: Q25, Q75).* ____ *12*

Figure 2.3: *pH (KCl) values at all non-calcareous soils in Hungary; values are presented as median with quartiles (Q25; Q75)* ____ *13*

Figure 2.4: *Percentage of base (M_b) and acid (M_a) cations of cation exchange capacity for two paired stands at Pusztavács forest district. Values are presented as medians (n=4 repetitions) quartiles.* ____ *17*

Figure 2.5: *Mean (± standard deviation) pH_{KCl} values, N-stocks and exchangeable base (M_b) and acid cations (M_a) for paired stands of Hungarian forest sites: Pusztavács (a), Baktalórántháza (b), Nagyatád (c) and Iván (d); four replicates for each horizon.* ____ *18*

Figure 2.6: *Mean nitrogen concentrations and stocks under black locust stands with different continuance of black locust influence. Values are presented as medians with quartiles (n=number of stands with four replications per horizon).* ____ *19*

Figure 2.7: *Mean concentrations of exchangeable Al^{3+} and H^+ under black locust stands of the first and second tree generation. Values are presented as medians with quartiles (n=number of stands with four replicates per horizon).* ____ *20*

Figure 2.8: *Mean pH_{KCl} values under black locust stands of the first and second tree generation. Values are presented as medians with quartiles (n=number of stands).* ____ *21*

Figure 3.1: *Experimental arrangement of the pot experiment.* ____ *28*

Figure 3.2: *Absolute height and diameter growth of black locust seedlings cultivated in soils with different starting pH and Rhizobium application in 2002 (first year), 2003 (second year) and for the complete observation period from May 2002 to October 2003. Data presented as medians (n=15) with quartiles (Q25, Q75). Bars denoted with different letters (a, b) are significantly different between inoculation treatments within the same lime treatment and measuring interval; bars denoted with * are significantly different in comparison to the unlimed soil variants within the same inoculation treatment and time interval.* ___ *32*

Figure 3.3: *N-concentrations and -accumulation in plant components of seedlings cultivated in soils with different acidity (starting pH values = 3.4, 4.8 and 6.2) and inoculation treatments (Inoc. = inoculated; Un-Inoc. = uninoculated). Data presented as medians with quartiles (n=15).* ___ *36*

Figure 3.4: *Relative distribution of N content among different plant parts at the beginning (Reference; n=5) and end (different starting soil pH and inoculation variants; n=15) of the experiment. Data presented as medians (± quartiles).* ___ *37*

Figure 3.5: *Mean pH-(KCl) values and base saturation (BS %) in the soils with different lime application [(a) = pH 3.4, (b) = pH 4.8, (c) = pH 6.2] and inoculation treatment (inoculated & uninoculated) at May 2002 and October 2003; values presented as medians with quartiles (Reference May 2002 and Control n=5; inoculated and non-inoculated planted pots n=15). Bars denoted with different letters (a, b, c) are significantly different between variants (inoculated, non-inoculated & controls) within the same soil pH variant; bars denoted with + or - show significantly higher or lower values compared to the Reference in May 2002.* _ *42*

Figure 3.6: *Mean stocks of K_2SO_4 extractable N fractions in soils (0-60cm) with different $CaCO_3$ application [(a), (b), (c)] and inoculation treatment at the end of the experiment in October 2003 (n=30) in comparison to the values before replanting in May 2002 (n=5); data presented as medians with quartiles.* ___ *45*

Figure 3.7: *Mean soil water efflux per month for the whole observation period from June 2002 to October 2003 under the soils with different initial pH values and inoculation treatment (Inoc. = Inoculated, Un-Inoc. = Uninoculated, Ctrl. = unplanted Controls). Values presented as medians with quartiles (n=16 months).* ___ *47*

Figure 3.8: *Mean (± quartiles) leaching losses of NO_3^- [mg] and base cations (M_b) [$mmol_c$] soils with different starting pH values and inoculation treatments (Inoculated; Uninoculated) in pots planted with Robinia pseudoacacia for the observation period from June 2002 to September 2003 (planted variants n=15, unplanted controls n=5).* ___ *49*

Figure 4.1: *Arrangement of rhizotrons in the insulated wagon (left) and a rhizotron unit with two single rhizotrons (right).* ___ *63*

Figure 4.2: *Cumulative root length increment over the complete experimental period (calculated as differences from reference plants) and average root diameter at the end of the experiment for seedlings grown in soils with different initial pH_{KCl} values (a, b, c); data presented as medians with quartiles (n=10).* ___ *69*

Figure 4.3: *Relationships between excess cations in the plant tissue and nitrogen accumulation in the plants and between excess cations and dry matter production of plants*

cultivated from May to October 2003 in soils with different $CaCO_3$ application (□ unlimed soil; ■ moderately limed soil; ▲ strongly limed soil). 73

***Figure 4.4:** Content of the different K_2SO_4 extractable N fractions per pot (mmol) in May (Start) and October (End) 2003 of soils with different initial pH values; data presented as medians with quartiles (Start n=5; End n=10).* 75

***Figure 4.5:** Measured pH values in bulk soil (3mm in front of the root tip) and in the rhizosphere (from 2mm in front of to 8mm behind the root tip) in soils with different initial pH_{KCl} values (a, b, c); data presented as medians with quartiles (n=6).* 77

***Figure 4.6:** Average (n=10) growth development of the root surface areas (cm^2) of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)] for 120 days.* 81

***Figure 4.7:** Average (n=10) cumulative Ca^{2+} uptake (µmol $plant^{-1}$) of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)] for 120 days* 82

***Figure 4.8:** Calculated radial pH gradients (curves) and calculated rhizosphere pH_{RH} values (unfilled spots) as well as measured mean pH values (filled spots) in the rhizosphere of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 83

***Figure 4.9:** Change of equivalent fractions of protons on the exchanger complex for the three soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 84

***Figure 4.10:** Correlation between measured and calculated pH differences in the rhizotrons cultivated with seedlings in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 85

***Figure 4.11:** Relation between measured and calculated pH differences (based on uptake of non-nitrogenous ions) in the rhizosphere of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.* 86

***Figure 5.1:** Effects of soil sterilization on black locust regeneration on a long-term cultivated site in Hungary. In the front left without and right with soil sterilization.* 96

***Figure 5.2:** Origin of the study soils from Robinia stands in Hungary.* 97

***Figure 5.3:** Cumulative relative height and diameter growth rates for sterilised (+) and non-sterilised (-) soils derived from three different sites in Hungary. Data provided as arithmetic means (n=5).* 100

***Figure 5.4:** N-concentrations (mg N/g Dry Weight), N-content (mg N) and C/N ratios 120 for plant compartments and the whole plants grown in soils sterilized with Basamid and in untreated soils. Data presented as mean values with standard deviation (n=4).* 103

1 General Introduction (background hypothesis)

On a global scale, the cultivation of the nitrogen-fixing tree *Robinia pseudoacacia* L. (black locust) gains more and more importance in forestry and agro-forestry systems. Black locust is native to south-eastern North America, yet worldwide the land area covered by black locust stands has enlarged drastically to about 3 million ha, an area only exceeded by that of Eucalyptus and Poplar (Hanover et al. 1991). In Europe, Hungary is the largest producer of black locust timber. The area under black locust stands there is projected to increase from 320.000 to 360.000 ha in the next twenty years (Molnár 1994).

Black locust is a multipurpose tree, though it primarily is used for erosion control and reclamation of disturbed areas due to its tolerance against drought and severe frosts, its high vitality and regeneration ability and the fixation of atmospheric nitrogen. It is also increasingly profitable and cultivated in short rotation plantations outside of its natural range because of its fast growth compared to other tree species, especially under nutrient deficient soil conditions, its easy and cheap stand regeneration from root suckers and stem sprouts, as well as its excellent wood properties. Moreover, black locust is considered to be extremely resistant to pests as well as fungal and bacterial decay. Only a few fungal pathogens are known to attack this neophytic tree in its introduced range, and most do not have severe impacts on its biological and economical yields. However, it has been observed that the repeated black locust cultivation on the same site leads to growth depressions in the second or in the third tree generation. Currently, the factors which might be responsible for this phenomenon are unknown and rarely researched.

Before the industrial age, rates of atmospheric N deposition were exceedingly low and nitrogen was a growth limiting factor in many forest ecosystems. Most of these inputs were as NO_3^-, generated by lightning-driven N_2 oxidation, and as particulate organic N fixed by microorganisms. Nitrogen deposition in form of fertilizer, animal husbandry and fossil fuel combustion have significantly increased emissions of NH_X (NH_3 and NH_4^+) and NO_X (NO and N_2O) in the last century. Transport and transformations of NH_X and NO_X in the atmosphere resulted in elevated deposition of biologically active N forms in forests, mostly as NH_4^+ and NO_3^-. The atmospheric inorganic N deposition to forest ecosystems in Europe through natural processes and predominately anthropogenic activity ranges from 5 to 80kg N $ha^{-1}yr^{-1}$ (mean 20-30kg N $ha^{-1}yr^{-1}$). In most cases, this exceeds the amount immobilized in the annual growth increment of the forest stands (5 to 20kg N ha^{-1} yr^{-1}). Depending on stand age and density as well as on chemical and biological soil properties, the nitrogen fixation of black locust stands ranges from 35 to 150kg N $ha^{-1}yr^{-1}$. Thus, atmospheric N deposition together with the symbiotic N_2 fixation can lead to a strong N-enrichment in the vegetation and soils under forest stands of *Robinia*.

Of essential interest in forest research is the consequence of additional nitrogen input through symbiotic N_2 fixation by black locust on soil fertility and site productivity. The question arises, what is the fate of the surplus N in these stands and how do the elevated nitrogen inputs influence the soil chemical and biological status and processes. Many experiments and surveys dealing with soil effects of N_2 fixing trees have been conducted for alder species (*Alnus spec.*) associated with symbiotic *Actinomycetes*, but little information is available for black locust associated with *Rhizobium* on the level of forest stands. From studies of mixed stands of trees with and without symbiotic nitrogen fixation and pure stands of N_2 fixing tree species, it can be concluded that net N-mineralization (NNM), net primary production (NPP) and the foliar nitrogen content especially of the non-N fixing trees increase, resulting in higher N concentrations and accumulation as well as lower C/N ratios in the litterfall and soil organic matter. N-mineralization rates that exceed the demands for tree nutrition cause intensive nitrification, nitrogen leaching and gaseous N losses. N transformations in soils under stands with N_2 fixing trees result in extremely high internal H^+ production due to elevated nitrification rates leading to strong soil acidification. Biomass accumulation also contributes to soil acidification, when the nitrogen taken up by plants has its origin from N-fixation, caused by nitrification and subsequent leaching of nitrate and base cations especially in the surface soil and lead to nutrient depletion and soil degradation. These chemical processes appeared consistently across the sites studied. However, the reactions of the systems differed significantly due soil chemical and soil physical conditions. For N_2 fixing plant species growing in soil with different inorganic N amendments, a strong decrease of the rhizosphere pH was detected when plants were cultivated under high NH_4^+ or N-free environments. Plants supplied with NH_4^+ or fixed dinitrogen take up more cations than anions and counterbalance the corresponding excess of positive charges in plant tissue by releasing equivalent amounts of H^+, acidifying their environment.

Soil acidification and elevated concentrations of cation acids (especially soluble Al-species) lead to the dominance of and infection with strains of *Rhizobium*, which are mostly ineffective in their ability to fix atmospheric dinitrogen.

The aim of the present study was to investigate the effects of black locust cultivation on chemical and biological soil parameters and additionally to deduce the processes responsible for the observed growth decline after long-term growth of *Robinia pseudoacacia* on the same site.

It was hypothesized that:

- the N_2 fixation rates of black locust stands are not regulated by the N demands for tree nutrition, leading to N enrichment of the organic layer and mineral soil
- N accumulation results in strong mineralization and nitrification processes coupled with strongly elevated internal H^+ production and leaching losses of nitrate and associated base cations
- the soil inoculation with effective *Rhizobium* strains elevates N_2 fixation rates, plant growth and nitrogen content as well as the above mentioned processes of soil N-enrichment and degradation
- H^+ excretion of the *Robinia* fine roots causes a strong decrease of the rhizosphere pH
- changes of soil chemical properties (acidification & nutrient depletion) due to N transformations coupled with decreased N_2 fixation rates through root infections with ineffective *Rhizobium* strains and enhanced appearance of soil-born pathogens are responsible for the growth depressions after long-term cultivation of black locust on the same site

The hypotheses partly are based on preliminary studies, which have been published elsewhere and can be found in the appendix (compare Goldacker and Berthold, 2002).

To determine the impacts of black locust on the sustainability of soil fertility, four different studies were conducted: (1) field sampling under pure black locust as well as under adjacent oak and mixed (*Robinia* & *Quercus* spec.) stands in Hungary to assess soil chemical changes due to N_2 fixation; (2) a greenhouse experiment with black locust seedlings grown in soils with different pH values and *Rhizobium* inoculation treatments to measure the effects on plant growth, plant nutrient status, chemistry of the bulk soil and chemistry of the soil solution; (3) rhizotron experiments to measure and to calculate nutrient and pH gradients in the rhizosphere; (4) sterilization of soils derived from forest stands of the second *Robinia* generation with observed growth decline to analyze biological factors of the yield decline after repeated black locust growth.

The results presented here were compiled in the form of four manuscripts which have the following titles and which will be submitted for publication soon:

a) Impact of cultivating black locust (*Robinia pseudoacacia* L.) on soils in Hungary
b) Effects of soil inoculation with *Rhizobium* and of lime application on plant development and nutrient status of black locust (*Robinia pseudoacacia* L.) seedlings
c) Root-induced pH gradients of black locust (*Robinia pseudoacacia* L.) seedlings grown in soils with different acidity
d) Effects of soil sterilization on the growth of black locust (*Robinia pseudoacacia* L.) seedlings

2 Impact of cultivating black locust (*Robinia pseudoacacia* L.) on soils in Hungary

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2.1 Abstract

Huge areas have been planted under *Robinia pseudoacacia* in the world, especially in Hungary. Due to its symbiotic N_2-fixation, black locust may improve N and C status of soils but may cause losses of cations through enhanced nitrification and leaching. The humus layer and the mineral soil to a depth of 50cm from five different forest sites of *Robinia pseudoacacia* L. growing area distributed over Hungary were studied for their chemical properties and compared with those under oak (*Quercus cerris and Q. pubescens*) and mixed stands (*Robinia* with *Quercus*). On non-calcareous sites the C, N, P and K stocks in the humus layer under *Robinia* were significantly higher than under oak and mixed stands. In the mineral soil significantly higher stocks of these elements were detected under mixed stands, whereas no differences were observed between black locust and oak stands. High content of soil organic matter (SOM) under mixed stands seemed to be the reason for elevated stocks of these elements. A significant decrease in soil pH under locust stands compared to oak stands was detected in the mineral soil (10-40cm depth), however lowest pH values were measured under mixed stands. Significantly higher proton concentrations and significantly lower Mg^{2+}-concentrations under locust stands confirmed the hypothesis of nutrient depletion by cultivation of tree legumes. On comparing several paired stands of locust and oak, spatial heterogeneity in soil properties was reduced and processes of soil degradation through black

locust forests became more distinct. Another important factor influencing the magnitude of soil degradation by N-fixing trees is the continued cultivation on the same site. Soils under *Robinia* stands of the second generation had significantly higher concentrations of H^+ and Al^{3+} than those of the first generation forests. Lower N-stocks in the humus layer of the second generation stands indicate the losses due to clear cutting for regeneration and decrease in N fixation.

2.2 Introduction

Black locust (*Robinia pseudoacacia* L.) is a nitrogen-fixing tree, native to south-eastern North America (Barett et al. 1990), which grows in two disjunct original areas between the 35^O and 43^O latitude (Little 1971; Fowells 1965; Huntley 1990). The land area covered by black locust plantations has increased in recent decades and is about 3 Mio. ha worldwide, only exceeded by Eucalyptus and Poplar (Hanover et al. 1991). China (1 Mio. ha) and South Korea (0.5 Mio. ha) are the most important producers of black locust wood in the world (Rédei 1998; Claasen 2001). In Hungary the area under black locust stands has increased from 201.000 ha in 1958 to 320.000 ha in 1994 and may increase by another 40.000 ha in the next twenty years (Molnár 1994).

Reasons for cultivating *Robinia pseudoacacia* are: erosion control and reclamation of disturbed areas (Boring et al. 1984a; Chang-Seok et al. 2003), its tolerance against drought, its excellent wood properties and its ability to fix atmospheric nitrogen (DeGomez 2001). It is easy to regenerate from root suckers, grows efficiently on poor sites and improves nitrogen supply and element turnover (Ntayombya et al. 1995). Depending on stand age and density as well as on climatic conditions *Robinia* fixes 35-150 kg N ha^{-1} a^{-1}, indicating a high capacity for N_2 fixation (Hoffmann 1960; Boring et al. 1984b; Danso et al. 1995). This amount of N if fixed for the long-term may exceed the demand for tree growth causing the excess nitrogen to partly accumulate but mostly lost through leaching and denitrification processes. During nitrification and leaching processes soil acidification and loss of base cations may occur causing a significant drop in the pH of the surface soil (van Migroet & Cole 1984). Moreover leaching of nitrate can contaminate ground water (van Migroet & Cole 1985).

During silvicultural treatment of black locust stands where stands are clear cut for regeneration from root suckers and stem sprouts, the N accumulated in the organic matter is likely to undergo fast release and the excess of nitrogen together with nutrient cations can be leached (Feller et al. 2000; Piirainen et al. 2001; Prescott et al. 2003), leading to growth losses in the second and later generations. Such processes and effects have been already described for stands with other N fixing tree, e.g., for red alder by Franklin et al. (1968), Bormann et al. (1981), van Migroet and Cole (1985) and Binkley (1992). Despite the huge area planted under black locust, there is scanty of information on the changes in soils and any effects on the sustainability of wood production on black locust stands. The growth of black locust has been observed to decline when cultivated for several generations on the same site.

Objective of this study is to determine the impact of black locust cultivation on soil properties, with emphasis on nitrogen, carbon and cations in the organic layer and mineral soil. For that purpose soils from black locust stands of Hungary will be compared with corresponding stands of oak and of black locust and oak in mixtures. Moreover the effects of long-term growth of black locust on the same soil for two tree generations are investigated.

2.3 Material and Methods

2.3.1 Study sites and soils

To cover a wide range of soil and climatic conditions *Robinia pseudoacacia* L., *Quercus cerris and/or Q. pubescens* and mixed stands (*Robinia* with *Quercus*) were selected from five different forest regions of Hungary described in (*Figure 2.1*): *Pusztavács, Baktalóránthazá, Kelebia, Nagyatád and Iván*. Altogether 21 stands were investigated (8 black locust, 8 oak and 5 mixed stands). All sites were characterized by low annual precipitation and high mean annual temperatures (*Table 2.1*). The dominant soil types were sandy Typic Cambisols and loamy Typic Chernozems.

Selection of forest sites was done in collaboration with the Institute of Soil Science of the Hungarian University of Sopron and the local Forest Departments. Information about stand characteristics and history (forest compartment, tree species, silvicultural treatment, age, tree-generation,) were provided by the responsible Forest Organizations. Size of forest compartments ranged between 3.0 and 30.0 hectare.

Figure 2.1*: Map of Hungary with the five experimental sites distributed over the whole country.*

The undergrowth of all stands was sparsely developed. Under black locust and mixed stands some nitrophilous species (*Urtica dioica, Chelidonium majus & Linum catharticum*) occurred, whereas oak stands in most cases did not have any shrubs or herbaceous plants.

2.3.2 Soil sampling and analysis

To avoid the influence of adjacent tree species soil samples were taken in a distance of 50m away from the plot border at four points randomly selected. In every stand samples of the organic layer and mineral soil horizons were collected in triplicate. Samples were taken by using a steel corer (diameter = 7.22cm) from 0-50cm depth in intervals of 10cm. Samples were dried at 40° C (mineral soil) or 60° C (organic layer), sieved (2mm) and ground. The total C and N contents of the forest floor and mineral soil were analysed by dry combustion with C/N analyser (Vario Elementar Analysensyteme, Hanau, Germany). Total element concentrations of P, S, K, Ca and Mg were measured using ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion of samples in 65% concentrated HNO_3. Soil samples (2,5g sieved material) were percolated with 1N NH_4Cl and exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+},Al^{3+}) were analysed by AAS 300 AA (Varian Inc., Darmstadt, Germany). pH was measured with a digital pH-meter (WTW GmbH, Wesel, Germany) in 0,1mol l^{-1} KCl.

Table 2.1: *Climatic and soil conditions of the five forest regions in Hungary.*

Site	Elevation (m)	Precipitation Annual/Growth-Period (mm)	Temperature Annual/Growth-Period (°C)	Period of sunshine (hours/year)	Soil type
Pusztavács	100-150	500 / 320	10.3 / 17.3	2050	sandy Cambisols
Bakta-lóránthazá	100-150	570 / 350	9.7 / 17.1	1970	sandy Cambisols
Kelebia	100-150	570 / 330	10.7 / 17.6	2170	loamy, calcareous Chernozems
Nagyatád	100-150	760 / 460	9.9 / 16.2	1990	sandy Cambisols
Iván	200	630 / 360	9.6 / 15.8	1860	loamy Cambisols (pseudogleyification)

The data for calcareous and non-calcareous soils are presented separately due to the differences in their proton buffering.

To reduce spatial heterogeneity of chemical soil parameters on a landscape level, four adjacent black locust and oak stands at non-calcareous sites were evaluated as pairs based on the total concentrations of P, S, K, Ca and Mg in the subsoil (30-50cm), assuming that the differences of variable chemical soil properties (e.g. base saturation) in the organic layer and the main rooting zone (0-30cm) were caused by plant influence. Due to their high proton buffering capacity calcareous sites were excluded. For each selected pair pH, N stocks, concentrations of exchangeable bases and acid cations were compared.

Long-term impact of black locust cultivation was investigated by comparing soil chemical parameters between stands of the first and second tree generations of the non-calcareous sites. Black locust stands of the first generation were 35 to 50 years old, and of the second generation they were 15 to 30 years. The second generation was regenerated through stem sprouts and root suckers after clear fell. Nitrogen status, pH_{KCl} values and concentration of exchangeable cations served as indicators to determine the effects of growing black locust for successive generations.

Analysis of variance (ANOVA) were performed by the program *STATISTICA version 6.0* and the means were tested by the Mann-Whitney U-Test at $p<0.05$ level.

2.4 Results and Discussion

2.4.1 Comparison of soil properties at the landscape level

Besides the vegetation the humus layer is the most important store for carbon and nitrogen. The mean dry mass of the litter layer under black locust stands (5.4 kg/m^2) was significantly higher than under oak (2.8 kg/m^2) or mixed stands (3.0 kg/m^2) at the non-calcareous sites. On calcareous sites no elevated accumulation of organic matter in the litter layer under black locust was observed when compared to other stands (*Table 2.2*). The accumulation of organic matter resulted in significantly higher stocks of nitrogen, phosphorous and potassium in the litter layers under black locust stands on non-calcareous sites (*Table 2.2)* than those of oak and in some cases, of mixed stands (P and K values were not significantly different). At the calcareous sites no significant differences in the storage and content of these elements in the litter layer for the three vegetation types were found.

Table 2.2: *Mean (± standard deviation) dry mass of the organic layers, bulk density of the soils and stocks of C, N, P and K for the non-calcareous and the calcareous sites (n=number of stands).*

Soil group / Stand type	Organic layer					Mineral soil (0-50cm)				
	Dry mass kg/m^2	C t/ha	N	P kg/ha	K	bulk density g/cm^3	C t/ha	N	P	K
					Non-calcareous soils					
Black locust (n=6)	5.4^{a}	8.3^{a}	0.6^{a}	31^{a}	153^{a}	1.2^{a}	32.9^{a}	3.0^{a}	1.4^{a}	21.4^{a}
	±3.1	±4.7	±0.3	±17	±93	±0.1	±18.8	±1.7	±0.2	±10.3
Oak (n=5)	2.8^{b}	4.7^{b}	0.3^{b}	19^{b}	79^{b}	1.3^{a}	33.4^{a}	2.7^{a}	1.4^{a}	18.4^{a}
	±1.7	±3.1	±0.2	±11	±50	±0.1	±9.2	±1.0	±0.4	±7.0
Mixed (n=3)	3.0^{b}	5.7^{b}	0.4^{b}	$23^{a,b}$	$100^{a,b}$	1.2^{a}	49.6^{b}	4.7^{b}	1.9^{b}	27.9^{b}
	±1.3	±3.2	±0.2	±9	±42	±0.1	±8.1	±0.6	±0.6	±5.8
					Calcareous soils					
Black locust (n=2)	3.9^{a}	4.3^{a}	0.3^{a}	29^{a}	172^{a}	1.2^{a}	98.6^{a}	7.2^{a}	2.6^{a}	23.9^{a}
	±2.6	±2.0	±0.2	±18	±151	±0.1	±13.4	±0.4	±0.2	±4.7
Oak (n=3)	$4.3^{a.b}$	8.7^{a}	0.5^{a}	48^{a}	181^{a}	1.3^{a}	150.6^{a}	9.7^{a}	4.4^{b}	31.9^{b}
	±2.8	±8.6	±0.4	±40	±120	±0.1	±78.9	±3.9	±2.3	±2.8
Mixed (n=2)	2.3^{b}	3.2^{a}	0.2^{a}	19^{a}	110^{a}	1.1^{b}	84.1^{b}	7.4^{a}	$2.8^{a,b}$	$24.2^{a,b}$
	±1.4	±1.5	±0.1	±8	±60	±0.1	±30.1	±1.3	±0.5	±7.4

Values with different superscript letters are significantly different ($p<0.05$) between stand types within the same soil group.

Carbon stocks in the organic layers were low due to C concentrations of only 16-20% in the samples. Fast decomposition of the organic matter complicated sampling of the litter layer and samples contained material from the surface mineral soil, too, which was approved by enhanced silicate residues in samples after analysis.

Bulk density of mineral soils did not differ among the three stand types. In the mineral soil of the non-calcareous sites significantly higher concentrations (data not shown) and stocks of C, N, P and K were determined under mixed stands than under oak and black locust sites, whereas no difference in these elements between black locust and oak stands was observed. No clear soil differences were observed among the three stands on calcareous sites. Total amounts of these elements in non-calcareous soils (from the organic layer to 50cm depth per hectare) under black locust stands were 41t C, 3.6t N, 1.5t P and 22t K which compared to 38t C , 3.0t N, 1.4t P and 18t K under oak and 55t C, 5.0t N, 1.9t P and 28t K under mixed stands. Stocks under mixed stands were significantly higher than under black locust and oak ($p<0.01$). On the calcareous sites no differences between the stand types were recorded, but the total amounts were much higher than on the non-calcareous sites with values of 90-160t C, 7-10t N, 1.6-3.5t P and 24-32t K ha^{-1}. For all Hungarian sites potassium concentrations (3-5mg K/g) and stocks (18-32t K/ha) in the mineral soils to a depth of 50cm can be considered as extremely high. For sandy soils planted with black locust and pine in eastern Germany (Goldacker et al. 2002) stocks of only 0.1 to 0.2t K/ha were detected for the mineral soils (0-50cm).

In previous soil inventories under different tree species (spruce, beech and pine and mixed deciduous stands) in Lower Saxony / Northern Germany Berthold & Beese (2002) found 12-50t C/ha stored in the organic layer. For black locust stands in eastern Germany Goldacker et al. (2002) reported 9-19t C ha^{-1} and 0.7-1.2t N in the litter layer which are similar to the values obtained in the present studies on non-calcareous Hungarian sites. Due to better climate and soil conditions Hungarian sites are frequently characterized by intense biological activity and fast mineralisation of the organic matter (Dénes 1988). Therefore humus layers are mostly very shallow and element stocks are relatively low. Considering 1.2 to 2.6t N ha^{-1} in the mineral soils under black locust stands in eastern Germany (Goldacker 2002) and 2.7 to 4.7t ha^{-1} of stored nitrogen in the non-calcareous Hungarian soils the amounts are low which may be due to low N fixation, low protection mechanisms and high mineralization of organic N due to coarse texture and favourable climatic conditions. According to Strong et al. (1999) the mineralisation of organic matter is generally more rapid in sandy/coarse textured than in loamy/fine textured soils. Mineralization of organic N may lead to excessive amount of nitrates which are lost either through leaching or denitrification. For a red alder (*Alnus rubra*) ecosystem with similar rates of N-fixation as under black locust stands van Miegroet and Cole (1984, 1985) observed NO_3^--N concentrations in the soil solution that exceeded drinking water standards of 10 mg L^{-1}.

High amount of organic matter in the investigated Hungarian black locust stands despite the favourable mineralization conditions (low C/N ratios, favourable climatic conditions) (see *Figure 2.2*) indicates low decomposability of locust litter, which has been attributed to high lignin content of black locust leaves (Bartuska et al. 1981; Hirschfeld et al. 1984). White et al. (1988) observed that *Robinia pseudoacacia* leaflets after 863 days of decomposition contained 81% of their original N. They concluded that this retention of N, coupled with its greater potential to form stabilized fractions during decomposition, explained the long-term effect of black locust on N storage in the forest floor and soil. According to Berg et al. (2003) it is not the lignin content per se that is retarding mineralization rate, but the lignin in combination with high N concentrations above a certain threshold level.

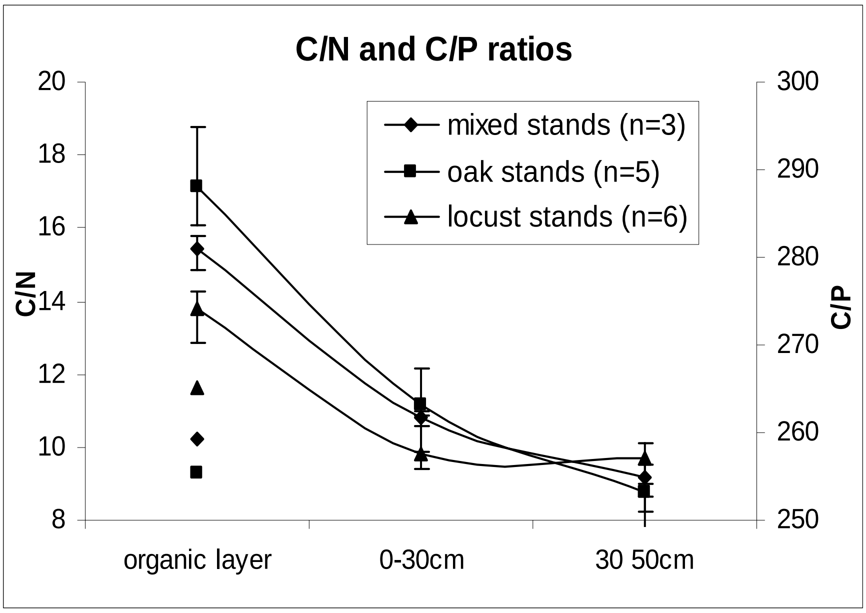

Figure 2.2: *Mean C/N ratios for the whole profile and C/P ratios for the organic layer at all non-calcareous sites in Hungary; values are presented as median (for C/N with quartiles: Q25, Q75).*

Low C/N ratios in the mineral soil indicate enhanced N-levels (*Figure 2.2*). For the non-calcareous sites significantly lower C/N ratios on pure black locust stands than on oak and mixed stands were observed for the litter layer and mineral soil (0-30cm). Density of root nodules is commonly high in the surface soil and declines with increasing depth (Hoffmann 1960). No significant differences between the three stand types were observed for C/P ratios in the organic layer and the mineral soil (data not presented). Contrary to the C/N ratios oak stands showed the lowest (C/P=254) and black locust stands the highest ratio (C/P=265). Due to high P demands for the N_2-fixing nodules (Marschner 1995), black locust trees need high amounts of phosphorous and acidification of the rhizosphere by acid root excretions of black locust can enhance the availability of dissolved P (Gillespie & Pope 1990).

In the mineral soil pH values under oak and locust stands were significantly higher ($p<0.05$) than under mixed stands (*Figure 2.3*). Humus layer pH was lower under mixed stands, but this difference was not significant. The comparison between pure stands of black locust and oak showed significantly lower pH values in the 10 - 40cm mineral soil under black locust.

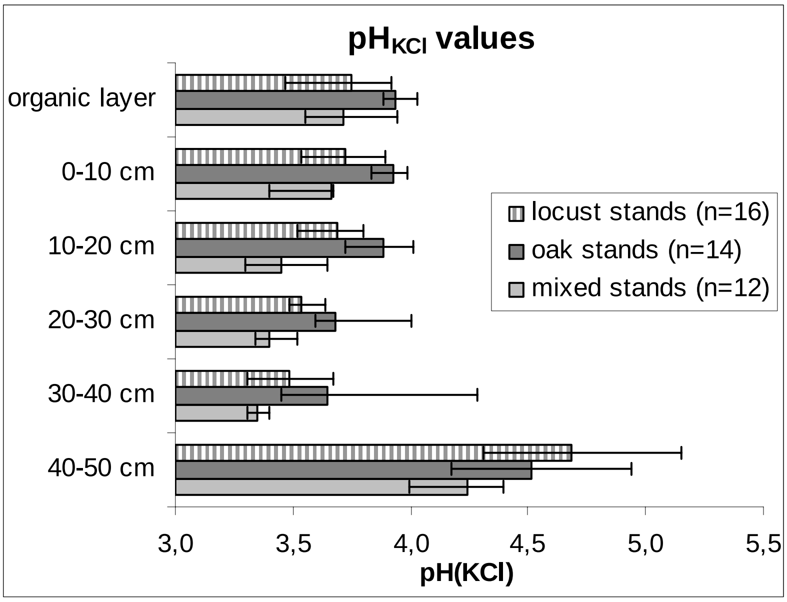

Figure 2.3: *pH (KCl) values at all non-calcareous soils in Hungary; values are presented as median with quartiles (Q25; Q75)*

For the non-calcareous sites the effective cation exchange capacity (CEC_e) was significantly higher ($p<0.05$) for all depths in the soils under mixed stands (*Table 2.3),* which may also was, besides higher element content and store, related to higher content of soil organic matter (SOM) in theses soils. No differences were observed between pure locust and oak stands. In the upper soil (0-30cm) under mixed stands a significantly lower amount of exchangeable base cations and higher concentrations of acid cations were observed.

***Table 2.3**: Mean (*± standard deviation) *cation exchange capacity (CEC_e), exchangeable base (M_b $\sum Na^+$, K^+, Ca^{2+}, Mg^{2+}) and acid cations (M_a $\sum H^+$, Mn^{2+}, Fe^{3+}, Al^{3+}) in the mineral soils under black locust, oak and mixed stands. Significantly different values ($p<0.05$) between stand types within the same depth are superscripted with different letters (^{a,b,c}).*

Stand type	Depth	CEC_e	Ca^{2+}	Mg^{2+}	H^+	Al^{3+}	M_b	M_a
	cm	$\mu mol_c/g$			%			
					non-calcareous soils			
Locust stands** (6 stands)	0-30cm	34.9^{a}	37.6^{a}	6.8^{a}	6.3^{a}	41.4^{a}	48.5^{a}	51.5^{a}
		±18.8	±25.1	±4.3	±5.6	±25.5	±28.5	±28.5
	30-50cm	59.9^{a}	44.7^{a}	8.9^{a}	2.1^{a}	38.8^{a}	57.1^{a}	42.9^{a}
		±58.4	±29.7	±5.2	±2.2	±31.0	±33.2	±33.2
Oak stands** (5 stands)	0-30cm	33.6^{a}	38.7^{a}	9.2^{b}	4.2^{b}	38.6^{a}	52.2^{a}	47.8^{a}
		±12.5	±24.4	±5.6	±4.3	±25.8	±29.4	±29.4
	30-50cm	31.8^{a}	40.9^{a}	12.3^{b}	1.8^{a}	39.1^{a}	57.0^{a}	43.0^{a}
		±16.4	±27.8	±7.9	±2.2	±31.9	±34.4	±34.4
Mixed stands** (3 stands)	0-30cm	45.8^{b}	18.5^{b}	4.3^{c}	7.7^{c}	58.5^{b}	26.2^{b}	73.8^{b}
		±13.5	±10.4	±1.9	±2.7	±13.1	±12.1	±12.1
	30-50cm	67.0^{b}	49.2^{a}	14.4^{b}	3.0^{b}	27.9^{a}	66.3^{a}	33.7^{a}
		±34.6	±21.2	±5.8	±1.4	23.7	±25.7	±25.7
					calcareous soils			
Locust stands** (2 stands)	0-30cm	347.7^{a}	93.3^{a}	5.5^{a}	0.0^{a}	0.1^{a}	99.8^{a}	0.2^{a}
		±107.7	±2.2	±1.6	±0.0	±0.1	±0.2	±0.2
	30-50cm	434.7^{a}	95.4^{a}	4.1^{a}	0.0^{a}	0.0^{a}	99.9^{a}	0.1^{a}
		±151.5	±1.3	±1.1	±0.0	±0.0	±0.1	±0.1
Oak stands** (3 stands)	0-30cm	415.1^{a}	91.8^{a}	7.1^{a}	0.0^{a}	0.1^{a}	99.8^{a}	0.2^{a}
		±196.1	±6.4	±5.6	±0.0	±0.1	±0.4	±0.4
	30-50cm	438.6^{a}	94.3^{a}	5.1^{a}	0.0^{a}	0.1^{a}	100.0^{a}	0.0^{a}
		±176.1	±3.4	±3.1	±0.0	±0.1	±0.1	±0.1
Mixed stands** (2 stands)	0-30cm	179.5^{b}	82.7^{b}	12.1^{b}	0.1^{a}	2.8^{b}	96.5^{b}	3.5^{b}
		±147.6	±6.7	±3.3	±0.5	±5.5	±6.0	±6.0
	30-50cm	213.5^{b}	83.1^{b}	13.1^{b}	0.0^{a}	2.6^{b}	97.2^{b}	2.8^{b}
		±202.4	±5.4	±3.7	0.0	±4.3	±4.5	±4.5

***Within the same stand type all values of non-calcareous and calcareous soil are significantly different ($p<0.01$)*

In the subsoil (30-50cm) these differences disappeared almost. Under pure black locust stands lower concentrations of exchangeable M_b- and higher concentrations of M_a-cations were detected in the upper part of the profile than under oak stands. However, only for Mg^{2+} and H^+

the differences were significant. Magnesium availability was significantly lower under black locust stands in the top- and subsoil. For the calcareous sites no differences of exchangeable cations between black locust and oak stands appeared. At these sites a significantly lower cation exchange capacity as well as significantly lower base cation and higher acid cation concentrations for the whole soil profile under mixed stands were found, but there were no dramatic differences between soil properties under the three stand types due to overall high base saturation of these sites.

It was hypothesised, that in comparison to non N_2-fixing oak stands black locust stands have high nitrogen sequestration, low pH and availability of nutritional elements in the mineral soils. Nitrogen was high in the humus layer and also in the whole profile (organic layer to 50cm depth) under *Robinia*, although the differences were significant for the organic layer, only. Significantly low pH values in the mineral soil, significantly low concentrations of exchangeable Mg^{2+} at all depths and high concentrations of H^+ in the surface soil under black locust stands compared to pure oak stands indicated soil degradation through cultivation of black locust.

Commonly, one would expect that site effects under mixed stands would lie between pure stands of oak and black locust (Binkley 1992), but this was not the case for most soil parameters measured under the Hungarian sites. For mixed stands of spruce and beech Rothe et al. (2002) concluded that site effects by mixed stands cannot be predicted as a summation of the corresponding monocultures. Mixed stands of *Robinia* and *Quercus* had high N levels in the mineral soil, probably indicating continual N_2 fixation by black locust when the fixed N is used by the other vegetation in the mixture. However, C/N and C/P ratios under mixed stands were lying between those under pure locust and oak stands.

To describe the effects of black locust cultivation on a landscape level, erroneous results may be obtained because of high spatial heterogeneity in soil parameters. Better comparisons among sites may be possible if sites are appropriately paired and pairs are considered for soil properties (compare Rothe et al. 2002; Berger et al. 2002).

2.4.2 Comparison between selected paired stands

Total elements (P, S, K, Ca and Mg) in the subsoil (30-50cm) of pure *Robinia* and *Quercus* stands at non-calcareous sites were selected as indicators of pairing (*Table 2.4*). The data showed that for most cases there were no differences between selected soils under black locust and oak. It was thus possible to consider other soil parameters such as pH, nitrogen status and base saturation in the organic layer and mineral soil, which may have been changed by plant influence.

Table 2.4: *Mean (± standard deviation) total element concentrations in the subsoil (30-50cm) for the selected pairs of Robinia and Quercus stands (n=4).*

Site	Pair	P	S	K	Ca	Mg
	Stand/Compartment			mg/g		
Pusztavács	Robinia/93a	0.22	0.07	2.16	1.81*	1.37
		±0.06	±0.03	±0.38	±0.40	±0.10
	Quercus/92b	0.21	0.06	1.95	2.71	1.48
		±0.03	±0.01	±0.07	±0.08	±0.04
Baktalóránthazá	Robinia/10e	0.23*	0.06	4.35	1.52	2.05
		±0.01	±0.01	±0.39	±0.10	±0.18
	Quercus/6a	0.33	0.05	4.91	1.45	2.41
		±0.04	±0.01	±0.74	±0.17	±0.44
Nagyatád	Robinia/4n	0.24	0.35	1.89	1.67*	1.15
		±0.01	±0.03	±0.27	±0.04	±0.15
	Quercus/4p	0.20	0.29	2.06	1.90	1.25
		±0.04	±0.10	±0.06	±0.09	±0.04
Iván	Robinia/2b	0.22	0.10	8.08*	2.12*	4.05*
		±0.02	±0.01	±0.62	±0.26	±0.33
	Quercus/3e	0.14	0.02	5.29	1.32	2.61
		±0.02	±0.03	±0.65	±0.17	±0.30

*Values with * are significantly different within pairs (p<0.05)*

The exigency of comparisons between paired stands of black locust and oak to study the effects of the N_2-fixing tree species on soil fertility can be demonstrated by a forest site in the lowland of middle Hungary (Pusztavács). At this site two pairs of adjacent stands of *Robinia* and *Quercus*, which were approximately 1km remote from each other, were investigated. For the comparison on the landscape level these stands were grouped into the non-calcareous sites due to comparable total element concentrations. But with respect to base saturation differences between the two pairs were determined, which covered the impact of black locust on soil chemical parameters (*Figure 2.4*). The comparison of the cation exchange capacity of the two paired stands revealed a significantly higher base saturation for pair 1 (compartments 199a & 201e). Therefore hypothesised effects concerning acidification and deterioration of nutritional status in consequence of black locust cultivation could not be detected for this pair. Whereas for the other two stands (compartments 92b and 93a) the base saturation of the mineral soil was significantly lower under *Robinia.* For the comparison of paired black locust and oak stands compartments 93a and 92b at the forest site of Pusztavács were included, compartments 199a and 201e of this site were neglected.

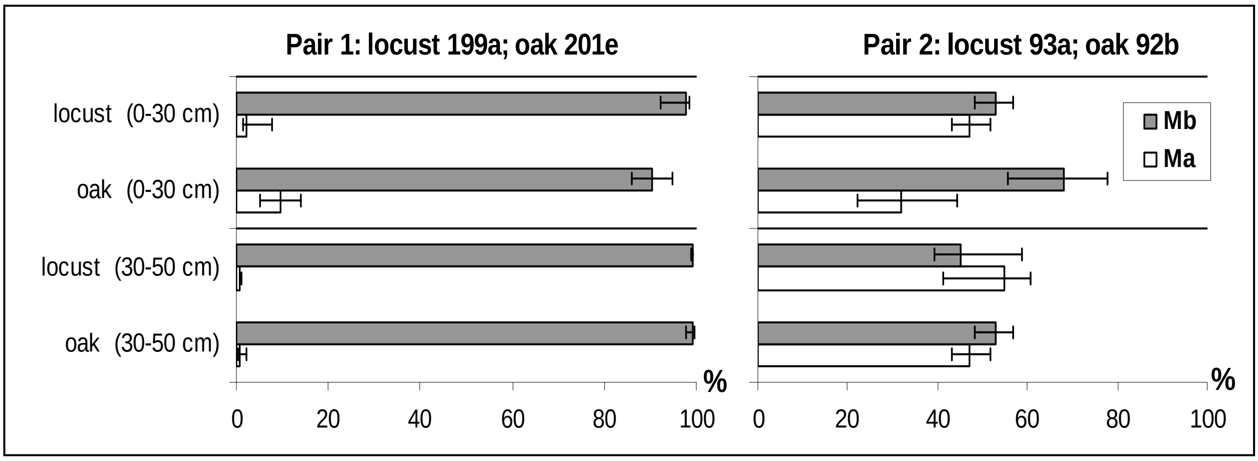

Figure 2.4: *Percentage of base (M_b) and acid (M_a) cations of cation exchange capacity for two paired stands at Pusztavács forest district. Values are presented as medians (n=4 repetitions) quartiles.*

In *Figure 2.5* the mean pH_{KCl} values, N-stocks and percentage of base (M_b) and acid cations (M_a) for the compared pairs are presented. For all the investigated pairs N accumulation in the organic layer under black locust stands was significantly higher. At the forest sites of Pusztavács (a) and Iván (d) significantly elevated nitrogen accumulation was detected for the mineral soil and the whole profile (Total = summation of all layers).

Significantly lower pH values and base saturation (M_b) in the mineral soil under black locust were found for adjacent stands at Pusztavács (a), Baktalóránthazá (b) and Nagyatád (c), indicating acidification and nutrient depletion through black locust cultivation. The changes were strongest in the main rooting zone of the mineral soil (0-30cm). The selected pair at the forest site of Iván was an exception: Due to a significantly higher base saturation under black locust and higher concentrations of acid cations (H^+, Fe^{3+}, Al^{3+}) under oak, processes of acidification and nutrient depletion could not be observed, despite a significantly higher nitrogen accumulation under the *Robinia* stand.

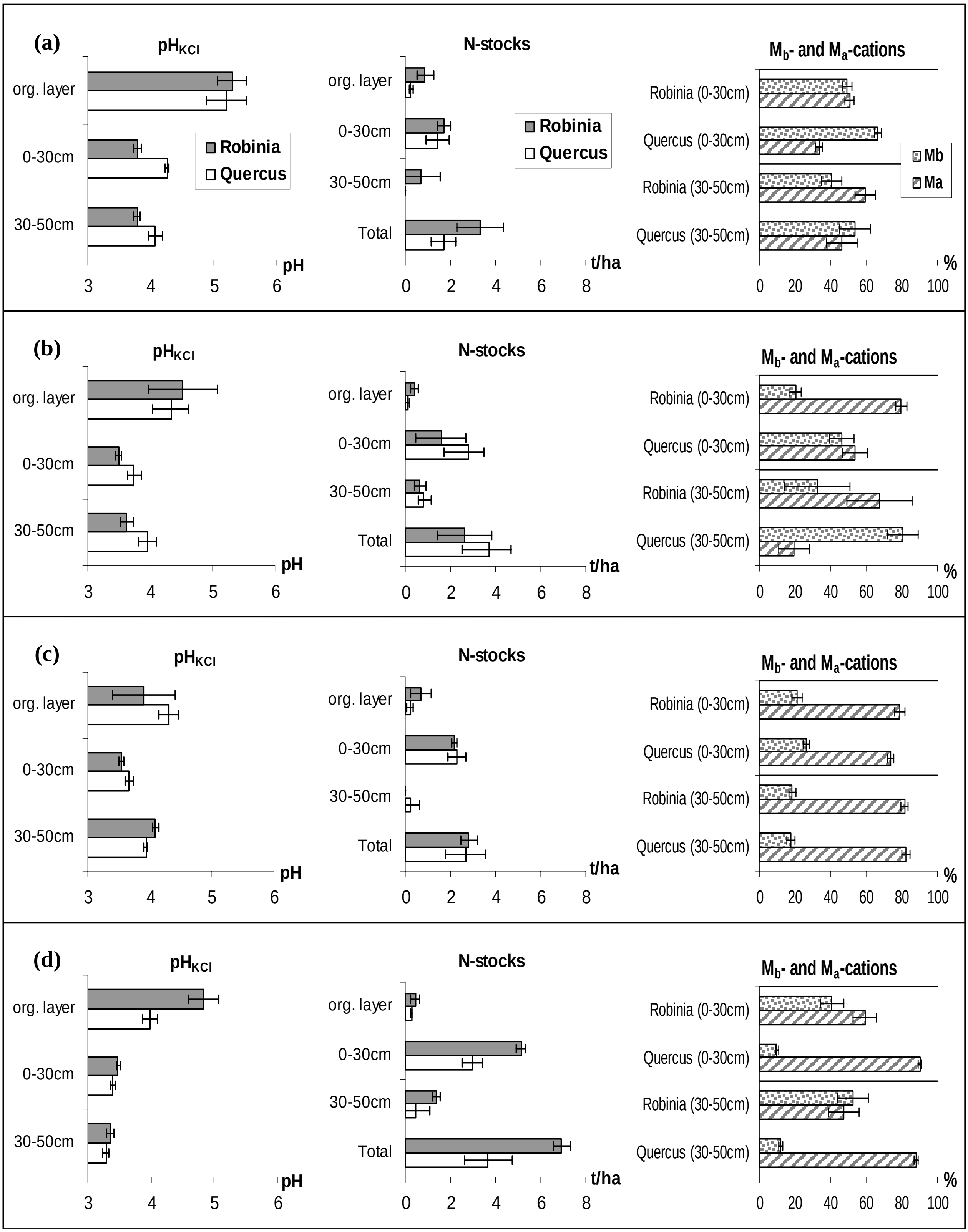

Figure 2.5: *Mean (± standard deviation) pH$_{KCl}$ values, N-stocks and exchangeable base (M$_b$) and acid cations (M$_a$) for paired stands of Hungarian forest sites: Pusztavács (a), Baktalórántháza (b), Nagyatád (c) and Iván (d); four replicates for each horizon.*

2.4.3 Effects of growing black locust for successive generations

A decline in stand yield after cultivation of *Robinia pseudoacacia* on the same site for several generations has been observed for stands in Hungary and eastern Germany. This decline may be related to biological or/and chemical processes in soil. It was hypothesised that long-term cultivation of black locust on the same site with fixation rates up to 150kg N ha^{-1} y^{-1} and leaching of excess nitrogen will cause soil degradation, which may be high depending on the period of tree growth.

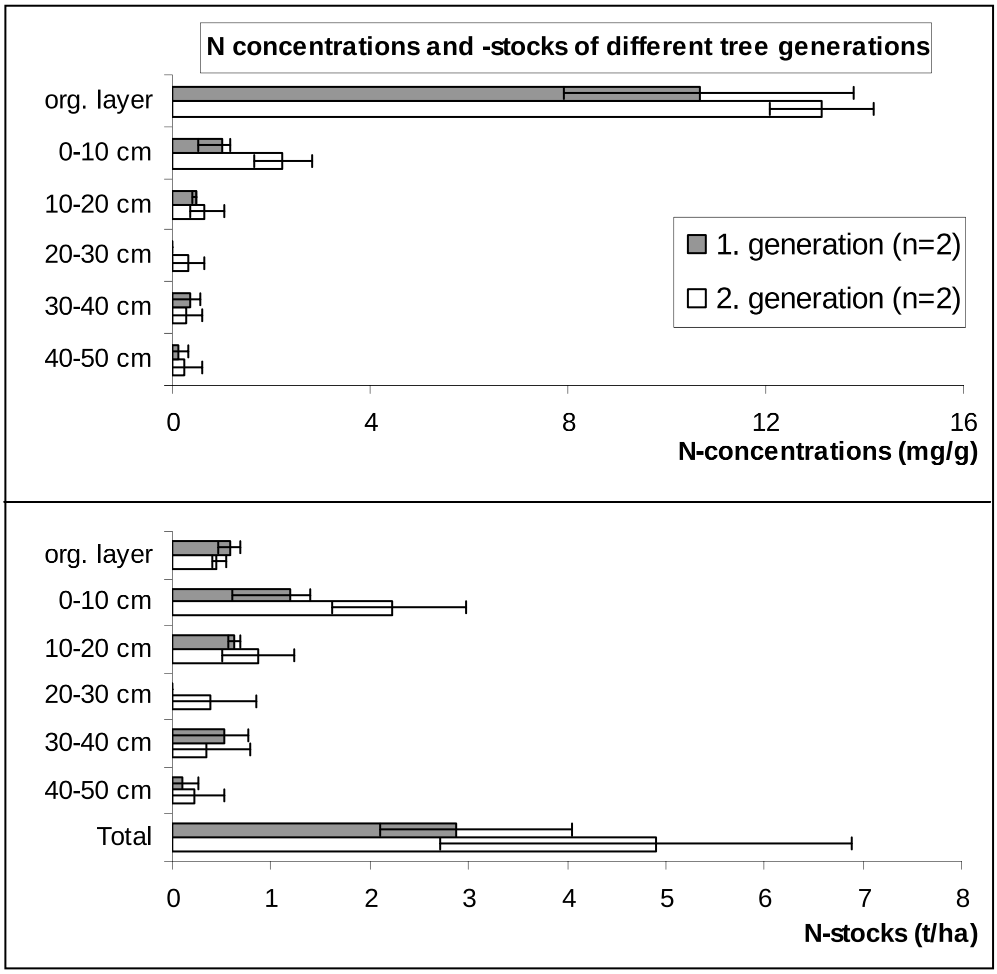

Figure 2.6: *Mean nitrogen concentrations and stocks under black locust stands with different continuance of black locust influence. Values are presented as medians with quartiles (n=number of stands with four replications per horizon).*

To analyse the effect of long-term growth of black locust on chemical soil parameters sites with stands of the first and second tree generation were compared. Stands of the second generation were established by clear cutting and regeneration by stem sprouts and root suckers. N-concentrations and -stocks under the second generation stands were higher in the surface mineral soil (*Figure 2.6*). However, due to high variation of the measured values, significant differences only occurred in the depth from 0-10cm. On average the total amount stored in soils (organic layer to 50cm) under locust stands of the first and second tree generation was 2,9t and 4,9t N ha^{-1}, respectively. The accumulated organic matter on the forest floor of the second tree generation was low (450kg under the first and 590kg N ha^{-1} under the second generation), probably due to decomposition during forest regeneration. With increasing time of locust growth on the same site a decrease of pH and increase of exchangeable acid cations (H^+- and Al^{3+}-ions) occurred (Figure 2.7).

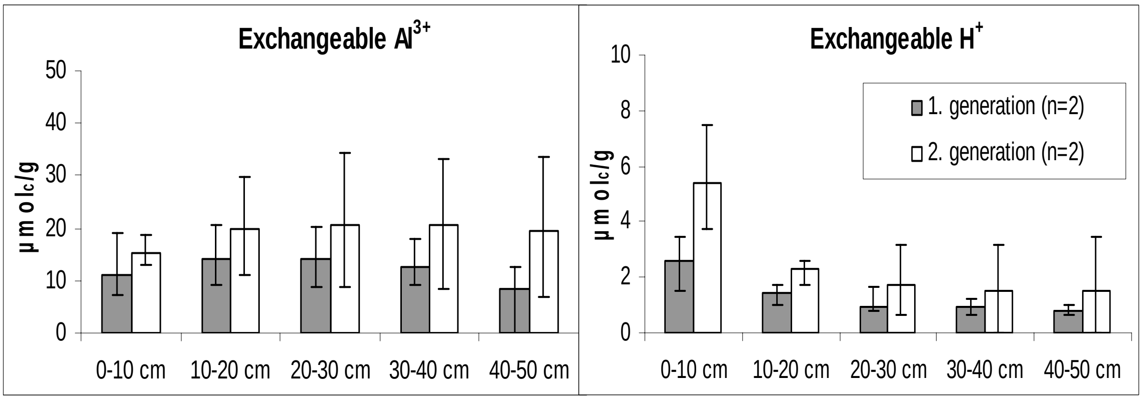

Figure 2.7: *Mean concentrations of exchangeable Al^{3+} and H^+ under black locust stands of the first and second tree generation. Values are presented as medians with quartiles (n=number of stands with four replicates per horizon).*

However, due the high variance of measured values significant differences were observed only in the depth from 0-20cm. Simultaneously base saturation decreased mostly in the surface mineral soil (data not presented), but differences were not significant. Similar results of a N_2-fixing tree species on chemical properties, especially in the surface mineral soil, were also reported by others (van Migroet et al. 1985).

The increase in exchangeable H^+- and Al^{3+}-ions after long-term growth of black locust did not always result in lowering pH values (*Figure 2.8*), due to proton buffering by mineral soil. However, soils from sites under the second generation of black locust showed lower pH values in the organic layer and the upper part of the mineral soil with significant differences observed only in the 10 to 20cm depth.

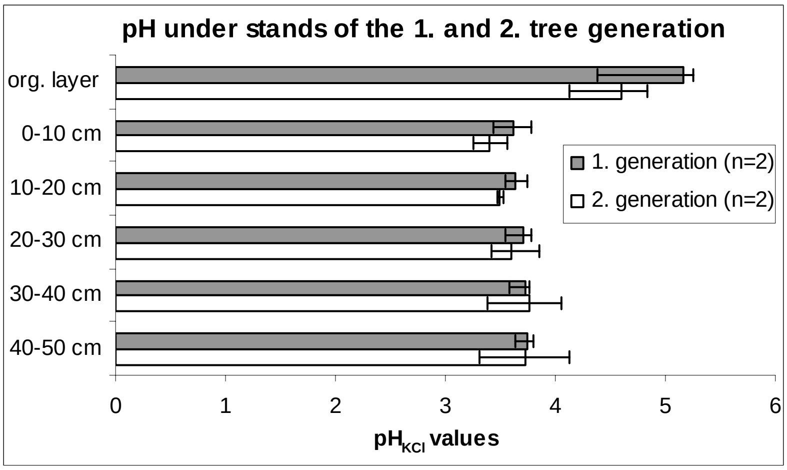

Figure 2.8: *Mean pH$_{KCl}$ values under black locust stands of the first and second tree generation. Values are presented as medians with quartiles (n=number of stands).*

2.5 Conclusions

The investigation of soils under *Robinia pseudoacacia* stands indicated an increase of soil N via symbiotic dinitrogen fixation. This resulted in soil acidification and loss of basic cations. However, the observed changes are not likely to decline growth even after long-term cultivation of black locust on the same site. Other soil parameters such as biological changes in soil would need additional attention to describe growth decline.

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3 Effects of soil inoculation with *Rhizobium* and of lime application on plant development and nutrient status of black locust (*Robinia pseudoacacia* L.) seedlings

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3.1 Abstract

High atmospheric nitrogen deposition rates to forest soils in Europe have led to local N saturation. Stands of *Robinia pseudoacacia* can provide an additional amount of 35-150kg N $ha^{-1}yr^{-1}$ through the symbiotic fixation of atmospheric nitrogen. The N_2 fixation efficiency of black locust can even be increased by liming the soil and inoculation with effective strains of symbiotic bacteria (*Rhizobium*). Soil N-enrichment can lead to strong nitrification, nutrient leaching and acidification. We conducted a greenhouse experiment using inoculated and uninoculated soils with different acidity ($pH_{(KCl)}$ 3.4, 4.8 and 6.2) to determine the effects of chemical and biological manipulations on black locust seedling growth, seedling and soil nutrient status, and the chemical composition of the soil solution under the different treatments. The development of plants was most strongly influenced by liming and less by inoculation. $CaCO_3$ application to soil resulted in 40% higher absolute height and diameter growth and in 70% higher biomass increment after two years. A moderate application of lime and the inoculation of this soil had the most beneficial effect on plant height (+55%) and formation of N_2 fixing nodules (+470%). Effects from the *Rhizobium* application on seedling growth occurred in the second year, indicating a delayed impact of that treatment. The lime and inoculation treatments had no observed effects on plant tissue nitrogen concentration, but due to higher biomass increment of seedlings cultivated in limed soils, the total accumulation of N and other nutrients were higher in plants in limed soils. After two growing seasons, pH in the bulk soil decreased (-0.3 to 0.6 units) in limed soil, but increased in unlimed soil. Similarly, base saturation decreased (-3 to 11%) in the limed bulk soil but again increased in the unlimed soil. Differences between inoculated and uninoculated treatments were not found in either limed or unlimed soils. Increased plant growth and a slightly enhanced buffer capacity in the moderately limed soil were responsible for the strong decrease of soil fertility observed after two years of *Robinia* growth in this soil. Plant uptake and leaching losses reduced the content of the mineral N fractions in all soils significantly. In the unlimed soils, the N_{tot}-N stocks decreased between 220 (controls) and 310kg ha^{-1} (planted pots), 230kg ha^{-1} in the moderately limed soils and 410kg in the strongly limed soils. Neither liming nor inoculation promoted the N_2 fixation of young black locust seedlings to a degree, at which more nitrogen was fixed than was lost through plant uptake and leaching. Leaching losses of nitrate and mineral nutrients due to enhanced N_2 fixation could not be confirmed in the experiment. Expected effects on the chemical soil parameters were probably covered by the preparation of soils at the beginning of the experiment. For future studies, longer seedling cultivation is recommended and leaching losses under *Robinia* stands should be investigated.

3.2 Introduction

Black locust (*Robinia pseudoacacia* L.) is considered to be one of the most effective nitrogen fixing tree species (Olesniewicz & Thomas 1999) and has been shown to increase soil nitrogen levels. Depending on stand age and density, *Robinia* is able to fix 35-150 kg N $ha^{-1}yr^{-1}$ (Hoffmann 1960; Boring & Swank 1984; Danso et al. 1995). Nitrogen-fixing trees are widely used in forest restoration and in agroforestry (Sprent & Parson 2000), thus more attention is being paid to improving the growth and survival rate of these species' seedlings (Tian et al. 2003). Growth and especially the rooting ability of black locust seedlings are commonly improved by inoculation with effective *Rhizobium* strains (Herrera et al. 1993; Balla et al. 1998).

Previous investigations of *Rhizobium* strains nodulating black locust have demonstrated a high variability in their nitrogen-fixing efficiency, and their effect on biochemical properties and plant biomass (Hoffmann 1964; Batzli et al. 1992; Han 1996; Ulrich & Zaspel 2000). One of the most important environmental factors influencing the phenotypic characteristic of

Rhizobium is soil pH (Munns 1978; Franco & Munns 1982). Exposure to low soil pH commonly results in a population that is mostly ineffective in N_2 fixation (Holdings & Lowe 1971). Ibekwe et al. 1997 found that isolated *Rhizobium* bacteria associated with clover (*Trifolium* sp.) were ineffective N_2 fixers in low-pH soils, but more effective in high-pH soils, regardless of metal content of the soils from which they originated. Furthermore, shoot weight, nodule weight and nodule number on plants grown in the low-pH soils were significantly lower than those in high-pH soils.

Together with high atmospheric deposition rates of mean 20-30 kg N ha^{-1} yr^{-1} (Berg & Matzner 1997; Gerstberger 2000; Stuhrmann 2000), elevated N_2 fixation due to inoculation with effective strains of *Rhizobium* may cause excess of N to accumulate, leading to enhanced leaching of nutrients and soil acidification. Legumes such as black locust may take up more cations than anions, hence releasing the excess of positive charges as H^+ and acidifying their rhizosphere (Liu et al. 1989; McLay et al. 1997; Tang et al. 1997). Acidification of soils under legumes has been shown to vary between species (Pierre & Banwart 1973; Jarvis & Robson 1983; McLay et al. 1997; Tang et al. 1997) and to be influenced by the availability of nutrients, growth conditions and growth stages (Pierre & Banwart 1973). The amount of N fixed may also exceed the demand for tree growth, resulting in excess soil nitrogen which may accumulate or be lost through leaching and denitrification. Leaching of nitrate can contaminate ground water (van Migroet & Cole 1985), and the loss of nitrate and base cations as a consequence of N saturation causes soil acidification (van Migroet & Cole 1984; Aber et al. 1998). Application of lime is a widely used technique to support *Rhizobium* and N_2 fixation (Brauer et al. 2002). However, it is unclear whether the increase in soil pH due to liming counters the effects of excessive N fixation and the subsequent leaching of nitrate.

The objectives of this study were to determine the effects of black locust cultivation on the chemical composition of the soil solution and the bulk soil in soils undergoing different liming and inoculation treatments. Furthermore, the influence of lime and inoculation on the development and nutrient status of the seedlings were investigated. We hypothesize that (i) *Robinia* growth rates can be enhanced through liming and inoculation with effective *Rhizobium* strains, (ii) increasing growth rates lead to increasing N_2 fixation and (iii) increasing N_2 fixation causes increasing losses of nitrate and base cations and soil acidification.

3.3 Materials and Methods

3.3.1 Experimental design

In 2001, seeds of black locust originating from Hungarian forest stands were germinated in commercial potting soil. In May 2002, after the seedlings had grown for one year they were transplanted into pots (Ø 14.4cm x 60cm) containing a non-sterilised soil, which was derived from an acidic spruce (*Picea abies* K.) site in Lower Saxony (Germany). To prepare the soils, they were dried and sieved to reduce stone content and to facilitate mixing of the soil with lime. After determination of the base neutralisation capacity (BNC) of the soil (Meiwes et al. 1984), three pH levels were obtained through liming: (**a**) Unlimed (0.0g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **3.4**]; (**b**) Moderately limed (2.1g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **4.8**] and (**c**) Strongly limed (5.9g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **6.2**]. Half of the pots were then inoculated with a 500ml suspension of mixed *Rhizobium* from commercial inocula (strains Rob8, Rob10, Rob11, Rob12 and Rob25; Institute for Plant Cultivation, Solkau, Germany), while the rest received no additional symbiotic soil bacteria. Five pots without plants served as references for each pH treatment.

Figure 3.1: *Experimental arrangement of the pot experiment.*

From spring to fall, plants were placed outside the greenhouse, while during winter they were kept inside the greenhouse to avoid frost damage and to ensure a continuous soil water efflux. Seedlings were watered by natural rainfall and with deionised water to satisfy plant water demands in times of low precipitation and in winter. *Figure 3.1* shows the experimental arrangement of the pots with the different soil treatments in the greenhouse.

3.3.2 Measurements during the observation period

For two growing seasons, the leachates from soils of each pot were collected continuously, stored at 4° C and analysed at intervals of two months. The collection of the soil solution under different soil variants and treatments started one month after planting the seedlings (June 2002). Analyses of soil solution included pH, cations (NH_4^+, H^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}) and anions (NO_3^-, PO_4^{3-}). Leachates of NH_4^+-N, NO_3^--N and total N were analysed with a continuous flow system spectrophotometer (Skalar Analytic GmbH, Erkelenz, Germany) (König et al. 1996), while total element concentration of the other elements in the samples were analysed by ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65% concentrated HNO_3.

3.3.3 Plant growth and analysis

Plant growth, height and diameter increment of seedlings was measured at the end of both growing seasons. At the end of the experiment, plants were harvested and the biomass of leaves, shoots and roots was determined. After washing the roots with deionised water, all visible nodules were collected, separated into three diameter classes (big: >5mm, medium: 2-5mm and small: <2mm), dried and weighed. To analyse differences in the activity of nodules from the different experimental treatments, fresh nodules were incubated for 15 minutes in gas-tight syringes directly after harvesting and the CO_2 production (mg CO_2 g^{-1} DW h^{-1}) was measured with a gas chromatograph (GC 11, DELSI).

For chemical analysis, the different plant compartments were dried at 60 °C and ground. The total N content was determined by dry combustion with a C/N auto analyser (Vario Elementar Analysensyteme, Hanau, Germany) and the total element concentrations of P, S, K, Ca, Mg, Mn, Fe and Al was determined by ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65% concentrated HNO_3.

3.3.4 Soil analysis

Soil samples were collected from the upper (topsoil, 0-30cm) and lower part of the pots (subsoil, 30-60cm), dried at 40° C, sieved (2mm) and ground. The total N content of the mineral soil was analysed by dry combustion with a C/N analyser (Vario Elementar Analysensyteme, Hanau, Germany). Total element concentrations of P, K, Ca, Mg, Mn, Fe and Al were measured using ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion of samples in 65% concentrated HNO_3. pH was measured with a digital pH-meter (WTW GmbH, Wesel, Germany) in 0.1M KCl. After addition of 1N NH_4Cl to samples (2.5g sieved material), exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, H^+, Mn^{2+}, Fe^{3+}, Al^{3+}) in soils were percolated and analysed using an AAS 300 AA (Varian Inc., Darmstadt, Germany). The base saturation (%) was calculated as the percentage of base cations ($\sum$Na, K, Ca, Mg) to the effective cation exchange capacity. To determine different N fractions (NO_3^--N, NH_4^+-N, N_{org}-N, N_{tot}-N) soil samples were extracted with 0.5 M K_2SO_4 (~ 5:1 ratio of solution to dry mass soil) for 18 hours in a batch experiment. The extracted N fractions (NO_3^--N, NH_4^+-N, N_{tot}-N) were analysed by a continuous flow system spectrophotometer (Skalar Analytic GmbH, Erkelenz, Germany), and dissolved organic nitrogen (DON = N_{org}-N) was calculated as the difference of N_{tot}-N to combined inorganic nitrogen (NH_4-N + NO_3-N).

Plant biomass, element concentrations of plant components and soils were also determined at the beginning of the experiment to serve as initial values.

Statistical analysis of the measured data was performed by the program Statistica version 6.0 by using the Mann-Whitney U-Test at the $p<0.05$ level.

3.4 Results and Discussion

3.4.1 Plant growth and biomass increment

At the beginning of the experiment seedlings transplanted into the different soil treatments showed no significant differences in plant height, diameter and biomass. During the two growing seasons, liming strongly affected plant development, whereas effects due to inoculation were less pronounced. The cultivation of seedlings in limed soils resulted in significantly higher plant growth and biomass increment after two years (*Fig. 3.2, Tab. 3. 1).* The inoculated seedlings in the unlimed soil grew significantly slower than uninoculated seedlings in the first vegetation period, but due to higher growth (not significant) in the second year, no differences between the two treatments were observed for the complete growing period. Also, in the soil with moderate $CaCO_3$ application (treatment b) the inoculation with *Rhizobium* resulted in significantly higher seedling growth in the second growing season, but not in the first year or for the cumulative growth over two years. For soil treatment c (strongly limed soil), only the diameter increment of seedlings in inoculated pots was significantly higher in the second vegetation period. The height and diameter growth of inoculated seedlings in all soils was observed to be lower in the first year of cultivation than in soils without *Rhizobium* addition. The fixation of atmospheric nitrogen is a highly energy consuming process. The carbon costs for the fixation are between 4mg (Warembourg & Roumet 1989) and 6mg C/mg N fixed (Werner 1987). Therefore growth of plants in an early stage is often lower, when soil nitrogen is limited and plants rely on fixation as the only N source (Johnsen & Bongarten 1992), which was not the case in this study. Balla et al. (1998) reported that a beneficial growth stimulation of black locust seedlings can occur later, depending on the strains of *Rhizobia* used for soil inoculation.

The highest nodule biomass increment and total nodule number, especially the number of small nodules (<2mm) of all treatments were found in the moderately limed soil with inoculation (b / Inoc.) (*Table 3.1*). Compared to the uninoculated treatment of this soil, the increment of the leaf biomass and the number of nodules with a diameter of 2-5mm (medium size) were significantly higher. Significant differences in the total number of nodules between unlimed and strongly limed soil were found for the uninoculated treatments.

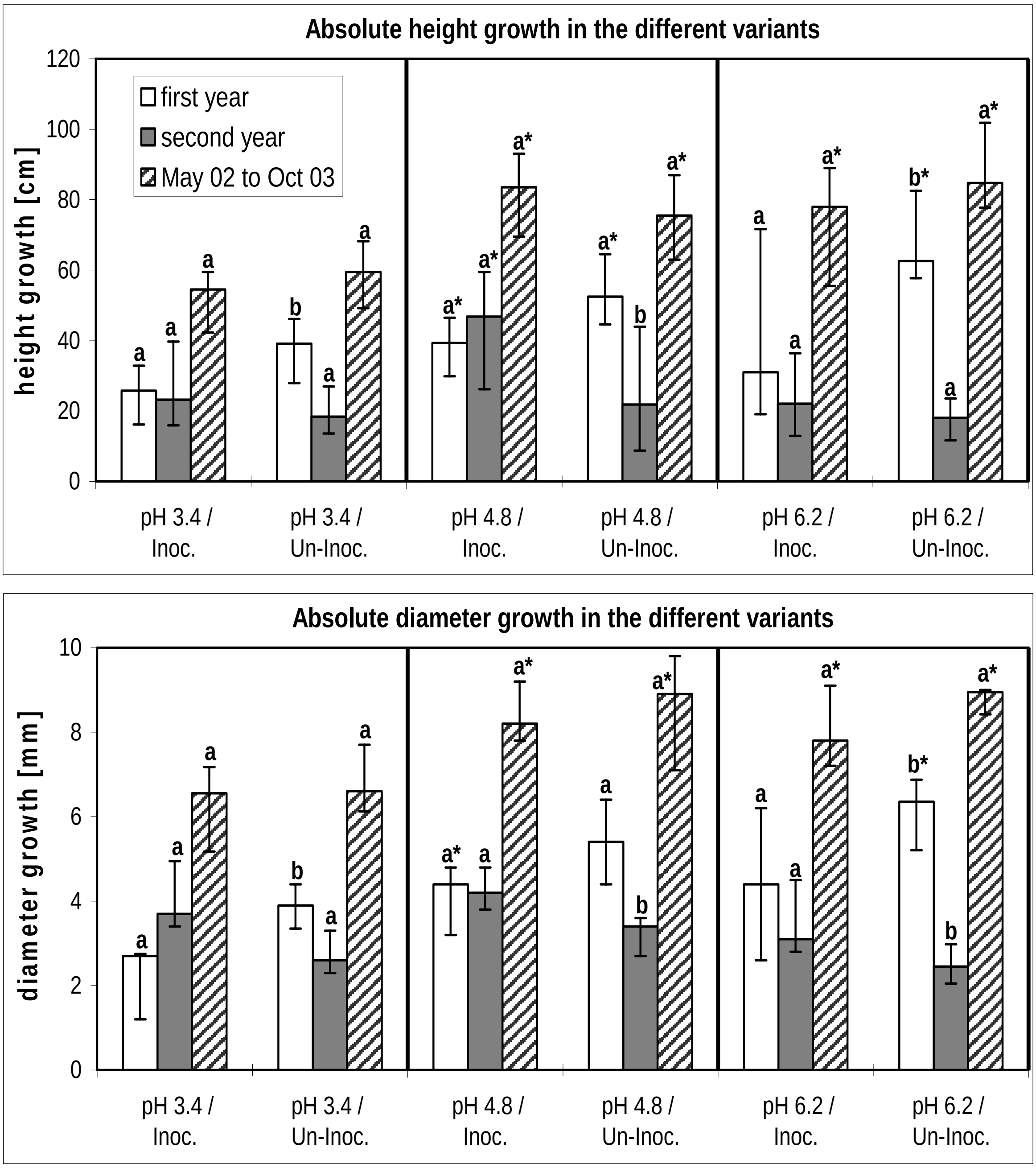

Figure 3.2: *Absolute height and diameter growth of black locust seedlings cultivated in soils with different starting pH and Rhizobium application in 2002 (first year), 2003 (second year) and for the complete observation period from May 2002 to October 2003. Data presented as medians (n=15) with quartiles (Q25, Q75). Bars denoted with different letters (a, b) are significantly different between inoculation treatments within the same lime treatment and measuring interval; bars denoted with * are significantly different in comparison to the unlimed soil variants within the same inoculation treatment and time interval.*

Liming of acid soils with moderate amounts of $CaCO_3$ may promote the development of and infection with *Rhizobium* bacteria. According to Marschner (1995), N_2 fixation of legumes can be affected indirectly or directly by mineral nutrients. Root infection and nodule initiation have a much higher calcium requirement than the root and shoot growth of the host plant.

Table 3.1: *Mean (± standard error) absolute biomass increment of Robinia seedlings in soils with different starting pH and treatments (Inoc. = inoculated; Un-Inoc. = uninoculated) from May 2002 to October 2003 and mean number of nodules per plant in October 2003 (n=15).*

Initial Soil pH / Treatment	**Absolute biomass increment May 2002-October 2003**					**Nodule number in October 2003**			
	Leaves	Stems	Roots	Nodules_{tot}	Biomass_{tot}	Big	Normal	Small	Total
	———		(g)		———	> 5mm	2-5 mm	< 2mm	> 0mm
pH 3.4 / Inoc.	2.66^{a}	6.36^{a}	12.55^{a}	0.59^{a}	20.62^{a}	13^{a}	59^{a}	87^{a}	160^{a}
	±0.43	±1.04	±1.59	±0.06	±3.11	±2	±9	±15	±21
pH 3.4 / Un-Inoc.	2.16^{a}	8.78^{a}	15.45^{a}	0.63^{a}	26.91^{a}	14^{a}	66^{a}	90^{a}	169^{a}
	±0.36	±1.14	±2.06	±0.07	±3.33	±2	±9	±16	±18
pH 4.8 / Inoc.	$5.97^{+,b}$	15.63^{b}	20.52^{b}	0.97^{b}	43.21^{b}	10^{a}	$276^{+,b}$	628^{b}	914^{b}
	±0.35	±1.01	±1.15	±0.09	±1.79	±2	±43	±70	±91
pH 4.8 / Un-Inoc.	3.30^{a}	15.72^{b}	19.41^{a}	0.73^{a}	39.37^{b}	7^{b}	153^{b}	618^{b}	779^{b}
	±0.74	±1.67	±3.04	±0.10	±4.83	±2	±28	±216	±230
pH 6.2 / Inoc.	5.03^{b}	13.23^{b}	15.33^{a}	0.53^{a}	34.13^{b}	11^{a}	125^{a}	278^{a}	413^{a}
	±0.34	±1.81	±2.03	±0.13	±4.13	±4	±36	±80	±102
pH 6.2 / Un-Inoc.	$6.34^{+,b}$	18.52^{b}	19.72^{a}	0.62^{a}	45.28^{b}	14^{a}	135^{b}	259^{b}	408^{b}
	±0.64	±1.62	±1.39	±0.11	±2.82	±6	±25	±64	±80

Values denoted with $^{+}$ are significantly higher between different treatments within the same soil pH, values with different superscript letters ($^{a,\ b}$) are significantly different between soils of equal treatment ($p<0.05$)

Franco and Munns (1982 a, b) observed a decrease of soybean nodulation and a simultaneous decrease in root hair length by lowering the pH from 5.5 to 5.0. For white clover (*Trifolium repens*), changes in nodulation were more closely associated with changes in soil pH than soil Ca (Brauer 1998; Brauer et al. 2002). Furthermore, the availability of soil N (NO_3^-, NH_4^+) can enhance or depress nodulation and N_2 fixation, depending on plant genotypes and the form and level of the N supply. For black locust, Roberts et al. (1983) observed that seedlings grown in nutrient solution with combined nitrogen (5mM NH_4NO_3) had significantly lower N_2 fixation rates but higher relative growth rates than seedlings grown without additional nitrogen supply.

To determine differences in the activity of nodules in all treatments, the CO_2 production of fresh nodules was measured directly after harvesting (*Table 3.2*). Inoculation of soil had no effect on the measured CO_2 concentrations (mg CO_2 * g^{-1} DW * h^{-1}) or on CO_2 production per nodule biomass (mg CO_2 * h^{-1}) within the same soil pH treatment.

Table 3.2: *Mean CO_2 production (± standard error) of different sized nodules per mg nodule dry weight (mg CO_2* g^{-1} DW * $h^{-}1$) and plant (mg CO_2* h^{-1}) from soils with different starting pH and inoculation treatment, measured directly after harvesting the plants in October 2003 (n=15).*

Initial Soil pH / Treatment	Mean CO_2 production of nodules							
	Big > 5mm	Normal 2-5mm	Small > 2mm	Mean < 0mm	Big > 5mm	Normal 2-5mm	Small > 2mm	Total < 0mm
	mg CO_2* g^{-1} DW * h^{-1}				**mg CO_2* h^{-1}**			
pH 3.4 / Inoc.	1.08^{a} ±0.26	2.19^{a} ±0.91	1.76^{a} ±0.39	1.71^{a} ±0.60	0.22^{a} ±0.06	0.32^{a} ±0.05	0.14^{a} ±0.03	0.65^{a} ±0.13
pH 3.4 / Un-Inoc.	1.25^{a} ±0.21	1.28^{a} ±0.25	1.49^{a} ±0.17	1.34^{a} ±0.21	0.27^{a} ±0.06	0.36^{a} ±0.05	0.15^{a} ±0.02	0.73^{a} ±0.08
pH 4.8 / Inoc.	0.55^{a} ±0.07	1.30^{a} ±0.14	1.79^{a} ±0.32	1.27^{a} ±0.25	0.06^{b} ±0.02	0.70^{b} ±0.10	0.71^{b} ±0.14	1.45^{b} ±0.16
pH 4.8 / Un-Inoc.	1.31^{a} ±0.38	$1.65^{a,b}$ ±0.25	1.50^{a} ±0.31	$1.50^{a,b}$ ±0.30	0.11^{b} ±0.05	$0.61^{a,b}$ ±0.11	0.50^{b} ±0.13	1.19^{a} ±0.23
pH 6.2 / Inoc.	1.88^{b} ±0.28	2.32^{b} ±0.30	2.16^{a} ±0.27	2.12^{b} ±0.28	0.22^{a} ±0.07	$0.62^{a,b}$ ±0.17	0.33^{a} ±0.10	$1.17^{a,b}$ ±0.27
pH 6.2 / Un-Inoc.	1.66^{a} ±0.33	1.85^{b} ±0.18	2.07^{b} ±0.19	1.86^{a} ±0.23	$0.21^{a,b}$ ±0.07	0.67^{b} ±0.11	$0.25^{a,b}$ ±0.04	1.13^{a} ±0.15

Values with different superscript letters (${}^{a,\ b}$) are significant different between soils of equal treatment ($p<0.05$)

Respiration of nodules from the strongly limed soil was higher than from the other two lime treatments, but these differences were not always significant. Liming of soil elevated the number and biomass of nodules (medium, small, and total) and therefore in some cases also the total CO_2 production of the nodules per plant.

Measuring the CO_2 production of fresh nodules as an indicator for nodule activity and efficiency of N_2 fixation is just a rough technique to get an impression of possible differences due to variable growth conditions of the host plant. According to Hoffmann (1964), nodule number and biomass are not correlated to the nodule activity and efficiency of N_2-fixation. Statements about the efficiency of nodules are possible through the analysis of the N-content of the complete plant, only. In recent studies, nodule activity and amount of fixation are determined by the acetylene reduction assay (Roberts et al. 1983; Ibekwe et al. 1997) or the 15N isotope dilution method (Danso et al. 1995; Olesniewicz & Thomas 1999).

3.4.2 Chemical analysis of plants

Inoculation of soil with strains of symbiotic *Rhizobium* did not result in higher N concentrations and accumulation in plant tissue (*Figure 3.3*). For uninoculated seedlings cultivated in the unlimed soil (a), significantly higher nitrogen concentrations in leaves and roots and elevated N accumulation in shoots and roots were detected. In the limed soil treatments, a significantly higher N accumulation was found only in the leaves of seedlings derived from the uninoculated strongly limed soil (c / Non-Inoc.). In this soil pH treatment, the accumulation of nitrogen was high in the shoots and roots of the uninoculated seedlings. The absolute nitrogen concentrations (mg N/g) in the single plant components of seedlings cultivated under different lime and inoculation soil variants were similar and ranged for leaves from 14 to 19, shoots 13-15, roots 20-23 and nodules 47-53. As expected, the fixation of N_2 by root associated nodules led to the highest concentrations in the tissue of these compartments. Significantly elevated nitrogen concentrations in plants from soils with different $CaCO_3$ application were only observed in leaves (18mg N/g) and nodules (53mg N/g) from seedlings of the unlimed soils.

The elevated biomass increment of seedlings from soils with lime application resulted in higher N accumulation in these plants. For the complete plant the following mean (±standard error) amounts of N (mg) were accumulated over the experimental period of two years (inoculated/uninoculated): (a) unlimed soils: 357±69 / 550±68, (b) moderately limed soils: 744±31 / 627±110 and (c) strongly limed soils: 611±90 / 804±53. Compared to seedlings from the unlimed soil within the same inoculation treatment, significantly more nitrogen was stored in plants from soil variants (b) / inoculated and (c) / uninoculated.

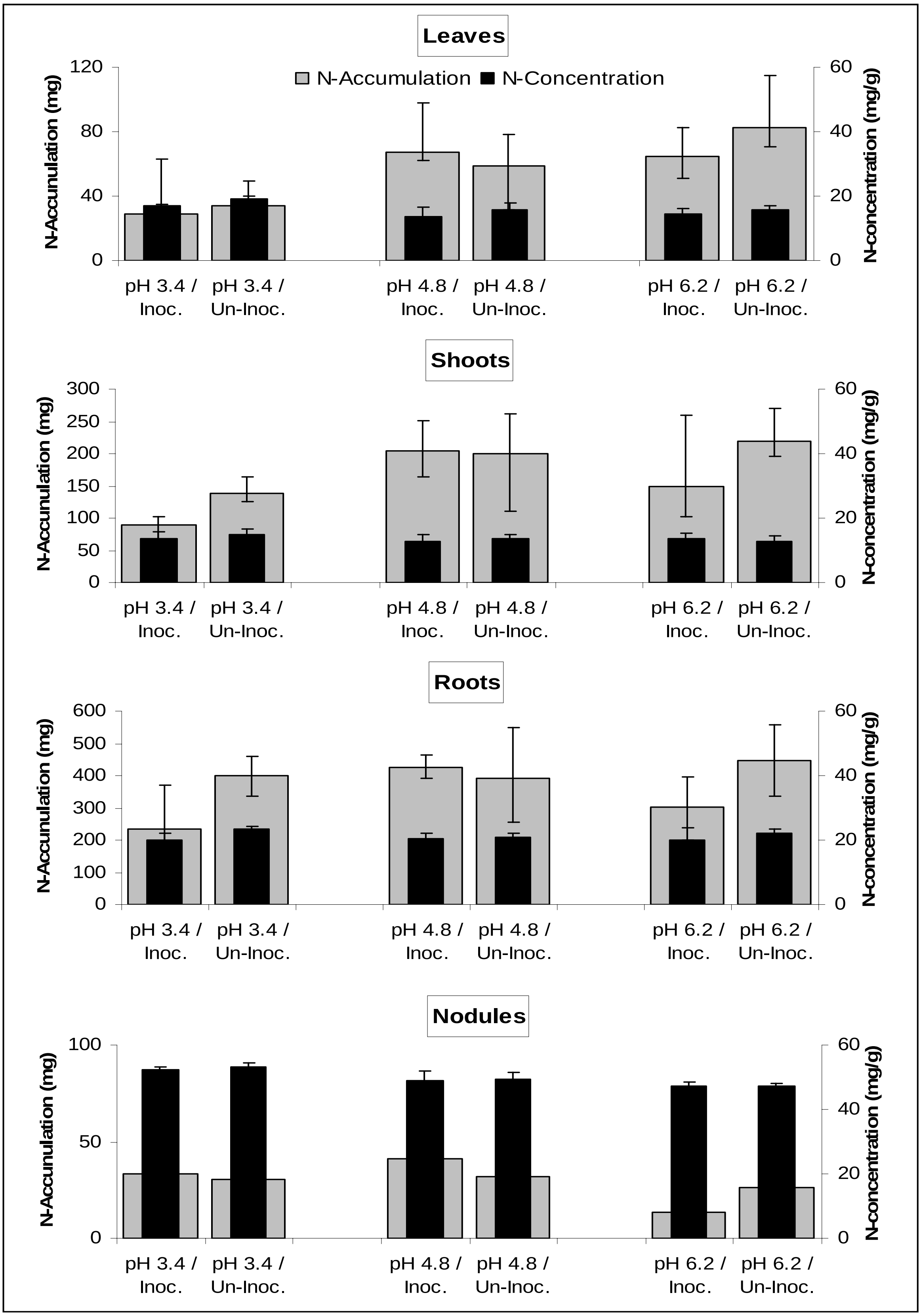

Figure 3.3: *N-concentrations and -accumulation in plant components of seedlings cultivated in soils with different acidity (starting pH values = 3.4, 4.8 and 6.2) and inoculation treatments (Inoc. = inoculated; Un-Inoc. = uninoculated). Data presented as medians with quartiles (n=15).*

Figure 3.4 presents the relative distribution of N in seedlings among the different plant parts at the beginning (Reference) and end (different soil variants) of the experiment. Nodulation of seedlings cultivated in commercial potting soil and after transplanting into the uninoculated soils can be explained by the widespread occurrence of *Rhizobium* in soils and by the non-specific infection of *Robinia* roots with different strains of symbiotic bacteria (Hoffman 1964; Turk et al. 1993). The comparison of the N accumulation in the single plant components between seedlings from pots with different soil pH and the same inoculation treatment partly revealed significantly higher values in the limed treatments than the unlimed treatment. In the moderately limed inoculated soil, significantly higher accumulation was found in leaves, shoots and nodules. In seedlings grown in the strongly limed soil significantly more nitrogen was stored in leaves and shoots (both inoculation treatments). Significant higher N accumulation in plants cultivated in the limed soils was detected in the roots and nodules of seedlings from variant (b) / inoculated.

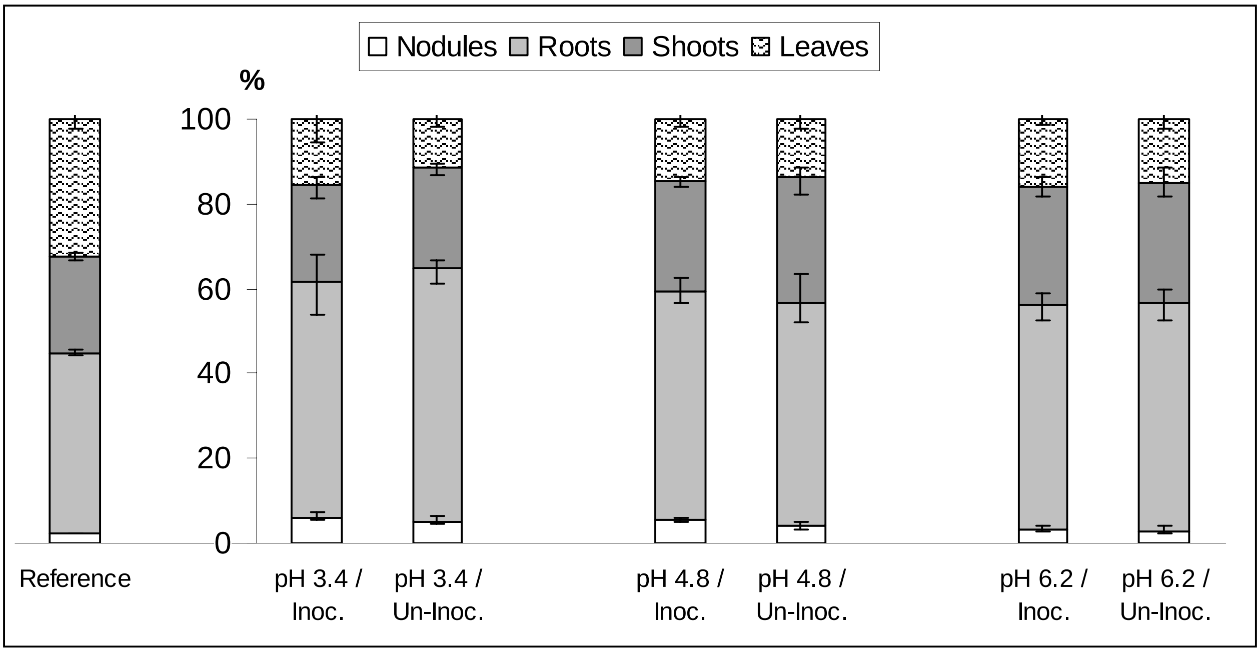

Figure 3.4: *Relative distribution of N content among different plant parts at the beginning (Reference; n=5) and end (different starting soil pH and inoculation variants; n=15) of the experiment. Data presented as medians (± quartiles).*

According to Hoffmann (1964), the percentage of N to the complete plant biomass is a suitable parameter for estimating the effectiveness of N_2 fixation. He detected neither an elevated biomass increment nor an elevated N accumulation of *Robinia* seedlings after inoculation with effective *Rhizobium* strains, but the proportion of N to the complete plant biomass was significantly higher and was 1.2 for uninoculated seedlings to 3.4% N for inoculated ones. After two growing seasons the percentage of N of the total plant biomass ranged in our study between 1.8 and 2.0%, without significant differences between the different soil pH and inoculation treatments. Obviously the application of commercial inocula did not enhance the fixation rates of seedlings through more effective symbioses.

Differences in concentrations and accumulation of other elements due to inoculation of soil with *Rhizobium* were obvious only in a few cases (*Table 3.3*). Inoculation resulted in significantly higher K and Mn concentrations in plants from the unlimed soil and in significantly elevated concentrations and accumulation of Ca, Mg and Mn in the moderately limed variants. Due to enhanced total biomass increment of uninoculated seedlings, significantly higher accumulation of S was detected in the plants from the unlimed soil and of Ca, Mg and Al from the strongly limed soils.

In contrast to the inoculation treatment, liming the soil had a strong impact on the chemical composition of plant tissue. Significant differences were most strongly pronounced between the unlimed (a) and strongly limed (c) soil variants. In comparison to the unlimed soils, significantly elevated P and Ca concentrations were measured in seedlings for both limed soils. For the strongly limed soils, significantly higher S and lower Mg, Mn and Fe concentrations were also detected in the plants.

No effect of liming on the plants´ K content was observed. Significantly higher values of Al concentrations were found for seedlings derived from the unlimed inoculated soil compared to strongly limed inoculated soil. Higher biomass increment and partly higher concentrations of nutrients in the plant tissues from limed soils resulted in significantly elevated accumulation of P, S, K, Ca and Mg compared to the soils without lime application. The accumulation of Mn, Fe and Al was highest, though not significant in every case, in seedlings from the moderately limed soils because these seedlings had a higher biomass increment than unlimed plants and higher element concentrations than strongly limed plants.

Table 3.3: *Concentrations (mg/g dry weight) and accumulation (mg/plant) of P, S, K, Ca, Mg, Mn, Fe and Al in black locust seedlings from soils with different initial pH values and treatments (inoculated, uninoculated). Data presented as mean values ± standard error (n=15).*

Soil variant	Treatment	P	S	K	Ca	Mg	Mn	Fe	Al
		Concentration [mg/g dry weight]							
unlimed (pH 3.4)	inoculated	1.03^{a} ±0.05	1.79^{a} ±0.06	4.66^{a+} ±0.55	7.38^{a} ±0.46	2.51^{a} ±0.15	0.08^{a+} ±0.01	0.91^{a} ±0.12	0.38^{a} ±0.05
	uninoculated	1.00^{a} ±0.04	1.83^{a} ±0.05	3.65^{a} ±0.40	6.40^{a} ±0.46	2.34^{a} ±0.10	0.06^{a} ±0.01	$0.75^{a,b}$ ±0.07	0.33^{a} ±0.03
moderately limed (pH 4.8)	inoculated	1.16^{b} ±0.04	1.77^{a} ±0.07	3.98^{a} ±0.23	13.31^{b+} ±0.47	2.52^{a+} ±0.05	0.08^{a+} ±0.01	$0.66^{a,b}$ ±0.07	$0.27^{a,b}$ ±0.03
	uninoculated	1.27^{b} ±0.11	$1.92^{a,b}$ ±0.09	3.51^{a} ±0.19	11.57^{b} ±0.64	2.19^{a} ±0.13	0.07^{a} ±0.01	0.90^{a} ±0.11	0.44^{a} ±0.11
strongly limed (pH 6.2)	inoculated	1.49^{c} ±0.05	2.02^{c} ±0.08	4.06^{a} ±0.27	15.85^{c} ±0.74	1.93^{b} ±0.07	0.03^{b} ±0.00	0.50^{b} ±0.09	0.23^{b} ±0.03
	uninoculated	1.41^{c} ±0.05	2.01^{b} ±0.06	3.86^{a} ±0.16	16.57^{c} ±0.58	2.02^{b} ±0.05	0.03^{b} ±0.00	0.60^{b} ±0.10	0.30^{a} ±0.03
		Accumulation [mg/plant]							
unlimed (pH 3.4)	inoculated	19.44^{a} ±2.86	25.98^{a} ±4.90	49.50^{a} ±11.67	26.47^{a} ±19.19	35.54^{a} ±9.94	1.63^{a} ±0.26	$25.14^{a,b}$ ±4.75	$10.88^{a,b}$ ±4.27
	uninoculated	25.10^{a} ±2.63	39.46^{a+} ±4.88	57.81^{a} ±8.73	42.41^{a} ±18.83	30.71^{a} ±4.46	1.30^{a} ±0.12	26.10^{a} ±4.06	10.24^{a} ±1.92
moderately limed (pH 4.8)	inoculated	45.09^{b} ±1.57	60.59^{b} ±2.00	127.57^{b} ±7.98	474.77^{b+} ±29.93	62.94^{b+} ±2.62	2.95^{b+} ±0.23	28.82^{a} ±3.11	13.48^{a} ±1.98
	uninoculated	39.41^{b} ±5.18	$51.86^{a,b}$ ±8.25	$88.90^{a,b}$ ±17.89	298.36^{b} ±55.74	41.93^{a} ±6.72	2.14^{b} ±0.29	30.14^{a} ±4.87	$14.73^{a,b}$ ±2.81
strongly limed (pH 6.2)	inoculated	51.39^{b} ±5.37	57.33^{b} ±4.86	96.02^{c} ±12.36	467.23b ±42.25	45.76^{c} ±4.49	0.94^{c} ±0.13	19.19^{b} ±4.89	7.92^{b} ±2.19
	uninoculated	58.15^{c} ±3.39	71.83^{b} ±4.84	107.87^{b} ±9.03	671.53^{c+} ±45.73	61.37^{b+} ±4.09	1.12^{a} ±0.11	28.54^{a} ±5.24	15.83^{b+} ±2.32

Values denoted with different letters ($^{a,\ b,\ c}$) are significantly different between seedlings from different soil pH variants within the same inoculation treatment; values denoted with $^{+}$ are significantly higher between inoculation treatments within the same soil pH variant ($p<0.05$).

3.4.3 Soil analysis

The effects of different amounts of lime application on the soil chemical properties are presented in *Table 3.4*. Compared to the unlimed soil (a), application of $CaCO_3$ reduced soil acidity (increase of pH between 1.4 and 2.8 units) and elevated the total concentrations of calcium significantly (~100/~300%). Moreover, the total N content was reduced significantly in these treatments (30%), probably due to enhanced nitrification through higher soil pH and leaching losses. Liming exerted no strong effect on the total concentrations of the other elements measured. Liming elevated the effective cation exchange capacity (+35-115$mmol_c$/kg) and the base saturation (50%) significantly. Due to the exchange by Ca^{2+}-ions, no H^+- and Al^{3+}-ions were found at the cation exchange complex and the concentrations of Fe^{3+}-ions were reduced significantly in the limed soils.

Table 3.4: *Mean (± standard error) pH (KCl) values, total element concentrations, exchangeable cation concentrations, cation exchange capacity (CEC_e) and base saturation (BS) in the soils with different lime applications [(a) = unlimed, (b) = moderately limed, (c) = strongly limed] at the beginning of the experiment in May 2002 (n=5).*

pH (KCl) and total element concentrations (mg/g) of soils with different lime application before replanting

Soil Variant	pH	N	P	K	Ca	Mg	Mn	Fe	Al
	(KCl)				(mg/g)				
(a)	3.35^{a}	2.23^{a}	0.27^{a}	1.49^{a}	0.93^{a}	0.54^{a}	0.04^{a}	7.63^{a}	5.82^{a}
	±0.02	±0.14	±0.01	±0.05	±0.20	±0.01	±0.00	±0.31	±0.16
(b)	4.76^{b}	1.54^{b}	$0.27^{a,b}$	$1.35^{a,b}$	1.62^{b}	0.45^{b}	0.04^{a}	$8.28^{a,b}$	5.10^{b}
	±0.03	±0.03	±0.04	±0.06	±0.07	±0.02	±0.00	±2.18	±0.22
(c)	6.18^{c}	1.58^{b}	0.24^{b}	1.26^{b}	3.55^{c}	$0.49^{a,b}$	0.03^{a}	6.23^{b}	4.81^{b}
	±0.03	±0.04	±0.00	±0.01	±0.10	±0.04	±0.00	±0.14	±0.04

Exchangeable cations ($mmol_c$/kg) and base saturation (%) of soils with different lime application before replanting

Soil Variant	K^+	Ca^{2+}	Mg^{2+}	H^+	Mn^{2+}	Fe^{3+}	Al^{3+}	CEC_e	BS
				($mmol_c$/kg)					(%)
(a)	0.81^{a}	16.01^{a}	6.88^{a}	9.71^{a}	0.20^{a}	4.44^{a}	12.28^{a}	50.81^{a}	47.39^{a}
	±0.05	±1.34	±0.41	±0.20	±0.03	±0.25	±0.48	±2.61	±1.14
(b)	0.98^{b}	75.45^{b}	7.67^{a}	0.00^{b}	0.21^{a}	0.45^{a}	0.00^{b}	85.36^{b}	99.23^{b}
	±0.02	±1.88	±0.55	±0.00	±0.01	±0.03	±0.00	±2.41	±0.05
(c)	0.89^{a}	154.89^{c}	7.57^{a}	0.00^{b}	0.19^{a}	0.31^{b}	0.00^{b}	164.48^{c}	99.70^{c}
	±0.01	±2.00	±0.00	±0.00	±0.01	±0.03	±0.00	±1.97	±0.02

Values with different superscript letters ($^{a,\ b,\ c}$) are significant different between soil variants ($p<0.05$).

The soil amelioration, especially the increased soil pH, was supposed to create favourable conditions for the survival and activity of symbiotic soil bacteria, for nodulation and for plant growth (Staley 2002; Brauer et al. 2002; Hartley et al. 2004).

After two growing seasons (May 2002 to October 2003) it was expected that cultivation of black locust resulted in soil acidification and nutrient depletion due to H^+-excretion by roots, nutrient uptake by plants and leaching. In unlimed soil, a significant increase of the pH and the base saturation in the planted pots was detected, whereas in the unplanted controls no significant change over the whole observation period occurred (*Figure 3.5*). There were no differences between inoculated and uninoculated soils. The increase of pH and base saturation was significantly higher in the topsoil than in the subsoil of the planted pots (0.3 to 0.1 units), also compared to the topsoil of the controls. In contrast to the unlimed soil, pH in planted pots with lime application decreased significantly after two growing seasons. Inoculation did not affect soil pH or base saturation. For the moderately limed soil (b), pH and base saturation of all variants and depths were significantly lower at the end of the experiment. In the planted pots the decrease of pH in 30-60cm (0.6 units) was significantly stronger than in 0-30cm (0.3 units), though the decrease of base saturation was not significantly different among the two soil depths. The base saturation in the whole soil column (0-60cm) dropped by 3% (inoculated), 9% (uninoculated) and 11% (Control) from May 2002 to October 2003. pH of the strongly limed treatments (c) decreased significantly in the inoculated (all depths; mean 0.3 units) and in the subsoil of the uninoculated variants (0.4 units). The acidification of the subsoil was again stronger than that of the topsoil. Due to high amounts of added $CaCO_3$, no changes of the base saturation were observed after two years of *Robinia* cultivation in this limed variant.

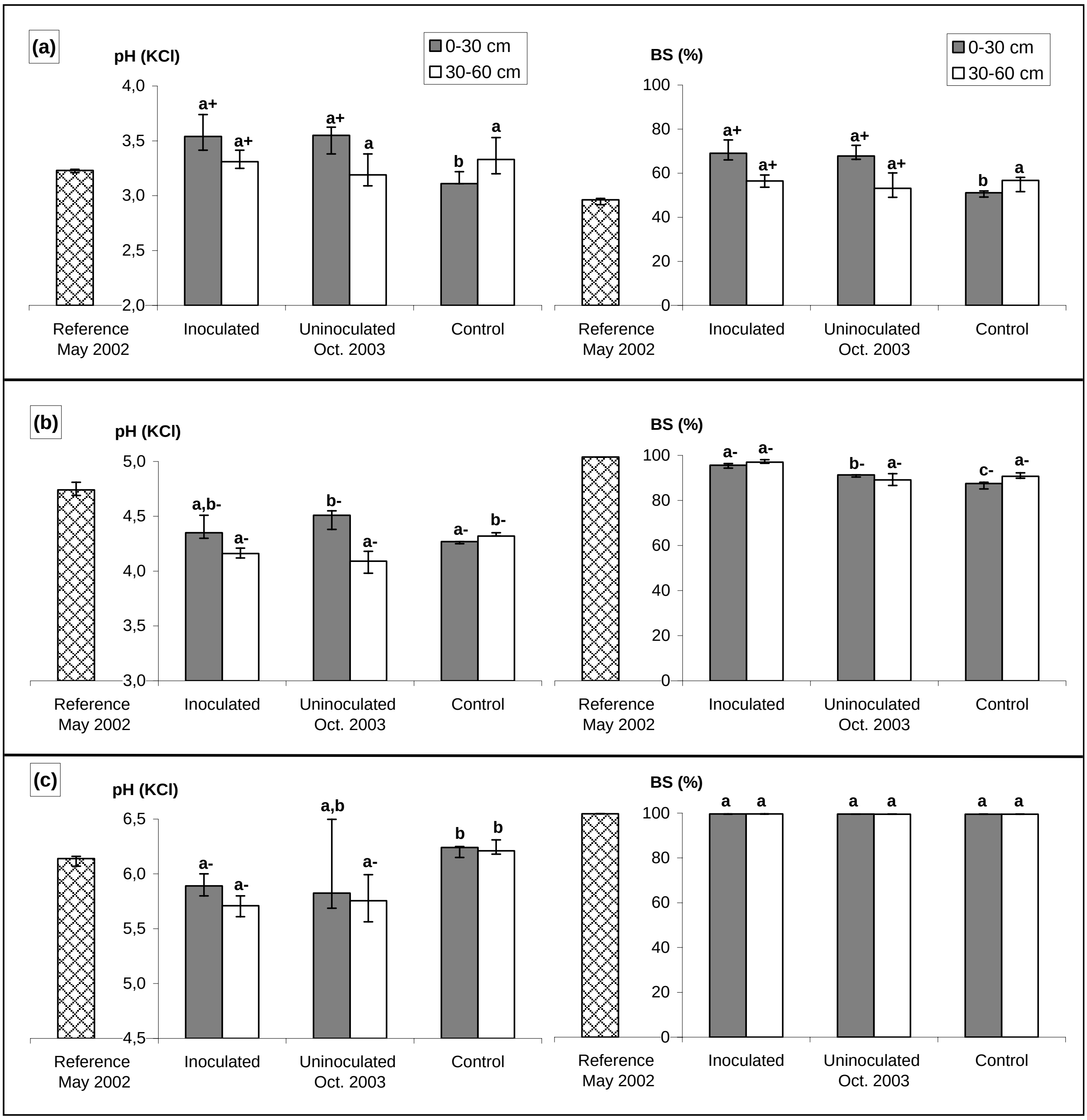

Figure 3.5: *Mean pH-(KCl) values and base saturation (BS %) in the soils with different lime application [(a) = pH 3.4, (b) = pH 4.8, (c) = pH 6.2] and inoculation treatment (inoculated & uninoculated) at May 2002 and October 2003; values presented as medians with quartiles (Reference May 2002 and Control n=5; inoculated and non-inoculated planted pots n=15). Bars denoted with different letters (a, b, c) are significantly different between variants (inoculated, non-inoculated & controls) within the same soil pH variant; bars denoted with + or - show significantly higher or lower values compared to the Reference in May 2002.*

To estimate the effects of black locust cultivation after two growing seasons on the level of forest stands, stocks of the main exchangeable cations ($kmol_c/ha$) in the different soil treatments were calculated for the profile from 0-60cm (*Table 3.5*). For the unlimed (a) and moderately limed (b) soils, no differences between inoculation treatments were detected. For the strongly limed variant (c) significantly lower stocks of K^+, Ca^{2+}, Mg^{2+}, Fe^{3+} and higher Mn^{2+} stocks were found in the inoculated soil.

Table 3.5: *Mean (± standard error) stocks ($kmol_c/ha$) of exchangeable cations in the soils with different starting pH values (pH 3.4, 4.8 and 6.2) and treatments (Inoculated, Uninoculated and unplanted Controls); planted variants n=15, controls n=5.*

Initial Soil pH / Treatment	K^+	Ca^{2+}	Mg^{2+}	H^+	Mn^{2+}	Fe^{3+}	Al^{3+}
				$kmol_c/ha$			
pH 3.4 / Inoc.	3.40^{a} ±0.15	122.87^{a+} ±5.95	41.54^{a+} ±1.71	40.91^{a} ±3.53	1.52^{a+} ±0.18	16.55^{a} ±1.26	37.01^{a-} ±1.13
pH 3.4 / Un-Inoc.	$3.56^{a,b}$ ±0.15	128.68^{a+} ±6.44	42.14^{a+} ±1.91	49.39^{a} ±3.67	1.29^{a} ±0.18	17.88^{a} ±1.30	39.16^{a-} ±1.17
pH 3.4 / Control	3.90^{b} ±0.11	98.63^{b+} ±2.60	33.13^{b} ±1.61	58.77^{a} ±8.39	1.58^{a} ±0.34	19.85^{a} ±1.95	46.78^{b} ±1.19
pH 4.8 / Inoc.	2.61^{a-} ±0.12	292.38^{a-} ±10.80	30.69^{a-} ±1.31	4.95^{a+} ±0.39	1.83^{a+} ±0.12	11.17^{a+} ±0.48	$7.90^{a,b+}$ ±1.57
pH 4.8 / Un-Inoc.	2.61^{a-} ±0.11	291.84^{a-} ±10.48	28.75^{a-} ±1.20	5.85^{a+} ±0.71	$2.02^{a,b+}$ ±0.21	11.62^{a+} ±0.67	6.51^{b} ±1.58
pH 4.8 / Control	2.55^{a-} ±0.14	269.94^{a-} ±6.14	27.12^{a-} ±0.73	4.95^{a+} ±0.90	2.80^{b+} ±0.23	9.72^{a+} ±1.33	12.01^{a+} ±1.41
pH 6.2 / Inoc.	2.39^{a-} ±0.11	579.92^{a-} ±13.44	28.31^{a-} ±1.09	0.00^{a} ±0.00	0.77^{a} ±0.06	1.90^{a+} ±0.10	0.71^{a} ±0.39
pH 6.2 / Un-Inoc.	2.78^{b-} ±0.09	670.33^{b} ±31.30	31.02^{b-} ±0.53	0.00^{a} ±0.00	0.59^{b-} ±0.09	2.53^{b+} ±0.10	0.60^{a} ±0.33
pH 6.2 / Control	2.78^{b-} ±0.10	671.24^{b-} ±17.34	26.33^{a-} ±0.60	0.00^{a} ±0.00	0.90^{a} ±0.18	$2.39^{a,b+}$ ±0.20	1.07^{a} ±1.07

Values denoted with different letters ($^{a,\ b,\ c}$) are significantly different between treatments within the same soil pH; values denoted with $^+$ or $^-$ are significantly higher or lower compared to the starting conditions in May 2002 ($p<0.05$).

Compared to the values at the beginning of the experiment (May 2002), significantly higher amounts of exchangeable Ca^{2+} and Mg^{2+} as well as lower amounts of Al^{3+} were found in the planted treatments of the unlimed soil. Unplanted controls had significantly higher stocks of K^+ and Al^{3+} and lower accumulation of Ca^{2+} than the planted variants. In the moderately limed soils the content of exchangeable base cations (K^+, Ca^{2+}, Mg^{2+}) decreased and the acid cations (H^+, Mn^{2+}, Fe^{3+}, Al^{3+}) increased significantly in all treatments (except for Al^{3+} in the uninoculated soil). Except for Mn^{2+} no significant differences between planted pots and control pots were found. Also in the strongly limed soil, stocks of base cations were lowered significantly in October 2003. Among acid cations only the amount of exchangeable Fe^{3+} was elevated at the end of the experiment. Distinct differences in the stocks of exchangeable cations between planted pots and unplanted controls were obvious in this pH treatment.

Liming stimulated nitrification and resulted in significantly elevated K_2SO_4-extractable nitrate (*Figure 3.6*). In May 2002 the strongly limed soil contained 310kg/ha NO_3-N, the moderately limed 120kg/ha NO_3-N and the unlimed soil 60kg ha/NO_3-N. In the unlimed soil, NH_4-N was the dominant N-fraction (280kg/ha), and liming reduced the concentrations of this fraction (130kg/ha in both limed soils) and elevated the amounts of N_{org}-N in these treatments (+ 40 to 60kg/ha). The lowest amount of N_{tot}-N (310kg/ha) was detected in the moderately limed variant (b), whereas the unlimed soil (a) contained 390kg/ha and the strongly limed soil (c) contained 550kg/ha. After two years of plant growth the soil content of the different N-fractions decreased significantly in each lime treatment. No significant differences according to the stocks of the extracted N-fractions between inoculated and uninoculated soils were found after two years.

In the unplanted pots of the unlimed soil (a), significantly more NH_4-N and N_{tot}-N was retained than in the planted pots in October 2003. Compared to the limed soils the remaining amounts of NH_4-N were significantly higher in soils without $CaCO_3$ addition. In the moderately limed soil (b) black locust cultivation resulted in lower NO_3-N, N_{tot}-N and higher N_{org}-N stocks compared to the unplanted pots. In the unlimed and strongly limed soils higher amounts of N_{tot}-N (not significant for planted variants in [a]) were found than in the moderately limed soils. In the strongly limed soils (c) planted variants showed lower NO_3-N and higher N_{org}-N stocks than the controls without plant influence. In this lime variant NO_3-N (especially in the controls) and N_{org}-N were the dominant N-fractions, whereas in the unlimed and moderately limed soils in pots with seedlings all fractions had nearly equal percentages.

Obviously N_2 fixation rates and litter input of *Robinia* seedlings were not high enough to balance or exceed the amount of nitrogen reduced by plant uptake and leaching losses during the observation period. Additionally, it has been shown that nitrogen excretion by legume roots deliver only a negligible contribution (1-2%) to the N enrichments of soils by N_2 fixing plants (Fedorow 1960; Hoffmann 1963; Uselman et al. 1999).

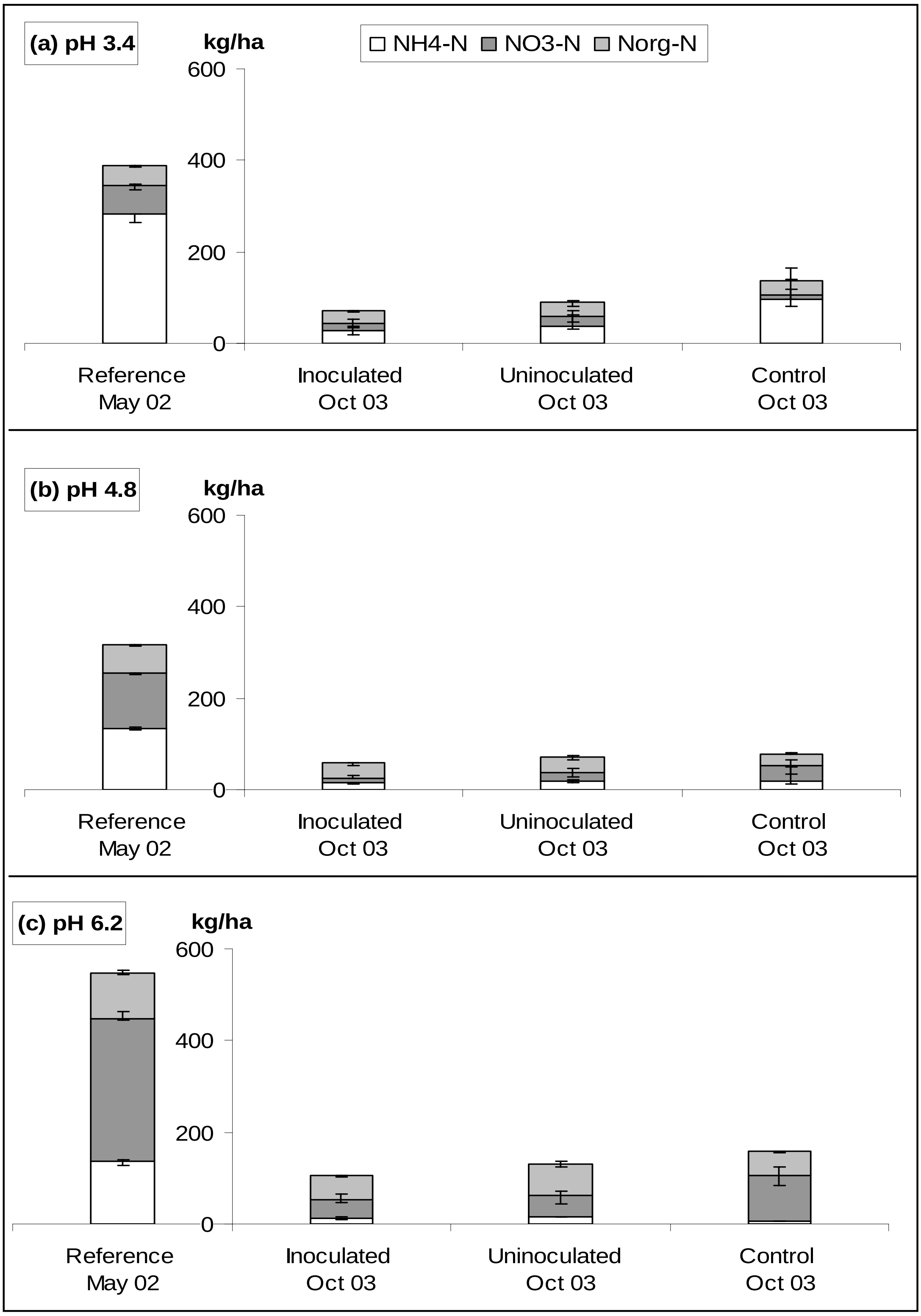

Figure 3.6: *Mean stocks of K_2SO_4 extractable N fractions in soils (0-60cm) with different $CaCO_3$ application [(a), (b), (c)] and inoculation treatment at the end of the experiment in October 2003 (n=30) in comparison to the values before replanting in May 2002 (n=5); data presented as medians with quartiles.*

The inoculation of soil with symbiotic bacteria (*Rhizobium*) and the expected promotion of N_2 fixation did not result in elevated soil degradation and nitrogen accumulation. The pre-treatment of soil (drying, sieving and re-wetting) and the relatively high content of soil organic matter (4-5%) were responsible for strong mineralization processes leading to elevated pH values and base saturation in the planted pots of unlimed soil treatments compared to the starting conditions and to the topsoil of the unplanted controls. Due to enhanced plant growth on the limed soils, pH in both limed variants and base saturation in the moderately limed soil decreased in the observation period of two years. Negative effects of black locust cultivation on soil acidity and nutrient availability were most strongly pronounced in the moderately limed soil (b). In this soil plant development was elevated (see *Fig. 3.2* & *Tab. 3.1*) and the buffer capacity was obviously not high enough to superimpose the effects of *Robinia* on soil chemical properties. In the control soils of the moderately limed soil, the absence of plant influence resulted in lower acidification and less decreased base saturation in the subsoil (30-60cm). In the soils of the strongly limed variant, the impact of black locust cultivation resulted in lower pH values under the planted pots compared to the Reference and to the unplanted soils. Changes of soil acidity in the control pots over the experimental period were not determined.

Figure 3.7 shows the mean soil water efflux per month for the collection period from June 2002 to September 2003. The absence of plant water uptake resulted in the highest efflux rates under the control pots, but due to the high variation of values, unplanted pots had significantly higher monthly water amounts than planted pots only in the strongly limed soil treatment. No significant differences were found between the planted pots with different soil pH treatments or with different inoculation treatments. On average, 75ml water per month was collected under the planted and 200ml under the unplanted soils. Differences in the water efflux rates between collection months or seasons were marginal (data not presented), because all pots received deionised water in periods of low precipitation and in the winter to satisfy plant demands and to guarantee soil water efflux.

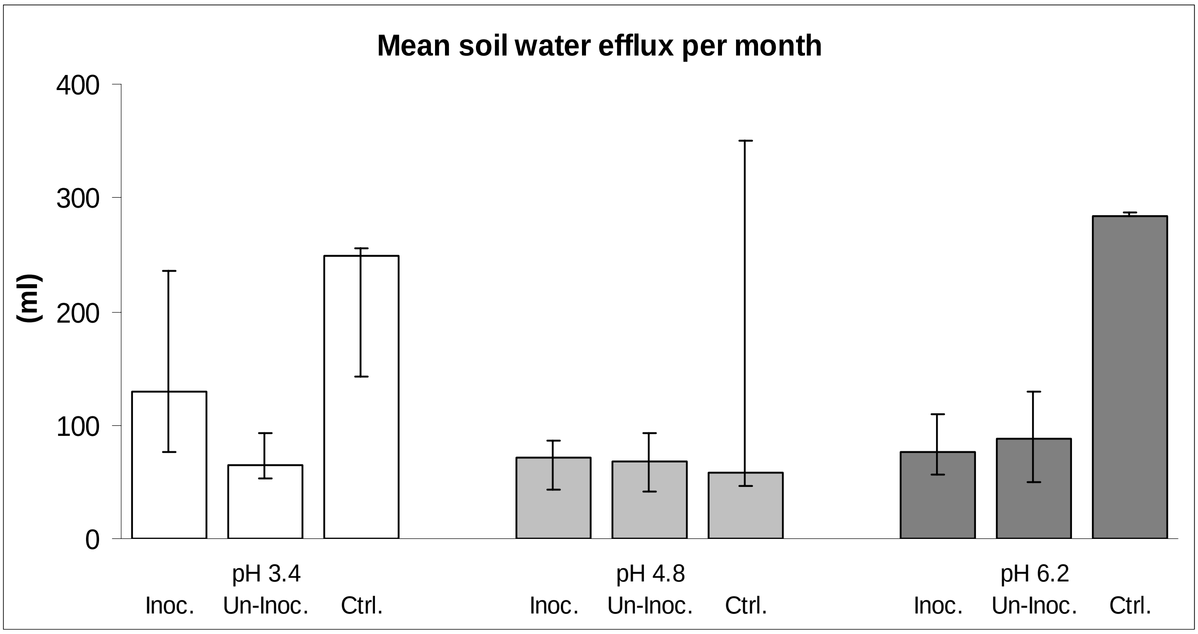

Figure 3.7: *Mean soil water efflux per month for the whole observation period from June 2002 to October 2003 under the soils with different initial pH values and inoculation treatment (Inoc. = Inoculated, Un-Inoc. = Uninoculated, Ctrl. = unplanted Controls). Values presented as medians with quartiles (n=16 months).*

Leachates from moderately limed and especially strongly limed soils contained higher Ca as well as lower K, Mg, Mn, Fe, Al, and H concentrations than the leachates from soils without $CaCO_3$ application (*Table 3.6*). Among the planted pots, a higher depletion of PO_4-P was detected under the unlimed soil variants. Under all treatments, the H^+-concentration in the leachate was very low (0.04 mg/L in the unlimed soils) or below the detection limit (limed soils). With the exception of lower Ca and Mg concentrations under the moderately limed (b) and inoculated soil, no effects of the inoculation concerning the chemical composition of the percolate was noticeable. Due to the plant uptake of nutritional elements the concentrations of these elements in the soil water were lower in nearly all cases under planted pots than under the control pots. Higher plant growth and biomass increment of seedlings in the limed soils than in the unlimed ones (compare *Fig. 3.2* & *Tab.* 3.1) seemed to be responsible for lower concentrations of nutritional elements (especially K & Mg) in the leachate of these variants. Liming may have reduced the solubility of these elements and decreased their concentrations in the soil solution in the case of acid cations. Elevated plant uptake and accumulation due to high requirement for nodule formation (Marschner 1995) may also be a reason for lower concentrations of Fe and PO_4-P in the soil solution in the limed and especially in the inoculated soils.

Table 3.6: *Mean PO_4-P, K, Ca, Mg, Mn, Fe, Al and H concentrations (± standard error) in the percolate under the pots with different initial soil pH and inoculation treatment for the whole observation period (n=16 months).*

Initial Soil pH / Treatment	PO_4-P	K	Ca	Mg	Fe	Mn	Al	H
					mg/L			
pH 3.4 / Inoc.	$0.91^{a,b}$	2.62^{a}	$19.24^{a,b}$	10.09^{a}	31.06^{a}	0.48^{a}	1.91^{a}	0.03^{a}
	±0.27	±0.29	±1.82	±0.87	±8.39	±0.07	±0.16	±0.01
pH 3.4 / Un-Inoc.	0.58^{a}	1.78^{b}	20.85^{a}	11.00^{a}	18.28^{a}	0.40^{a}	1.93^{a}	0.04^{a}
	±0.24	±0.22	±1.10	±0.57	±6.62	±0.04	±0.10	±0.01
pH 3.4 / Control	1.91^{b}	3.60^{a}	25.14^{b}	12.87^{a}	42.98^{a}	0.62^{a}	2.05^{a}	0.04^{a}
	±1.04	±0.39	±1.25	±0.88	±21.36	±0.13	±0.20	±0.02
pH 4.8 / Inoc.	0.30^{a}	1.76^{a}	31.44^{a}	5.23^{a}	9.36^{a}	0.22^{a}	0.60^{a}	0.00^{a}
	±0.05	±0.16	±2.33	±0.39	±2.23	±0.02	±0.04	±0.00
pH 4.8 / Un-Inoc.	0.45^{a}	$2.07^{a,b}$	41.50^{b}	6.66^{b}	19.67^{a}	0.39^{b}	$0.70^{a,b}$	0.00^{a}
	±0.09	±0.23	±3.72	±0.53	±4.73	±0.06	±0.08	±0.00
pH 4.8 / Control	1.52^{b}	2.46^{b}	64.17^{c}	9.40^{c}	54.35^{b}	0.71^{b}	0.86^{b}	0.00^{a}
	±0.25	±0.18	±2.76	±0.49	±5.27	±0.18	±0.12	±0.00
pH 6.2 / Inoc.	0.31^{a}	$1.70^{a,b}$	116.05^{a}	9.01^{a}	$3.84^{a,b}$	0.12^{a}	0.09^{a}	0.00^{a}
	±0.02	±0.29	±5.98	±0.53	±1.72	±0.03	±0.02	±0.00
pH 6.2 / Un-Inoc.	0.29^{a}	1.39^{a}	119.25^{a}	8.18^{a}	1.09^{a}	0.08^{a}	0.07^{a}	0.00^{a}
	±0.03	±0.42	±9.62	±0.88	±0.41	±0.04	±0.01	±0.00
pH 6.2 / Control	0.31^{a}	2.01^{b}	135.72^{a}	10.39^{a}	6.18^{b}	$±0.23^{a}$	0.07^{a}	0.00^{a}
	±0.02	±0.28	±7.11	±0.90	±3.68	0.09	±0.01	±0.00

Values with different superscripted letters ($^{a, b, c}$) are significantly different between treatments within the same soil pH variant ($p<0.05$).

Plant uptake of water and nutrients significantly decreased the cumulative leaching losses of all nutrients (summation over the whole experimental period) compared to the depletion under the controls (data not presented). Significantly higher leaching losses for the observation period of 16 months were detected for the inoculated variants in the unlimed pots. The differences resulted from higher mean amounts of soil water efflux (not significant) and not from higher element concentrations in the inoculated treatments (compare *Fig. 3.7* & *Tab. 3.6*).

No differences between inoculated and untreated variants were found for the two other planted soils.

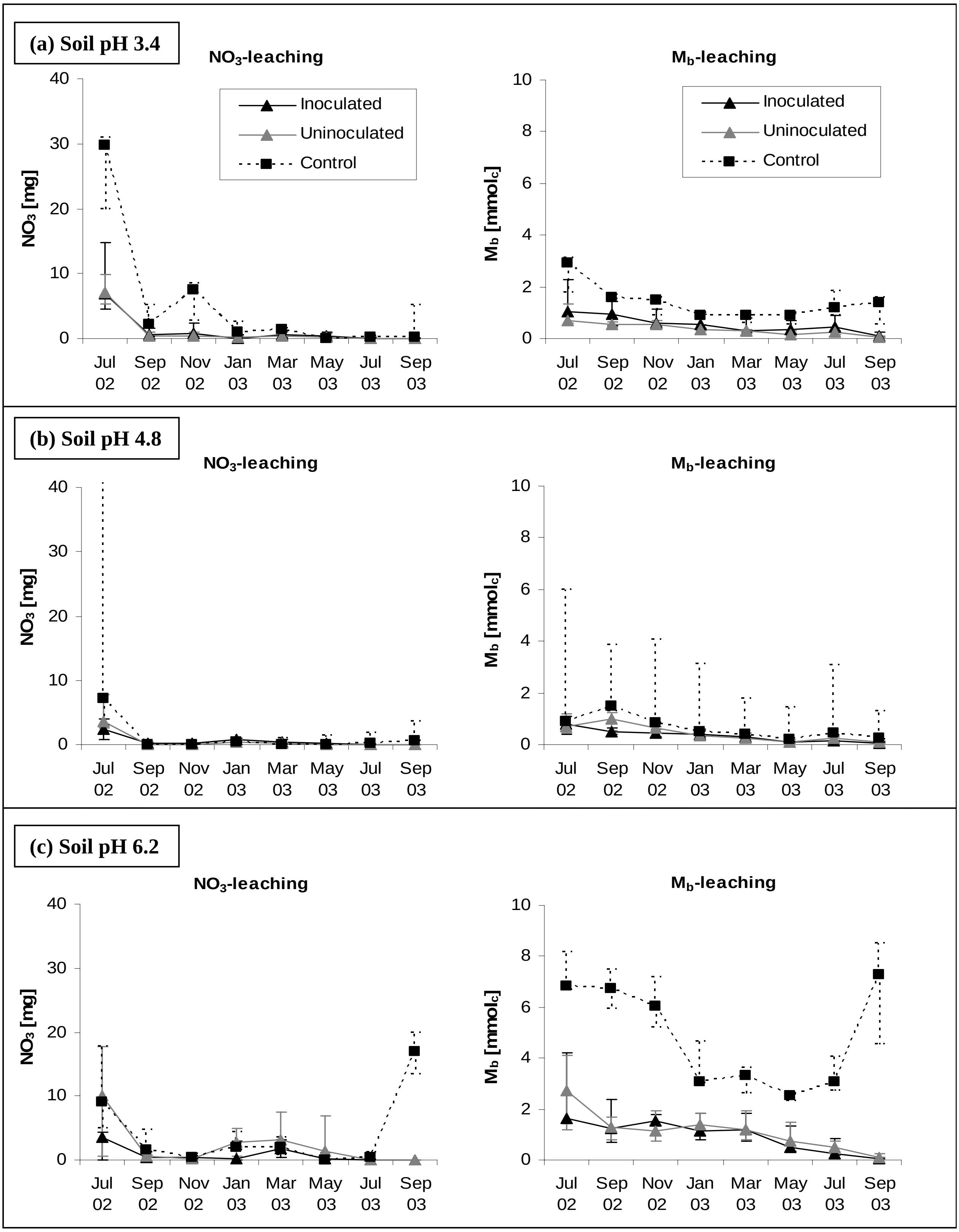

***Figure 3.8:** Mean (± quartiles) leaching losses of NO_3^- [mg] and base cations (M_b) [$mmol_c$] from soils with different starting pH values and inoculation treatments (Inoculated; Uninoculated) in pots planted with Robinia pseudoacacia for the observation period from June 2002 to September 2003 (planted variants n=15, unplanted controls n=5).*

The initial soil treatment caused intensive mineralization leading to high leaching losses of all nutrients, especially in the first sampling interval in June/July 2002 (*Figure 3.8*). No significant differences in the leaching of nitrate between inoculated and untreated seedlings in the three pH variants were observed for the single observation intervals. Significantly higher leaching losses of NO_3^- under the unplanted reference pots than the planted ones occurred when the soil was unlimed. In the limed control pots, nitrate leaching was high at the beginning (June/July 2002). Leaching losses of base cations from the unplanted pots was high for the unlimed treatment and for the one receiving high amounts of lime (initial soil pH 6.2). Significantly high losses of M_b cations from pots with inoculated plants in the unlimed soil were determined from September 2002 to July 2003. For the two other soil variants no significant difference between the two treatments was found. Leaching of base cations was enhanced in strongly limed soil in both planted and unplanted pots because of the large CaCO3 application. As expected, leachate of M_b cations under the strongly limed variant was dominated by Ca (65-85%).

As for PO_4-P and the different cations (*Table 3.6*) the highest N-concentrations and leaching losses were determined under the unplanted pots (*Table 3.7*). No significant differences in the mean N_{tot} concentrations (11-17mg/L) and in N_{tot} depletion (64-99mg) between reference soils of the three pH variants were found. Differences in the chemical composition of the leachate between planted and unplanted pots were most strongly pronounced in the unlimed soils. The concentrations of the N fractions between inoculated and uninoculated did not differ significantly within that lime treatment, but slightly higher NH_4-N, N_{org}-N and N_{tot}-N concentrations as well as higher water efflux under the inoculated soils led to significantly higher cumulative leaching losses of these fractions. For the moderately limed soil the concentrations of NH_4-N and N_{org}-N were higher under the uninoculated pots, whereas the NO_3-N concentrations were higher under the inoculated soils. No significant differences between the two treatments were found in respect to the total leaching losses. Higher NO_3-N concentrations under the uninoculated pots were the only significant difference among N fractions in the percolate under the strongly limed soils.

Table 3.7: *Mean (± standard error) concentrations (mg/L) in and absolute leaching losses (mg) of the different N fractions with the percolate under the soils with different initial pH values and inoculation treatments over the complete observation period (n=16 months).*

Initial Soil pH / Treatment	NH_4-N		NO_3-N		N_{org}-N		N_{tot}-N	
	mg/L	mg	mg/L	mg	mg/L	mg	mg/L	mg
pH 3.4 / Inoc.	$1.80^{a,b}$ ±0.50	5.50^{a} ±1.54	2.32^{a} ±0.99	$19.41^{a,b}$ ±5.88	5.94^{a} ±0.81	20.61^{a} ±5.37	10.53^{a} ±1.33	45.52^{a} ±12.28
pH 3.4 / Un-Inoc.	1.01^{a} ±0.31	1.28^{b} ±0.27	2.33^{a} ±0.60	11.10^{a} ±1.54	4.63^{a} ±0.54	6.15^{b} ±0.66	8.92^{a} ±0.80	18.53^{b} ±1.67
pH 3.4 / Control	3.04^{b} ±1.11	13.34^{a} ±6.50	6.15^{b} ±3.90	51.18^{b} ±8.11	7.77^{a} ±2.24	35.58^{a} ±14.01	16.92^{b} ±0.98	99.48^{c} ±23.43
pH 4.8 / Inoc.	0.43^{a} ±0.10	1.15^{a} ±0.16	1.29^{a} ±0.22	4.83^{a} ±0.87	4.08^{a} ±0.31	5.66^{a} ±0.63	7.50^{a} ±0.79	11.64^{a} ±1.32
pH 4.8 / Un-Inoc.	0.86^{b} ±0.16	$2.69^{a,b}$ ±1.00	0.59^{b} ±0.17	7.87^{a} ±2.12	5.39^{b} ±0.66	10.22^{a} ±3.49	$9.41^{a,b}$ ±1.18	20.78^{a} ±5.57
pH 4.8 / Control	0.95^{b} ±0.20	6.66^{b} ±3.14	$1.32^{a,b}$ ±0.57	29.62^{a} ±15.37	7.88^{c} ±0.22	27.39^{a} ±12.53	12.50^{b} ±0.71	63.67^{a} ±30.68
pH 6.2 / Inoc.	$1.07^{a,b}$ ±0.52	5.61^{a} ±3.10	1.29^{a} ±0.22	13.88^{a} ±4.70	4.30^{a} ±0.27	12.03^{a} ±3.19	8.92^{a} ±1.24	31.51^{a} ±8.68
pH 6.2 / Un-Inoc.	1.45^{a} ±1.10	11.78^{a} ±10.89	3.96^{b} ±0.90	$26.99^{a,b}$ ±4.83	4.23^{a} ±0.34	12.67^{a} ±5.31	11.15^{a} ±1.72	51.45^{a} ±18.21
pH 6.2 / Control	2.06^{b} ±1.08	19.83^{b} ±8.14	2.06^{a} ±0.43	39.78^{b} ±3.92	4.02^{a} ±0.23	25.24^{b} ±3.53	10.63^{a} ±2.72	84.84^{b} ±10.69

Values with different superscripted letters ($^{a, b, c}$) are significantly different between treatments within the same soil pH variant (p<0.05).

The comparison of the percolate between planted soils with different acidity and within the same inoculation treatment revealed significantly lower concentrations and leaching losses of all N fractions under soil variant (b) / inoculated than under the unlimed and inoculated variant (statistics not presented). For the uninoculated pots of these soil pH variants, only the NO_3-N concentration was significantly lower under the moderately limed soils. Comparing the unlimed and strongly limed soils, the NH_4-N concentrations were significantly lower under the inoculated and the concentrations and leaching losses were higher under the uninoculated pots of the strongly limed variants. For soil variant (c) significantly higher NO_3-N and N_{tot}-N losses were detected under the inoculated and higher concentrations and leaching losses of NH_4-N and NO_3-N as well a higher N_{tot} depletion under the uninoculated soils were determined in comparison to the moderately limed variants.

Leaching losses of nitrate and depletion of mineral nutrients from soils under *Robinia pseudoacacia* could not be verified in the short term pot experiment. This was probably due to the soil changes associated with their preparation for the experiment (drying, sieving and re-wetting) causing mineralization of soil organic components and thereby covering any expected effects of black locust cultivation on the chemical composition of soil solution. Plant uptake of nitrate and base cations reduced leaching losses compared to the unplanted references.

3.5 Conclusions

The hypothesised negative effects of black locust cultivation concerning soil acidification and nutrient depletion were partly confirmed in the pot experiment. Drying, sieving and re-wetting of soil caused intensive mineralization, presumably covering other soil processes. In moderately limed soils only, a decrease of pH and base saturation due to elevated seedling growth was found. Inoculation with Rhizobium did not promote N_2 fixation, plant growth, or the subsequent processes of soil degradation. Pre-treatment of soil enhanced the availability of mineral nitrogen and suppressed the formation of effective symbioses in soils with inocula supply. However, inoculated seedlings showed higher growth rates in the second vegetation period, indicating a delayed effect of the treatment with unknown consequences for plant growth and soil chemical status in the future. In future studies, longer seedling cultivation is recommended.

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4 Root-induced pH gradients of black locust (*Robinia pseudoacacia* L.) seedlings grown in soils with different acidity

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4.1 Abstract

The degree of rhizospheric acidification through the cultivation of the tree-legume black locust (*Robinia pseudoacacia* L.) depends on the quantity of N_2 fixation, the form and quantity of additionally absorbed mineral N fractions (NH_4^+ or NO_3^-) by plants, and the soil chemical status (i.e. the buffer capacity). Black locust seedlings were cultivated for six months in rhizotrons with different soil lime treatments [initial pH_{KCl} values of 3.4 (unlimed soil), 4.8 (moderately limed soil) and 6.2 (strongly limed soil)]. Main objectives of the study were to determine changes of chemical soil properties as a response to plant growth and to explain expected pH differences between bulk soil and rhizosphere. Measured data were used in a multispecies rhizosphere model to find correlations between measured and modelled pH values in the rhizosphere and plant nitrogen uptake.

Liming promoted plant growth significantly (+ 30-60% higher biomasses) and resulted in a root system with significantly higher proportions of fine roots and the highest root surface areas. Seedlings from both limed soils accumulated higher amounts of nutrients in their plant tissue, resulting in an excess of cations (+ 1.0 mmol) and in higher calculated root H^+ excretion rates (+ 2.3 to 3.3 mmol H^+ for ammonium nutrition). There was a high correlation between the amount of excess cations in plants and the dry matter production (r = 0.87) and the plant nitrogen content (r = 0.85), respectively. In all soils the concentrations of the exchangeable main base cations decreased significantly by the end of the experiment. Proton

concentrations increased in the unlimed (+ 3.6 µmol/g) and moderately limed (+ 4.9 µmol/g) soils, whereas no change was observed in the strongly limed soil. The effective cation exchange capacity (CEC_e) and the base saturation decreased significantly in the moderately limed soil (- 34%; - 20%), as did the CEC_e in the strongly limed soil (- 46%). Liming of the soils resulted in higher contents of NO_3^- (+ 64% in the moderately and + 430% in the strongly limed soil), whereas in the unlimed soil $NH_4^+$was the dominant N species (+ 250% compared to the limed soils). Soil liming and subsequent processes of mineralization and nitrification elevated leaching losses of N_{tot}-N (1.3 to 4.3 mmol per pot higher than in unlimed soils), base cations and protons, whereas a higher release of acid cations (Fe^{3+}, Al^{3+}) was observed from the unlimed soil. The calculated balance of nutrient contents (soil content at the beginning minus ∑leaching losses and plant accumulation) matched very well the measured values at the end of the experiment for most exchangeable cations. Distinctively higher measured than calculated N contents in the high-pH soils indicated additional N inputs via mineralization or in the case of plant uptake N_2 fixation. Higher measured than calculated H^+ contents (+ 6 to 11 mmol per pot) in all soils were attributed to the proton release by roots and the mineralization of soil organic matter. Measured rhizosphere pH values were 0.7 to 0.8 units lower compared to the bulk soil, without difference between treatments. However, differences in the increase of rhizosphere H^+ concentrations between soils were more distinct (44 to 240 µmol /L, unlimed soil; 11 to 71 µmol /L, moderately limed soil and 2 to 9 µmol /L, strongly limed soil).

The multispecies rhizosphere model calculated the rhizosphere pH values under the assumption of preferential NH_4^+ uptake. The calculated pH decreases in the three soil treatments were the consequence of proton excretions by the roots. Due to the extremely high affinity of protons to the soil exchanger the calculated pH decrease in the rhizosphere of the unlimed and moderately limed soils was higher than the measured one. For the unlimed and moderately limed soil treatments the model explained the rhizospheric pH decrease by nitrogen uptake exclusively in form of NH_4^+, whereas measured pH values in the rhizosphere of the strongly limed soil can be explained by various ratios of NH_4^+/NO_3^- uptake. But the model calculations were conducted at higher NH_4^+ and NO_3^- bulk concentrations compared to the low measured content of mineral N fractions in soils at the end of the experiment, which indicated that N_2 fixation must also have been an important factor for the plant nitrogen nutrition and the rhizospheric acidification.

For a better understanding of the mechanisms responsible for the measured rhizospheric pH decreases, experiments with labelled nitrogen (^{15}N) addition are required to determine the proportions of the different N species taken up by plants.

4.2 Introduction

The information of root-induced pH in the rhizosphere is of particular importance for soil chemical processes, which are strongly related to the proton activity (Gobran et al. 1998). The pH gradient from the rhizosphere to the bulk soil may range from nearly no changes to up to two pH units. The rhizosphere pH is driven by the amount of potentially proton producing substances excreted by the roots and the availability of H^+ buffering substances in the rhizospheric soil. Hinsinger et al. (2003) reviewed root induced pH changes and suggested following aspects: root respiration and exudation of organic carbon and corresponding release of CO_2 (Schachtschabel et al. 1992; Lambers et al. 1996); root exudation of organic substances leading to enhanced C and N mineralization or to deprotonation of organic acids (Jones & Darrah 1994; Jones & Brassington 1998; Jones et al. 2002); enhanced H^+ efflux resulting from iron and phosphorous deficiency (Marschner et al. 1982; Marschner et al. 1986; Gillespie & Pope 1990; Tang et al. 2001; Wang et al. 2004); imbalance in cation-anion uptake, particularly dependent on the nitrogen source (Marschner & Römheld 1983; Häussling et al. 1985; Römheld, 1986). The important processes causing pH changes are attributed to the H^+ or OH^- excretion by the roots due to unbalanced cation/anion uptake of nutrients (Haynes 1990). Nitrogen plays the most important role in the cation-anion balance, because its amount taken up by most plant species is high (Marschner, 1995; Mengel et al., 2001) and it occurs as a cation (NH_4^+), as an anion (NO_3^-) or in the case of N_2-fixing plants (e.g. black locust) as an uncharged species (N_2). Plants supplied with NO_3^- will counterbalance the corresponding excess of negative charges in plant tissue by releasing equivalent amounts of HCO_3^- into the rhizosphere and thereby increase rhizosphere pH, whereas roots react on the uptake of NH_4^+ with the release of H^+ leading to rhizospheric acidification (Le Bot et al., 1990; Gahoonia et al., 1992). Legumes, e.g. black locust, using fixed atmospheric dinitrogen as an additional N source, take up more cations than anions, and hence release the excess of positive charges as H^+ and acidify their rhizosphere (Liu et al., 1989; McLay et al., 1997; Tang et al., 1997). The rate of acid efflux through black locust roots is determined by the quantity of N_2 fixation and the form and quantity of soil N absorbed during N_2 fixation. In the presence of high amounts of mineral soil N legumes do not rely wholly on N_2 fixation for plant nutrition. For example, Sanford et al. (1993) reported that the proportion of N obtained from fixation by pasture legumes ranged from 0 to 100%. The availability of soil NH_4^+ and NO_3^- could affect acid production and the uptake of non-nitrogenous excess cations (Tang et al. 1999). For black locust seedlings grown in sand culture with 5mM NH_4-NO_3-supplemented nutrient solution Roberts et al. (1983) detected 74% lower nitrogen-fixation rates and 3 times greater relative growth rates of seedlings than for those grown without nitrogen fertilization. Consequently, in environments with high mineral N availability legumes preferentially utilize mineral nitrogen species (NH_4^+, NO_3^-) for plant growth and reduce or stop fixation to avoid carbon costs involved with the chemical reactions of N_2 fixation (compare Werner 1987; Warembourg & Roumet 1989).

In a previous model the rhizosphere pH was described as a result of the movement of the acid-base pair H^+-HCO_3^- (Nye 1981) under constant predefined H^+/$HCO3^-$ root excretion rates. But a quantitative analysis of the interrelation of H^+/OH^- root excretion and rhizosphere pH under consideration of buffering reactions is still missing. An outstanding characteristic of the rhizosphere is the existence of ion-specific gradients of all ions involved in the bulk soil. The uptake of some nutrients in high rates usually causes a depletion in the rhizosphere soil solution and leads to the desorption of those cations from the soil exchanger (e.g. K^+ or NH_4^+; Claassen et al. 1986). Highly available ions usually accumulate on the root surface via the water flux towards the root. As the actual uptake rates of nutrients may change temporally the H^+/OH^- root excretion rates may change during the root uptake process. Modelling of the rhizospheric pH should consider the uptake of all major nutrients, which can be realized by a multispecies model, only. In an appropriate model approach the H^+/OH^- root excretion rates can be formulated as a response to the actual uptake of the nutrients involved.

The objectives of this study were to determine the degree of rhizospheric acidification for black locust seedlings cultivated in soils with different buffer capacities and to explain the processes responsible for the decrease of the pH. We present (1) measured values of the rhizosphere pH and of the chemical status of the bulk soil and the plants as well as nutrient balances under experimental environments, (2) calculations of the rhizosphere pH with a multispecies model, (3) comparisons of measured and calculated pH changes in the rhizosphere as affected by various liming levels of an acid forest soil and (4) assessments of the uptake of mineral soil N as NH_4^+ or NO_3^-.

4.3 Model-Theory

It was assumed that the soil solution of the rhizosphere contains the following set of elements:

$$\Omega = \Omega_{cation} \cup \Omega_{anion} = \left\{ H^+, Al^{3+}, Ca^{2+}, Mg^{2+}, K^+, Na^+ NH_4{}^+ \right\} \cup \left\{ NO_3{}^-, SO_4{}^{2-}, HCO_3{}^-, OH^-, Cl^- \right\}$$

4.3.1 *Ionic flux equation*

The ionic flux equation in the rhizosphere was described by the Nernst-Planck equation, which was extended by a convection term. In accordance with the previous models the steady state condition of water flux in the rhizosphere is assumed to be valid (Nye & Tinker 1977). The flux of each ion M_i in Ω was defined by

$$J_i = -D_i \left(\frac{\partial C_i}{\partial r} + \frac{F}{RT} z_i C_i \frac{\partial \Phi}{\partial r} \right) - \frac{q_0}{r_0} C_i \qquad (1)$$

where J_i is the flux of M_i, $D_i = \theta * fi * D_i^0$ its soil diffusion coefficient, D_i^0 its self diffusion coefficient, C_i its solution concentration, z_i its valancy, θ is the volumetric water content and fi is the soil impedance factor accounting for the tortuosity of the diffusion path. *T* is the absolute temperature, *R* is the *gas*, *F* the Farraday´s constant, q_0 is the transpiration induced root water uptake rate inducing a water flux towards the root and *r* is the radial position in the rhizosphere.

The flux of ions is modified by various soil chemical reactions, which are treated as equilibrium reactions or as kinetical constrained reactions.

4.3.2 *Equilibrium reactions with CO_2*

The equilibrium reaction of water, $H^+ + OH^- \leftrightarrow H_2O$, is characterized by

$$C_H * C_{OH} = K_W \qquad (2a)$$

where p_{KW} = 14.0. The protolysis of H_2CO_3, expressed by the equilibrium reaction $H^+ + HCO_3 \leftrightarrow H_2CO_3^-$, is described by the law of mass action. By setting pCO_2 equal to a constant, the relation between H^+ *and* HCO_3^- is given by

$$C_H * C_{HCO_3} = K_S * K_H * p_{CO_2} \qquad (2b)$$

where K_S and K_H are the dissociation constant of H_2CO_3 and the Henry constant, respectively.

4.3.3 Cation exchange

Exchange reactions between the soil solution and the soil exchanger, which has a fixed cation exchange capacity, involve all cations. Previous investigations of proton buffering in various soils showed that the short-time buffering of the pH is traceable to the exchange of protons against cations (Schaller & Fischer 1985; Schaller 1987). Hence, a slow buffer reaction as realized in Nye´s model (1981) was not considered here. Following Prenzel and Schulte-Bisping (1991), the exchange reactions may be represented by algebraic equilibrium expressions. The stoichiometric cation exchange reactions between the soil solution and the soil exchange phase may be presented in accordance with thermodynamically convention. If there are two cations M_i and M_j with valences z_i and z_j, the exchange reactions can be described as $\frac{1}{z_i} M_i^{+z_i} + \frac{1}{z_j} M_j X_{zj} \leftrightarrow \frac{1}{z_j} M_j^{+z_j} + \frac{1}{z_i} M_i X_{zi}$, where X denotes one unit of charge of the cation exchanger with a fixed cation exchange capacity of $\overline{C}_T$ (compare Prenzel & Schulte-Bisping 1991). In analogy to the law of mass action one defines the selectivity coefficient for the equation above (Bolt 1979) as

$$K_{M_i/M_j}^{sel} = \frac{C_i^{\frac{1}{z_i}} * m_j^{\frac{1}{z_j}}}{C_j^{\frac{1}{z_j}} * m_i^{\frac{1}{z_i}}} \tag{3}$$

where C_i and C_j are the soil solution concentrations of M_i and M_j; $m_k = \frac{\overline{C}_k}{\overline{C}_T}$, $k = i,j$ is the equivalent fraction of M_k on the sorbed phase, $\overline{C}_k$ its exchanger concentration and K_{M_i/M_j}^{sel} is the selectivity coefficient. Generally, it is sufficient for a system of n_l cations to specify n_l-1 equilibrium relations of type (3) for different pairs of cations and the additional condition that the exchange capacity is composed by the relative fractions of equivalent exchanger phase concentrations of all cations involved, which means $\sum_{\mathrm{M_i} \in \Omega_{\mathrm{cation}}} m_i = 1$.

4.3.4 Root mineral nutrient uptake

The root ion uptake was modelled formally according to the widely accepted Michaelis-Menten-kinetics. The flux of ions except protons was defined by a parameter expression in analogy to the Michaelis-Menten kinetics

$$\tilde{J} = \tilde{J}_{\max} * \left(\frac{C(r_0, t) - C_{\min}}{K + (C(r_0, t) - C_{\min})} \right) \tag{4a}$$

where $\tilde{J}_{\max}$ is the maximum influx rate, $C(r_0, t)$ the solution concentration at the root surface, C_{min} the minimum concentration below no influx occurs and K the Michaelis-Menten affinity constant.

4.3.5 H^+/OH^- excretion

The efflux rates of H^+ or OH^- were calculated implicitly via electro-neutrality (see Nye & Tinker 1977). The compensation of excess cation uptake by H^+ and OH^- in the case of excess anion uptake is evident. The excretion is also stoichiometrically equivalent to the charge imbalances (Haynes 1990). Based on these definitions, a cation-anion balance of uptake rates of various ions can be calculated. Let $C = \sum_{M_i \in \Omega_{cation} / \{H^+\}} z_i \tilde{J}_i$ and $A = \sum_{M_i \in \Omega_{anion} / \{HCO_3^-, OH^-\}} |z_i| \tilde{J}_i$ be the sum of cation and anion fluxes across the root surface. As a result, the fluxes of the H^+- or OH^--ions at the root surface are defined as

$$\tilde{J}_H = \begin{cases} -(C - A) & \\ & \text{if } C\text{-}A > 0; \\ 0 & \end{cases}$$

$$\tilde{J}_{OH} = \begin{cases} -(A - C) & \\ & \text{if } A\text{-}C > 0; \\ 0 & \end{cases} \tag{4b}$$

4.3.6 Boundary conditions

At the root surface the general flux equation is defined via $J_i\big|_{r=r_0} = -\tilde{J}_i$ for each of the ions involved, where $\tilde{J}_i$ is a parameter expression (4a) and (4b), respectively, for the flux at the root surface.

4.4 Materials and Methods

4.4.1 Experimental design and measurements

Clones of black locust seedlings (clone 2466, Waldsieversdorf, Germany) were cultivated in sterile vermiculite and transplanted in soil, which was derived from an acidic spruce site (*Picea abies* K.) in Lower Saxony, Germany (Wiedey 1991). After determination of the base neutralisation capacity (BNC) of the soil (Meiwes et al. 1984), three pH levels were obtained through liming: (**a**) Unlimed (0.0g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **3.4**]; (**b**) Moderately limed (2.1g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **4.8**] and (**c**) Strongly limed (5.9g $CaCO_3$ / kg soil) [**pH**$_{(KCl)}$ **6.2**].

Figure 4.1: *Arrangement of rhizotrons in the insulated wagon (left) and a rhizotron unit with two single rhizotrons (right).*

Prior to pH adjustment and planting the seedlings the soil was dried and sieved. Seedlings of the N_2 fixing black locust tree were cultivated with 10 replicates per soil treatment in thin layer rhizotrons (70.0 X 10.0 X 1.5cm) for six month from May to October 2003 (*Figure 4.1*). Root growth and nodule development along a transparent Plexiglas plate was given by fixing the rhizotrons in a 45° angle inside closed and shaded wagons. To simulate natural soil temperatures insulated wagons were connected to a cooling system, providing a constant soil temperature of 6-8° C. The seedlings were watered with deionized water supplied by flexible-tube pumps. The water amounts were adjusted to the plant demands (mean 20ml per day). No further nutrients were added to the solution, plant nutrient demands had to be satisfied by the element content of the soil and by nitrogen fixation.

To quantify leaching losses of nitrogen, anions and cations the percolate under the rhizotrons was collected continuously, stored at 4° C and analyzed for two observation intervals (August & September 2003). Mean leaching losses over the whole observation period were calculated from these two sampling periods. Analysis of the soil solution comprised pH, cations (NH_4^+, H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}), anions (NO_3^-, SO_4^{2-}, PO_4^{3-}) and DON (N_{org}). Leachates of NH_4^+-N, NO_3^--N and total N were analysed with a continuous flow system spectrophotometer (Skalar Analytic GmbH, Erkelenz, Germany) (König et al., 1996), total element concentration of the other elements in the samples were analysed by ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65%concentrated HNO_3.

After six month, pH in the rhizosphere along the root tips and in the bulk soil (in a distance of 3 mm to the root tips) was measured using a combined needle pH electrode with a tip diameter of 0.8 mm (Combination Needle pH Electrode No. 818, Diamond General, Michigan, USA). At the end of the experiment (October 2003) plants were harvested and separated into single compartments (stems, roots, nodules). After that, the biomass and the nutrient contents of the compartments as well as the soil nutrients were measured. To investigate the development of the root system of black locust seedlings in soils with different acidity, roots of seedlings at the beginning of the experiment as well as roots of plants cultivated for six month were scanned. Data were analysed with the *WinRhizo* software (Régent Instruments Inc., Québec, Canada). The *WinRhizo* software calculated the cumulative length, surface area and volume of the complete roots for defined diameter classes (0.2mm) as well as the average diameter of the rooting system.

Chemical analysis of plant tissue comprised measurements of total N content by dry combustion with a CN auto analyser (Vario Elementar Analysensyteme, Hanau, Germany) and the total element concentrations of P, S, Na, K, Ca Mg by ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65%concentrated HNO_3. Excess cations were calculated from the individual elements as the sum of charge concentrations of Na^+, K^+, Ca^{2+} and Mg^{2+} ions minus the sum of $H_2PO_4^-$ and SO_4^{2-} ions. Differences in the excretion of H^+ or OH^- ions through plant roots result from mechanisms by which plants control their ionic budget (Raven & Smith, 1976; Smith, 1979; Franco & Munns, 1982). Depending on the source of nitrogen for plant nutrition (NO_3^-, NH_4^+ or N_2) the efflux of H^+ or OH^- was estimated after the chemical analysis of the whole plant (Beese, 1986; Tang et al., 1997).

Uptake of nitrate (NO_3^-) as the only N source:

$$\boldsymbol{OH^- \ excretion = N_{org} - (\sum Na^+, K^+, Ca^{2+}, Mg^{2+} - \sum H_2PO_4^-, SO_4^{2-})}$$

Uptake of ammonium (NH_4^+) as the only N source:

$$\boldsymbol{H^+ \ excretion = N_{org} + (\sum Na^+, K^+, Ca^{2+}, Mg^{2+} - \sum H_2PO_4^-, SO_4^{2-})}$$

For legumes relying on N_2 fixation as the only N source the proton excretion by roots can be calculated without consideration of the N content in the plant tissue:

$$H^+ \text{ excretion} = (\sum Na^+, K^+, Ca^{2+}, Mg^{2+} - \sum H_2PO_4^-, SO_4^{2-})$$

After addition of 1N NH_4Cl to samples (2,5g sieved material) exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, H^+, Mn^{2+}, Fe^{3+}, Al^{3+}) in soils were percolated and analysed by AAS 300 AA (Varian Inc., Darmstadt, Germany). To determine differences in the content of N fractions in soil (NO_3^-, NH_4^+, N_{org}, N_{tot}) soil samples were extracted with 0.5 M K_2SO_4 (~ 5:1 ratio of solution to dry mass soil) for 18 hours in a batch experiment. The extracted N fractions (NO_3^-, NH_4^+, N_{tot}) were analysed by a continuous flow system spectrophotometer (Skalar Analytic GmbH, Erkelenz, Germany), dissolved organic carbon (DON = N_{org}) was calculated as the difference of N_{tot} to combined nitrogen (NH_4 + NO_3) content.

Plant biomass, element concentrations of plant components and soils were also determined at the beginning of the experiment and served as references and to calculate biomass increment and changes of element concentrations and accumulation.

Statistical analysis of the measured data was performed by the program *Statistica version 6.0* by using the Mann-Whitney U-Test at $p<0.05$ level.

4.4.2 Parameter values used for model calculations

For the calculation of the root surface increment the growth of the root system was assumed to follow an exponential pattern:

$$L_t = L_0 * e^{g*t} \qquad (6a)$$

where L_0 and L_t are the length of the root system at the beginning and at the time t, respectively. The growth parameters were estimated by using the equation (6b) and have been calculated on the basis of the root length measurements conducted for the individual rhizotrons.

$$g = \frac{\ln L_{t_{\max}} - \ln L_0}{t_{\max}} \qquad (6b)$$

where $L_{t_{\max}}$ and L_0 are the length of the root system at the beginning and end of the experiment. An average initial root length of 589.2cm with an average diameter of 0.06925cm was used for all calculations. Corresponding to the volumetric water content, which determines the conducting tube, for the calculation of the cumulative nutrient influxes 30% of the potentially absorbing root surface was used.

For the calculation of the average mineral uptake rates it was assumed that the measured total amount of mineral *M*, A_M (µmol plant^{-1}), taken up by plants during the duration of the experiment, can be presented as cumulative influxes of daily nutrient uptake rates into the growing root system. In detail, for each nutrient M the daily nutrient uptake rate U_M (µmol d^{-1} cm^{-2} root surface) was calculated according to following equation (Barber 1984; Darrah & Rose 2001)

$$N_M = 2 * \pi * r_0 * L_0 \int_0^{t_{\max}} U_M \, dt + 2 * \pi * r_0 \int_0^{t_{\max}} \frac{de^{gt}}{dt} \int_0^{t_{\max}-t} U_M \, dt \qquad (6c)$$

where r_0 (mm) is the root radius, *t* is the time (d) and t_{max} the duration of the experiment (120 days).

Rhizosphere model calculations were realised on the basis of measured soil solution concentrations in the bulk soil (compare *Table 4.1*). The concentrations of OH^- and HCO_3^- were implicitly determined via the bulk soil proton concentrations (see equation (2a) and (2b), respectively) and pCO_2=0.035 kPa.

Table 4.1: *Ion-concentrations in the solution of bulks soils with different starting pH values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.*

Ion	Concentration [mmol L^{-1}]		
	Soil 1	Soil 2	Soil 3
K^+	0.05	0.05	0.05
Ca^{2+}	0.15	0.25	0.50
Mg^{2+}	0.05	0.50	0.05
NH_4^+	0.50	0.35	0.19
Na^+	0.025	0.025	0.025
SO_4^{2-}	0.05	0.05	0.05
$H_2PO_4^-$	0.005	0.005	0.005
NO_3^-	0.05	0.35	0.50

As ionic composition of the soil exchanger complex over the complete observation period measured values at the end of the experiment were used. The pK-values of the cation selectivity coefficients correspond to the range of pK-values determined for a huge variety of acidified soils by Prenzel and Schulte-Bisping (1991).

The volumetric water content was defined to θ=0.3, which implies an impedance factor (f_l) of 0.1 (Beese, 1986). The measured soil density was 0.9 g cm^{-3}. The self diffusion coefficients of the different ions involved are reported by Li & Gregory (1974). As root radius the average value of the measured root diameters of the individual plants in every single rhizotron were used for the calculations. Rhizosphere model calculations were introduced based on rhizotron-specific measured values of bulk soil chemistry and assumed uptake parameters. The potential root uptake rates of mineral nutrients ($\widetilde{J}_{\max}$-values) were adjusted to the actual daily root uptake rates. The Michaelis-Menten constant was set to 0.1 mmol L^{-1} for Ca^{2+}, Mg^{2+} and Na^{+} and to 0.05 mmol L^{-1} for K^{+}, NH_4^{+} and NO_3^{-} (Barber 1984). The C_{min} values were set to $2.0*10^{-3}$ mmol L^{-} for all ions. The average transpiration-induced water uptake rate was set to $0.5*10^{-5}$ L m^{-2} s^{-1}.

4.4.3 Comparison of model calculation results and measurements

For model calculations the parameter values described above were used. Calculations were conducted for a simulation period of five days. From the calculated nutrient influxes into a single root during the simulation period the actual daily mineral uptake rates $\widetilde{U}_M$ (µmol cm^{-2} d^{-1}) for each mineral nutrient were calculated. The potential nutrient uptake rates [$\widetilde{J}_M$ (equation 4a)] in the model calculations were changed until $\widetilde{U}_M$ values agreed with the measurement-related daily uptake rates U_M (µmol cm^{-2} d^{-1}) for each mineral nutrient, as determined via equation (6c).

It was assumed that the measured rhizosphere pH represents a spatial average value, which can be related to a soil volume of 0.8mm (diameter of the pH electrode) distance from the root. The calculated proton concentration gradients had spatially high resolution with a distance of up to 0.8mm from the root and were used to calculate an average concentration value. Hence, the average proton concentration $\widetilde{C}_H$ is given by

$$\widetilde{C}_H = 2 * \pi * \frac{1}{V} \cdot \int_{r_0}^{0.08+r_0} r * C_H(r,t) * dr \qquad (6d)$$

where $V = \pi * \left((r_0 + 0.08)^2 - r_0^2 \right)$ is the volume of an unit cylinder of 0.08cm distance from the root. The rhizosphere pH is then defined as: $pH_{Rh} = -\log \widetilde{C}_H$. The measured pH values in a distance of 1.0 cm to the root surfaces were defined as bulk soil pH (pH_{Bulk}).

4.5 Results and Discussions

4.5.1 Measured parameters

4.5.1.1 Plant analysis

Liming of the soil promoted the growth of black locust seedlings in the rhizotrons (*Table 4.2*). Seedlings grown in the limed soils were significantly taller and had a higher dry weight by the end of the experiment. The same positive effect of liming was found for the number of produced nodules. The nodule dry weight and the length of the main root system were also enhanced through liming, however, these effects were not significant.

Table 4.2*: Mean dimension (± standard deviation), number (nodules) and biomass (dry weight) of plant compartments at the end of the observation period (October 2003); n=10.*

Soil treatment / initial pH_{KCl}	Stems		Roots (length of main root system)		Nodules		Plant biomass
	cm	g	cm	G	number	mg	g
(a) / pH_{KCl} 3.4	15.9^{a}	0.7^{a}	62.0^{a}	1.7^{a}	22^{a}	53.1^{a}	2.4^{a}
	±4.1	±0.2	±15.7	±0.8	±11	±24.5	±1.1
(b) / pH_{KCl} 4.8	20.05^{b}	1.2^{b}	67.4^{a}	2.6^{a}	63^{b}	86.3^{a}	3.9^{b}
	±3.5	±0.5	±7.3	±1.2	±38	±45.9	±1.7
(c) / pH_{KCl} 6.2	18.19$^{a.b}$	1.0^{b}	70.2^{a}	2.2^{a}	82^{b}	58.2^{a}	3.2$^{a.b}$
	±3.9	±0.3	±5.3	±0.9	±36	±26.0	±1.2

Values denoted with different indices (^{a,b}) are significantly different between treatments ($p<0.05$)

To precisely define the root formation under different chemical soil conditions the root system of every single plant was scanned at the end of the experiment (*Figure 4.2*). High pH values promoted root branching significantly (cumulative length increment of 2713 cm in the unlimed soil compared to 4275 and 5239 cm in the moderately and strongly limed soils). Obviously liming exerted a positive effect on root development, although the differences between the moderately and strongly limed soils were not significant. The average root diameter at the end of the experiment was significantly lower of roots from the limed soils (mean 0.41mm) than from the unlimed soil (0.55mm), indicating that the alkalisation of the soil resulted in root systems with significantly higher proportions of fine roots. In *Table 4.3* root length, surface area and volume grouped into two diameter classes (≤ 2.0mm; >2.0mm) for the three treatments are presented. It is obvious that liming resulted in a root system dominated by fine roots (D≤ 2.0mm), whereas in the acid soil (a) significantly higher proportions of medium and coarse roots were found. These results were also found in similar studies (Bakker et al. 1999; Rodrigues et al. 2001) and field observations (Puhe 1994; Nowotny et al. 1998).

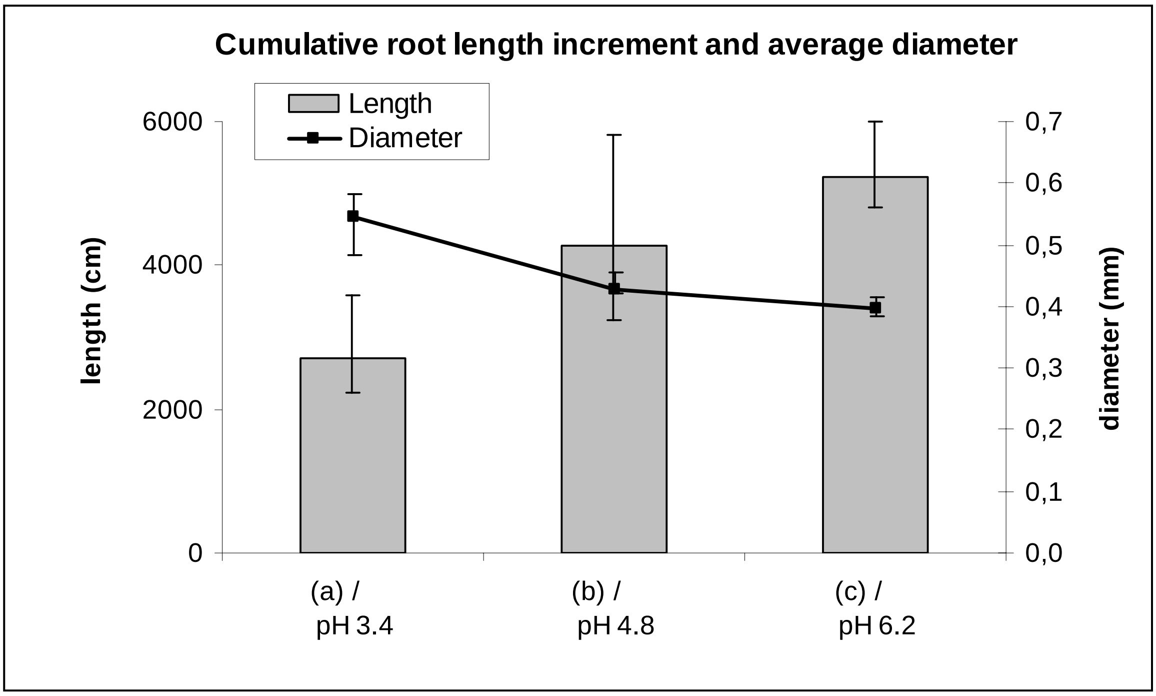

Figure 4.2: *Cumulative root length increment over the complete experimental period (calculated as differences from reference plants) and average root diameter at the end of the experiment for seedlings grown in soils with different initial* pH_{KCl} *values (a, b, c); data presented as medians with quartiles (n=10).*

Seedlings showed significant differences in element concentrations and contents between the beginning and end of the experiment as well as between the three soil treatments [(a) pH_{KCl} 3.4; (b) pH_{KCl} 4.8; (c) pH_{KCl} 6.2] (*Table 4.4*). Concentrations of N, P and acid cations (∑Mn, Fe, Al) increased significantly after six month. For base cations (∑Na, K, Ca, Mg) concentrations decreased significantly at the end of the observation period. The biggest differences of element concentrations from the beginning to the end of the experiment were detected for nitrogen and acid cations. Carbon concentrations remained unaltered, but C content of plants was significantly elevated through plant growth. Also the accumulation of N, P and ∑Mn, Fe, Al was significantly higher compared to the plants at the beginning of the experiment. Due to relatively lower concentrations of Na, K, Ca and Mg, the total content of these elements in black locust seedlings was not enhanced at the end of the study.

***Table 4.3**: Mean (± standard deviation) cumulative root length, surface area and volume of fine roots (D ≤ 2.0mm), medium and coarse roots (D>2.0mm) per plant after cultivation of black locust seedlings in soils with different initial pH values [(a), (b), (c)] for one growing season (n=10).*

diameter class / soil treatment	length (cm)		surface area (cm^2)		volume (cm^3)	
	D<=2mm	D>2mm	D<=2mm	D>2mm	D<=2mm	D>2mm
(a) / pH_{KCl} 3.4	$3335,3^a$ ±858,1	$94,1^a$ ±63,8	$433,1^a$ ±116,9	$111,3^a$ ±81,0	$3,4^a$ ±1,0	$3,9^a$ ±2,7
(b) / pH_{KCl} 4.8	$5201,2^b$ ±1769,8	$35,0^b$ ±14,2	$676,2^b$ ±229,9	$38,7^b$ ±16,9	$6,2^b$ ±2,0	$1,7^b$ ±0,8
(c) / pH_{KCl} 6.2	$6167,6^b$ ±1386,3	$28,3^b$ ±12,5	$734,9^b$ ±186,1	$32,4^b$ ±14,1	$6,1^b$ ±1,7	$1,6^b$ ±0,7

Values denoted with different letters (^{a,b}) are significantly different between soil treatments ($p<0.05$)

Liming of soil exerted a strong effect on plant growth and therefore on nutrient accumulation, but also on element concentrations in plants. Compared to the unlimed soil significantly higher concentrations and accumulation of nearly all elements were measured in black locust seedlings from the limed soils. The concentration of base cations (especially Ca) was highest in the strongly limed soil treatment.

***Table 4.4**: Mean (± standard deviation) element concentrations (mg/g) and content (mg) of black locust seedlings at the beginning of the experiment (reference; n=5) and after cultivation under different soil environments [(a), (b), (c)] for six month (n=10).*

soil treatment / initial pH_{KCl}	C	N	P	∑Na, K, Ca, Mg	∑Mn, Fe, Al
			mg/g		
reference	458,1 ±7,5	19,3 ±0,3	0,4 ±0,1	18,1 ±2,4	0,8 ±0,7
(a) / pH_{KCl} 3.4	453,1^{a} ±1,8	30,0$^{a\,*}$ ±0,5	1,2$^{a\,*}$ ±0,1	11,7$^{a\,*}$ ±0,5	2,2$^{a\,*}$ ±0,3
(b) / pH_{KCl} 4.8	460,8^{b} ±2,5	32,4$^{b\,*}$ ±1,0	1,5$^{b\,*}$ ±0,1	13,2$^{b\,*}$ ±0,9	2,6$^{b\,*}$ ±0,4
(c) / pH_{KCl} 6.2	459,7^{b} ±2,2	32,3$^{b\,*}$ ±1,5	1,5$^{b\,*}$ ±0,1	15,9$^{c\,*}$ ±0,8	2,3$^{a,b\,*}$ ±0,3
			Mg		
reference	346,9 ±201,1	14,5 ±7,8	0,3 ±0,1	13,2 ±6,0	0,6 ±0,5
(a) / pH_{KCl} 3.4	1128,3$^{a\,*}$ ±482,1	52,5$^{a\,*}$ ±24,1	2,3$^{a\,*}$ ±1,1	10,6^{a} ±4,1	2,3$^{a\,*}$ ±1,2
(b) / pH_{KCl} 4.8	1795,3$^{b\,*}$ ±747,8	84,3$^{b\,*}$ ±33,2	4,7$^{b\,*}$ ±1,6	18,5^{b} ±7,7	4,7$^{b\,*}$ ±2,4
(c) / pH_{KCl} 6.2	1453,4$^{a,b\,*}$ ±522,1	69,6$^{a,b\,*}$ ±25,5	3,9$^{b\,*}$ ±1,4	18,7^{b} ±6,6	3,5$^{b\,*}$ ±1,5

*Values denoted with different letters (a,b,c) and with * are significantly different ($p<0.05$) between treatments and in comparison to the reference, respectively.*

Application of $CaCO_3$ to soils elevated the concentrations of anions and cations (data not presented) and also the content of nitrogen (N_{org}), anions ($\sum H_2PO_4^-$, SO_4^{2-}), cations ($\sum Na^+$, K^+, Ca^{2+}, Mg^{2+}) and excess cations (∑cations minus ∑anions) per plant significantly (*Table 4.5*). Assuming that symbiotic fixation was the only N source for plant nutrition, the accumulation of excess cations in the plant tissue can be equated to the rate of H^+ excretion by roots over the whole observation period. The significantly higher amount of excess cations in the tissue of plants grown in limed soils would have resulted in significantly enhanced proton excretion by roots. Liming increased the proton buffer capacity of these soils and therefore proton excretion did not result in a stronger decrease of the rhizosphere pH (*compare pH measurements; Figure 4.5).* Provided that NH_4^+ uptake was the only form of nitrogen acquisition (100%) the resulting H^+ excretion into the rhizosphere would also be significantly higher for plants growing in limed soil (90%). For the uptake of NO_3^- as the only nitrogen source for plant nutrition the amount of OH^- excretion rates would not have been significantly different between the soil treatments.

***Table 4.5**: Mean (± standard deviation) accumulation of N_{org}, anions ($\sum H_2PO_4^-$, SO_4^{2-}) and cations ($\sum Na^+$, K^+, Ca^{2+}, Mg^{2+}) in the whole plant, as well as calculated excretion of H^+ or OH^- ions from May to October 2003 depending on the N species taken up by plants (n=10).*

soil treatment/ initial pH_{KCl}	N_{org}	∑ anions	∑ cations	excess cations = H^+ excretion (N_2 fixation)	H^+ excretion (NH_4^+ uptake)	OH^- excretion (NO_3^- uptake)
				Mmol		
(a) /	2.9^a	0.2^a	0.9^a	0.7^a	3.6^a	2.2^a
pH_{KCl} 3.4	±1.7	±0.1	±0.6	±0.5	±2.2	±1.3
(b) /	5.2^b	0.5^b	2.2^b	1.7^b	6.9^b	3.5^a
pH_{KCl} 4.8	±2.4	±0.2	±1.2	±1.0	±3.3	±1.4
(c) /	$4.1^{a,b}$	0.4^b	2.2^b	1.8^b	5.9^b	2.3^a
pH_{KCl} 6.2	±1.8	±0.2	±1.0	±0.8	±2.6	±1.0

Values denoted with different letters (^{a,b}) are significantly different between plants from different soil treatments ($p<0.05$)

Accumulation of excess cations was 1.5-fold higher in plants from limed soils than from unlimed soils. The calculated excess cation accumulation over the experimental period matched very well the nitrogen accumulation as well as the dry matter production (*Figure 4.3*). In a solution culture experiment with twelve pasture legumes Tang et al. (1997) found out, that total H^+ production of herbaceous legumes was also highly correlated with the accumulation of excess cations and total nitrogen accumulation during the study period. In our experiment the correlation between accumulated excess cations and nitrogen seemed to be an effect of seedling growth and not of higher N concentrations in the plant tissue.

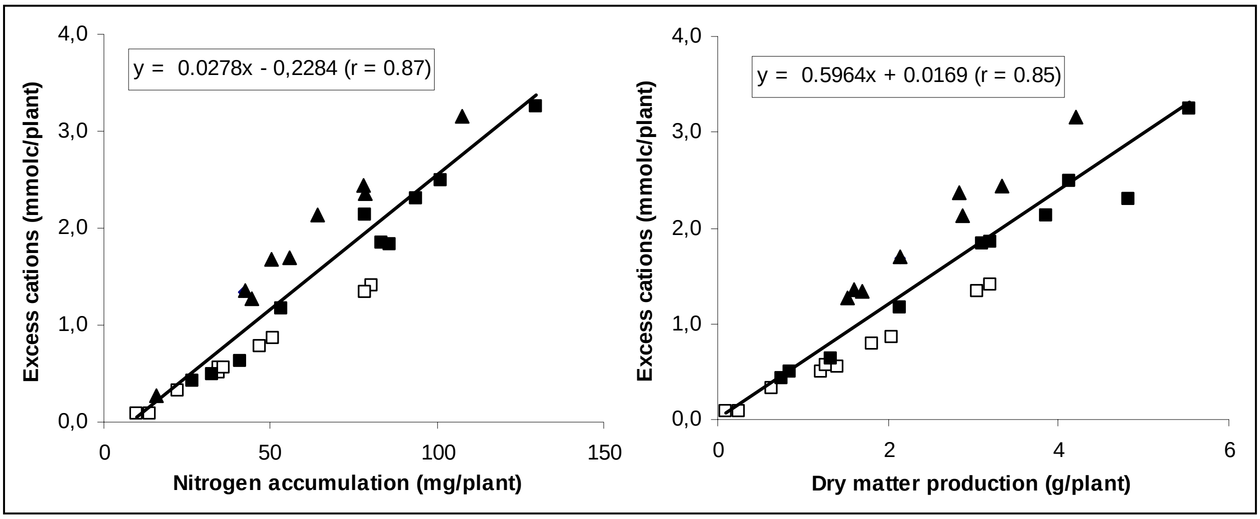

Figure 4.3: *Relationship between excess cations in the plant tissue and nitrogen accumulation in the plants and between excess cations and dry matter production of plants cultivated from May to October 2003 in soils with different* $CaCO_3$ *application (□ unlimed soil; ■ moderately limed soil; ▲ strongly limed soil).*

4.5.1.2 Soil analysis

Significant changes in chemical properties after the cultivation of black locust seedlings for one growing season (*Table 4.6*) were observed. The concentrations of the main exchangeable base cations (K, Ca, Mg) in the bulk soil decreased significantly for all soil treatments during the observation period [except for Ca^{2+} in the unlimed soil (a)]. Proton concentrations increased significantly in the unlimed (a) and moderately limed (b) soils after six month of black locust cultivation. Concentrations of exchangeable Fe and Al decreased significantly in the unlimed soil (a), whereas for the limed soils (b) and (c) a significant increase was observed. The effective cation exchange capacity (CEC_e) decreased significantly during the six months in both limed soils. Also the base saturation dropped significantly after liming, however a drastically lowered base saturation was observed for the moderately limed soil (-20%), only. Soil leaching and plant uptake decreased the concentrations of exchangeable base cations and increased the concentrations of protons and acid cations in soils. However, only in the unlimed (a) and moderately limed (b) soils enhanced concentrations of protons were measured, but not in the strongly limed (c) treatment due to high proton buffering in this soil. At the beginning of the experiment not-dissolved lime residues in the soils with $CaCO_3$ application may have elevated the measured concentrations of exchangeable Ca^{2+}. Hence, the determined decrease of exchangeable Ca^{2+} at the end of the experiment may not only be attributed to the impact of black locust cultivation. Moreover, mineralization of soil organic matter during the observation period may have also been a reason for the detected elevated proton concentrations after six months.

Table 4.6: *Mean (± standard deviation) exchangeable cation concentrations, cation exchange capacity (CEC_e) and base saturation (BS) in soils with different initial soil pH values (a, b, c) at the beginning (Start; n=5) and end (End; n=10) of the experiment.*

soil treatm./ initial pH_{KCl}	Time	Na^+	K^+	Ca^{2+}	Mg^{2+}	H^+	Mn^{2+}	Fe^{3+}	Al^{3+}	CEC_e	BS
		$\mu mol_c/g$									%
(a) / pH_{KCl} 3.4	Start	0.5	0.8**	16.0	6.9**	9.7*	0,2	4.4**	12.3**	50.8	47.4
		(0.1)	(0.1)	(3.0)	(0.9)	(0.5)	(0,1)	(0.6)	(1.1)	(5.8)	(2.5)
	End	0.4	0.4	15.8	4.7	13.3	0,2	3.2	8.6	46.6	45.9
		(0.2)	(0.0)	(1.1)	(0.6)	(1.7)	(0,0)	(0.4)	(0.5)	(2.9)	(1.7)
(b) / pH_{KCl} 4.8	Start	0.6	1.0**	75.5**	7.7**	0.0*	0,2	0.5**	0.0**	85.4**	99.2**
		(0.2)	(0.0)	(4.2)	(1.2)	(0.0)	(0,0)	(0.1)	(0.0)	(5.4)	(0.1)
	End	0.6	0.5	39.3	4.6	4.9	0,2	2.1	4.9	57.1	78.9
		(0.2)	(0.1)	(3.9)	(0.4)	(1.6)	(0,0)	(0.2)	(0.8)	(4.2)	(4.0)
(c) / pH_{KCl} 6.2	Start	0.6	0.9**	154.9*	7.6**	0.0	0,2	0.3**	0.0**	164.5	99.7**
		(0.1)	(0.0)	(4.4)	(0.0)	(0.0)	(0,0)	(0.1)	(0.0)	(4,4)	(0.0)
	End	0.6	0.5	83.5	4.8	0.0	0,2	0.5	0.2	90.3	99.0
		(0.2)	(0.1)	(6.2)	(0.8)	(0.0)	(0,0)	(0.1)	(0.5)	(6.6)	(0.6)

*Values denoted with **($p<0.01$) are significantly different within the same soil treatment*

A significant decrease in the soil content of K_2SO_4 extractable nitrogen fractions was observed at the end of the growing period (*Figure 4.4*). In six months the content of N_{tot}-N decreased (per pot) at 5.0mmol (±0.2) in the unlimed soil, 3.2mmol (±0.1) in the moderately limed soil and 6.4mmol (±0.2) in the strongly limed soil. The highest absolute decline of NO_3-N was found under the moderately (b) and the strongly limed (c) soils, whereas in the unlimed soil a higher decrease of NH_4-N was observed. Liming of soil stimulated nitrification and resulted in elevated nitrate concentrations [especially in the high-pH soil (c)] in comparison to the unlimed soil (data not presented). At the end of the experiment the contents of the extracted nitrogen species were significantly lower in all soil treatments. In the unlimed soil no NO_3^--N was measured after the observation period. Low amounts of fixed dinitrogen, plant uptake and leaching seemed to be responsible for the observed depletion of K_2SO_4 extractable nitrogen species. On the other hand, it has to be critically stressed that the determination of the mineral nitrogen status of soils at two points of time (start and end of the experiment) can not reproduce the N dynamics of the system within the experimental duration.

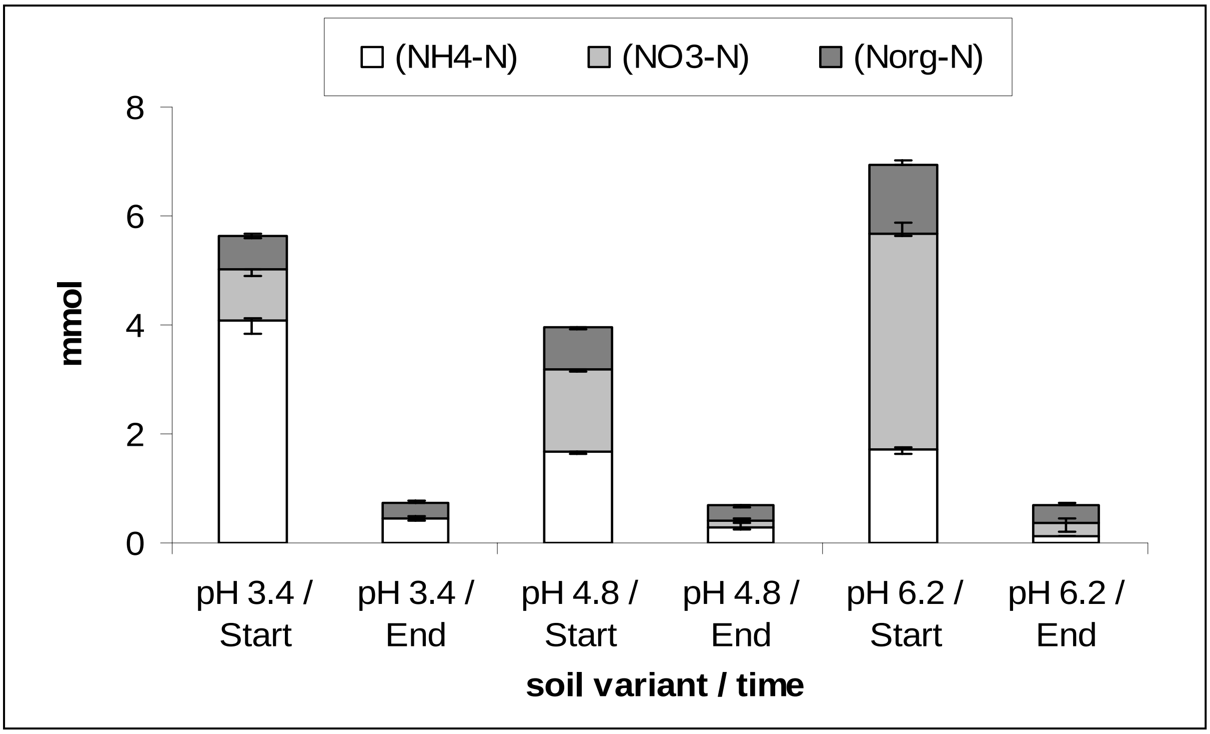

Figure 4.4: *Content of the different K_2SO_4 extractable N fractions per pot (mmol) in May (Start) and October (End) 2003 of soils with different initial pH values; data presented as medians with quartiles (Start n=5; End n=10).*

Liming and subsequent elevated processes of mineralization and nitrification resulted in the highest release of NO_3-N with the soil water under the moderately (b) and the strongly limed (c) soils, whereas NH_4-N release was the same in all treatments (*Table 4.7*). Due to elevated decomposition of the soil organic matter after $CaCO_3$ application, leaching of dissolved organic nitrogen (N_{org}-N) was also higher under the limed treatments compared to the unlimed soil, but a significant difference was detected for the strongly limed soil, only. After six months the following mean amounts of N_{tot}-N (mmol per pot) were released with the percolate from the three soil treatments: (a) 2.18 ±0.55 mmol, (b) 3.53 ±1.61 mmol and (c) 6.46 ±4.78 mmol.

***Table 4.7:** Mean (± standard deviation) cumulative leaching losses (mmol per pot) of the mineral N fractions under soils with different initial soil pH values (a b, c) from May to October 2003 (n=10).*

soil treatment/ initial pH_{KCl}	NH_4-N	NO_3-N	N_{org}	N_{tot}
		mmol		
(a) / pH_{KCl} 3.4	0.26^a ±0.24	1.28^a ±0.90	0.64^a ±0.40	2.18^a ±0.55
(b) / pH_{KCl} 4.8	0.22^a ±0.37	2.41^a ±1.72	0.90^a ±0.44	3.53^b ±1.61
(c) / pH_{KCl} 6.2	0.31^a ±0.41	4.84^b ±4.41	1.31^b ±0.35	6.46^b ±4.78

Values with different superscript letters (^{a,b}) are significant different between soil treatments (p<0.05).

Table 4.8 shows the cumulative discharge of anions (∑PO_4^-, SO_4^{2-}) and cations (∑Na^+, K^+, Ca^{2+}, Mg^{2+}, H^+, Mn^{2+}, Fe^{3+}, Al^{3+}) with the percolate under the rhizotrons with different initial soil pH values from May to October 2003. Compared to the unlimed soil (a), a higher depletion of H^+, Ca^{2+} and Mg^{2+} [for Mg^{2+} in treatment (c)] and of SO_4^{2-} in both lime treatments was detected. Significantly higher leaching losses of Fe^{3+} and Al^{3+} were detected for the unlimed soil (a). Liming enhanced the leaching of base cations (K^+, Ca^{2+}, Mg^{2+}) and protons and decreased the depletion of Fe^{3+} and Al^{3+}.

***Table 4.8:** Mean (± standard deviation) cumulative leaching losses of anions (SO_4^{2-}, PO_4^{3-}) and cations (H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}) per pot under the different soil treatments from May to October 2003 (n=10).*

soil treatm./ initial pH_{KCl}	SO_4^{2-}	PO_4^{3-}	H^+	Na^+	K^+	Ca^{2+}	Mg^{2+}	Mn^{2+}	Fe^{3+}	Al^{3+}
	mmol					$mmol_c$				
(a) / pH_{KCl} 3.4	1.31^a ±0.77	0.02^a ±0.01	5.37^a ±3.35	0.43^a ±0.09	0.13^a ±0.04	4.24^a ±1.65	1.91^a ±0.65	0.04^a ±0.01	1.65^a ±1.98	0.46^a ±0.29
(b) / pH_{KCl} 4.8	1.75^a ±1.14	0.02^a ±0.01	7.50^a ±3.69	0.49^a ±0.14	$0.15^{a,b}$ ±0.06	9.88^b ±3.11	$2.07^{a,b}$ ±0.67	$0.03^{a,b}$ ±0.02	$0.85^{a,b}$ ±1.08	0.18^b ±0.09
(c) / pH_{KCl} 6.2	2.21^a ±1.50	0.03^b ±0.01	10.90^b ±2.91	0.51^a ±0.18	0.17^b 0.04	21.08^c ±5.37	2.75^b ±0.69	0.02^b ±0.01	0.40^b ±0.31	0.06^c ±0.02

Values with different superscript letters (^{a,b,c}) are significant different between soil treatments (p<0.05).

Black locust seedlings growing in soils with considerable amounts of NH_4^+ or NO_3^- obtain part of their N demands from the soil and part from N fixation (Johnsen & Bongarten 1992; Danso et al. 1995). To quantify N accumulation from soil and atmospheric sources these studies used the ^{15}N isotope dilution method. With high levels of soil NO_3^- and NH_4^+ seedlings satisfied most of their N demands from soil N, whereas in soils without these N species the fixation of atmospheric dinitrogen was the dominant N source. These processes are of great importance for the ratio of H^+ and OH^- excretion by roots and therefore for the acidification of the rhizosphere. In our study seedlings were not supplied with labelled nitrogen (^{15}N), therefore the ratio of N uptake by fixation to the uptake of soil N can be estimated by the nitrogen balance (change of N content in soil, leaching of N with the soil water and N accumulation in plants) and the degree of rhizospheric acidification, only.

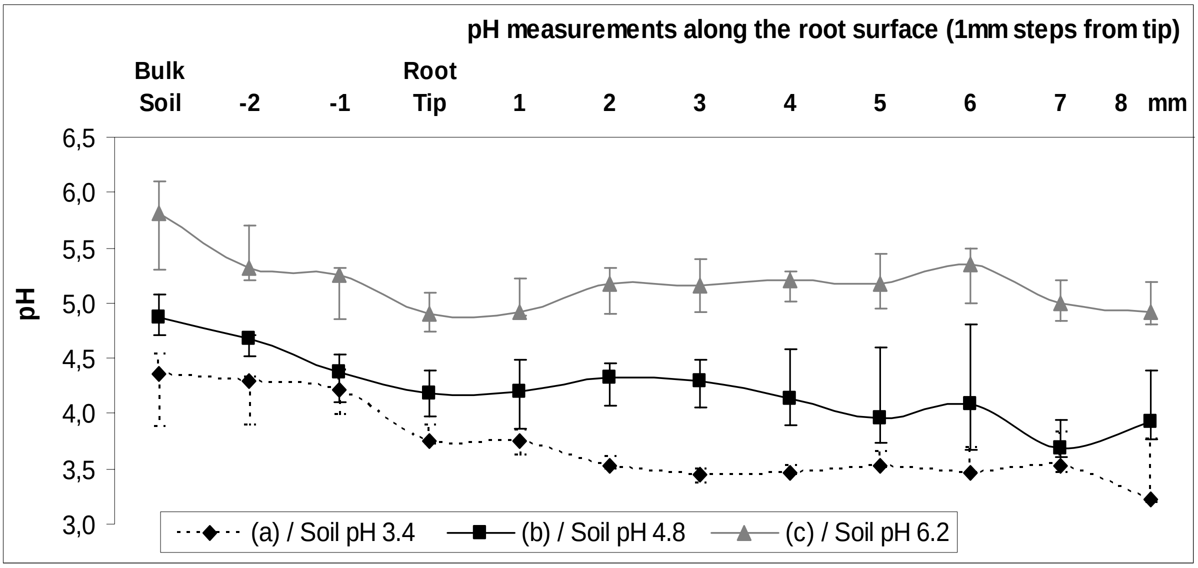

Figure 4.5: *Measured pH values in bulk soil (3mm in front of the root tip) and in the rhizosphere (from 2mm in front of to 8mm behind the root tip) in soils with different initial* pH_{KCl} *values (a, b, c); data presented as medians with quartiles (n=6).*

Depending on the amount of applied $CaCO_3$ and therefore on the different buffer capacity of the soils, measured pH values differed between treatments. However, pH values along the surface of black locust roots (8mm along the root surfaces until 3mm in front of the root tip in steps of 1mm) were constantly for all treatments on average 0.7 to 0.8 units lower than in the bulk soil (*Figure 4.5*). In the unlimed soil (a), the pH value dropped from bulk soil to rhizosphere from pH 4.3 to pH 3.6, in the moderately limed soil (b) from pH 5.0 to pH 4.2 and in the strongly limed soil (c) from pH 5.7 to pH 5.0, on average. In terms of H_3O^+

concentrations the differences were more obvious. Calculated concentrations of H^+ ions in the bulk soil and rhizosphere for the different soil treatments were (bulk soil/rhizosphere): (a) 44/240 µmol/L; (b) 11/71 µmol/L; (c) 2/9 µmol/L. As the rhizosphere comprises a restricted volume of soil around roots, it is rather difficult for in situ measurements to access the actual values of rhizospheric pH (Jaillard et al. 2003). Besides different destructive (e.g. sampling the soil adhering to the roots) and non-destructive (e.g. by use of rhizoboxes or agar-indicators) approaches for rhizosphere pH measurements, the most direct method for measuring H^+ activity in the rhizosphere is potentiometry. pH measurements with microelectrodes in soils are accompanied by several problems, which influence the accuracy and expressiveness of the results. By the determination of the soil suspension pH (solution plus solids) interactions between the soil solution and the soil matrix (e.g. exchange reactions) can lead to erroneous results (Essington 2004). It is a well-established characteristic of soil pH measurements that the pH of a clear supernatant solution sitting on top of soil suspensions will differ from the pH measured in the sediment. This characteristic is termed the suspension effect. Furthermore, soil is a rather dry medium, which raises the problem of liquid continuity between the probe and the soil. With decreasing soil water content also the measured pH values decrease due to simultaneous measurement of the protons in the solution and of those absorbed at the soil exchanger sites. Therefore it can not be excluded that the detected pH decrease in the rhizosphere of black locust seedlings was overestimated due to these problems of pH measurements in soils.

Modelling the results by a multispecies model (see below) delivered possible explanations for the measured pH decrease in the rhizosphere of these soils with different bulk soil pH for different scenarios of N uptake for plant nutrition.

4.5.1.3 Nutrient balances

The measured mean element content (N and exchangeable cations) in soils at the beginning and at the end of the experiment, the element losses through leaching and plant uptake over the whole study period as well as the calculated element content of soils at the end of the experiment (element content at the beginning minus leaching losses and plant uptake) are presented in *Table 4.9*. Lower (and negative) calculated values than measured soil content of elements at the end of the experiment indicate nutrient release by mineralization and/or N_2 fixation (concerning nitrogen uptake), whereas higher estimated values suggest (not-measured) element losses. Differences between calculated and measured values may also refer to inaccuracies in measurements. For the unlimed soil with an initial pH_{KCl} of 3.4 (a), the biggest difference between measured and calculated element content in soil at the end of the experiment was detected for H^+. Measured H^+ content was more than twice as high (11.4 compared to 5.0 mmol) than the calculated one, indicating a strong release of protons by plant roots or mineralization processes (ammonification) in this soil. Also for the moderately limed soil [pH_{KCl} 4.8 (b)] elevated excretion of protons into the soil was obvious. Due to the high proton buffering capacity in soil (c) and leaching no accumulation of H^+ was detected within this treatment after six month. Concerning the other exchangeable cations, the differences between measured and calculated end-content were low for the unlimed soil. In the limed soils a high discrepancy was detected for Ca^{2+} as well as for the acid cations Fe^{3+} and Al^{3+}.

Table 4.9: *Measured mean element content in soils at the beginning (May 03) and at the end (Oct 03) of the experiment, element losses through leaching and plant uptake over the whole study period as well as the calculated element content of soils in October 2003 (n=10).*

soil treatment / compartment and time	N	H	K	Ca	Mg	Mn	Fe	Al
	mmol	mmol$_c$						
(a) soil pH 3.4								
soil content May 03	5.52	8.37	0.69	13.74	5.62	0.15	3.71	10.47
leaching May-Oct 03	2.18	3.40	0.11	3.33	1.44	0.03	0.65	0.27
plant accumulation May-Oct 03	2.91		0.20	0.42	0.05	0.00	0.06	0.05
measured soil content Oct 03	**0.73**	**11.41**	**0.36**	**13.66**	**4.16**	**0.15**	**2.67**	**7.75**
calculated soil content Oct 03	***0.43***	***4.96***	***0.38***	***10.00***	***4.14***	***0.12***	***2.99***	***10.14***
(b) soil pH 4.8								
soil content May 03	4.26	0.00	0.85	65.75	6.68	0.18	0.39	0.00
leaching May-Oct 03	3.53	6.24	0.12	8.19	1.76	0.02	0.39	0.14
plant accumulation May-Oct 03	5.18		0.45	1.31	0.25	0.00	0.17	0.15
measured soil content Oct 03	**0.71**	**4.46**	**0.42**	**34.01**	**4.09**	**0.17**	**1.83**	**4.26**
calculated soil content Oct 03	***-4.45***	***-6.24***	***0.28***	***54.87***	***4.21***	***0.17***	***-0.16***	***-0.28***
(c) soil pH 6.2								
soil content May 03	6.93	0.00	0.77	133.94	6.60	0.16	0.26	0.00
leaching May-Oct 03	6.46	8.82	0.14	15.87	2.12	0.02	0.22	0.06
plant accumulation May-Oct 03	4.14		0.35	1.23	0.22	0.00	0.08	0.11
measured soil content Oct 03	**0.68**	**0.00**	**0.44**	**72.63**	**4.37**	**0.13**	**0.42**	**0.00**
calculated soil content Oct 03	***-3.67***	***-8.82***	***0.28***	***116.84***	***4.26***	***0.14***	***-0.04***	***-0.16***

Leaching losses with the soil water were measured only for two months (August & September 2003) and served as the basis of leaching losses calculation for the duration of the complete study. Especially at the beginning of the experiment (May & June 2003) soil pre-treatment and liming may have elevated mineralization of soil organic matter and leaching of elements. Therefore, the calculated losses with the percolate could have been underestimated resulting in these remarkable differences.

In respect to the balance of nitrogen (for the soil content the pool of K_2SO_4 extractable mineral N fractions is considered), differences between measured and calculated values were high for the limed soils. In these soils leaching losses and plant uptake exceeded the initial soil N content (resulting in negative calculated values), but nitrogen was still measurable in soils at the end of the experiment. Therefore, the mineralization of the soil organic matter and/or the fixation of atmospheric N_2 in the case of plant nutrition must have delivered additional nitrogen into the system. Cumulative N inputs over the complete experimental period of 72.3 mg in the moderately and 64.5 mg in the strongly limed soil per rhizotron must have been derived from these processes. Assuming that this additional nitrogen originated completely from the decomposition of soil organic matter, mineralization rates of 0.64 and 0.54 µg N g^{-1}soil and d^{-1} were calculated. In the unlimed soil the difference between measured and calculated N content was very low (0.3 mmol), indicating a lower stimulation of the N mineralization and/or the N_2 fixation due to not altered soil acidity without $CaCO_3$ application.

4.5.2 Multispecies rhizosphere model

4.5.2.1 Model results

Figure 4.6 shows the growth development of the root surface areas of black locust seedlings grown with different soil treatments. The growth courses base on the average values of the measured data (root length and root diameter) at both, the beginning and the end of the experiment. There is a clear increase of the measured total root length (all diameter classes) from the unlimed soil to the extremely limed soil {3429.2 cm in soil 1 [unlimed soil (a)], 5236.0 cm in soil 2 [moderately limed soil (b)] and 6302.3 cm in soil 3 [strongly limed soil (b)]}, but a decrease of the average diameter from the unlimed to the limed soil treatments (average diameters of 0.054, 0.044 and 0.040 in soils 1, 2 and 3, respectively; compare *Fig. 4.2 & Tab. 4.3*). In relation to the root development in the unlimed soil, the potentially absorbing root surface increased at 24.7% (soil 2) and 36.8% (soil 3) in the limed soils.

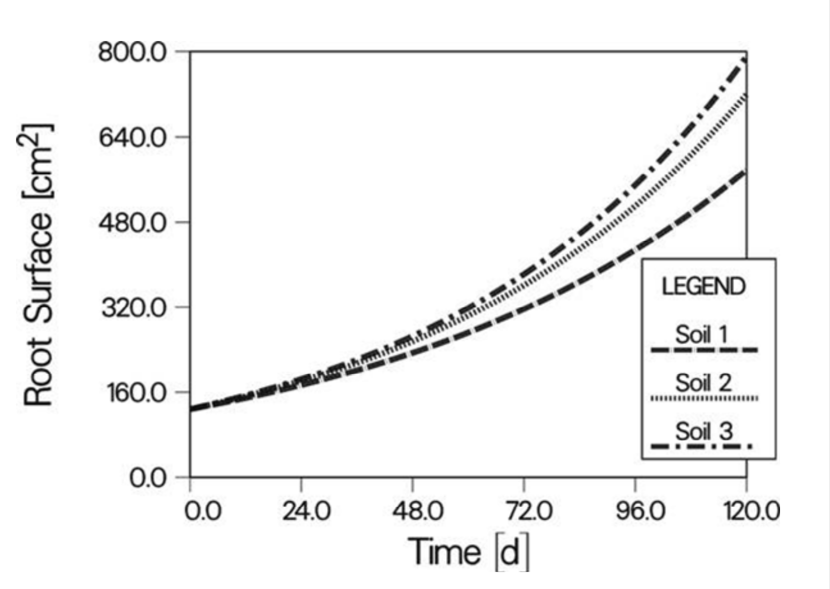

Figure 4.6: *Average (n=10) growth development of the root surface areas (cm^2) of seedlings cultivated in soils with different initial pH$_{KCl}$ values [Soil 1 = pH$_{KCl}$ 3.4 (a); Soil 2 = pH$_{KCl}$ 4.8 (b); Soil 3 = pH$_{KCl}$ 6.2 (c)] for 120 days.*

The temporal development of the cumulative ion uptake calculated according to equation (6c) is exemplarily demonstrated for the uptake of Ca^{2+} (*Figure 4.7*). The measured Ca^{2+} uptake rate N_{Ca} in the rhizotrons of soil 1 [unlimed soil (a)] was significantly lower compared to the plant uptake in the other soil treatments. The N_{Ca} values (µmol per plant) for the three soil treatments were following: soil 1 = 180, soil 2 = 560 and soil 3 = 600.

Daily uptake rates of Ca^{2+} (U_{Ca}) and of the other ions were calculated by the quantity of the measured cumulative uptake rates and the growth development of the root system in the different soil treatments (*Table 4.10*). Compared to the uptake rates in the unlimed soil, liming increased the average daily Ca^{2+}-influxes per unit root surface at 66.0% (soil 2) and 22.8% (soil 3). Similar to Ca^{2+}, the calculated average daily uptake rates of the other ions were highest for plants in the moderately limed soil (soil 2), whereas the uptake rates of seedlings in the strongly limed soil ranged between those of the other two treatments. As documented in detail for Ca^{2+}-ions, the higher N_M values of ions in the limed soil treatments can not be attributed to the elevated absorbing root surface only.

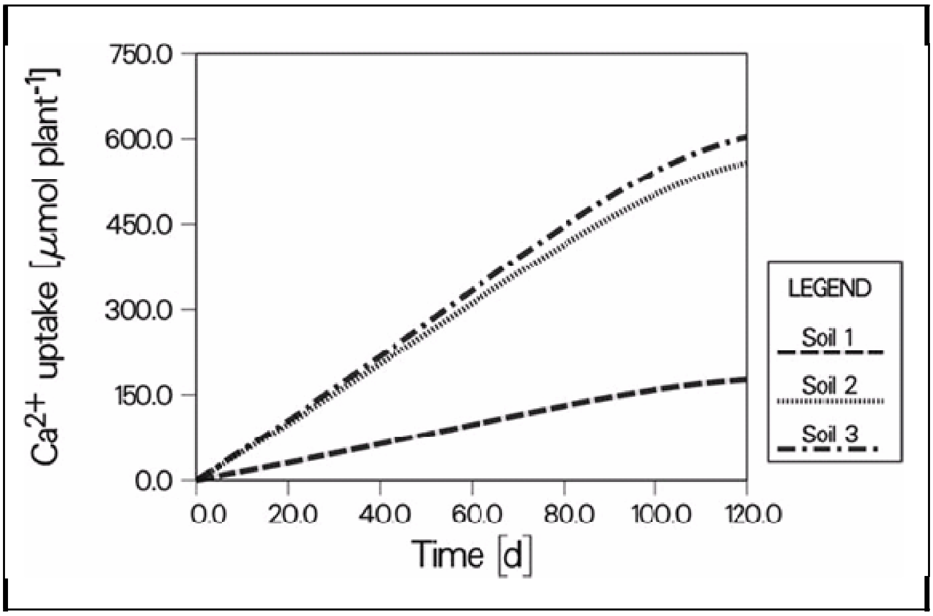

Figure 4.7: *Average (n=10) cumulative Ca^{2+} uptake (µmol plant^{-1}) of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)] for 120 days*

Table 4.10: *Calculated average daily nutrient uptake rates of black locust seedlings cultivated in soils with different starting pH values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.*

Nutrient	Calculated daily nutrient uptake rates [10^{-2} µmol cm^{-2} d^{-1}]		
	Soil 1	Soil 2	Soil 3
K^+	2.47	3.75	3.32
Ca^{2+}	1.84	5.15	5.33
Mg^{2+}	0.56	1.21	1.12
N	28.32	47.04	34.80
Na^+	0.35	0.44	0.32
SO_4^{2-}	0.95	1.78	1.33
$H_2PO_4^-$	1.84	3.10	2.49

4.5.2.2 Modelled pH changes and comparisons with measurements

The radial pH gradients in the rhizosphere were calculated according to the chemical bulk soil conditions of each soil treatment and to the particular root uptake rates of minerals (*Figure 4.8*). The calculated pH changes in the rhizosphere were attained by the nutrient uptake at daily rates, which were identical to those presented in *Table 4.10*. In contrast to the measured values detected at the end of the experiment, the modelled soil solution concentrations of nitrogen were increasing (*see Table 4.1*) to a level, which guaranteed daily uptake rates of total nitrogen (see *Table 4.10*). All model calculations were conducted under the assumption of preferential NH_4^+ uptake, i.e. the roots acted as zero sinks with an extremely high maximum influx rate ($J_{NH_{4,\max}} = 15.0 * 10^{-5}$ mmol m^{-2} s^{-1}) and the uptake of NO_3^- was adjusted for the adaption to the measured rhizosphere pH values.

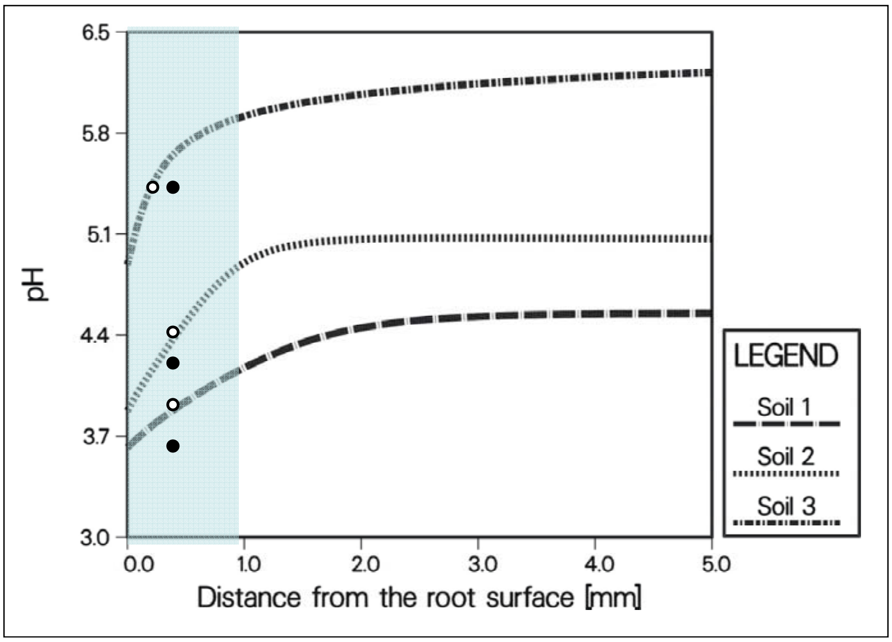

Figure 4.8: *Calculated radial pH gradients (curves) and calculated rhizosphere pH_{RH} values (unfilled spots) as well as measured mean pH values (filled spots) in the rhizosphere of seedlings cultivated in soils with different initial pH_{KCl} values [Soil 1 = pH_{KCl} 3.4 (a); Soil 2 = pH_{KCl} 4.8 (b); Soil 3 = pH_{KCl} 6.2 (c)]; n=10.*

The spatial distribution of the proton concentrations did not exceed a volume of more than about 1.5 mm distance around the root. The single unfilled spots in the graph represent the modelled rhizospheric pH values (pH_{RH}) of the average proton concentrations [equation (6d)] in the defined measurement volume around the root (see shaded area in *Figure 4.8*). The pH_{Rh}

values in these calculation examples were: Soil 1 = 3.91 pH_{RH} compared to 4.56 pH_{Bulk}, soil 2 = 4.39 pH_{RH} compared to 5.09 pH_{Bulk} and soil 3 = 5.59 compared to 6.34 pH_{Bulk}. The filled spots represent the measured rhizosphere pH values [pH 3.59 (soil 1), pH 4.22 (soil 2) and pH 5.53 (soil 3)]. Especially in the unlimed, but also in the moderately limed soil the calculated pH decreases were not as high as the measured pH drops. We attribute this to the extremely high affinity of protons to the soil exchanger sites in the soil treatments 1 and 2 [unlimed (a) and moderately limed treatments (b)], which was documented by the ratio between the concentrations of exchangeable H^+-ions in the soil and the H^+ solution concentrations. Results of the simulation with lower sorption strength showed stronger pH decreases and larger acidification areas around roots and had therefore a much better agreement with the measured values.

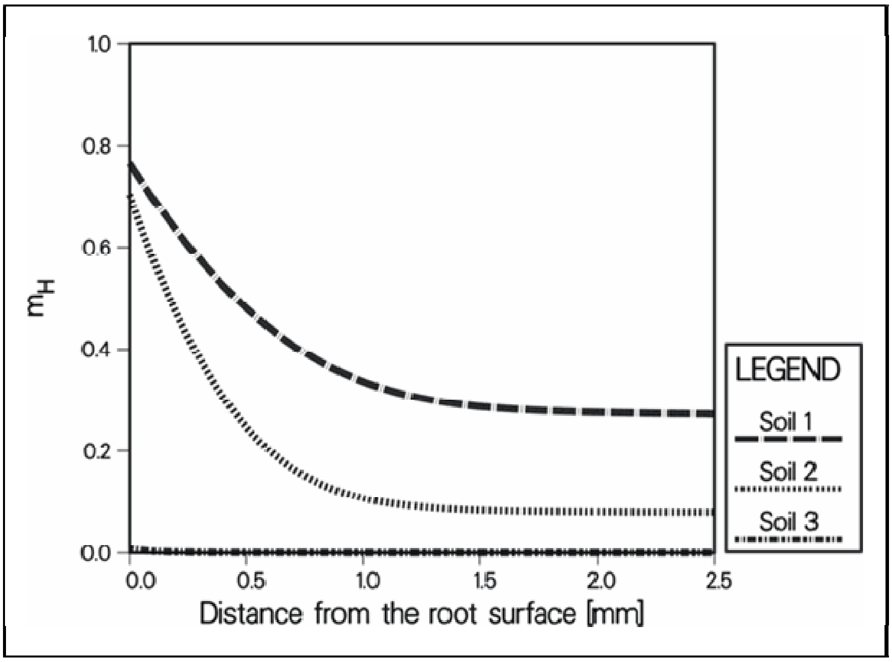

Figure 4.9: *Change of equivalent fractions of protons on the exchanger complex for the three soils with different initial* pH_{KCl} *values [Soil 1 =* pH_{KCl} *3.4 (a); Soil 2 =* pH_{KCl} *4.8 (b); Soil 3 =* pH_{KCl} *6.2 (c)]; n=10.*

Figure 4.9 presents the change of the equivalent fractions of protons on the soil exchanger complex. In the unlimed and moderately limed soil [soils 1 (a) and 2 (b)] the increase of proton concentrations at the exchanger sites is extremely high. In the strongly limed soil [soil 3 (a)] nearly no increase of the already low exchangeable proton bulk soil concentration was determined. This was ascribed to the lower affinity of protons for the soil exchanger sites, which was affected by Ca saturation, and to the reduced root H^+ excretion rate in this treatment.

The calculated pH decreases were the consequence of proton excretions in the three soil treatments (data not presented). The uptake of NH_4^+ in soils 1 (a) and 2 (b) agreed with the daily uptake rate of total nitrogen as listed in *Table 4.10*, i.e. not nitrate was taken up. In soil 3 (c) the calculated daily uptake of nitrogen had a NH_4^+/NO_3^- ratio of 0.64. Therefore, the excretion rate of protons was reduced compared to with the other treatments, because the ratio of mineral N uptake caused reduced additional acidification.

In *Figure 4.10* the correlation between measured and modelled pH differences is shown. The correlation bases on the rhizosphere pH values of pH_{Rh} calculated according to equation (6d). This correlation was introduced under the assumption that NH_4^+ is taken up completely and the rate of NO_3^- uptake was adjusted in order to adapt modelled to measured pH values in the rhizosphere.

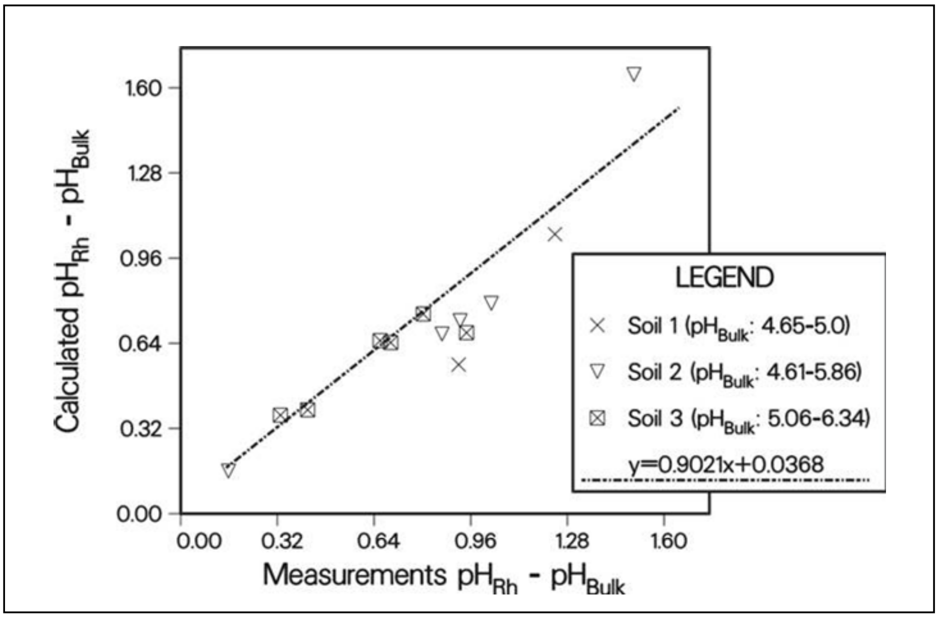

Figure 4.10: *Correlation between measured and calculated pH differences in the rhizotrons cultivated with seedlings in soils with different initial* pH_{KCl} *values [Soil 1 =* pH_{KCl} *3.4 (a); Soil 2 =* pH_{KCl} *4.8 (b); Soil 3 =* pH_{KCl} *6.2 (c)]; n=10.*

In the unlimed [soil 1 (a)] and moderately limed soil [soil 2 (b)], nitrogen was taken up in form of NH_4^+ in nearly all cases (an exception was the case with a low pH difference of 0.15 units). Hence, in the soils 1 and 2 the uptake of M_b cations and NH_4^+ ions produced H^+ root excretions leading to clear pH decreases, which in most cases agreed with the measured pH values. In the rhizotrons filled with the strongly limed soil [soil 3 (c)] nitrogen was taken up in various NH_4^+/ NO_3^- ratios and the calculated percentage of NO_3^- uptake to the actual nitrogen uptake ranges between 75.0 and 35.0 % .

Figure 4.11 shows the relation between measured pH differences and calculated pH differences based on the uptake of Mb cations, SO_4^{2-} and $H_2PO_4^-$, only. For the calculations the same bulk soil concentrations as for the calculated values in *Figure 4.10* were used. The maximum influx rates for NO_3^- and NH_4^+ ions in equation (4a) were set to zero and the uptake parameters of the other nutrients remained unchanged. Obviously the uptake of non-nitrogen ions in soil 1 (data pairs marked with open crosses (x) in *Figure 4.11*) produced only small pH decreases of 0.23 to 0.3 units.

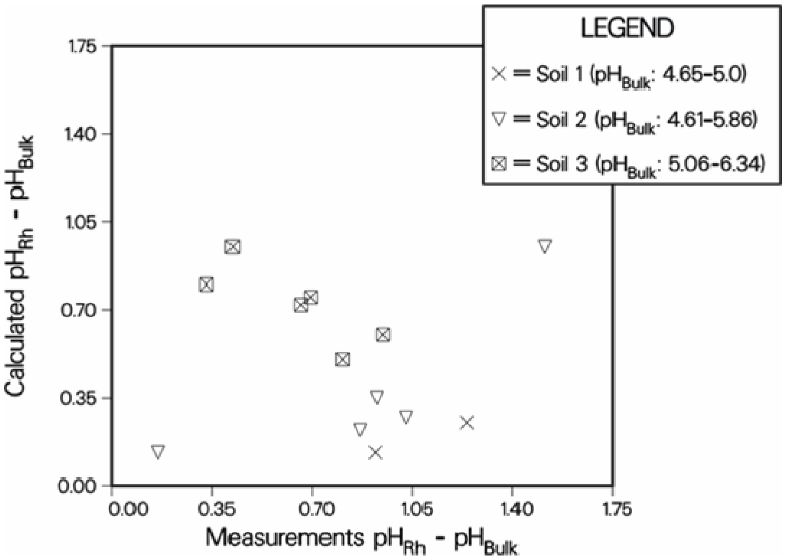

Figure 4.11: *Relation between measured and calculated pH differences (based on uptake of non-nitrogenous ions) in the rhizosphere of seedlings cultivated in soils with different initial* pH_{KCl} *values [Soil 1 =* pH_{KCl} *3.4 (a); Soil 2 =* pH_{KCl} *4.8 (b); Soil 3 =* pH_{KCl} *6.2 (c)]; n=10.*

The calculated pH decreases in soil 1 and 2 (a & b) were only 15-60% of the measured values (with one exception in which the uptake of NO_3^- was involved). In soil 3 (c) the calculated pH decreases were irregular. In some cases a higher pH decrease was calculated than measured, which indicated a NH_4^+/NO_3^- uptake ratio lower than 1. In other cases a higher acidification was measured compared with the modelled pH induced by the uptake of non nitrogen-nutrients. This indicated an additional input of protons in the rhizosphere due to a NH_4^+/NO_3^-

uptake ratio higher than 1. In some other cases no additional inputs of protons are caused by the uptake of nitrogen ions, i.e. the uptake of NH_4^+ and NO_3^- was balanced.

According to the previously applied one-component models (Nye & Tinker 1977; Barber 1984) the ion dynamics in the rhizosphere are characterized by ion-specific concentration changes including accumulations and depletions. But these concentration changes occur simultaneously and their calculation can be addressed by a multispecies model only. The multispecies model applied here, calculates the distribution dynamics of all ions involved in the rhizosphere soil under consideration of the ionic interactions caused by the competitive cation exchange and the diffusion potential. The root excretion of protons was calculated implicitly via the charge balance of the cations and anions actually taken up (Haynes 1990). Hence, their rates are affected by the availabilities of nutritional ions. During the root uptake process the distribution patterns of nutrients follow up an individual pattern, which determines their actual root uptake rates. As the actual root uptake rates may change temporarily the excretion rate of protons may change including a possible change from H^+ to OH^- excretion or vice versa.

The scenarios calculated here and presented in *Figures 4.8* and *4.10* demonstrate that the measured pH changes can be attributed to the root-induced pH changes based on the measured amounts of the nutrients taken up. The model results also indicate that for an understanding of the fate of protons in the rhizosphere the sources and sinks of protons have to be considered. This especially includes the determination of the pH-buffering power of the soil, which is mostly traceable to cation exchange processes in accordance to previous investigations (Schaller & Fischer 1987). But the buffering of protons in the rhizosphere may be affected by the concentration changes of the other cations involved. The rhizospheric conditions are characterized by the fact that the uptake-induced depletion of some cations in the soil solution mostly correspond to depletions on the soil exchanger. The soil exchanger sites becoming free during the root uptake process are occupied by protons and by other cations, which are taken up in low rates only (e.g. aluminium ions). But these H^+ buffering mechanisms caused by cation depletion on the soil exchanger are not considered by the experimental systems usually used for the determination of proton buffering capacity.

It is documented by the previous investigations that the spatial extent of the pH changes occupies a soil area of maximum 3.0 mm around the root (Marschner 1995). The results of model calculations showed that the diffusive movement of protons is considerably reduced in dry soils and highly retarded by a high affinity for the soil exchanger sites, thus resulting in an increased acidification which does not exceed 1-1.5 mm around the root (Nietfeld 2000). Also the calculation results presented here (*Figure* 4.*8*) show a significant pH change only up to 1.5 mm around the root, which can be attributed to high sorption strength of protons on the soil exchanger. The high affinity of protons may be attributed to the high amount of organic matter of 4-5% in the soils (Berthold et al. 2005). Hence, pH measurement data obtained by the use of a pH sensor with a diameter of 0.8 mm in this extremely small soil area may have a high degree of uncertainity. As shown in *Figures* 4.*8 & 4.10* the disagreements between model results and the measurements (soil 1 and partially in soil 3) can be originated from these problems.

The model results shown in *Figures* 4.*8 & 4.10* demonstrate that for a reasonable well agreement with the observed pH decreases [in soils 1 (a) and 2 (b)] high amounts of protons are required. Assuming that the rhizospheric pH is only affected by the root uptake activity, high H^+ root excretion rates are required to produce these measured pH drops. These rates are only attainable if nitrogen taken up by the plant is involved in the plant-driven H^+ excretion rates. Neglecting the possibility that rhizobial fixation of nitrogen produces an excretion of protons directly or via plant-internal physiological processes the uptake of the mineral nitrogen forms is obligatory. The uptake of mineral nitrogen by legumes is well-documented (e.g. Roberts et al. 1983; Johnsen & Bongarten 1992; Sanford et al. 1993; Danso et al. 1995; Marschner 1995), but a preference for NH_4^- or NO_3^+ ions is unknown. The simulation results of the rhizospheric pH in the rhizotrons of soil 3 (*Figure 4.11*) indicate that an uptake of NO_3^- by the root may at least be hypothesized in some cases. The uptake of non-nitrogen ions only produces a higher or a lower acidification of the rhizosphere (*Figure 4.11*), which indicates that nitrogen is taken up in various NH_4^+/NO_3^- ratios but not completely as NH_4^+.

On the other hand, the low concentrations of NH_4^+ and NO_3^- in the bulk soil at the end of the experiment can not match the daily uptake rates of nitrogen (*Table 4.10*), which was an assumption in the modelling concept. This indicates that also high amounts of the nitrogen taken up by the plant were fixed by rhizobial microorganisms. This is supported by the appearance of rhizobial nodules in all soil treatments. Considering the mentioned uncertainties of pH measurements, it can be assumed that the pH decreases in the strongly limed soil can be attributed to the exclusive uptake of non-nitrogen nutrients, but the clear pH decreases in the soils 1 and 2 can not be explained in this way. These pH decreases are explainable only by H^+ excretion mechanisms caused by N_2 fixation. This was supported thereby that the highest rhizobial biomass and the strongest influence of black locust on soil chemical properties were found in the moderately limed soil.

4.6 Conclusions

The hypothesized strong pH decrease in the rhizosphere of black locust seedlings was confirmed in the rhizotron experiment. However, measured differences between bulk soil and rhizosphere pH values have always to be interpreted in respect to the actual bulk soil pH and the calculated H^+ concentrations in the rhizosphere. Otherwise the results may lead to erroneous conclusions concerning the impacts of lower rhizosphere pH values on the soil chemical conditions around roots. The multispecies rhizosphere model explained the measured rhizosphere pH values through different uptake scenarios of soil mineral N fractions. However, the measured low contents of these fractions in the soils and the detected nodules at the end of the experiment indicated that the pH drops must also have been the result of N_2 fixation by the plants. In order to determine the uptake rates of the different N forms (mineral N fractions and atmospheric N_2) by black locust plants and to correlate them with the detected rhizospheric acidification, experiments with soils of low organic matter content and with labelled N fractions (^{15}N) are required.

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5 Effects of soil sterilization on the growth of black locust (*Robinia pseudoacacia* L.) seedlings

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5.1 Abstract

Growth decline after long-term repeated cultivation of a crop on the same site is a very common phenomenon in agriculture and forestry. While soil sterilization methods to control pathogens are often used in agriculture, their effect on tree growth is still poorly understood. The objective of this study is to relate seedling growth and nutrient status of N_2-fixing black locust (*Robinia pseudoacacia* L.) to soil sterilization. Soils from 3 different and repeatedly cultivated *Robinia* stands in Hungary were sterilized with the biocide *Basamid*® and replanted with *Robinia* seedlings. The seedlings were grown for one growing season in the greenhouse. Height and diameter increment were monthly measured. Sterilization had no effect on seedling growth in the calcareous soil. In the two non-calcareous soils, relative height (0.4 $\ln_{cm}$) or diameter (0.2 $\ln_{mm}$) growth rate was increased by the application of *Basamid*. However, there was no significant effect on total biomass increment. Plant nutrient content was significantly increased through sterilization, whereas nodulation was decreased. A chemical soil analysis before and after the observed growing season revealed that differences in growth and nutrient content of the seedlings were not only the result of a pathogen effect. There was some evidence for a strong release of nutrients from the fumigated soil fauna and flora that may have affected the plant growth in addition to the inhibition of nodulation. It was concluded that pathogen effects of soil sterilization can be covered by

several other effects, which might not be intended. Hence, this soil treatment can not be recommended in general to improve the growth of tree seedlings, as long as growth affecting pathogens have not clearly been identified.

5.2 Introduction

Local forest managers in Hungary and eastern Germany have observed that the continuous cultivation of black locust (*Robinia pseudoacacia* L.) for several generations on the same site led to depression of yields. The results of a chemical inventory of soils from Hungarian forests showed a correlation between the period of black locust cultivation and soil acidification and availability of soil nutrients (Berthold et al. 2004), which may have caused the black locust growth decline. However, it is likely that there is also an increasing impact of pathogens. The long-term cultivation of legumes on the same site is related to an enhanced occurrence of soil-borne pathogens, and pathogenic fungi can survive in soil for several years as saprophytes without their hosts (Levenfors 2003). A progressive enrichment of soil-borne pathogens after cultivation of agricultural legumes for several generations on the same field is a well-known phenomenon (Kleinhempel et al. 1989; Bødker et al. 1993; Laszlovszky-Zmarlicka et al. 1998). While *Robinia* is known to have several biological antagonists in its original North American habitat (Buchmann 1964; Farr et al. 1995), in Europe it has only a few fungal pathogens (Kehr et al. 1999; Michalopoulos-Skarmoutsos et al. 1999). However, as a result of silvicultural treatment by means of clear cutting for stand regeneration through stem sprouts and root suckers, these pathogens can ingress in plants and cause symptoms of disease as well as growth depressions (Butin 1989; Gupta et al. 1992; Zaspel & Ulrich 2002). Evidence for the biological processes involved comes from a study from Hungary, in which growth of black locust seedlings was improved after soil sterilization with *Basamid® Granular Soil Fumigant* (99% Dazomet, tetrahydro-3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione; 1% inert ingredients). Unfortunately, no data were collected in this study and results were documented only visually (*Figure 5.1*).

Especially for N_2-fixing plants, other effects of long-term cultivation are likely. For example, interactions between chemical and biological changes, such as nodulation and rates of symbiotic N_2-fixation, which are low under highly acidic conditions (Munns 1978; Franco & Munns 1982; Marschner 1986). Moreover, the infection of roots with strains of the nodule forming bacteria (*Rhizobium spec.*) may be affected reducing their ability to fix atmospheric nitrogen (Holdings & Lowe 1971; Glenn & Dilworth 1991; Ibekwe et al. 1997; Ulrich & Zaspel 2000). The fixation of atmospheric nitrogen is a highly energy-consuming process. Approximately 6g C/g N (Werner 1987), i.e. for a fixation rate of 150kg N ha^{-1} 900kg C, are required. Therefore the infection of roots with ineffective strains with constant energy costs for the symbiosis can result in a decreased primary production (Batzli et al. 1992; Han 1996).

Figure 5.1: *Effects of soil sterilization on black locust regeneration on a long-term cultivated site in Hungary. In the front left without and right with soil sterilization.*

Apparently together with changes of chemical soil properties, both changes in the infecting population of *Rhizobium* and the enhanced occurrence of soil-borne pathogens are responsible for the observed decrease in growth increment of locust stands.

This paper addresses the impact of soil sterilization on the growth of black locust seedlings. Growth should increase after sterilization if the pathogen impact is important, whereas it should decrease if the effect of lost symbionts is more important. Plant growth and nutrient contents were compared for black locust seedlings growing in sterilized and non-sterilized soils, respectively. Additionally, we compared soil N concentrations before and after the experiment. Soils were collected from Hungarian forest sites, which were cultivated with stands of *Robinia pseudoacacia* for two tree generations. Our study should help to assess whether soil sterilization is a useful technique to avoid black locust growth decline or not.

5.3 Materials and Methods

5.3.1 *Soil origins and treatments*

Soils from 3 different Hungarian forest sites were collected in October 2002 and used in a greenhouse experiment. Prior to the experiment the soil was stored at 4° C to inhibit processes of chemical and biological conversions. Soils originated from Kelebia (South Hungary), Pusztavács (Central Hungary) and Baktalórántháza (North-East Hungary) (*Figure 5.2*).

Figure 5.2: *Origin of the study soils from Robinia stands in Hungary.*

According to the total element analysis (*Table 5.1*, measured after pressure digestion in 65% concentrated HNO_3) soils from Pusztavács and Baktalórántháza were non-calcareous. Soil types were 'sandy Typic Cambisols'. The soil from Kelebia had a calcareous origin and was a 'loamy Typic Chernozems'. To illustrate the influence of biological factors on plant growth, half of the soil of each site was sterilized with *Basamid® Granular Soil Fumigant* (BASF AG, Ludwigshafen, Germany) whereas the rest was used as untreated controls. Basamid is a non-specific agent used for pre-planting control of most weeds, nematodes and soil diseases. According to the instructions for use soils were watered (at least 50% of field capacity) for one week and subsequently treated with 200g *Basamid* per m^3 soil. When *Basamid* is correctly incorporated into moist soils, the active ingredient is transformed into substances that possess soil-sterilizing properties. The gas that is formed diffuses upwards through the soil, killing nearly all living organisms (BASF 1998). Soil microorganisms and plant pathogens were supposed to be killed by the sterilization. A cress (*Lepidium sativum* L.) test 14 days after the *Basamid* treatment indicated the end of toxicity for plant growth.

Table 5.1: *Chemical soil characteristics of the Hungarian Robinia sites. Values are arithmetic means with standard deviation for the whole soil profile (0-50cm), n=5.*

	Total element concentrations				
	pH	P	K	Ca	Mg
	KCl		mg/g		
Baktalórántháza	3,9±0,1	0,2±0,0	4,0±0,1	1,4±0,0	1,8±0,1
Pusztavács	4,5±0,1	0,3±0,0	2,8±0,1	3,5±0,1	2,0±0,1
Kelebia	7,3±0,1	0,6±0,0	4,9±0,3	19,0±2,9	4,7±0,1
	Exchangeable cations				
	K	Ca	Mg	Fe	Al
			$\mu mol_c/g$		
Baktalórántháza	1,2±0,1	22,8±5,0	4,3±0,5	0,0±0,0	4,1±1,3
Pusztavács	1,0±0,1	35,4±7,1	5,7±0,9	0,0±0,0	0,2±0,5
Kelebia	2,4±0,6	468,1±60,3	18,9±0,8	0,2±0,0	0,0±0,0

5.3.2 Plant material and plant growth

Seedlings of *Robinia pseudoacacia* L. (Clone 2466, Waldsieversdorf, Germany) that had been cultivated in sterile vermiculite were planted in PVC pots (Ø14.4cm x 60.0cm) with 5 replicates per soil treatment. Plant height and diameter were measured at the beginning of the experiment and monthly for one growing season (May to October 2003). For the analysis of plant growth relative growth rates were calculated. Absolute growth rates are unbiased by initial seedling size and associated differences of plant increment. Therefore detected results are often the product of a different basis of comparison and not of the experimental treatment (Causton & Venus 1981; Hunt 1982). Under distinct experimental treatments forest researchers frequently use *Mean Relative Growth Rates* (*MRGR*) to compare growth of seedlings that differ in initial size (Van den Driesche 1982; Harrington & Tappeiner, 1991). The *Mean Relative Growth Rate* can be calculated by using plant size at two points in time. The equation for calculating the *MRGR* is written as:

$$MRGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad (1)$$

where W_1 and W_2 are the plant size at the beginning (t_1) and end (t_2) of the sampling period, and ln is the natural logarithm.

5.3.3 *Plant and soil analysis*

Plant biomass, element concentrations of plant components and concentrations of N fractions of all soil treatments were measured at the beginning of the experiment and served as references. At the end of the experiment plants were harvested and separated into leaves, shoots and roots including nodules. Total C and N of plant tissues were measured by dry combustion with a C/N autoanalyser (Vario Elementar Analysensyteme, Hanau, Germany). N-accumulation in plant tissue was calculated as the difference of mean N content of reference plants at the beginning of the experiment (May 2003) to the N content of seedlings from different soils and treatments at the end of the experiment (October 2003). The elements P, S, K, Mg, Mn, Fe were measured with an ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65% concentrated HNO_3. To quantify differences of N content in soil, samples were extracted with 0.5 M K_2SO_4 (~ 5:1 ratio of solution to dry mass soil) for 18 hours in a batch experiment. The extracted N fractions (NO_3^-, NH_4^+, N_{tot}) were analysed by a continuous flow system spectrophotometer (Skalar Analytic GmbH, Erkelenz, Germany) (König & Fortmann 1996).

Statistical analysis of the data was performed using the Mann-Whitney U-Test ($p<0.05$, *Statistica 6.0, StatSoft*).

5.4 Results and Discussion

5.4.1 *Plant growth and biomass increment*

Apart from seedlings transplanted into soil derived from Baktalórántháza, no significant differences in plant height and diameter occurred at the beginning of the experiment (May 2003). Seedlings grown in soil of Baktalórántháza were significantly taller in the non-sterilised soil treatment. Therefore relative increment (mean relative growth rate) for the different soils and treatments is illustrated (*Figure 5.3*).

For the calcareous soil from Kelebia no significant sterilization effect on plant height and diameter development was observed. Obviously the high nutrient content buffered treatment effects. However, for seedlings cultivated in sterilized sandy soils from non-calcareous sites, significantly higher growth rates of height or diameter were found. Sterilization of the soil from Baktalórántháza (Bakta) led to a significantly greater relative height growth and plants cultivated in treated soil of Pusztavács (Puszta) responded with a significantly higher relative diameter growth.

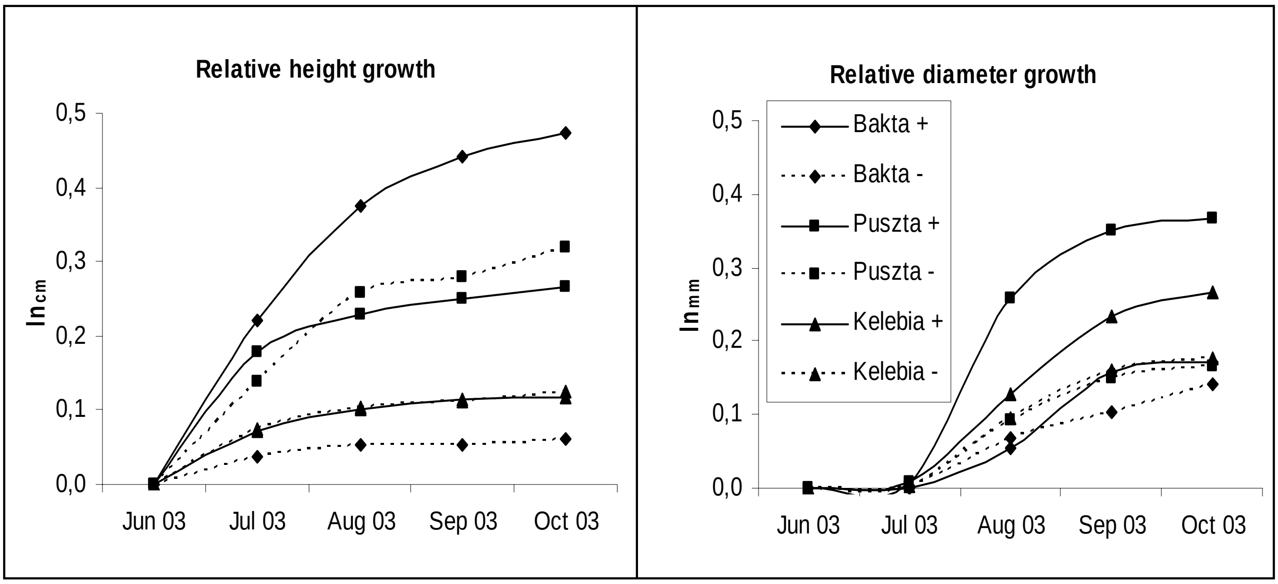

Figure 5.3: *Cumulative relative height and diameter growth rates for sterilised (+) and non-sterilised (-) soils derived from three different sites in Hungary. Data provided as arithmetic means (n=5).*

The observed significant differences of height and diameter increment were not validated by the comparison of biomass increment between the different soil treatments (*Table 5.2*). For all soils, effects were not significant, although the average leaf and root biomass was constantly higher after sterilization.

No direct identification of soil-borne pathogens was carried out for this experiment, the existence of pathogens was just assumed by the fact that the soils originated from black locust stands with a documented growth decline. Therefore it can not be excluded that, as mentioned above, the decline of plant growth was caused by chemical factors, only. Furthermore, the soil sterilization with *Basamid* also killed the nodule forming bacteria (*Rhizobium*), resulting in a reduced nodulation (*Table 5.2*). In the sterilised soils of Baktalórántháza and Kelebia no nodules were formed and only a few in the untreated soils (mean 2 nodules per plant). The strongest nodulation was observed in the soil derived from Pusztavács, with distinct differences in nodule number and biomass between *Basamid* treated and untreated soils. On average 10 nodules per plant with a dry mass of 3.8mg were built in sterilised soils compared to 22 nodules and 25.6mg in soils without *Basamid* application. Very similar effects of soil sterilization were found by Moffat (1994). In this study growth of alder (*Alnus glutinosa*) seedlings was stimulated and nodulation was reduced after soil sterilization, too. However, reduced N_2 fixation as well as reduced mycorrhizal colonization due to sterilization can also have a negative effect on black locust growth (Olesniewicz & Thomas 1999; Tian et al. 2003).

Table 5.2: *Mean and standard deviation of biomass increment (dry weight) during the observation period (June-October 2003) and number and dry weight of nodules in October 2003 for the soils of different origin and treatment (n=5).*

Forest site	Treatment	**Biomass increment (g) June-October 2003**				**Nodules October 2003**	
		Leaf	Stem	Root	Total	Number	Dry weight (mg)
Baktalórántháza	Basamid	1.3±0.8	0.3±0.4	5.3±1.1	6.9±1.8	0±0	0.0±0
	Untreated	1.2±0.6	1.4±0.8	4.5±0.7	7.1±1.8	1±1	6.0±0
Pusztavács	Basamid	3.9±1.9	2.4±1.8	6.6±3.7	12.9±7.0	10±14	3.8±4.7
	Untreated	2.9±0.9	2.1±0.5	4.9±1.2	9.9±2.4	22±29	25.6±17.7
Kelebia	Basamid	2.7±1.2	2.4±1.1	4.8±1.9	9.9±4.1	0±0	0.0±0
	Untreated	1.9±0.5	2.0±0.7	3.1±0.7	7.0±1.4	2±3	9.3±9.0

5.4.2 Plant nutrient content

At the beginning of the experiment (May 2003) nitrogen was predominantly allocated to the roots and to the leaves of the seedlings (*Table 5.3*). After one growing season, significantly higher N-concentrations and lower C/N ratios in plants from sterilized soils were detected, whereas the *Basamid* treatment resulted in significantly higher N-accumulation only in the roots of seedlings cultivated in soil from Kelebia (*Figure 5.4*). For seedlings cultivated in sterilized soil from Baktalórántháza (Bakta) significantly higher N-concentrations (mean 12%) and lower C/N ratios were detected in leaves, shoots and for the complete plant (average of all single plant components). No differences concerning the N-accumulation in plants cultivated in pots with and without sterilization were observed for this soil.

Table 5.3: *N-status of seedlings at the beginning of the experiment mean and standard deviation, n=4).*

Compartment	N (mg/g DW)	N (mg)	C/N
Leaves	21.9±1.7	26.0±2.0	21.1±1.6
Stems	13.2±1.9	12.3±1.8	35.8±5.5
Roots	24.4±1.2	23.9±1.1	18.5±1.0
Plant	20.2±2.2	65.3±3.2	21.3±1.5

In the soil from Pusztavács (Puszta) significantly higher N-concentrations (mean 15%) and lower C/N ratios after the *Basamid* treatment were determined for the shoots, only. However, the N-accumulation in all plant compartments and for the whole plant (summation of the N-accumulation in all compartments) was also elevated (300mg N ±100mg for the complete plant) compared to the untreated control. Plants cultivated in sterilized soil from the calcareous site of Kelebia possessed significantly lower C/N ratios in leaves and higher N-concentrations and lower C/N ratios in the whole plant. Basamid application also elevated the accumulation of N in the plant tissue, but only for the roots this effect was significant.

For both nodulation and effective N_2-fixation the supply of mineral nutrients is an important factor. For a good nodulation a high availability of P, K and Fe is required. Nodulation demands a higher P supply than root or shoot growth (Cassman et al. 1980) and Fe is an important ingredient of the leghemoglobin and the nitrogenase complex, which are key enzymes for the fixation reaction (Marschner 1986). Moreover, the initial nodulation depends on high potassium availability (Hoffmann 1960; Marschner 1986). High concentrations Mn restrict nodulation (Whelan & Alexander 1986). *Table 5.4* shows many significant differences in the concentrations of these elements in the single compartments of black locust seedlings between the treatments.

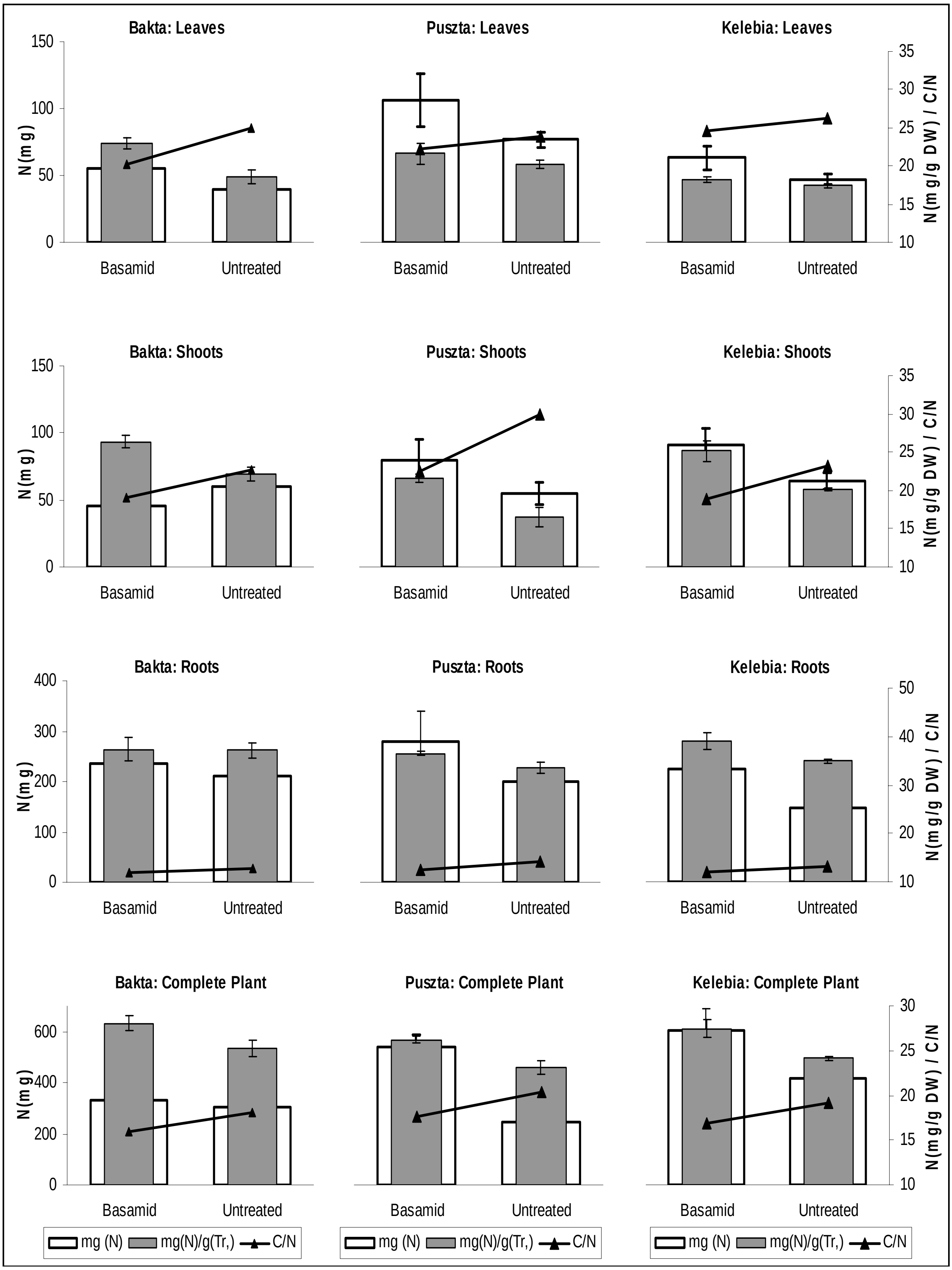

Figure 5.4: N-concentrations (mg N/g Dry Weight), N-content (mg N) and C/N ratios 120 for plant compartments and the whole plants grown in soils sterilized with Basamid and in untreated soils. Data presented as mean values with standard deviation (n=4).

Seedlings grown in untreated soils from Kelebia and especially Pusztavács formed significantly more nodules than in the sterilized soils (*Table 5.2*). Due to high P and K content of nodules this seems to have been resulted in higher concentrations of these elements in roots of seedlings from the untreated soils. However, only in the case of soils from Pusztavács the difference was significant. Enhanced Fe-concentrations in nodulated roots were not detected. Elevated concentrations of Mn were found in all plant components of seedlings cultivated in *Basamid*. The highest Mn-concentrations were observed in pots with soil from Baktalórántháza. This could have been one reason for the absence of nodulation in both soil treatments.

Table 5.4: *Total concentration of P, K, Mn and Fe (mg/g dry weight) in the separated plant compartments from soils of different sites and treatments. Data presented as mean values with standard deviation (n=5).*

Plant compartment	Treatment	P	K	Mn	Fe
		mg/g dry weight			
		Baktalórántháza			
Leaves	Basamid	0.95±0.14	9.33±1.63	6.61±1.57**	0.20±0.03
	Untreated	0.75±0.10	11.04±1.60	2.38±0.20	0.18±0.09
Shoots	Basamid	0.99±0.05**	5.27±0.45*	0.34±0.07**	0.17±0.04*
	Untreated	0.79±0.06	5.04±0.35	0.13±0.02	0.09±0.03
Roots including nodules	Basamid	1.29±0.22	6.83±0.87	0.80±0.28**	2.46±1.67*
	Untreated	1.28±0.12	6.89±1.03	0.36±0.07	0.86±0.32
		Pusztavács			
Leaves	Basamid	0.93±0.16	10.51±1.32	0.26±0.09**	0.20±0.06**
	Untreated	1.00±0.10	8.75±1.63	0.05±0.01	0.06±0.00
Shoots	Basamid	0.88±0.08	4.86±0.17*	0.01±0.00**	0.08±0.01
	Untreated	0.93±0.15	5.48±0.32	0.01±0.00	0.08±0.02
Roots including nodules	Basamid	1.52±0.14*	7.69±0.50*	0.06±0.02**	1.27±0.53*
	Untreated	1.86±0.21	9.05±0.74	0.03±0.01	0.59±0.18
		Kelebia			
Leaves	Basamid	0.82±0.11*	11.68±1.09*	0.08±0.02**	0.11±0.03
	Untreated	0.97±0.08	13.80±1.22	0.04±0.00	0.09±0.03
Shoots	Basamid	1.16±0.21	5.27±0.64	0.02±0.00**	0.11±0.02*
	Untreated	1.09±0.10	4.80±0.11	0.01±0.00	0.09±0.01
Roots including nodules	Basamid	1.83±0.23	7.08±1.45	0.09±0.02**	0.85±0.13
	Untreated	1.98±0.19	7.23±0.52	0.05±0.01	0.84±0.17

*Values denoted with *(p<0.05) or **(p<0.01) are significantly different between treatments.*

5.4.3 Soil N content

The sterilization of soils with *Basamid* enhanced the concentrations of K_2SO_4-extractable N fractions compared to the untreated controls, especially in soils from the non-calcareous sites (*Table 5.5*). Analogous to the CFE-method (chloroform fumigation extraction) for the determination of microbial C, N and P (Joergensen 1995) the fumigation of soils with *Basamid* killed soil microorganisms and released microbial N into soils. In legumes N (NO_3^-, NH_4^+) can enhance or depress nodulation and N_2 fixation, depending on plant genotypes and the form and level of the N supply. The contribution of N_2 fixation to the total nitrogen content per plant is increased by moderate levels of soil or mineral fertilizer nitrogen (~ 25 kg N ha^{-1}) but declines at high levels (Marschner, 1986). For *beans* George et al. (1992) found out that low levels of mineral fertilizer supply as `Starter-N´ increased nodulation and total amount of nitrogen derived from N_2 fixation, but high levels of fertilizer nitrogen drastically decreased nodulation and even prevented fixation. Therefore the decreased nodulation of seedlings cultivated in *Basamid* fumigated soils could also have been the result of enhanced available N levels from lysed microbial biomass.

Table 5.5: *Changes of N-status in soils after sterilization with Basamid before replanting soils with black locust seedlings (May 2003); data presented as mean values (n=5) with standard deviation.*

Site	Treatment	NH_4	NO_3	N_{org}	N_{tot}
		mg/kg dry weight			
Baktalórántháza	Basamid	41.8±2.1**	32.6±1.1**	21.4±1.9	95.8±3.1**
	Control	20.1±3.3	12.2±1.9	28.0±1.6**	60.3±5.8
Pusztavács	Basamid	41.0±0.8**	25.4±1.4**	20.8±1.2**	87.3±2.0**
	Control	7.1±0.8	17.8±2.7	28.1±4.0	53.0±6.9
Kelebia	Basamid	54.6±2.2**	36.7±1.9	31.2±5.4	122.6±9.0
	Control	7.8±1.4	47.6±11.6	43.8±5.6**	99.2±18.0

*Values denoted with *(p<0.05) or **(p<0.01) are significantly different between treatments within soils from the same site.*

At the end of the experiment (October 2003), sterilized soils still had higher NH_4 concentrations than untreated soils (*Table 5.6*). However, in the treated soils from Pusztavács and Kelebia the ammonium concentrations decreased drastically between May and October.

Apart from the control soil from Baktalórántháza, a strong decrease was also detected for NO_3 concentrations. A significantly higher drop of nitrate concentrations was observed in sterilized soils.

With respect to the relative change of N in soils after one growing season, concentrations decreased probably due to plant assimilation and leaching. It was obvious that soil sterilization had more than just a pathogen effect on the soil-plant interaction. This point was also critically stressed by Troelstra et al. (2001). Destroying a complex system of mutualism, parasitism and competition can lead to various total effects, and the time span needed for recovery of single components may be different. We observed the growth of black locust seedlings for one growing season and the initial total effect of sterilization lasted until the end of the experiment. To separate single effects it would be helpful to observe plant growth for a longer period and to identify the most affected soil organisms. Our study showed that soil sterilization has a slightly positive effect on the growth of *Robinia* seedlings, although we cannot attribute it to a certain reason. Furthermore, growth effects were proven for nutrient poor soils, only. Soil fumigation with biocides like *Basamid* is commonly used in nurseries to enhance the growth performance of tree seedlings (Moffat 1994; Fraedrich & Dwinell 2003). There is some evidence that the pathogen effect on tree growth resulting from soil sterilization is overestimated so far (see also Fraedrich & Dwinell 2003).

Table 5.6: *Concentrations of nitrate and ammonium at the end of the experiment (October 2003) and absolute change of concentrations during the observation period from May to October 2003, data presented as means with standard deviation (n=4).*

Site	Treatment	October 2003 NH_4^+	 NO_3^-	Difference May-October 2003 NH_4^+	 NO_3^-
		mg/kg dry weight			
Baktalórántháza	Basamid	46.1±21.1*	2.5±0.9**	+4.2±22.8	-30.1±0.7**
	Control	18.3±12.3	27.0±10.2	-1.8±10.4	+14.8±11.8
Pusztavács	Basamid	14.7±9.5**	4.7±1.9	-26.3±9.4**	-20.8±1.7**
	Control	0.7±0.4	3.9±1.0	-6.3±0.8	-13.9±2.1
Kelebia	Basamid	2.9±1.7*	18.9±8.3	-51.8±3.4**	-17.8±8.4**
	Control	1.2±0.1	19.0±7.4	-6.7±1.5	-28.6±15.8

*Values denoted with *(p<0.05) or ** (p<0.01) are significantly different between treatments within soils from the same site.*

5.5 Conclusions

The observed differences of growth and N-status of black locust seedlings cultivated in sterilized and non-sterilized soils from different forest regions in Hungary is likely to be related to an increased nutrient release from dead soil organisms and to a decreased nodulation after the *Basamid* treatment. No direct identification of soil-borne pathogens was conducted, therefore it can not be proven whether observed growth depressions of stands were related to an enhanced occurrence of pests or not. To determine the influence of biological factors on the sustainability of black locust yield after long-term cultivation, the identification of pathogens under stands with observed growth depressions has to be done. Soil sterilization can not be recommended to improve the growth of tree seedlings in general.

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6 Summary

Worldwide the land area covered by stands with the neophytic tree legume *Robinia pseudoacacia* L. (black locust) has expanded to about 3 Mio. ha and is predicted to extend further in the future. Besides the rising atmospheric inorganic N deposition (mean 20-30 kg N ha^{-1} yr^{-1}) to forest ecosystems in Europe, N_2 fixation rates (35-150 kg N ha^{-1} yr^{-1}) in black locust stands can enhance the problems associated with increasing N inputs into forest ecosystems. The strong N-enrichment in the vegetation and soils and the subsequent processes of N transformation under forest stands of *Robinia* can result in high nitrification rates in the mineral soil. Moreover, leaching of nitrate and base cations may occur, causing a significant drop in the pH of the surface soil that can lead to nutrient depletion as well as to nitrate contamination of the groundwater. After long-term cultivation of *Robinia* stands on the same site decreases of their yield performance are observed. However, the involved processes responsible for this phenomenon are currently unknown.

The present work contributes to the discussion of the implementation of the neophytic tree *Robinia pseudoacacia* L. according to its impact on chemical and biological soil parameters. Additionally, the aim of the study was to clarify the processes responsible for the observed growth decline after repeated black locust cultivation on the same site.

It was hypothesized that:

I. The N_2 fixation rates of black locust stands are not regulated by the N demands for tree nutrition, leading to surplus N-fixation and subsequent N enrichment of the organic layer and mineral soil.

II. N accumulation results in strong mineralization and nitrification coupled with elevated internal H^+ production and leaching losses of nitrate and associated base cations. Moreover, N supply by via fixation of atmospheric dinitrogen leads to an acidification of the rhizosphere.

III. Soil chemical degradation due to elevated acidification and concentrations of soluble Al-species affects soil microorganisms and leads to the dominance strains of nodule-forming *Rhizobium*, which are mostly ineffective to fix atmospheric dinitrogen. Soil inoculation

with effective *Rhizobium* strains elevates N_2 fixation rates, plant growth and nitrogen content as well as the above mentioned processes of soil N-enrichment and degradation.

IV. H^+ excretion of *Robinia* fine roots causes a strong decrease of the rhizosphere pH.

V. Changes of soil chemical properties (acidification & nutrient depletion) due to N transformations coupled with decreased N_2 fixation rates through root infections with ineffective *Rhizobium* strains and enhanced appearance of soil-born pathogens are responsible for the reduction in growth after long-term cultivation of black locust on the same site.

These hypotheses were verified by the results of the four studies:

I. In addition to the vegetation, the organic layer is an important store for nitrogen under forest stands. A higher dry mass of the litter layer under pure black locust compared to oak stands in Hungary (first paper) resulted in significantly higher nitrogen stocks on non-calcareous sites. High amounts of organic matter in the black locust stands despite favourable mineralization conditions due to high annual temperature and low C/N ratios indicate high litter production rates as well as low decomposability of *Robinia* litter. This was attributed to the combination of high lignin and N concentrations above a certain threshold level that is retarding the mineralization rate. Higher N-concentrations and -stocks as well as lower C/N ratios in the mineral soils under black locust stands revealed the influence on the soil nitrogen status. Differences between N_2 fixing and non-fixing trees became even more distinct, when the spatial heterogeneity of the data was reduced by the comparison of adjacent black locust and oak stands. Depending on the period of black locust cultivation on the same site, the nitrogen sequestration under black locust was observed to increase in the second tree generation.

In the second study pre-treatment of the soil (drying, sieving and re-wetting) and liming caused strong mineralization and nitrification and led to high inorganic N-fractions at the beginning of the experiment. However, a strong decrease of the soil mineral N-fractions was detected at the end of the experiment. Obviously at this stage N_2 fixation rates and litter input of young *Robinia* seedlings were not yet high enough to balance or exceed the amount of nitrogen reduced by plant uptake and leaching losses during the observation period.

The nitrogen enrichment hypothesized in the organic layer and mineral soils under black locust can be confirmed by the data of the soil inventory under forest stands in Hungary. In the greenhouse experiment the period of *Robinia* cultivation under different chemical and biological soil conditions obviously was too short to increase the soil nitrogen status.

II. Processes of soil acidification and nutrient depletion as a consequence of N_2 fixation and black locust growth were found under pure stands in Hungary. Compared to oak forests, lower pH values and base saturation as well as higher concentrations of M_a cations (H^+, Fe^{3+} and Al^{3+}) were measured in the topsoil under *Robinia* stands. Under mixed stands of *Robinia pseudoacacia* and *Quercus* spec. it was expected that effects on soil chemical properties due to black locust would exist between pure stands of these tree species, but this was not the case for most soil parameters in the Hungarian sites. Obviously site effects by mixed stands of N-fixing and not N-fixing trees cannot be predicted as a summation of the corresponding monocultures.

In the greenhouse study [experiment (2)] impacts of black locust cultivation on soil chemistry were again affected by strong mineralization processes at the beginning of the experiment, resulting in elevated pH values and base saturation in the planted pots of the unlimed soil treatments. However, due to enhanced plant growth in the limed soils, pH in both limed treatments and base saturation in the moderately limed soil decreased in the observation period of two years. The most negative effect of *Robinia* cultivation on soil pH and nutrient availability was found in the moderately limed soil, probably due to enhanced plant growth and limited buffer capacity.

III. Liming and inoculation of soil with effective *Rhizobium* strains was intended to create favourable conditions for the survival and activity of symbiotic soil bacteria, for nodulation, N_2 fixation and for plant growth. During the observation period of two growing seasons in the greenhouse experiment, liming strongly promoted plant growth and nodulation, whereas effects due to inoculation were less pronounced. The height and diameter growth of inoculated seedlings was lower in the first and higher in the second year than in soils without inoculation, indicating a delayed impact of that treatment due to high carbon costs for the establishment of the symbiosis. Inoculation led to elevated plant biomass and formation of nodules only in the moderately limed soil (soil pH 4.8). The nodule activity, measured as CO_2 production rates of incubated fresh nodules, was not influenced by inoculation. Furthermore, no effects of inoculation on nitrogen concentrations and accumulation in plant tissue were found. With regard to changes of soil chemical properties, the inoculation treatment did not result in elevated soil degradation and nitrogen accumulation. This clearly indicates that the “natural” inoculum of the soil provide strains adapted to different soil chemical conditions.

IV. Depending on the availability of inorganic nitrogen fractions in soils, black locust plants can take up N in forms of NO_3^- and NH_4^+, or they rely on N_2 fixation. The uptake of mineral N and the process of fixation can occur at the same time with unknown rates, respectively. Plants supplied with NO_3^- will counterbalance the corresponding excess of negative charges in plant tissue by releasing equivalent amounts of HCO_3^- into the rhizosphere and thereby increase rhizosphere pH, whereas roots react on the uptake of NH_4^+ with the release of H^+ leading to rhizospheric acidification. When relying fully on atmospheric N_2, legumes like black locust take up more cations than anions, and hence release the excess of positive charges as H^+ and acidify their rhizosphere. Measured pH values along the surface of black locust roots [experiment (3)] were 0.7 to 0.8 units lower than in the bulk soil. H_3O^+ concentrations were high (2 to 11 µmol/L in the limed and 44 µmol/L in the unlimed soils), indicating a strong proton release by the fine roots. A multispecies rhizosphere model explained the measured decrease of pH values by predominantly NH_4^+ uptake in the unlimed and moderately limed soils, whereas measured pH values in the rhizosphere of the strongly limed soil were explained by various ratios of NH_4^+/NO_3^- uptake. Strong mineralization due to soil pre-treatment and elevated nitrification after $CaCO_3$ addition and the subsequent high content of inorganic nitrogen in soils resulted in a lowered fixation and uptake of atmospheric dinitrogen. Therefore measured and modelled pH decreases were primarily the result of NH_4^+ and NO_3^- uptake and not of symbiotic N_2 fixation. On the other hand, measured low soil content of mineral N fractions at the end of the experiment, indicated that N_2 fixation was an important factor for the nitrogen nutrition and the rhizospheric acidification.

V. Growth decline after repeated cultivation of a crop on the same site for soil chemical and biological reasons is a well-known phenomenon in agriculture and forestry. The impact of *Rhizobium* strains and soil-born pathogens on the growth decline of black locust was studied on soils originating from sites with observed yield decreases after long-term cultivation. Therefore the growth of black locust seedlings cultivated in sterilized and non-sterilized soils as well as the nitrogen content of plant tissue and the soils after one vegetation period were compared. Sterilization of non-calcareous soils increased the relative height and diameter growth rate. However, there was no significant effect on the total plant biomass. Plant nitrogen content was significantly elevated through sterilization, whereas nodulation was reduced. The non-specific soil fumigation killed all microorganisms and released high amounts of microbial N into soils, which enhanced plant growth and inhibited nodulation. The experiment therefore was not a proof of the hypothesis of yield depressions by biological parameters. To investigate the influence of modified *Rhizobium* strains and enhanced occurrence of soil pathogens due to repeated black locust cultivation, an identification of these organisms under natural site conditions is required.

The studies showed that the cultivation of *Robinia pseudoacacia* L. has a clear impact on soil chemistry. Depending on the soil nitrogen status and buffer capacity this negatively influences the trees. Effects on biological soil parameters however can not be excluded. As a general outcome, the cultivation of *Robinia* in monospecific stands is not recommended. The potential of black locust for soil degradation on nutrient poor and mesic sites alone is reason enough to silvicultural integrate this tree species into mixed stands. The importance of black locust on rich sites is low due to the preference of tree species with higher nutrient demands.

7 Zusammenfassung

Die mit Beständen der neophytischen Baumart *Robinia pseudoacacia* L. (Robinie) bestockte Fläche wurde weltweit auf ca. 3 Mio. ha ausgeweitet. Auch in Europa und Deutschland ist mit einem steigenden Anbau dieser anspruchslosen und für ihre hochwertige Holzqualität geschätzten Baumart zu rechnen. Als Leguminose ist die Robinie in der Lage mit Hilfe von symbiontischen Bakterien (*Rhizobium* spec.) Luftstickstoff zu fixieren. Die N_2-Fixierung in Robinienbeständen (35-150kg N ha^{-1} a^{-1}) kann jedoch zusätzlich zu der hohen Deposition anorganischer N-Verbindungen aus der Atmosphäre (Ø in europäischen Waldökosystemen 20-30 kg N ha^{-1} a^{-1}) zu Problemen führen. Die starke Anreicherung der Vegetation und der Böden mit Stickstoff und die anschließenden N-Umwandlungsprozesse unter Robinienbeständen haben hohe Nitrifikationsraten im Mineralboden zur Folge. Dadurch kann es zur Auswaschung von Nitrat und M_b-Kationen kommen, was zu einem signifikanten Abfall des pH Wertes im Oberboden, zur Nährstoffverarmung und zur Nitrat-Kontamination des Grundwassers führen kann. Beobachtungen aus der forstlichen Praxis zeigen außerdem, dass der Anbau der Robinie über mehrere Generationen auf demselben Standort häufig zu einem starken Zuwachsrückgang der Bestände führt. Die Zusammenhänge der für diese Beobachtungen verantwortlichen Prozesse sind jedoch gegenwärtig weitgehend unbekannt.

Die vorliegende Arbeit liefert einen Beitrag zur Diskussion über die Einführung des Baum-Neophyten *Robinia pseudoacacia* L. in Bezug auf dessen Auswirkungen auf bodenchemische und -biologische Größen. Weiterhin war es Ziel dieser Studie, die für den beobachteten Zuwachsrückgang nach wiederholtem Robinienanbau auf demselben Standort verantwortlichen Prozesse aufzuklären.

Folgende Hypothesen wurden getestet:

I. Die N_2-Fixierung durch die Rhizobien der Robinie ist nicht so fein reguliert, dass lediglich der Bedarf der Bäume gedeckt wird, so dass es zu einer N-Anreicherung der organischen Auflagen und Mineralböden kommt.

II. Die Stickstoffanreicherung führt zu höheren Mineralisations- und Nitrifikation-Raten, welche mit erhöhter interner H^+-Produktion sowie Auswaschungsverlusten von Nitrat und Begleitkationen verbunden sind. Hinzu kommt, dass es bei der N-Versorgung über die Fixierung von Luftstickstoff zu einer Versauerung der Rhizosphäre kommt.

III. Die bodenchemische Degradation aufgrund von Versauerung und erhöhten Konzentrationen gelöster Al-Spezies beeinträchtigt allgemein die Bodenmikroorganismen und führt zur Dominanz von Rhizobienstämmen, die hinsichtlich ihrer N_2-Bindungsraten wenig effektiv sind. Die Impfung des Bodens mit effektiven Rhizobienstämmen erhöht daher die Bindungsrate atmosphärischen Stickstoffs, das Wachstum und den N-Gehalt der Pflanzen und verstärkt die oben angeführten Prozesse der N-Anreicherung und Degradation der Böden.

IV. Die Exudation von H^+-Ionen durch die Feinwurzeln der Robinie verursachen einen starken Abfall des pH-Wertes in der Rhizosphäre.

V. Veränderungen bodenchemischer Parameter (Versauerung & Nährstoffverarmung) als Folge der N-Umsetzung im Boden zusammen mit abnehmenden N-Fierungsraten durch die Infektion mit wenig effektiven Rhizobienstämmen und dem verstärkten Auftreten bodenbürtiger Pathogene sind Faktoren, welche für den Wachstumsrückgang nach Langzeitkultivierung der Robinie auf dem gleichen Standort verantwortlich sind.

Die Ergebnisse der Untersuchungen werden Bezug nehmend auf die Hypothesen zusammenfassend dargestellt:

I. Neben der Vegetation ist die organische Bodenauflage ein wichtiger Speicherort für Stickstoff in Wäldern. Auf kalkarmen Standorten in Ungarn wurden in Robinien-Reinbeständen signifikant höhere organische Auflagemengen gemessen als in Eichen-Reinbeständen [Untersuchung (1)]. Dies spiegelte sich auch in signifikant höheren Stickstoff-Vorräten wider. Die vergleichsweise hohe Akkumulation organischer Substanz unter Robinien weist auf hohe Streuproduktionsraten und, trotz theoretisch günstiger Abbaubedingungen aufgrund hoher Jahresmitteltemperaturen und enger C/N-Verhältnisse, auf eine schlechtere Zersetzbarkeit der Robinienstreu hin. Dieses Phänomen wurde auf die Kombination von hohen Lignin- und N-Konzentrationen zurückgeführt, welche die Mineralisation verzögert. In den Mineralböden unter Robinienbeständen waren nicht nur die N-Vorräte höher als in Eichenbeständen, sondern auch die N-Konzentrationen, wiederum verbunden mit engeren C/N Verhältnissen. Diese Ergebnisse bestätigen den beträchtlichen Einfluss der Robinie auf den N-Status der Böden. In Bezug auf die Dauer des Robinienanbaus auf dem selben Standort wurde beobachtet, dass die N-Akkumulation in Beständen der zweiten Baumgeneration höher ist als in Beständen der ersten Generation. In der zweiten Untersuchung (Gewächshausversuch) verursachte die Vorbehandlung des Bodens (Trocknen, Sieben, Wiederbefeuchten) eine starke initiale Mineralisation der organischen Bodensubstanz und anschließende Nitrifikation, was zu hohen anorganischen N-Gehalten des Substrats zu Versuchsbeginn führte. Am Ende der Untersuchung wurde jedoch eine starke Absenkung des Gehalts mineralischer N-Fraktionen festgestellt. Offensichtlich waren die Raten der N_2-Fixierung und der N-Eintrag mit der Streu junger Robinien noch nicht hoch genug, um die Stickstoffverluste durch die Pflanzenaufnahme und Auswaschung während des Beobachtungszeitraumes auszugleichen bzw. zu übertreffen. Die postulierte N-Anreicherung der organischen Auflagen und des Mineralbodens in Robinienbeständen kann durch die Ergebnisse der chemischen Bodeninventur ungarischer Standorte bestätigt werden. Für den Gewächshaus-Versuch war die Dauer des Robinienwachstums unter den verschiedenen chemischen und biologischen Bodenvarianten offenbar zu kurz, um den Stickstoffgehalt der Böden zu erhöhen.

II. Die N-Akkumulation in Robinienbeständen Ungarns hatte eine Bodenversauerung verbunden mit einer Verarmung anderer Nährstoffe zur Folge. Im Vergleich zu Eichenbeständen wurden tiefere pH-Werte und Basensättigungen sowie höhere Konzentrationen von M_a-Kationen (H^+, Fe^{3+} und Al^{3+}) im Oberboden gemessen. Für Robinien-Eichen-Mischbestände wurde angenommen, dass die Effekte auf den Boden zwischen denen der jeweiligen Reinbestände lägen. Dies konnte jedoch für die meisten Bodenparameter der ungarischen Standorte nicht bestätigt werden. Wie auch durch andere Untersuchungen bestätigt, können Standortsveränderungen durch den Anbau von Mischbeständen nicht durch lineare Verknüpfung der ermittelten Effekte unter Monokulturen bestimmt werden. Im Gewächshausversuch [Experiment (2)] wurden Effekte des Robinienanbaus auf den Bodenchemismus anfänglich durch die oben genannte erhöhte Mineralisation überdeckt. Jedoch führte ein gesteigertes Pflanzenwachstum auf beiden gekalkten Böden nach zwei Vegetationsperioden zur pH-Absenkung und auf dem mäßig gekalkten Boden zu einer Verringerung der Basensättigung. Aufgrund von gesteigertem Pflanzenwachstum und eingeschränkter Pufferkapazität im mäßig gekalkten Boden (pH_{KCl} 4,8) zeigten sich die stärksten Auswirkungen des Robinien-Wachstums in Bezug auf Boden-Versauerung und -Nährstoffverluste in dieser Variante.

III. Bodenkalkung und Beimpfung mit effektiven Rhizobium-Stämmen sollten günstige Bedingungen für das Überleben und die Aktivität der Bakterien, die Knöllchenbildung, die N_2-Fixierung und für das Pflanzenwachstum schaffen. Während des Untersuchungszeitraumes von zwei Vegetationsperioden [Experiment (2)] wurden das Wachstum der Robinien und die Knöllchenbildung durch die Bodenkalkung stark gefördert, wohingegen Auswirkungen der Inokulation weniger stark auftraten. Der Höhen- und Durchmesserzuwachs geimpfter Robinien war im ersten Jahr geringer und im zweiten Jahr höher als in Böden ohne Inokulation, was auf einen verzögerten positiven Effekt dieser Behandlung aufgrund des anfänglich hohen Kohlenhydrat-Bedarfs zur Etablierung der Symbiose hindeutet. Lediglich in der mäßig gekalkten Boden-Variante (pH_{KCl} 4,8) führte die Impfung zu erhöhter Biomasse- und Knöllchenbildung. Die Knöllchenaktivität, welche anhand von CO_2-Produktionsraten frisch inkubierter Knöllchen bestimmt wurde, war durch die Inokulation nicht beeinflusst. Weiterhin konnte kein Einfluss der Impfung auf die Stickstoff-Konzentration und -Akkumulation im Pflanzengewebe nachgewiesen werden. Hinsichtlich der postulierten Veränderungen bodenchemischer Parameter führte die Inokulation mit effektiven Bakterienstämmen weder zu verstärkter Bodendegradation, noch zu erhöhter N-Anreicherung.

IV. In Abhängigkeit von der Verfügbarkeit anorganischer N-Fraktionen im Boden können Robinien ihren N-Bedarf sowohl durch die Aufnahme von NO_3^-, NH_4^+ als auch durch die Fixierung atmosphärischen Stickstoffs decken. Die Aufnahme von mineralischem Stickstoff und der Prozess der Fixierung können gleichzeitig stattfinden, wobei die jeweiligen Raten nicht direkt messbar sind. Pflanzen mit NO_3^--Ernährung gleichen den hierdurch entstehenden negativen Ladungsüberschuss durch die Ausscheidung von HCO_3^--Ionen in die Rhizosphäre aus und erhöhen dadurch den pH-Wert der Rhizosphäre, wohingegen die Aufnahme von NH_4^+ und der hieraus resultierende positive Ladungsüberschuss mit einer H^+-Abgabe verbunden ist, welche die Rhizosphäre versauert. Wenn Leguminosen, wie z.B. *Robinia pseudoacacia*, auf die N_2-Fixierung als einzige Stickstoffquelle für das Pflanzenwachstum angewiesen sind, dann nehmen sie mehr Kationen als Anionen aus dem Boden auf und gleichen den resultierenden Überschuss an positiven Ladungen durch die Ausscheidung von H^+-Ionen aus und versauern somit die Rhizosphäre ebenfalls. pH Messungen entlang der Wurzeloberfläche junger Robinien [Experiment (3)] ergaben um 0,7 bis 0,8 pH-Einheiten niedrigere Werte als im Boden. Die errechneten H_3O^+-Konzentrationen betrugen 2 bis 11 µmol/L in den gekalkten und bis zu

44 µmol/L in den ungekalkten Böden, was eine hohe Protonenabgabe anzeigt. Durch die Anwendung eines Rhizosphären-Modells konnten die gemessenen pH-Absenkungen durch überwiegende NH_4^+-Aufnahme in den ungekalkten (pH_{KCl} 3,4) und moderat gekalkten (pH_{KCl} 4,8) Böden erklärt werden, sowie durch variierende NH_4^+/NO_3^--Aufnahmeraten in den stark gekalkten Böden (pH_{KCl} 6,2) beschrieben werden. Die starke Mineralisation der organischen Bodensubstanz aufgrund der Boden-Vorbehandlung, die erhöhte Nitrifikationsrate nach der Kalkung und die durch diese Prozesse stark erhöhten Gehalte anorganischen Stickstoffs beeinträchtigten die Fixierung und Aufnahme von atmosphärischem N_2. Folglich ließen sich die gemessenen und modellierten pH-Absenkungen in der Rhizosphäre vor allem durch die Aufnahme von NH_4^+ oder NO_3^- und weniger durch die symbiontische N_2-Fixierung erklären. Andererseits weisen die gemessenen geringen Gehalte mineralischer Stickstofffraktionen des Bodens zu Versuchende daraufhin, dass auch die Fixierung atmosphärischen Stickstoffs einen bedeutenden Einfluss auf die N-Bilanz und die Bodenversauerung hatte. Die jeweiligen Anteile der, von den jungen Robinien aufgenommenen unterschiedlichen N Spezies, können im Rhizotron-Experiment nicht quantifiziert werden. Daher konnten für die in der Rhizosphäre beobachtete Versauerung nur Szenarien berechnet werden, die jedoch plausible Erklärungen ergaben.

V. Wuchsdepressionen nach wiederholtem Anbau einer Pflanzenart auf dem selben Standort aus bodenchemischen und -biologischen Gründen sind ein bekanntes Phänomen in der Land- und Forstwirtschaft. Im Rahmen der vierten Untersuchung wurde keine direkte Identifikation der vorhandenen Rhizobienstämme und der bodenbürtigen Pathogene vorgenommen. Die Beteiligung dieser bodenbiologischen Faktoren wurde indirekt ermittelt, indem die für diese Studie verwendeten Böden von Standorten mit dokumentierter Wuchsdepression stammten. Es wurde das Wachstum junger Robinien auf sterilisierten und unbehandelten Böden sowie der Stickstoffgehalt dieser Pflanzen nach einer Vegetationsperiode verglichen. Auf kalkarmen Böden erhöhte die Bodensterilisation den relativen Höhen- und Durchmesserzuwachs der Robinien. Allerdings konnten keine signifikanten Effekte auf den Biomassezuwachs beobachtet werden. Die Sterilisation erhöhte den N-Gehalt des Pflanzengewebes und verringerte die Knöllchenbildung der Robinien signifikant. Die unspezifische Fumigation mit *Basamid* tötete viele Bodenorganismen ab und führte somit zu einer Freisetzung von leicht mineralisierbarem Stickstoff in den Boden, welcher das Pflanzenwachstum anregte und die Knöllchenbildung hemmte. Die Ergebnisse dieses Versuchs konnten die Hypothese des Zuwachsrückgangs durch die Beteiligung biologischer Parameter nicht einwandfrei bestätigen. Zur Überprüfung des Einflusses veränderter Rhizobiengemeinschaften und des verstärkten Auftretens bodenbürtiger Pathogene als Folge wiederholten Robinien-Anbaus ist in einem weiteren Versuch eine Bestimmung dieser Organismen unter natürlichen Umweltbedingungen vorgesehen.

Die Untersuchungen verdeutlichen, dass der Anbau von *Robinia pseudoacacia* L., in Abhängigkeit von Stickstoffgehalt und Pufferkapazität des Bodens, negative Auswirkungen auf bodenchemische Eigenschaften haben kann. Direkte Effekte auf bodenbiologische Größen konnten aber nicht einwandfrei nachgewiesen werden. Als allgemeine Schlussfolgerung aus dieser Studie ist der dauerhafte Anbau der Robinie in Reinbeständen nicht ohne Probleme zu bewerten. Neben anderen ökologischen Risiken verbunden mit Monokulturen ist das Potential der Robinie zur Bodendegradation auf ärmeren und mittleren Standorten Grund genug, sie waldbaulich nur als Mischbaumart zu behandeln. Dabei können der N-Überschuss besser ökologisch verwertet und die negativen Folgen reduziert werden. Auf guten Standorten ist ihre Bedeutung ohnehin gering, da dort anspruchsvolleren Baumarten Vorrang gegeben wird. In Gebieten, in denen sich die Robinie als invasive Baumart darstellt, sollte verhindert werden, dass sie Waldbestände dominiert.

8 Appendix

8.1 List of recent Publications

8.2 Berthold, D., Vor, T. & Beese, F. (2005): Soil degradation by Black locust (Robinia pseudoacacia L.). *In*: Nentwig, W. et al. (Eds.): “Biological Invasions - from Ecology to Control”, NEOBIOTA 6, pp. 67-78, Berlin (2005).

8.3 Berthold, D. & Vor, T. (2003): Robinie – Fluch oder Segen für den Waldboden. *In*: „Tagungsband, Jahrestagung der Sektion Waldbau im Deutschen Verband Forstlicher Forschungsanstalten („Biologische Rationalisierung im Waldbau“), pp. 55-61.

8.4 Goldacker, S., Berthold, D. & Beese, F. (2002): Bodenversauerung unter Robinie – Potenzielle Ursache von Wachstumsminderungen?. AFZ Der Wald, 19/2002, pp.1003–1006.

8.5 Berthold, D & Beese, F. (2002): Kohlenstoffspeicherung in Böden nach Aufforstung in Abhängigkeit von der Bewirtschaftungsform. Forst und Holz, 57 (13/14), pp.417-420.

8.2 Soil degradation by *Robinia pseudoacacia* L. (Black Locust) in Hungary

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In: Nentwig, W. et al. (Eds.): "Biological Invasions - from Ecology to Control", NEOBIOTA 6, pp. 67-78, Berlin (2005).

Summary

(1) Soil inventories under adjacent stands of black locust (Robinia pseudoacacia L.) and oak (Quercus cerris, Quercus pubescens) in Hungary were conducted to determine the impact of black locust cultivation on chemical soil characteristics. Under N_2 fixing black locust stands higher amount of nitrogen, lower pH values and lower base saturation were detected indicating excess of nitrate which was leached together with base cations (K^+, Ca^{2+}, Mg^{2+}) causing soil acidification and a decrease of soil fertility.

(2) To assess N leaching in association with N fixation on effectiveness of plant development, black locust seedlings were cultivated in soils with different pH and *Rhizobium* inoculation treatments for two vegetation periods. Due to the pre-treatment (drying, sieving, re-wetting) of soil strong organic matter mineralization affected leaching losses under all variants. Plant growth was promoted on high pH soils (pH 4.8 and 6.2) but less by inoculation of soil with *Rhizobium*. A moderate application of $CaCO_3$ and inoculation of soil with symbiotic bacteria showed highest plant survival, growth and nodule development.

(3) pH measurements at the root surface and in the rhizosphere of black locust seedlings growing in thin layer rhizotrons for one vegetation period confirmed the hypothesis of rhizospheric acidification through N_2 fixation. Rhizosphere acidification is attributed to the release of protons following uptake of cations in excess to those of anions. Depending on the pH of bulk soil, an increase of the concentrations of H^+ ions between 7 to 200µmol H^+/L in the rhizosphere was detected. Moreover decrease in exchangeable base cations and pH in unlimed and moderately limed soils was observed during the observation period.

Key words: acidification, base cations, excess nitrogen, leaching, N_2 fixation, rhizosphere, soil fertility

Introduction

Robinia pseudoacacia L. (black locust) is a nitrogen-fixing tree, native to south-eastern North America (Barett et al. 1990) in two disjunct original areas between the 35^{O} and 43^{O} latitude (Fowells 1965; Little 1971; Huntley 1990). Worldwide the land area covered by black locust plantations has increased in recent decades and is about 3 Mio. ha, only exceeded by that of Eucalyptus and Poplar (Hanover et al. 1991). China (1 Mio. ha) and South Korea (0.5 Mio. ha) are the most important producers of black locust wood in the world (Rédei 1998; Claasen 2001). In Hungary, the area under black locust stands has increased from 201.000 ha in 1958 to 320.000 ha in 1994 and may increase by another 40.000 ha in the next twenty years (Molnár et al. 1994).

Reasons for cultivating *Robinia pseudoacacia* are: erosion control and reclamation of disturbed areas (Boring & Swank 1984a; Chang-Seok et al. 2003), its tolerance against drought, the excellent properties of its wood and its ability to fix atmospheric nitrogen (DeGomez & Wagner 2001). It is easy to regenerate from root suckers, grows efficiently on poor sites and improves nitrogen supply and element turnover (Ntayombya & Gordon 1995).

However, cultivation of black locust may have usual problems associated with introduction of a neophyte. On dry and poor sites it is very competitive and invasive; its vigorous spread can cause a decline in native drought adapted plant species. Depending on stand age and density as well as on climatic conditions, *Robinia* fixes 35-150 kg N ha^{-1} a^{-1}, indicating a high capacity for N_2 fixation (Hoffmann 1960; Boring & Swank 1984b; Danso et al. 1995). Thus, the amount of N fixed may exceed the demand for tree growth causing the excess nitrogen to be lost through leaching and denitrification and to be accumulated partly. During nitrification and leaching processes soil acidification and loss of base cations may occur causing a significant drop in the pH in the surface soil (van Migroet & Cole 1984). Moreover, leaching of nitrate can contaminate ground water (van Migroet & Cole 1985). Where black locust stands are regenerated from root suckers and stem sprouts, N accumulated in the organic matter is likely to undergo fast release and the associated deleterious effects due to excess of nitrogen (Feller et al. 2000; Piirainen et al. 2001; Prescott et al. 2003).

The ratio of cation uptake to anion uptake determines the rhizosphere pH change, with excretion of H^{+} or HCO_3^{-} ions to balance the excess uptake of cations or anions, respectively. The chemical form of N compound absorbed is a major factor in determining this ratio. When through the uptake of fixed N uptake of anions (nitrate) is reduced causing imbalance in electrical neutrality whereby excessive H^{+} ions are excreted by plant roots, which in turn acidify the rhizosphere (Nyatsanga & Pierre 1973; van Beusichem 1981, 1982; Gillespie & Pope 1990).

We describe three different experiments to measure the effects of black locust on chemical soil properties: (1) field sampling to assess soil changes, (2) laboratory experiment to measure changes in soil solution composition, and (3) microrhizoplate experiments to measure pH changes in the rhizosphere.

Materials and Methods

(1) Field sampling

***Fig. 1**: Map of Hungary with the four experimental sites distributed over the whole country.*

To determine the effects on chemical soil properties, a soil inventory under adjacent stands of *Robinia pseudoacacia* and oak (*Quercus cerris, Quercus pubescens*) at four forest sites in Hungary was conducted: Pusztavács, Baktalórántháza, Nagyatád and Iván (*Figure 1*). All sites were characterized by low annual precipitation and high mean annual temperatures. The dominant soil types were sandy Typic Cambisols and loamy Typic Chernozems (*Table 1*). Stands of *Robinia* were 28 to 56 years old and of *Quercus* 88 to 120 years (compare *Table 2*).

In every stand samples of the organic layer and mineral soil horizons were collected in triplicate. Samples were taken by using a steel corer with a defined volume (Ø 7.2cm). Soil samples were extracted from 0-50cm depth in intervals of 10cm. Samples were dried at 40° C (mineral soil) or 60° C (organic layer), sieved (Ø 2mm) and milled. Total C and N contents were analysed by dry combustion with a C/N auto analyser (Vario Elementar

Analysensyteme, Hanau, Germany). Total element concentration (P, S, K, Ca and Mg) in the samples were analysed by ICP-AES (Spectro Analytic Instruments, Kleve, Germany) after pressure digestion in 65%-concentrated HNO_3. Soil samples were percolated with 1N NH_4Cl for measuring exchangeable cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+},Al^{3+}) using AAS 300 AA (Varian Inc., Darmstadt, Germany). pH was measured in 0,1mol l^{-1} KCl.

Tab. 1: *General clime and soil conditions of the four forest regions in Hungary.*

Site	Elevation (m)	Precipitation Annual/Growth-Period (mm)	Temperature Annual/Growth-Period (°C)	Period of sunshine (hours/year)	Soil type
Pusztavács	100-150	500 / 320	10.3 / 17.3	2050	sandy Cambisols
Bata-lóránthháza	100-150	570 / 350	9.7 / 17.1	1970	sandy Cambisols
Nagyatád	100-150	760 / 460	9.9 / 16.2	1990	sandy Cambisols
Iván	200	630 / 360	9.6 / 15.8	1860	loamy Cambisols (pseudogleyification)

(2) Laboratory experiment to measure soil solution composition

Cloned seedlings of black locust (clone 2466, Waldsieversdorf, Germany) were cultivated in vermiculite and transplanted in soil, which was derived from an acidic spruce site (*Picea abies* K.) in Lower Saxony (Germany). After determination of the base neutralisation capacity (BNK) of an acid soil (Meiwes et al. 1984) three pH variants were obtained through liming: (**a**) 0.0g $CaCO_3$/ kg soil → unlimed [**pH 3.4**]; (**b**) 2.1g $CaCO_3$/ kg soil → moderately limed [**pH 4.8**]; (**c**) 5.9g $CaCO_3$/ kg soil → strongly limed [**pH 6.2**]. Before pH adjustment and planting of seedlings into pots (Ø 14.4cm X 60cm) soil was dried and sieved to reduce stone content and to facilitate mixing of lime. For each treatment half of the pots were inoculated with a 500ml suspension of mixed *Rhizobium* from commercial inocula (strains Rob8, Rob10, Rob11, Rob12 and Rob25; Institute for Plant Cultivation, Solkau, Germany), whereas the rest received no further symbiotic soil bacteria. Five pots without plants served as references for each pH treatment.

Over two years the leachate from soils of each pot was collected continuously, stored at 4° C and analysed at intervals of two months. Analysis of soil solution comprised pH, cations (NH_4^+, H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}) and anions (NO_3^-, SO_4^{2-}, PO_4^{3-}). Plant growth was measured at the end of the first and second vegetation periods. At the end of the experiment plants were harvested and the biomass of the leaves, stems, roots and nodules was determined.

(3) Rhizotron experiment to measure pH changes in the rhizosphere

Seedlings of *Robinia pseudoacacia* were grown in thin layer rhizotrons (70.0 X 10.0 X 1.5cm) for six month with the same three soil pH treatments as in the laboratory experiment (10 replicates for each pH variant). A needle pH electrode with a tip diameter of 0.8 mm (Combination Needle pH Electrode No. 818, Diamond General, Michigan, USA) was used for measuring pH at the root surface and in the rhizosphere along the roots, around nodules and in bulk soil. Bulk soil was defined as soil at a distance of 3mm from root tips, root and nodule surfaces. pH in the rhizosphere was measured up to a distance of 3mm in front of root tips and in steps of 1mm along the root surface for a root section of 8mm. Changes in exchangeable cation concentrations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+},Al^{3+}) was monitored by measuring soil samples collected initially and at the end of the experiment.

Statistical analysis of the data was performed by the program *Statistica version 6.0* by using the Mann-Whitney U-Test at $p<0.05$ level. pH values were used directly without log transformation for statistical analysis.

Results

Field studies

Table 2 shows the selected pairs of pure *Robinia pseudoacacia* and *Quercus sp.* stands at the Hungarian forest sites with similar total element concentrations (P, S, K, Ca and Mg) in the subsoil (30-50cm). For these paired stands it was hypothesised that any difference of pH, nitrogen status and base saturation in the organic layer and mineral soil was due exclusively to plant influence. Besides the vegetation the humus layer is the most important sink for nitrogen. Mean dry weight of the organic layer under black locust stands (5.4 kg/m^2) was significantly higher than under oak (2.8 kg/m^2). For all paired stands N accumulation in the organic layer under black locust was significantly higher (5% level) *(Figure 2*). At the forest sites of Pusztavács (a) and Iván (d) significantly larger nitrogen accumulation was also detected for the mineral soil and the whole profile depth. As shown in *Figure 2*, significantly lower pH values and base saturation (M_b) in the mineral soil under black locust were found than in adjacent *Quercus* stands in Pusztavács (a), Baktalórántháza (b) and Nagyatád (c). These indicators for acidification and nutrient depletion through black locust cultivation were

***Tab. 2:** Total element concentrations in the subsoil (30-50cm) for selected pairs of Robinia and Quercus stands in Hungary. Data provided as mean values ± standard error (n=4).*

Site	Stand (Age)	P	S	K	Ca	Mg
				mg/g		
Pusztavács	Robinia	0.22	0.07	2.16	1.81*	1.37
	(56 years)	±0.03	±0.01	±0.19	±0.20	±0.05
	Quercus	0.21	0.06	1.95	2.71	1.48
	(103 years)	±0.01	±0.00	±0.03	±0.04	±0.02
Baktalórántháza	Robinia	0.23*	0.06	4.35	1.52	2.05
	(65 years)	±0.01	±0.00	±0.20	±0.05	±0.09
	Quercus	0.33	0.05	4.91	1.45	2.41
	(120 years)	±0.02	±0.00	±0.37	±0.08	±0.22
Nagyatád	Robinia	0.24	0.35	1.89	1.67	1.15
	(30 years)	±0.01	±0.02	±0.13	±0.02	±0.08
	Quercus	0.20	0.29	2.06	1.90	1.25
	(96 years)	±0.02	±0.03	±0.03	±0.05	±0.02
Iván	Robinia	0.22	0.10	8.08	2.12	4.05
	(28 years)	±0.01	±0.01	±0.31	±0.13	±0.17
	Quercus	0.14	0.02	5.29	1.32	2.61
	(88 years)	±0.02	±0.02	±0.46	±0.12	±0.21

*Values denoted with * are significantly different within pairs (p<0.05)*

pronounced in the main rooting zone of the mineral soil (0-30cm) with Iván (d) pair forming an exception where significantly higher base saturation under *Robinia* than under the *Quercus* stand was observed, despite a significantly higher N accumulation under the black locust stand.

The comparison of chemical properties under older (pairs a & b: approximately 60 years old) and younger (pair d: approximately 30 years old) stands indicated a significant influence of the period of black locust cultivation on acidification and base saturation in the mineral soil. At the forest sites of Pusztavács and Baktalórántháza (pairs a & b) the long-term cultivation resulted in lower pH values and base saturation in the whole mineral soil, whereas it occurred only in the topsoil (0-30cm) at Nagyatád (c).

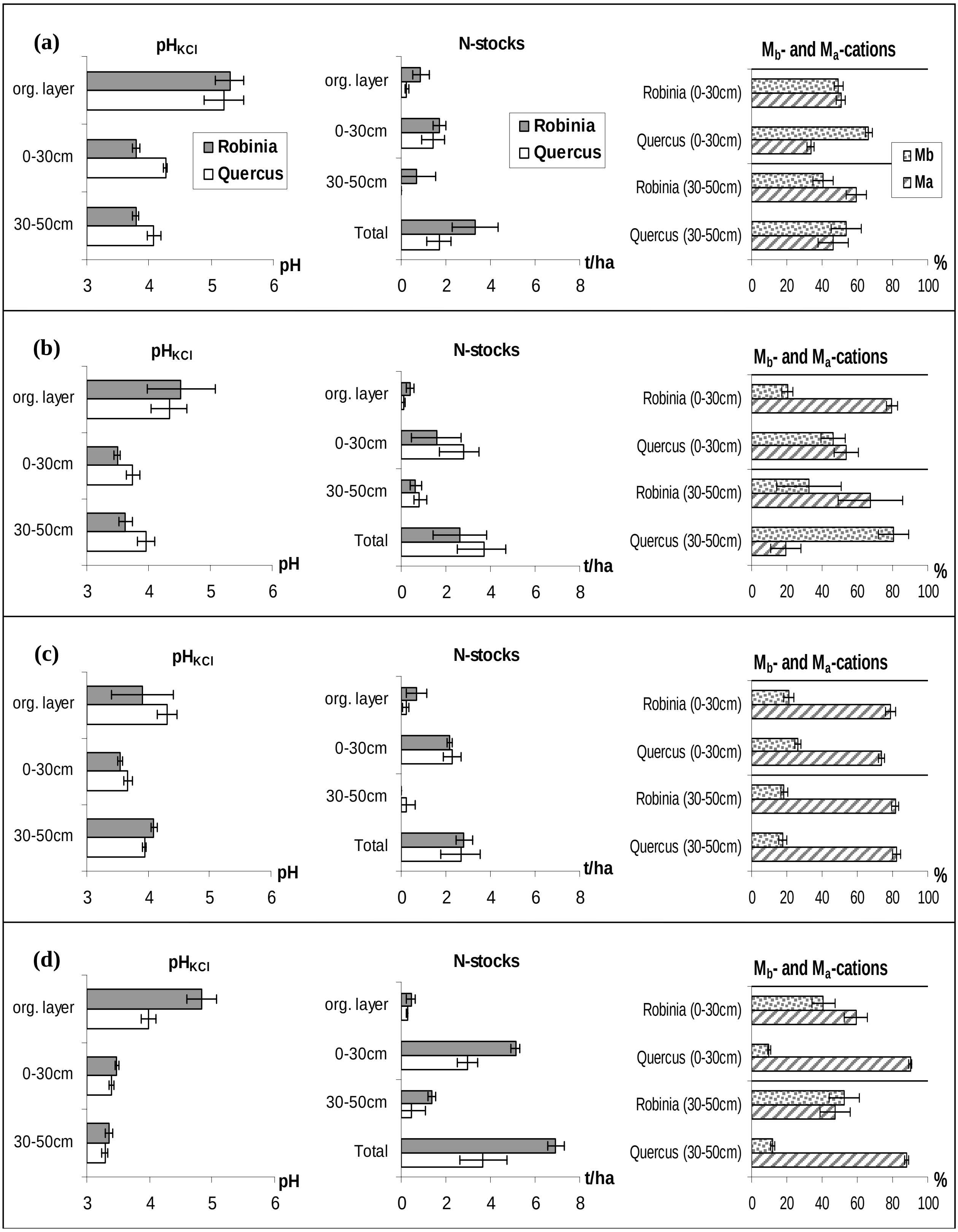

Fig. 2: *Mean (± quartiles) pH$_{KCl}$ values, N-stocks and exchangeable base (M_b) and acid cations (M_a) for paired stands of Hungarian forest sites: Pusztavács (a), Baktalórántháza (b), Nagyatád (c) and Iván (d); four replicates for each horizon.*

Laboratory experiment to measure soil solution composition

Initial soil treatment caused intensive mineralization (*Figure 3)* leading to high leaching losses of all nutrients, especially in the first sampling interval (June/July 2002). No significant differences in the leaching of nitrate between inoculated and untreated seedlings in the three pH treatments were observed. Significantly higher leaching losses of NO_3^- under the unplanted reference pots than the planted ones occurred when the soil was unlimed. In the limed reference pots nitrate leaching was high at the beginning (June/July 2002). Leaching losses of base cations from the unplanted pots was high for unlimed treatment and for the one receiving high amounts of lime (pH 6.2). Significantly high losses of M_b cations from pots with inoculated plants in the unlimed soil were determined from September 2002 to July 2003. For the two other soil variants no significant difference between the two treatments was found. Due to high application of $CaCO_3$ leaching of base cations from the high Ca variant soil, of reference and planted pots was enhanced compared to those from other pH variants. As expected, leachate of M_b cations under the strongly limed variant was dominated by Ca (65-85%).

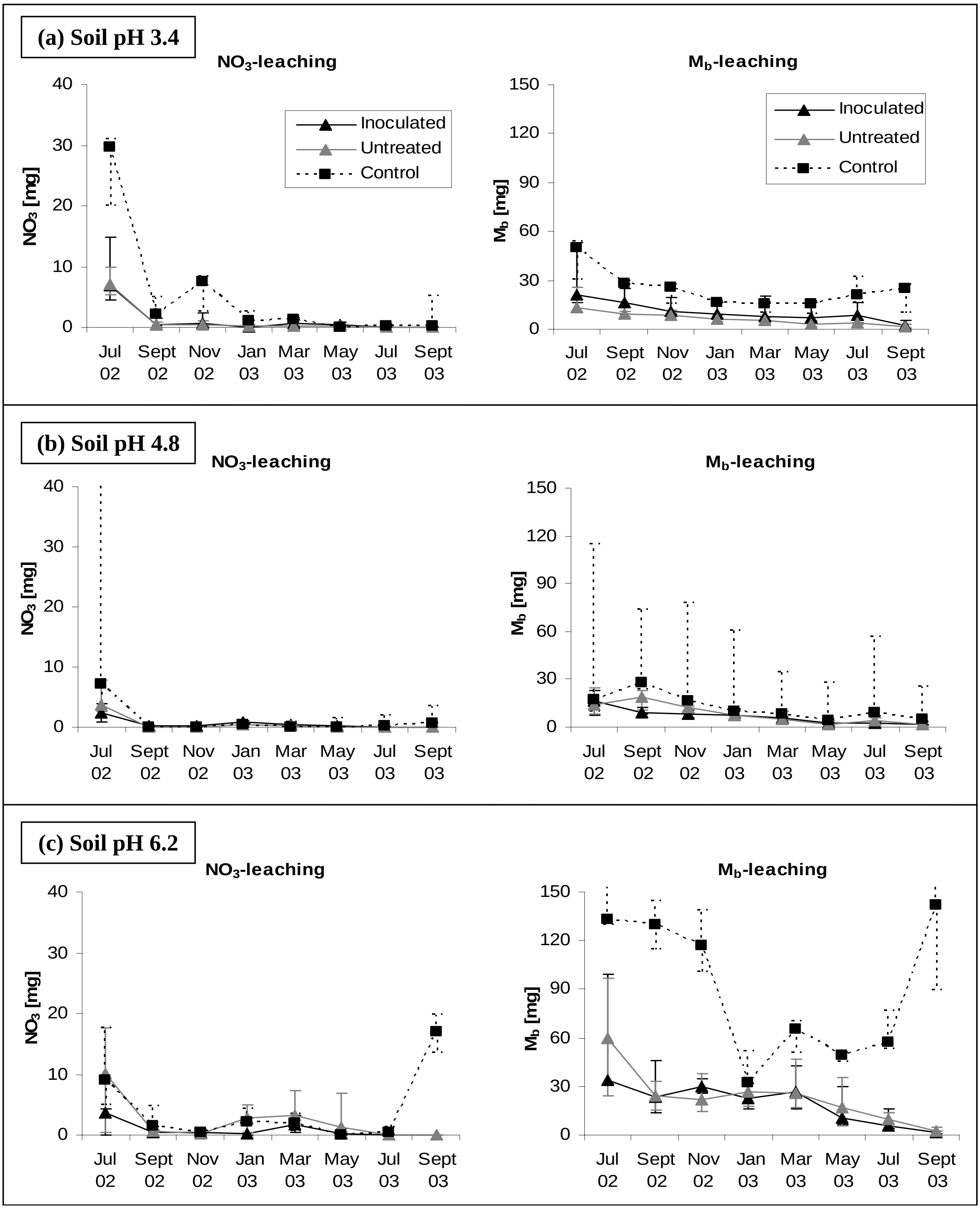

Fig. 3: Mean (± quartiles) leaching losses [mg] of NO_3^- and base cations (M_b) under soils with different starting pH values and inoculation treatments (Inoculated; Untreated) in pots planted with Robinia pseudoacacia for the observation period from June 2002 to September 2003 (planted variants n=15, unplanted controls n=5).

Liming affected strongly plant development, whereas effects due to inoculation were less pronounced (*Table 3*). The comparison between unlimed and limed soil revealed significantly higher plant growth, biomass formation and nodule development (except for the nodule biomass in variant c and the untreated soil of variant b) for seedlings cultivated in soils with high pH. The moderate application of $CaCO_3$ (variant (a) soil pH 4.8) promoted plant development most strongly. Within the different soil pH variants inoculation with *Rhizobium* resulted in enhanced height growth, biomass increment, nodule number and nodule biomass in the moderately limed variant (b) only. For the two other pH variants, significantly elevated biomass and higher plant growth were detected for seedlings from non-inoculated soils.

Tab. 3: *Mean (± standard error) absolute increment of plant height, diameter and biomass of Robinia pseudoacacia seedlings in soils with different starting pH (a, b, c) and treatments (Inoc. = inoculated; Ut. = untreated) from May 2002 to October 2003 and mean number of nodules and dry weight per plant in October 2003 (n=15).*

Soil pH / Treatment	Absolute plant growth and biomass increment May 2002 to October 2003			Nodules October 2003	
	height	diameter	biomass/plant	nodules/plant	
	cm	mm	g	number	dry weight (g)
(a) 3.4 / Inoc.	$50,7^{a}$	$6,1^{a}$	$18,8^{a}$	160^{a}	$0,6^{a}$
	±3,8	±0,5	±3,5	±21	±0,1
(a) 3.4 / Ut.	$58,3^{a}$	$7,0^{a}$	26,8*^{a}	169^{a}	$0,7^{a}$
	±4,4	±0,4	±3,4	±18	±0,1
(b) 4.8 / Inoc.	$82,7^{b}$	$8,3^{b}$	$43,1^{b}$	914^{b}	1,0*^{b}
	±3,5	±0,3	±1,6	±84	±0,1
(b) 4.8 / Ut.	$74,5^{b}$	$8,3^{b}$	$35,8^{a}$	779^{b}	$0,7^{a}$
	±4,9	±0,5	±5,4	±214	±0,1
(c) 6.2 / Inoc.	$73,2^{b}$	$7,9^{b}$	$34,1^{b}$	413^{c}	$0,6^{a}$
	±5,2	±0,5	±3,7	±94	±0,1
(c) 6.2 / Ut.	$87,1^{b}$	$8,8^{b}$	45,2*^{b}	408^{c}	$0,7^{a}$
	±4,7	±0,2	±2,8	±78	±0,1

*Values denoted with * are significantly different between different treatments within the same soil pH, values with different superscript letters (^{a,b,c}) are significant different between soils of equal treatment ($p<0.05$)*

Rhizotron studies - pH changes in the rhizosphere

The pH values measured along the surface of black locust roots were 0.7 to 0.8 units lower than in the bulk soil i.e., 2mm in front of the root tip and in soil at the root surface (*Figure 4*). For the unlimed variant (a) pH along the root was 0.7 units lower than in bulk soil (pH 3.6 to pH 4.3), for the moderately limed variant (b) 0.8 units (4.2 to 5.0) and for the strongly (c) limed soil 0.7 (5.0 to 5.7) units lower than in bulk soil. In terms of H_3O^+ concentration the differences were big. Calculated concentrations of H^+ ions in the bulk soil and rhizosphere for the different soil variants were (bulk soil/rhizosphere): **(a)** 44/240 µmol/L; **(b)** 11/71 µmol/L; **(c)** 2/9 µmol/L. Most differences were observed in front of the root tip and around the root surface, but lower pH values were also measured around nodules than in the bulk soil pH (data not presented).

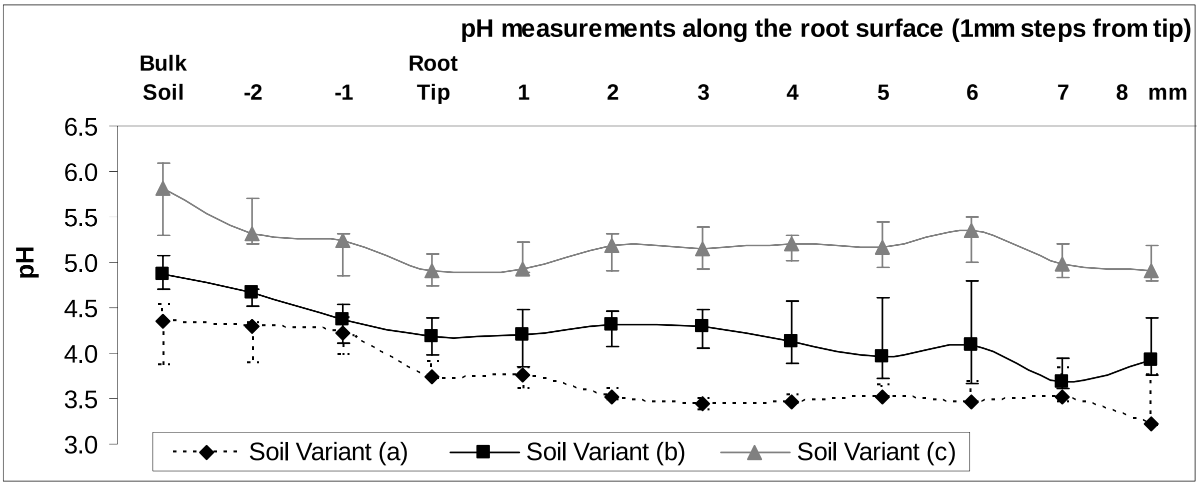

***Fig. 4**: Measured pH values in bulk soil (3mm in front of the root tip) and in the rhizosphere (from 2mm in front to 8mm behind the root tip) in soils with different starting pH values (a, b, c). Data presented as medians with quartiles (n=6).*

Base saturation decreased whereas the concentrations of protons and acid cations in the bulk soil increased after six months of black locust growth *(Table 4)*. However, in the unlimed and moderately limed treatments significantly elevated concentrations of protons were measured, but not in the strongly limed treatment due to high proton buffering.

Tab. 4: *Mean (± standard error) concentrations of the main exchangeable cations in soils of different starting pH values (a, b, c) in rhizotrons planted with Robinia pseudoacacia at the beginning (Start) and end (End) of the experiment (n=10).*

pH variant / Time	K	Ca	Mg	H	Fe	Al
		µmolc/g			µmolc/g	
a/Start	0.8** ±0.1	16.0 ±1.3	6.9** ±0.4	9.7 ±0.2	4.4** ±0.3	12.3** ±0.5
a/End	0.4 ±0.0	15.8 ±0.4	4.7 0.2	13.3** ±0.5	3.2 ±0.1	8.6 ±0.2
b/Start	1.0** ±0.0	75.5** ±1.9	7.7** ±0.6	0.0 ±0.0	0.5 ±0.0	0.0 ±0.0
b/End	0.5 ±0.0	39.3 ±1.3	4.6 ±0.1	4.9** ±0.5	2.1** ±0.1	4.9** ±0.3
c/Start	0.9** ±0.0	154.9** ±2.0	7.6** ±0.0	0.0 ±0.0	0.3 ±0.0	0.0 ±0.0
c/End	0.5 ±0.0	83.5 ±2.0	4.8 ±0.3	0.0 ±0.0	0.5** ±0.0	0.2 ±0.2

*Values denoted with ** ($p<0.01$) are significantly different between the date of measurement (Start/End) within the same soil variant.*

Discussion

High amount of organic matter under black locust stands in Hungary may be due to high litter production and high symbiotic N_2 fixation (Boring & Swank 1984b; White 1986), indicating high productivity of these stands. Part of the fixed N may be lost by leaching of NO_3^- especially after clear cutting for the regeneration of stands. However decomposability of black locust litter may be relatively low, despite low C/N ratios, which is attributed to high lignin content of black locust leaves (Bartuska & Lang 1981; Hirschfeld et al., 1984). White et al. (1988) detected that *Robinia pseudoacacia* leaflets contained 81% of their original N after 863 days of decomposition. They concluded that this retention of N explains the long-term effect of black locust on N storage in the forest floor and soil. According to Berg & McClaugherty (2003) it is not the lignin content per se that is rate retarding, but the lignin in combination with N concentrations above a certain level.

Although the differences were only significant for the organic layer of all paired sites and for two paired sites for the mineral soil, cultivation of black locust is expected to lead to N enrichment in the soil causing nitrification and NO_3^- leaching, which in turn results in acidification and leaching of base cations. Significantly lower pH values in the mineral soil, significantly lower concentrations of exchangeable bases and higher concentrations of acid cations (especially in the topsoil) under black locust stands than under pure oak stands, partly indicated soil degradation by *Robinia pseudoacacia*. Soil acidification was higher under long-term cultivated *Robinia pseudoacacia.* Soil acidification was measured in the surface and sub-surface soils. Similar results were detected for stands of *Robinia pseudoacacia in* eastern Germany (Goldacker et al. 2002).

Leaching losses of nitrate and depletion of mineral nutrients from soils under *Robinia pseudoacacia* could not be verified in the short term pot experiment. This was probably due to the soil changes associated with their preparation for the experiment (drying, sieving and re-wetting) causing mineralization of soil organic components and thereby superimposing any expected effects of black locust cultivation on the chemical composition of soil solution. Plant uptake of nitrate and base cations reduced leaching losses compared to the unplanted references.

Liming of acid soil with moderate amounts of $CaCO_3$ promoted the development of and infection with *Rhizobium* bacteria. According to Marschner (1995) N_2 fixation of legumes can be affected indirectly or directly by mineral nutrients. Root infection and nodule initiation have a much higher calcium requirement than the root and shoot growth of the host plant. Franco and Munns (1982 a, b) observed a decrease of soybean nodulation and a simultaneous decrease in root hair length by lowering the pH from 5.5 to 5.0. For white clover changes in nodulation were more closely associated with changes in soil pH than soil Ca (Brauer 1998; Brauer et al. 2002).

The rhizotron experiment confirmed the acidification of the rhizosphere by legume roots, which was largely attributed to the release of protons following the uptake of cations in excess to those of anions. Although decrease in rhizosphere pH was small and dependent on the initial pH values, the calculated increase of H^+ concentrations indicated possible effects on soil fertility, especially in the unlimed soil. The acidification around nodules was lower compared to the root surface and probably is due to acidic excretions from roots and not directly from nodules. Lower acidification around living nodules can be attributed to progressive proton buffering due to a larger distance from the root surface (nodule diameter between 1 to 3mm).

Conclusion

The comparison of paired *Robinia* and *Quercus* stands for soil chemical characteristics and the rhizotron experiment confirmed soil acidification through black locust cultivation.

Especially on poor less buffered sites, which are most commonly used for black locust cultivation in forestry, the long-term growth of this neophyte can decrease of soil fertility restricting the establishment of native tree species. Moreover, *Robinia* is known to be very competitive and invasive on dry and poor sites and can easily spread to adjacent biotopes, causing a decline in native drought adapted plant species.

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8.3 Robinie – Fluch oder Segen für den Waldboden?

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Einleitung

Der Anbau der Robinie (Robinia pseudoacacia L.) wurde weltweit in den vergangenen Jahrzehnten sprunghaft erweitert. Neben Eukalyptus- und Pappelarten ist die Robinie heute mit einer Anbaufläche von ca. 3,25 Millionen ha die weltweit am dritthäufigsten kultivierte Laubbaumart (Claasen, 2001). 1 Million ha der Gesamtfläche befinden sich in China und 0,5 Millionen ha in Südkorea, wo die Robinie meist auf für die Landwirtschaft ungeeigneten Böden angepflanzt wird (Dengg, 1994). In Europa hat Ungarn mit einer Fläche von 350.000 ha und einem Vorrat von 38 Mio. m^3 das bedeutendste Robinien-Vorkommen (Bidlo, 2001). Für Deutschland schätzt man die Robinienfläche auf 14.000 ha bei einem Vorrat von knapp 2 Millionen m^3 (Grüning, 1995; Seeling, 1997). Ursachen für die zunehmende Bevorzugung der Robinie liegen in ihrer Anspruchslosigkeit und ihrer Trockenresistenz sowie ihrer ausgezeichneten Holzeigenschaften. Dies prädestiniert sie zum einen zum Anbau auf Problemstandorten und degradierten Böden, zum anderen wird Robinienholz zunehmend zu einem Tropenholzersatz. Als Leguminose zeigt die Robinie auch auf ärmeren Standorten, besonders humusarmen Standorten noch vergleichsweise gute Wuchsleistungen. Durch die N_2-Fixierung (50-150kg N ha^{-1} a^{-1}) und die leicht abbaubare Streu vermag sie die Nährstoffverhältnisse der Standorte zu verbessern.

Das Holz der Robinie weist vorzügliche physikalische und mechanisch-technologische Eigenschaften auf und kann vielseitig verwendet werden (Hapla, 1998). Vor allem die natürliche Dauerhaftigkeit und Abbauresistenz ihres Kernholzes macht sie zu einem universell im Außenbereich einsetzbaren Werkstoff, der keiner chemischen Imprägnierung bedarf.

Hypothesen

Trotz der allgemein positiven Eigenschaften, die der Robinie zugesprochen werden, ist der Robinienanbau aus stofflicher Sicht nicht ohne Risiko:

1. Die Versorgung der Pflanze mit fixiertem atmosphärischen Stickstoff führt zu einer höheren Kationen- als Anionenaufnahme, die mit einem Export von H^+-Ionen in die Rhizosphäre und dadurch mit einer Versauerung des Bodens verbunden ist.
2. Die N_2-Fixierung der Robinie ist nicht so fein gesteuert und rückgekoppelt, dass nur der Bedarf der Bäume gedeckt wird. Daraus resultiert, in Abhängigkeit von der Fixierungseffektivität, eine erhöhte Akkumulation von Stickstoff im Boden und die Auswaschung von Nitrat und Begleitkationen aus dem Boden.

Material und Methoden

Zur Untersuchung des Stoffaustrages unter Robinien wurden drei ungarische Herkünfte in 60 cm tiefe PVC-Säulen gepflanzt und über zwei Vegetationsperioden beobachtet. Als Substrat dient ein saurer und nährstoffarmer Oberboden (Aluminiumpuffer), der im niedersächsischen Hils gewonnen wurde. Um den Einfluss der Robinie auf die Lösungschemie bei unterschiedlicher Pufferkapazität des Bodens zu analysieren, wurde das Substrat auf drei Pufferbereiche – Aluminium-, Silikat- und Carbonatpufferbereich – eingestellt. Die hierfür benötigten Kalkmengen ergaben sich aus der Basenneutralisationskapazität des Hilsbodens (vgl. Meiwes et al., 1984).

Da das Wachstum der Pflanzen und die N-Anreicherung im Boden eng an die N-Bindungseffektivität der Symbiose Robinie-Rhizobium gekoppelt ist, wurde jeweils eine Variante pro Herkunft und Pufferbereich mit Rhizobien geimpft, welche eine erhöhte Bindung atmosphärischen Stickstoffs garantieren soll.

Als Referenz zu den bepflanzten Säulen dient für jede Puffervariante je eine unbepflanzte Kontrolle. Um die Beobachtungen statistisch absichern zu können ist jede Variante mit jeweils 5 Wiederholungen vertreten, so dass an insgesamt 105 Bodensäulen Lösungsproben für die chemischen Analysen gewonnen werden.

An den Perkolat-Proben werden monatlich der pH-Wert, DOC, DON, Kationen (Na^+, K^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Al^{3+}, NH_4^+, H^+) und Anionen (Cl^-, NO_3^-, SO_4^{2-}, PO_4^{3-}) gemessen.

Neben diesen chemischen Erhebungen werden jeweils zu Anfang und zu Ende der Vegetationsperioden die Pflanzenhöhe und der Wurzelhalsdurchmesser aufgenommen. Hierdurch kann der Einfluss der verschiedenen Boden-Pufferbereiche und der Inokulation des Bodens mit einem definierten Rhizobiengemisch auf das Wachstum der Pflanzen dokumentiert werden.

Ergebnisse

- pH-Wert der Bodenlösung

Abb. 1 zeigt die Entwicklung der pH-Werte in der Bodenlösung für die verschiedenen Pufferbereiche gegliedert nach bepflanzter Variante (P) und Kontrolle (K). Man erkennt in allen Varianten zunächst einen kontinuierlichen Anstieg der pH-Werte bis November 2002. Dieser Prozess ist zum einen durch die bis dahin anhaltende Kalkauflösung (Silikat- und Carbonatpufferbereich), zum anderen aber auch durch einen behandlungsbedingten Mineralisationsschub des verwendeten Materials (alle Puffervarianten) zu erklären.

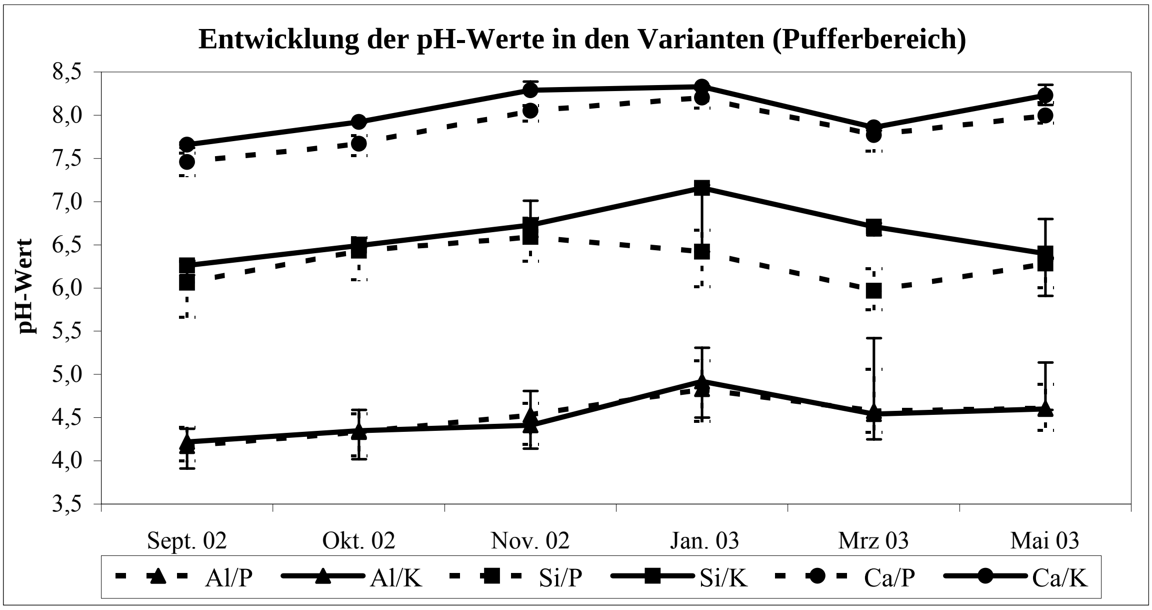
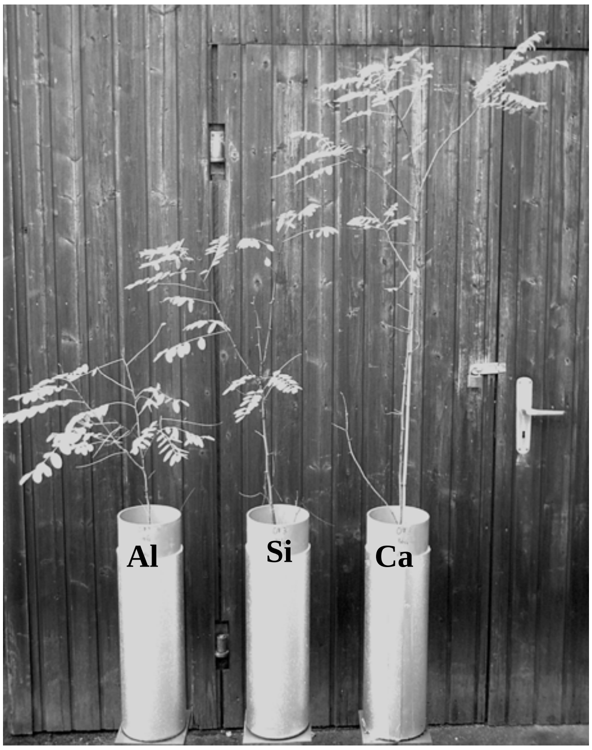


***Abb.1**: Entwicklung der pH-Werte nach Puffervariante*

Bei der ungekalkten Aluminiumpuffervariante zeigt sich über den gesamten Beobachtungszeitraum kein Unterschied zwischen bepflanzter und unbepflanzter Variante. Hingegen zeichnet sich bei der Silikatpuffervariante ein deutlicher Einfluss des Robinienbewuchses auf den pH-Wert des Perkolats ab. Hier findet man um bis zu 0,7 pH-Einheiten tiefere Werte unter den mit Robinie bepflanzten Säulen. Auch in der Carbonatpuffervariante wird in der Lösung des mit Robinie bestockten Bodens ein geringerer pH-Wert als in den Kontrollen gemessen. Vergleicht man für die Puffervarianten das Wachstum der Robinien (siehe auch Foto 1) mit der Entwicklung der pH-Werte, so ist eine

Versauerung offensichtlich an einen starken Zuwachs bei gleichzeitig moderater Pufferkapazität gekoppelt. Dies scheint v.a. für die Silikatpuffervariante zuzutreffen.

Foto 1*: Unterschiedlicher Höhenzuwachs in der Al-, Si- und Ca-Puffervariante*



Die Impfung des Bodens mit einem Rhizobiengemisch sollte zu einem effektiveren Einbau atmosphärischen Stickstoffs in die Pflanze und letztendlich zu einer stärkeren Versauerung führen. In Abbildung 2 ist die zeitliche Entwicklung der pH-Werte in der Bodenlösung für die geimpften (g) und ungeimpften (ug) Varianten dargestellt. Für alle drei Pufferbereiche ist in der Bodenlösung unter den beimpften Robinien im Vergleich zu den ungeimpften kein signifikant tieferer pH-Wert feststellbar. Ab Januar 2003 zeigt sich dagegen - v.a. in der Al-Puffervariante - unter den ungeimpften Pflanzen ein tendenziell tieferer pH-Wert des Perkolats. Dies scheint mit dem in der ersten Vegetationsperiode beobachteten Wachstumsverlauf in Einklang zu stehen (vgl. auch „Wuchsdaten“ ; Foto 2).

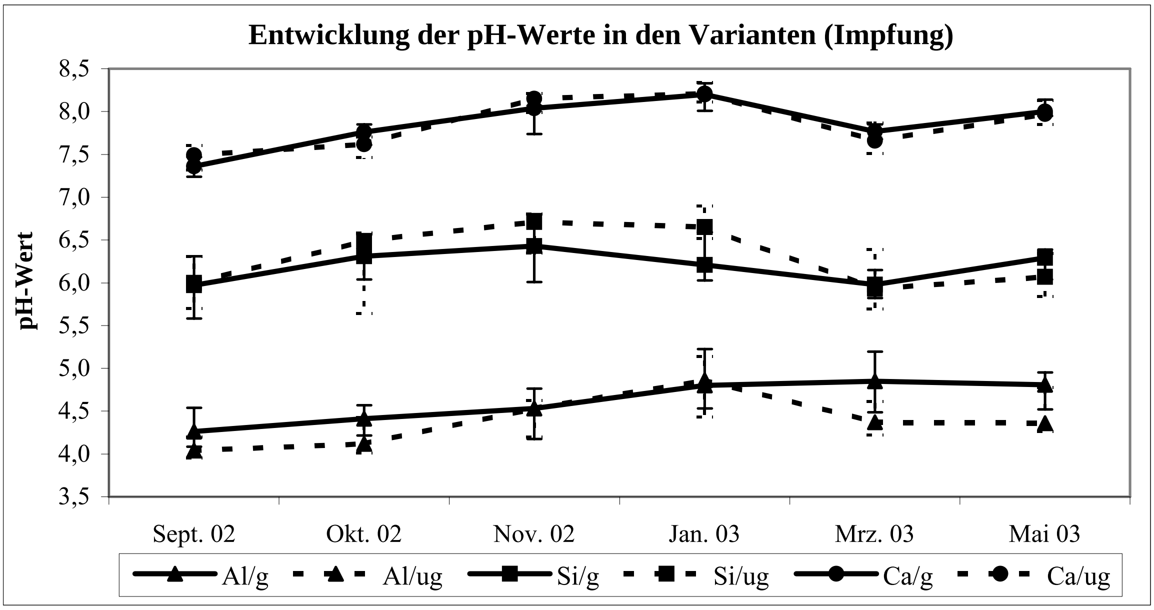


Abb. 2: Entwicklung der pH-Werte im Perkolat nach Impfung und Nichtimpfung

- Austrag von Nitrat und basischen Kationen (M_b)

Aus Abbildung 3 geht hervor, dass in den drei untersuchten Puffervarianten bisher noch kein erhöhter Nitrataustrag durch den Anbau der Robinie zu beobachten ist.

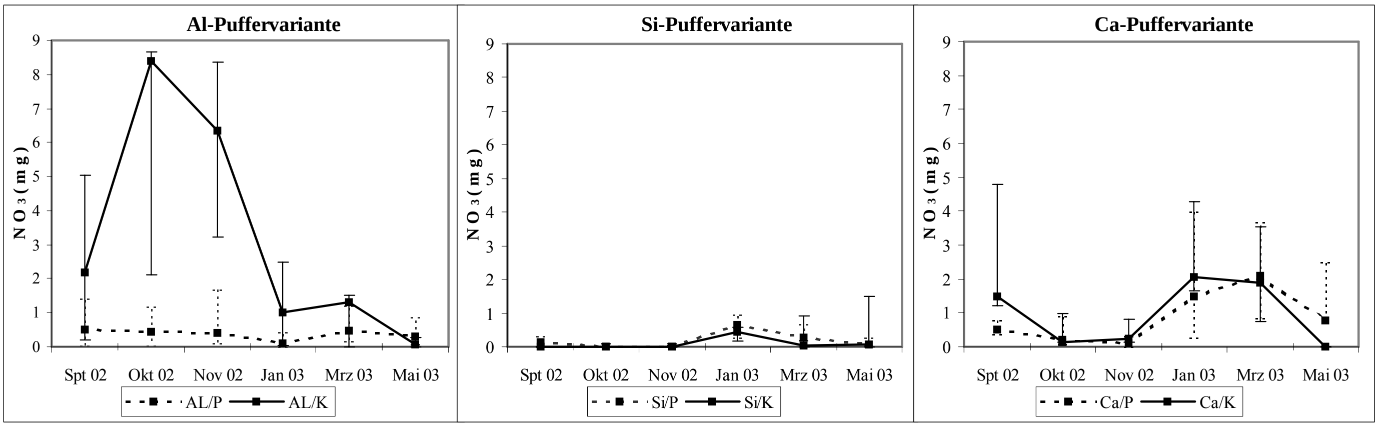


Abb. 3: Nitrat-Austrag in den Puffervarianten

In der ungekalkten Al-Puffervariante ist in den ersten Versuchsmonaten im Perkolat der Kontrollen sogar ein deutlich stärkerer Austrag als in den bepflanzten Säulen dieser Variante festzustellen.

Vergleicht man die geimpften und die ungeimpften Varianten (ohne Abbildung) so zeigt sich, dass auch Behandlung bzw. unterschiedliches Wachstum bisher keinen Effekt auf den Nitrat-Austrag ausübt.

Auch in Bezug auf die basischen Kationen (vgl. Abb. 4) führt der Anbau von Robinien im Vergleich zu unbepflanzten Referenzsäulen bisher zu keinem erhöhten Austrag.

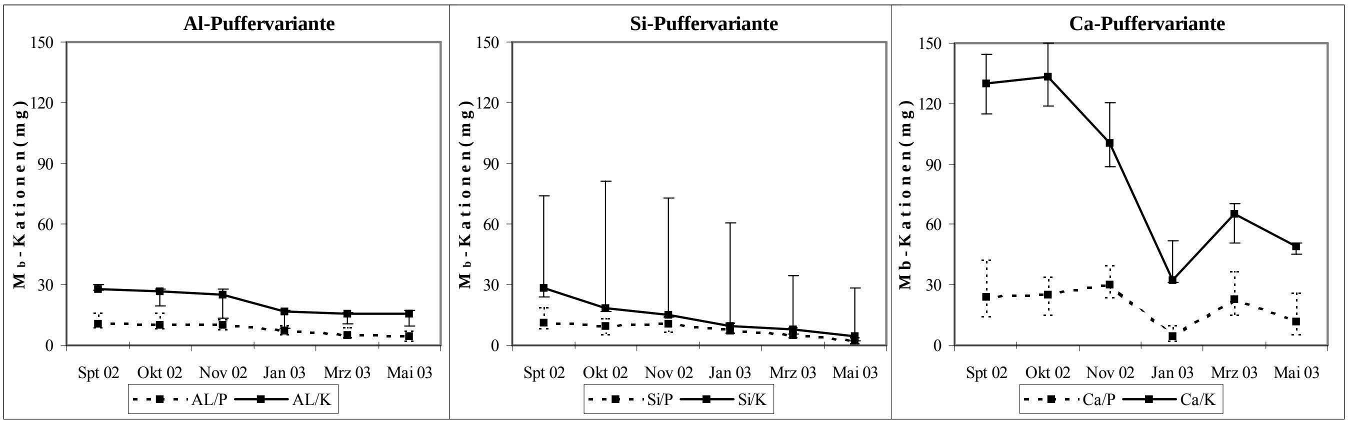


Abb.4 : Austrag basischer Kationen (M_b) in den Puffervarianten

Die z.T. signifikant höheren Austräge aus den Kontrollen können durch die Stickstoff- bzw. Kationenaufnahme in den bepflanzten Säulen erklärt werden.

- Wuchsdaten

In Abbildung 5 ist der absolute Höhenzuwachs in Abhängigkeit der Pflanzenhöhe zu Versuchsbeginn für die verschiedenen Puffervarianten und Behandlungen

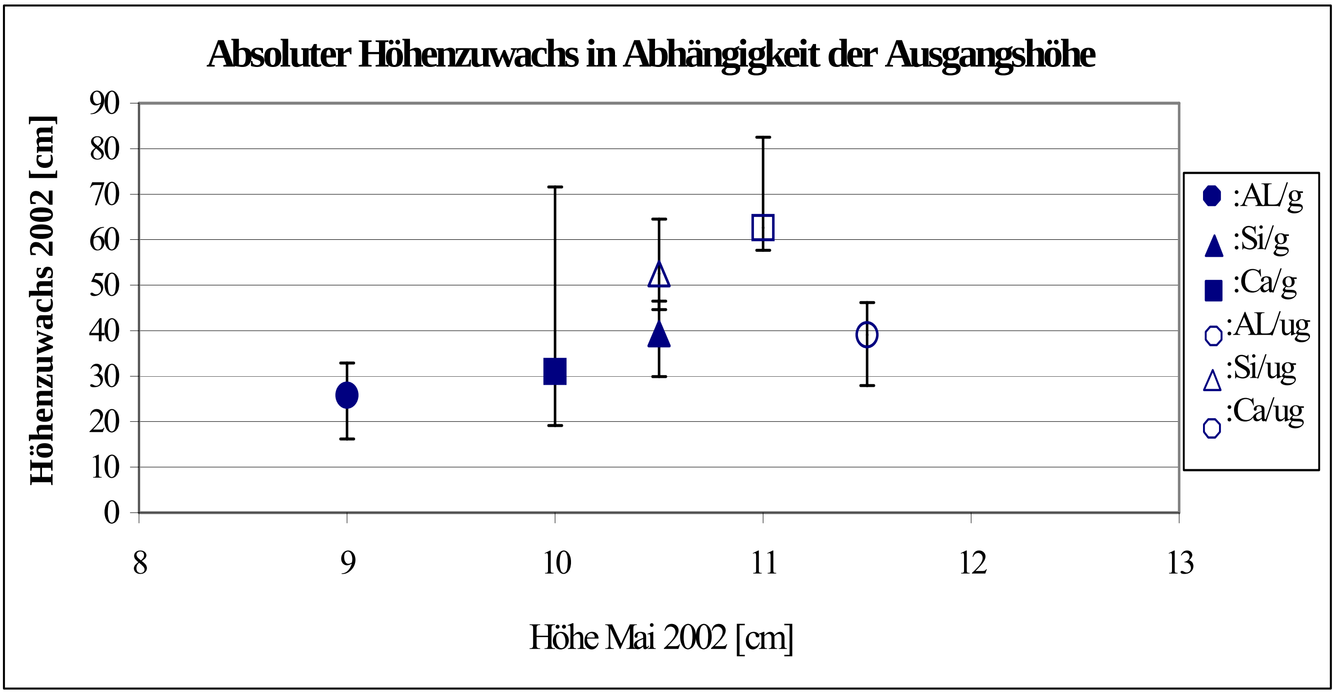


Abb. 5: Absoluter Höhenzuwachs in Bezug auf die Ausgangshöhe in den Varianten

(geimpft/ungeimpft) dargestellt. Das ausgepflanzte Pflanzenmaterial unterschied sich zu Beginn der ersten Vegetationsperiode in der Wuchshöhe nicht signifikant voneinander. Aus der Abbildung geht hervor, dass auch die Robinie auf verbesserte chemische Bodenbedingungen durch Kalkung mit einem erhöhten Wachstum reagiert (s. a. Foto 1).

Foto 2: Höhenwachstum geimpfter und ungeimpfter Robinie im gleichen Pufferbereich

Besonders auffällig an dieser Abbildung ist, dass in der ersten Vegetationsperiode in jeder der drei Puffervarianten die ungeimpften Pflanzen ein deutlich stärkeres Wachstum als die geimpften aufwiesen (vgl. auch Foto 2). Diese Differenz ist zwar nicht in jedem Fall signifikant, doch lassen sich aus diesem Trend Erklärungsansätze ableiten. Laut Werner (1987) liegen die Energiekosten für die N-Fixierung bei ca. 6g C/g N, d.h. bei einer Fixierung von 150 kg N/ha werden ca. 900 kg C benötigt. Bevor die Pflanze also von der Symbiose profitiert muss sie Energie investieren, welche dann für das Wachstum fehlt. Ein Indiz auf die erfolgreiche Inokulation geben auch die folgenden Blattspiegelwerte.

- Blattspiegelwerte

Eine effektivere N_2-Fixierung zeigt sich in höheren N-Konzentrationen in der gesamten Pflanze. Um erste Hinweise auf den Erfolg der Impfung zu erlangen, wurden im Spätsommer der ersten Vegetationsperiode Blattproben genommen.

Abbildung 6(a) deutet daraufhin, dass die Impfung mit einem Rhizobiengemisch zur Ausbildung eines effektiveren Fixierungsapparats geführt hat. In fast allen Puffervarianten finden sich signifikant höhere N-Gehalte in den Blättern der geimpften Pflanzen.

Ein weiteres Indiz hierfür liefern die Fe-Gehalte in den Blättern (Abb. 6b). Eisen ist als Bestandteil des Leghämoglobins und des Nitrogenasekomplexes ein wichtiges Element für den Aufbau der Symbiose Bakterium-Pflanze (Marschner, 1995). Die für alle Varianten signifikant geringeren Fe-Konzentrationen in den Blättern der geimpften Robinien deuten auf eine vermehrte Einbindung dieses Elements in die Knöllchen hin.

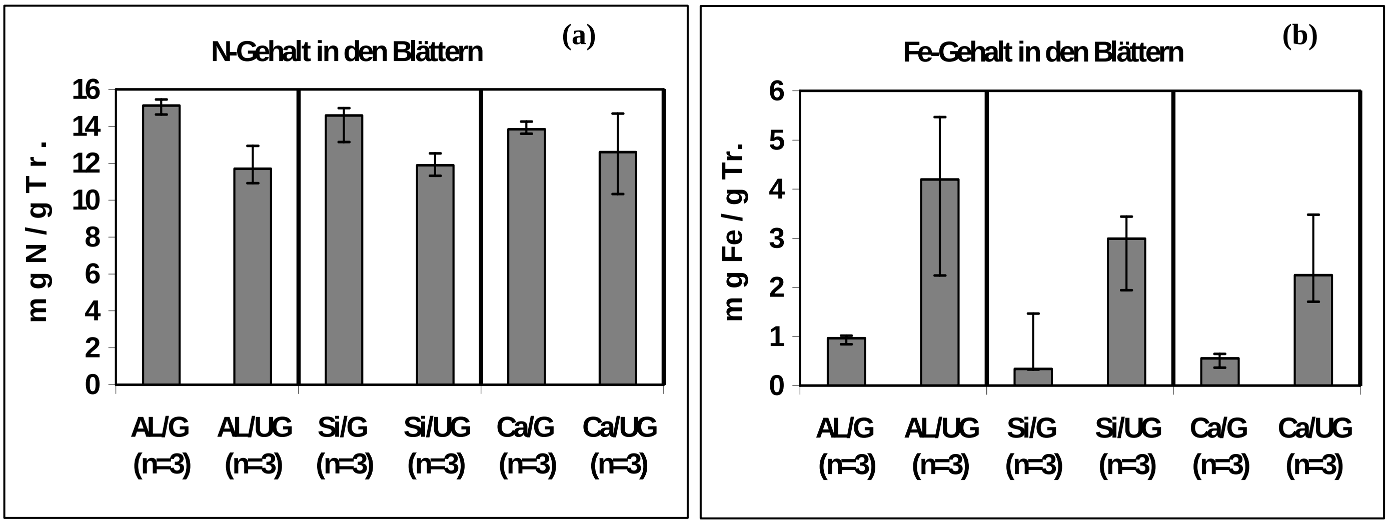


Abb. 6: *N- und Fe Gehalte der Blätter in der ersten Vegetationsperiode*

Zusammenfassung und Schlussfolgerungen

- Der anfängliche Anstieg des pH-Wertes in der Bodenlösung steht mit einem behandlungsbedingten Mineralisationsschub und der Kalkauflösung in Zusammenhang. Eine pH-Absenkung durch den Anbau der Robinie scheint v.a. an ein starkes Wachstum infolge erhöhter N_2-Fixierung bei gleichzeitig moderater Boden-Pufferkapazität gekoppelt zu sein.

- Bisher ist keine Erhöhung des Austrages von Nitrat und basischen Kationen durch die N-Fixierung der Robinien nachweisbar.

- Im Vergleich zu den ungeimpften Robinien zeigten die geimpften in der ersten Vegetationsperiode ein deutlich stärkeres Wachstum. Dies könnte mit den „Kosten“ für die Ausbildung eines effektiveren "Fixierungsapparats" in Zusammenhang stehen, da 6g C/g N verbraucht werden, was gerade bei jungen Pflanzen zu anfänglich verringertem Wachstum führen kann. Auch verlangt das Knöllchenwachstum einen hohen Versorgungsgrad mit anderen Nährstoffen, die dann ebenfalls für die Pflanzenentwicklung stärker limitiert werden.

Fazit

Ob die Robinie ein „Fluch“ oder „Segen“ für den Waldboden ist, lässt sich erst nach Beendigung des Projektes beantworten. Nur die komplette Analyse der Pflanzen, des Bodens und der bis dahin gewonnenen Perkolatproben und die Verknüpfung dieses Ergebnisse können zu einer abschließenden Beurteilung des Robinienanbaus aus stofflicher Sicht führen.

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8.4 Bodenversauerung unter Robinie – Potenzielle Ursache von Wachstumsminderungen?

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Einleitung

Das standörtliche Spektrum der Robinie (*Robinia pseudoacacia* L.) ist sehr weit. In den letzten Jahrzehnten wurde sie daher weltweit verstärkt angebaut. Sie ist heute neben Eukalyptus und Pappel eine der am häufigsten in Plantagen kultivierten Baumarten [8], [19]. Die aus Nordamerika stammende Leguminose kann aufgrund ihrer positiven Eigenschaften bei der Aufforstung landwirtschaftlicher Flächen, zur Wiederbegrünung von Problemstandorten [17] oder als Unterbau von Kiefermonokulturen eingesetzt werden. Außerdem wird die Robinie als „Teakholz Europas“ zunehmend zu einem Tropenholzersatz. Als schnell nachwachsender Rohstoff dient sie auch zur Energiegewinnung. Früher wurde die Robinie in Deutschland wie die Erle aufgrund ihrer Stickstofffixierung als Meliorationsbaumart eingesetzt [18].

Zielsetzung

Bei längerfristigem Anbau der Robinie ist eine Verminderung ihrer Produktivität festzustellen [6], [7], [21]. Zum einen kann eine Vermehrung von Krankheits- und Schadenserregern zu Wuchsminderungen führen. Dies ist für landwirtschaftliche Leguminosen nachgewiesen [22] und wurde auch in Ungarn auf sterilisierten Robinienversuchsflächen im Vergleich zu nicht behandelten Böden beobachtet [13]. Zum anderen erfolgt über das weitstreichende Wurzelsystem und den hohen Nährstoffbedarf eine Nährstofferschöpfung armer Böden [9]. Eine erhebliche Bodenverarmung wird nach zwei- bis dreimaligem Niederwaldumtrieb vorausgesagt [5], [7]. Eine weitere Ursache kann in der erhöhten Stickstofffixierung der Robinie liegen. Die N_2-Fixierung durch die mit der Robinie in Symbiose lebenden Knöllchenbakterien (*Rhizobium spec.*) ist nicht so feingesteuert, als dass sie nur den N-Bedarf der Bäume deckt. Der im Boden verbleibende Stickstoff kann entweder organisch gebunden und angereichert werden oder er wird mineralisiert und nitrifiziert. Das dabei entstehende

Nitrat unterliegt der Auswaschung. Dies führt zu einer Basenverarmung und Versauerung des Bodens [2]. Daten über stickstofffixierende Baumarten wurden bisher jedoch wenig erhoben [20]. In dieser Arbeit sollten die nachfolgenden Hypothesen geprüft werden.

1. Mit längerfristigem Robinienanbau vergrößert sich der Vorrat an organisch gebundenem Stickstoff im Mineralboden.
2. Durch die N_2-Fixierung der Rhizobien bereitgestellter aber nicht von der Robinie aufgenommener Stickstoff wird überwiegend nitrifiziert und unterliegt der Auswaschung. Damit verbunden sind Versauerung und Basenverarmung des Mineralbodens mit einer möglichen Minderung der Produktivität.

Material und Methoden

Auf sandigen, mäßig nährstoffversorgten Böden Ostbrandenburgs wurden die chemischen Bodeneigenschaften nach unterschiedlich langem Robinienanbau im Vergleich zu einem Schwarzkieferbestand betrachtet. Auf jeder Fläche wurden bis zu 50 cm Bodentiefe je Tiefenstufe 12 volumengerechte Proben (Ø 7,2 cm) genommen, und zu jeweils 4 Mischproben zusammengefasst. Tabelle 1 zeigt die wichtigsten Kenngrößen der unterschiedlichen Bestände.

Tabelle 1: Beprobte Bestände in den Revierförstereien der Oberförsterei Seelow

Gesamtanbau-zeit mit Ro/SK	**Bestandes-alter**	**Größe [ha]**	**Vorrat [m³/ha]**	**Boni-tät**	**Boden-typ**	**Humusart**	**Nähr-kraft**	**Wasser-versorgung**
Variante SK - Revier Altfriedland								
SK: >50 Jahre	17 Jahre	1,08	40-50	1,0	SB	Rohhumusart. Moder	Mittel (M)	Mittelfrisch (T2)
Variante Ro18 - Revier Altfriedland								
Ro: 18 Jahre	18 Jahre	0,64	92	1,5	SB	Rohhumusart. Moder	Mittel (M)	Mittelfrisch (T2)
Variante Ro32 - Revier Reitwein								
Ro: 32 Jahre	32 Jahre	1,92	230	1,8	BSB	Feinhumusr. Moder	Mittel (M)	Trocken (T3)

Ro	Robinie
SK	Schwarzkiefer
SB	Sandbraunerde
BSB	Bändersandbraunerde

Die Elementanalyse wurde nach dem HNO_3-Druckaufschluss durchgeführt. Die pH-Werte wurden in Wasser und in KCl gemessen, der Gesamtgehalt an Kohlenstoff und Stickstoff mittels Elementaranalyse und die effektiv austauschbaren Kationen nach Perkolation mit

NH_4Cl bestimmt. Ausführliche Beschreibungen der in dieser Arbeit angewandten Untersuchungsmethoden finden sich in der Literatur [12], [15].

Vergleichbarkeit der Standorte

Der HNO_3-Druckaufschluss gibt Hinweise über die potentiell nachlieferbaren Nährstoffe aus den Böden. Der in Lösung gegangene Anteil der Minerale entspricht etwa dem Anteil der Primär- und der Sekundärsilikate [14], [24]. Es treten nur geringe Unterschiede zwischen den untersuchten Böden auf (Tab. 2).

Tabelle 2: Gelöster Anteil und Elementkonzentrationen in 10-50 cm Bodentiefe der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median (n=4).[1]

	SK	**Ro18**	**Ro32**
Gelöster Anteil	4,1 ^{a}	3,9 ^{a}	3,3 ^{b}
K	0,9 ^{a}	0,8 ^{a}	0,9 ^{a}
Ca	0,9 ^{a}	0,8 ^{ab}	0,7 ^{b}
Mg	0,7 ^{a}	0,6 ^{a}	0,6 ^{a}
Mn	0,2 ^{a}	0,1 ^{a}	0,1 ^{a}
Fe	4,9 ^{a}	4,8 ^{a}	4,4 ^{b}
Al	5,7 ^{a}	5,3 ^{a}	5,4 ^{a}

[1] Mediane mit nicht übereinstimmenden Buchstaben unterscheiden sich signifikant ($p \leq 0{,}05$) zwischen den Beständen.

Kohlenstoff, Stickstoff und Phosphor

Die Konzentrationen an Kohlenstoff unterschieden sich nicht signifikant zwischen den Varianten. Aufgrund der unterschiedlich mächtigen Humusauflagen lag der C-Vorrat des Schwarzkieferbestandes bei 26,7 t ha^{-1}. Der C-Vorrat (Humusauflage) im jüngeren Robinienbestand (Ro18) war mit einem Wert von 18,7 t ha^{-1} doppelt so hoch wie der C-Vorrat von Ro32 mit 9,4 t ha^{-1}. Im Mineralboden wurden Kohlenstoff-Vorräte von 35 t C ha^{-1} (Ro18 und Ro32) und 46 t C ha^{-1} (SK) (Abb. 1) ermittelt. Vergleichbare Kohlenstoffmengen in Mineralböden von Robinien, die den ökologischen Bewertungsstufen gering, bis z. T. sehr gering zuzuordnen sind, werden in der Literatur beschrieben [3], [9].

Tabelle 3: Prozent-Anteile der C-, N-, P-Vorräte im Mineralboden (0-50 cm) der Robinienbestände (Ro18 und Ro32) im Vergleich zu einem Standort ohne Robinieneinfluss (SK); Median (n=4), die mit * markierten Werte unterscheiden sich signifikant ($p < 0{,}05$) von SK.

Bestand	**Tiefenstufe**	**Vorrat in % von SK**		
	cm	**C**	**N**	**P**
Ro32	0-50	77	71*	104*
Ro18	0-50	76	56*	105
SK	0-50	100	100	100

Der Stickstoffvorrat in der Humusauflage wurde durch den verstärkten Humusabbau mit zunehmendem Robinienalter ebenfalls reduziert. Unter SK wurde ein Vorrat von 1218 kg N ha^{-1}, unter Ro18 ein Vorrat von 1138 kg N ha^{-1} und unter Ro32 ein Vorrat von 665 kg N ha^{-1} ermittelt (Abb. 2). Die N-Vorräte im Mineralboden von 2066 kg N ha^{-1} in 0-50 cm Bodentiefe unter der Schwarzkiefer überschritten diejenigen unter den Robinienbeständen (Ro18: 1155 kg N ha^{-1}; Ro32: 1465 kg N ha^{-1}) ($p<0,05$, Abb. 2; Tab. 3). Die N-Vorräte der Variante SK lagen noch im Bereich für M2-Standorte mit Kieferbestockung [11].

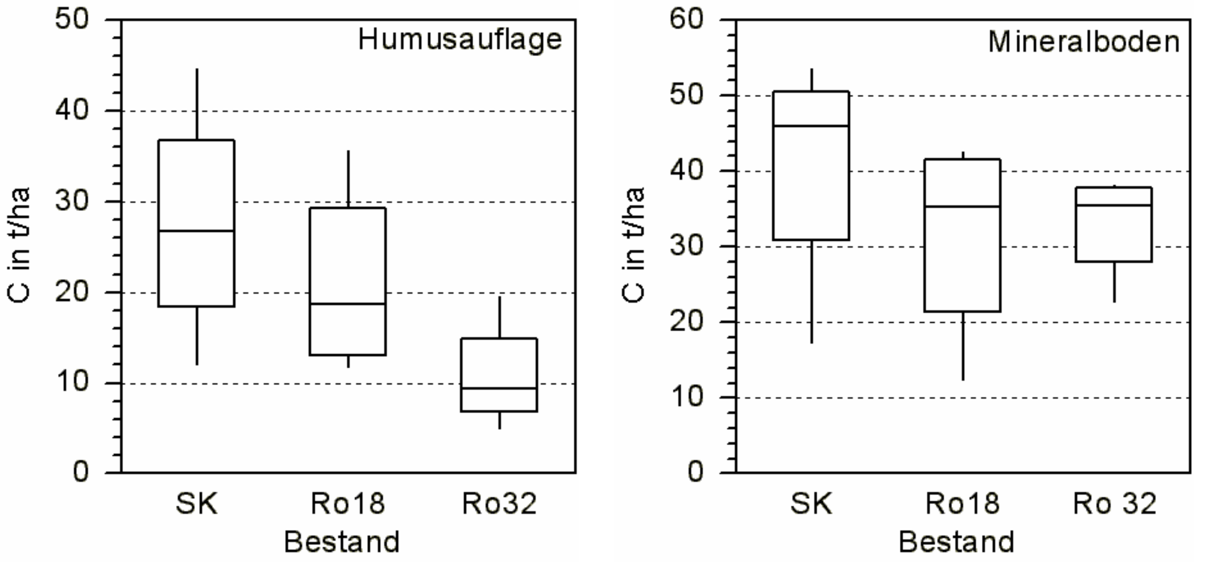

Abb. 1: C-Vorräte in der Humusauflage (links) und im Mineralboden (0-50 cm, rechts) der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median (n=4).

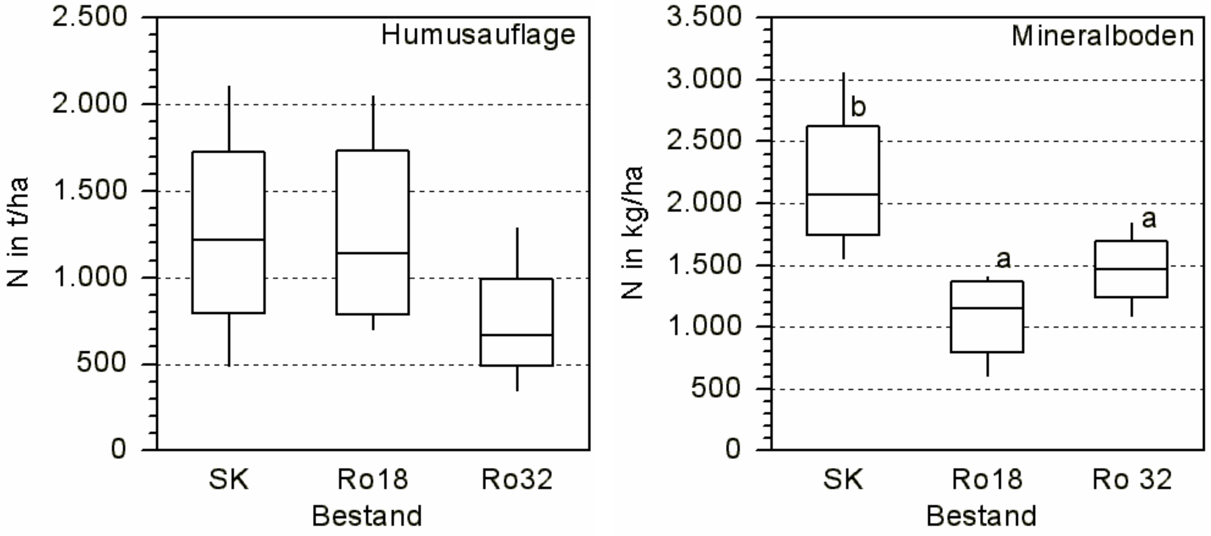

Abb. 2: N-Vorräte in der Humusauflage (links) und im Mineralboden (0-50 cm, rechts) der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median (n=4), Mediane mit nicht übereinstimmenden Buchstaben unterscheiden sich signifikant ($p \leq 0,05$).

Bei Untersuchungen auf Kipprohböden konnte signifikant mehr Stickstoff (um 60%) im Mineralboden von Robinien als im Mineralboden von Kiefernbeständen gefunden werden [10]. Auch andere Autoren fanden, dass die N-Konzentration in Böden unter

Robinienbeeinflussung höher ist als unter nicht stickstofffixierenden Baumarten [26]. Bei den untersuchten Beständen war dies jedoch nicht der Fall. In den Robinienbeständen weist allerdings eine nitrophile Artenzusammensetzung der Krautschicht (*Chelidonium majus* L., *Urtica dioica* L.) auf eine hohe Stickstoffverfügbarkeit hin. Außerdem erfolgt eine sechs- bis siebenfach höhere N-Festlegung im Robinienholz im Vergleich zur Kiefer [7]. Die geringere Stickstoffakkumulation im Robinienbestand ist aber wahrscheinlich vor allem durch die verstärkte Auswaschung von Nitrat mit dem Sickerwasser zu erklären, auch wenn aufgrund geringer Niederschlagsmengen von 490 bis 515 mm pro Jahr im Untersuchungsgebiet dem Austrag von Nitrationen Grenzen gesetzt sind.

Die Humusauflage des Kiefernbestandes weist mit 22,0 ein mäßig weites [1] und signifikant höheres C/N-Verhältnis gegenüber den Robinienbeständen auf ($p<0,02$). In der Humusauflage der Robinienbestände verengten sich die C/N-Verhältnisse mit zunehmender Dauer der Robinienbestockung ($r_s=-0,77$). Signifikante Unterschiede waren zwischen Ro18 und Ro32 zu erkennen. Von einem mittleren C/N von 16,6 (Ro18) verengte sich der Quotient auf 14,6 (Ro32). Die C/N-Verhältnisse der Mineralböden (0-20 cm) sind Abb. 3 zu entnehmen.

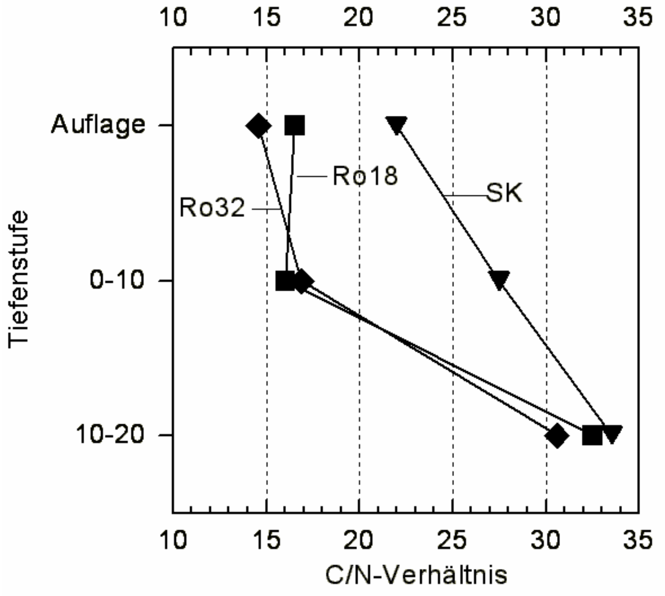

Abb. 3: C/N-Verhältnisse in der Humusauflage und im Mineralboden (0-20 cm) der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median (n=4).

Die Phosphorkonzentrationen der Humusproben variierten zwischen 0,3 und 0,5 mg g^{-1}. Zwischen den Robinienbeständen traten jedoch keine signifikanten Unterschiede auf. Aufgrund langsameren Streuabbaus lagen die Phosphorvorräte der Schwarzkieferauflage mit rund 90 kg ha^{-1} über denen der Robinienbestände ($p=0,02$) (Ro18: 44 kg ha^{-1}, Ro32: 27 kg ha^{-1}). Die Untersuchungen der Mineralböden (0-50 cm) ergaben bezüglich der Ausstattung mit Phosphor kaum Unterschiede zwischen den Beständen (Tab. 3). Unter der Schwarzkiefer wurden 1280 kg P ha^{-1} gemessen, in den Robinienbeständen 1340 kg P ha^{-1} (Ro18) und 1320 kg P ha^{-1} (Ro32).

Bodenacidität

Die in KCl gemessenen pH-Werte der Humusauflagen der Bestände lagen 0,5 bis 0,7 pH-Einheiten unter den in Wasser gemessenen. Die pH-Werte (KCl) der Humusauflagen der Robinienbestände lagen im Mittel bei 3,5 (Ro18) und 3,6 (Ro32). Zwischen dem Schwarzkieferbestand und den beiden Robinienbeständen waren keine signifikanten Unterschiede in den pH-Werten der Humusauflagen messbar (Abb. 4).

Die Mineralböden befanden sich ab 10-20 cm Bodentiefe im Austauscherpufferbereich und im Oberboden im Aluminiumpufferbereich [1]. Die durchschnittlichen pH-Werte der Mineralböden (alle 5 Tiefenstufen) aller Bestände lagen durchgängig unter 4,2 und über 3,3 (pH KCl) bzw. unter 4,6 und über 3,9 (pH H_2O). Der durchschnittliche pH-Wert des Kiefernbestandes von 4,0 in 0-50 cm unterschied sich von Ro18 und Ro32 nicht signifikant. Er lag aber in allen Tiefenstufen leicht über den pH-Werten des Mineralbodens der Robinienvarianten (Abb. 4).

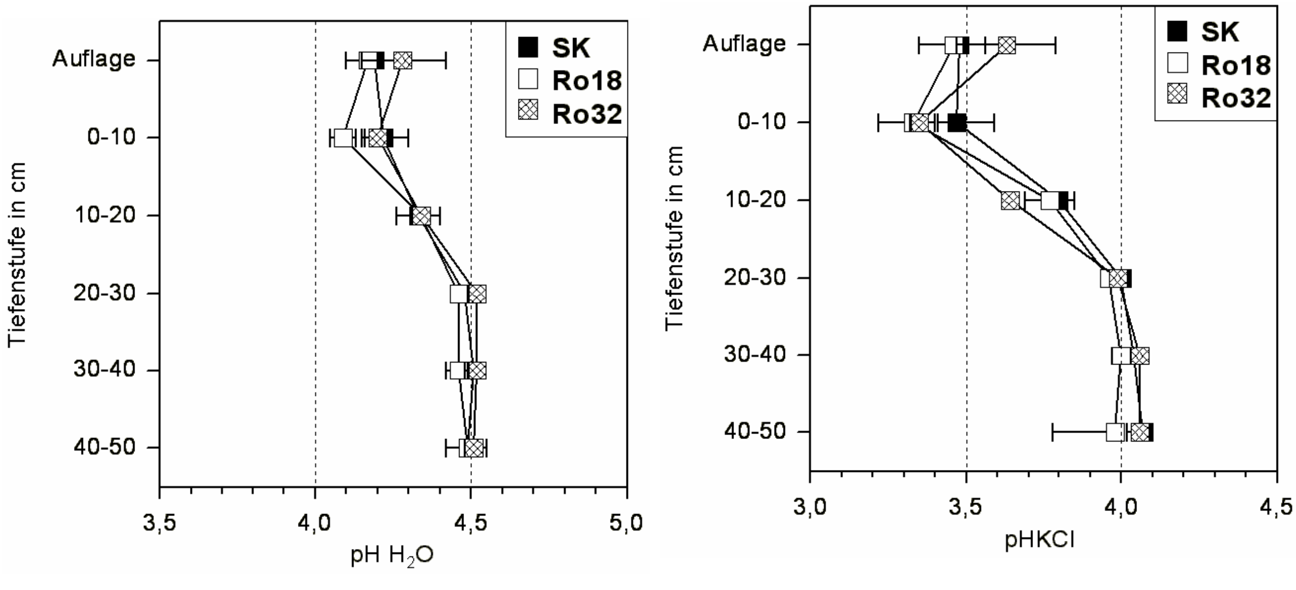

Abb. 4: pH-Werte (pH H_2O links, pH KCl rechts) in der Humusauflage und im Mineralboden der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median sowie 25%- und 75%-Quartil (Q25; Q75), (n=4).

Der längerfristige Einfluss stickstofffixierender Baumbestände auf den pH-Wert wurde bisher in Erlenbeständen untersucht. Es werden Absenkungen des pH-Wertes um 0,4 Einheiten in den oberen 15 cm des Mineralbodens unter einem 50-jährigen Erlenbestand im Vergleich zu einem gleichaltrigen Douglasienbestand beschrieben [25]. Diese eher geringe Versauerung führen die Autoren auf die hohe Pufferkapazität des Bodens zurück. Es wird ebenfalls von einer Versauerung des Oberbodens (0-20 cm) verbunden mit verstärkter Basenauswaschung und Nitratbelastung des Sickerwassers in Erlenbeständen auf terrestrischen Böden im Vergleich zu Fichtenbeständen berichtet [18], sowie von einer Absenkung des pH-Wertes mit zunehmendem Alter von Erlenbeständen [4]. Die Autoren begründen dies mit einer höheren Dekompositionsrate, größerer organischer Säureproduktion und einer höheren Nitrifikationsrate. Die Abschwächung des Versauerungseffektes der hohen Nitrifikationsrate wird mit hoher Basensättigung und hoher Ca-Konzentration erklärt [16].

Effektive Kationenaustauschkapazität und Basensättigung

Im Mineralboden waren im Vergleich der drei Bestände Unterschiede in der als gering einzustufenden effektiven Kationenaustauschkapazität [1] in den oberen 30 cm Bodentiefe zu erkennen, in den unteren Tiefenstufen jedoch nicht (Tab. 4).

Tabelle 4: Effektive Kationenaustauschkapazität (AKe) im Mineralboden der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); Median[1] (n=4).

Tiefenstufe	**AKe in mmol$_c$/kg**		
cm	**SK**	**Ro18**	**Ro32**
0-10	36,6 ^{a}	27,4 ^{a}	31,3 ^{a}
10-20	16,3 ^{b}	14,1 ^{ab}	14,2 ^{a}
20-30	13,9 ^{b}	11,7 ^{ab}	10,5 ^{a}
30-40	11,3 ^{a}	12,3 ^{a}	10,9 ^{a}
40-50	9,9 ^{a}	11,9 ^{a}	11,6 ^{a}

[1] Mediane mit nicht übereinstimmenden Buchstaben unterscheiden sich signifikant ($p \leq 0,05$) zwischen den Beständen (nicht zwischen den Tiefenstufen).

Die Basensättigung (BS) beschreibt den prozentualen Anteil der Summe der austauschbaren basischen Kationen Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} (M_b-Kationen) an der effektiven Austauschkapazität [23]. Die Böden sind aufgrund ihrer Basensättigung als mäßig elastisch gegenüber weiteren Säureeinträgen einzustufen [3]. In den Varianten Ro18 und Ro32 wurden mittlere Basensättigungen von 16% und 18% gemessen ($p<0,05$). Der Referenzbestand SK lag mit einer mittleren BS von 21% (0-50 cm) signifikant über der Basensättigung der Bestände Ro18 und Ro32 ($p>0,05$). Robinienbestände entziehen dem Boden die größten Mineralstoffmengen im Vergleich zu anderen Baumarten [5], [7], [9], [16]. Der Vergleich der Standorte Ro18 und Ro32 mit SK bestätigen die Annahme der Basenverarmung durch die Robinie (Abb. 5). Aus dem Verlust an Stickstoff von 65 kmol$_c$ N bei Ro18 und 43 kmol$_c$ N bei Ro32 wird eine äquivalente Menge an Protonen gebildet, die ebenfalls zur Versauerung beigetragen haben.

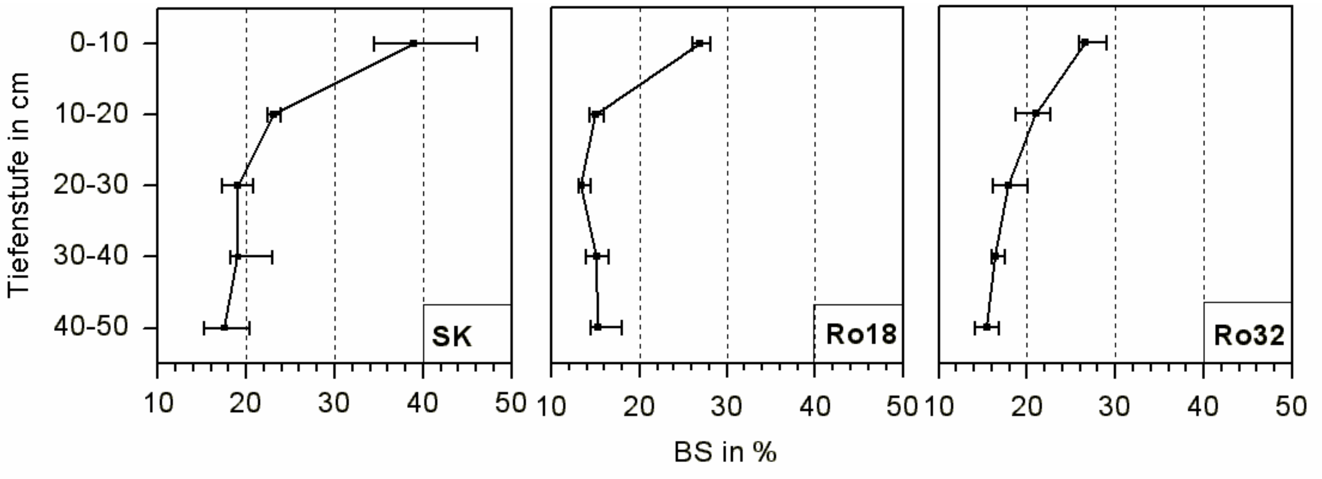

Abb. 5: Basensättigung in den Tiefenstufen des Mineralbodens der verschiedenen Bestände; Median sowie Quartile Q25, Q75 (n=4).

Der bei den Basensättigungen sichtbare Trend wird bei der Betrachtung der Vorräte an M_b-Kationen noch deutlicher. Die Vorräte im Boden des Schwarzkieferbestandes lagen mit 3026 kg ha^{-1} signifikant über denen der Robinienbestände (Ro18: 1795 kg ha^{-1}, Ro32: 1922 kg ha^{-1}). In Abb. 6 sind auch die Anteile der in der Humusauflage gespeicherter M_b-Kationen gemessen am Gesamtvorrat dargestellt. Im Vergleich zum 18-jährigen Robinienbestand, in dem die Humusauflage einen großen Nährstoffpool bildete (32%), waren unter Ro32 nur 16% der M_b-Kationen in der Auflage gespeichert. Der Schwarzkieferbestand reicherte 28% seines gesamten Vorrates im Auflagehumuspool an.

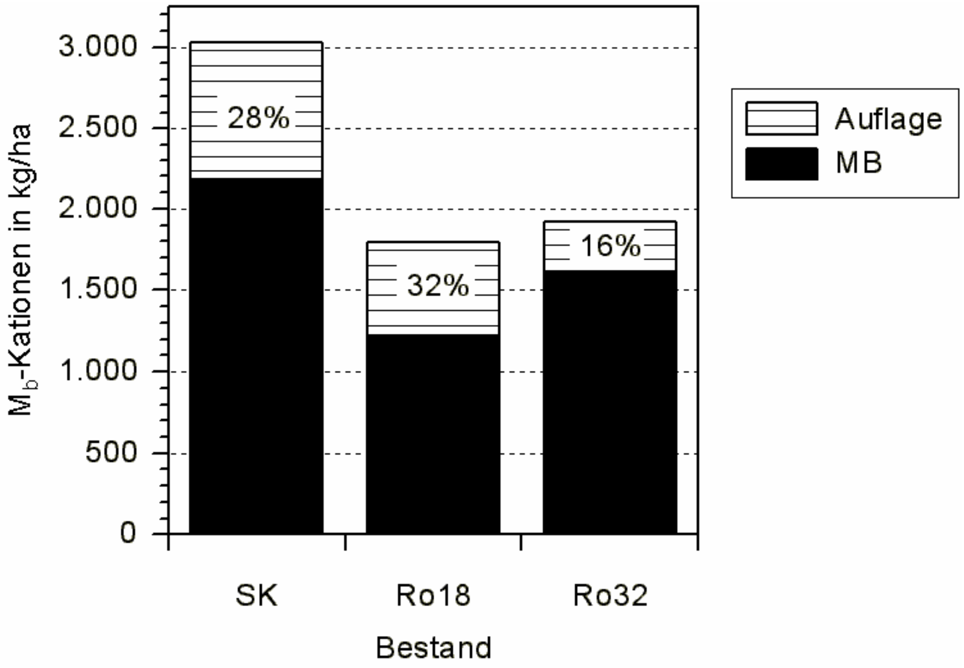

Abb. 6: Vorräte kurz- bis mittelfristig verfügbarer M_b-Kationen (Humusauflage und Mineralboden) der Robinienbestände (Ro18 und Ro32) und des Schwarzkieferbestandes (SK); (n=4), Werte mit nicht übereinstimmenden Buchstaben unterscheiden sich signifikant (p≤0,05).

Nach 18-jährigem und 32-jährigem Robinienanbau wird der Mineralboden stärker an Nährstoffen, vor allem an Calcium, erschöpft. Die Verminderungen des kurz- bis mittelfristig mobilisierbaren Vorrates an Mb-Kationen liegen bei 21% bzw. 35% im Vergleich zum Schwarzkieferbestand. Diese Minderung ist nicht allein durch die stärkere Aufnahme erklärbar, sondern weist auf eine auswaschungsbedingte Abnahme hin.

Fazit

Die von anderen Autoren gefundene N-Anreicherung unter Robinie konnte bei den untersuchten Beständen nicht bestätigt werden [9], [26]. Nach Überführung von Kiefernbeständen in Robinienbestände sind in einer Initialphase auch gegenteilige Entwicklungen möglich. Die Zufuhr nährstoffreicher Streu führt zum weitgehenden Abbau des Auflagehumus ohne gleichzeitig die Humusanreicherung im Mineralboden zu fördern. Dieser Abbau verbunden mit der Freisetzung und Nitrifizierung von N führt zur Freisetzung von Protonen, die nur partiell durch die Alkalinität der organischen Substanz kompensiert wird. Dieser Prozess im Verein mit hoher Nährstoffaufnahme und mit der Bindung von nicht durch die Robinie verwerteten Stickstoff und dessen Auswaschung als Nitrat führen zu einer Versauerung und Nährstoffverarmung (Entbasung) der Böden. Auf sorptionsschwachen, bereits versauerten und entbasten Böden kann der wiederholte Anbau der Robinie zu einer chemischen Degradation der Böden und einer damit verbundenen Wuchsdepression führen.

Die Ergebnisse zeigen, dass bei den beobachteten Wuchsminderungen der Robinie Prozesse der internen Versauerung und Basenverarmung als Ursache nicht ausgeschlossen werden können.

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8.5 Kohlenstoffspeicherung in Böden nach Aufforstung in Abhängigkeit von der Bewirtschaftungsform

Carbon storage in soils after afforestation in relation to management practices

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In: *Forst und Holz (2002), 57 (13/14), Seite 417-420*

Einleitung

Die Verbrennung fossiler Energieträger sowie die ungeregelte Landnutzung, und hier insbesondere die unkontrollierte Rodung der Wälder, führen zu einer globalen CO_2-Emission von jährlich ca. 23,1 Mrd. t CO_2 (Böswald et. al 2001). Die Bundesrepublik Deutschland, mit einer Gesamtfläche von 36 Mio. ha, gehört mit einer jährlichen CO_2-Emission zwischen 0,9 und 1,0 Mrd. t CO_2 zu den größten CO_2-Emittenten der Welt (UBA 2001). Das 1997 in Kyoto abgeschlossene Protokoll (als Folge der Rio-Konferenz von 1992) verlangt von den Industriestaaten, ihre Emissionen an CO_2 und anderen Treibhausgasen im Zeitraum 2008 bis 2012 um durchschnittlich 5% gegenüber 1990 zu reduzieren. Im Rahmen der Lastenverteilung innerhalb der EU hat sich die Bundesrepublik Deutschland verpflichtet, die Treibhausgasemissionen bis 2010 um mindestens 21% zu senken (Schlagheck 2001). Aus Sicht der Forst- und Holzwirtschaft stellt sich die Frage, welche Rolle Waldprojekte im globalen CO_2-Reduktions-Geschehen einnehmen, d.h. ob und in welchem Umfang sie zur Erfüllung der Emissionsreduktionsverpflichtung herangezogen werden können. Wälder weisen von allen terrestrischen Waldökosystemen in ihren Biomassen die größten Kohlenstoffvorräte auf, aber auch in den Waldböden sind erhebliche C-Massen gespeichert. Entsprechend wirken sich Eingriffe in Waldökosysteme besonders stark aus; dies gilt sowohl für die C-Freisetzung als auch für die C-Speicherung. Es gilt daher abzuschätzen, ob und in welchem Umfang die Wälder in Deutschland eine Senke für CO_2 darstellen und wie diese Senke gegebenenfalls vergrößert werden kann.

Legt man die Daten des Berichts des BMELF (1996) zugrunde, so betragen die Derbholzvorräte zurzeit im Mittel etwa 277 m^3 ha^{-1}. Dies entspricht bei einer über die

Baumarten gemittelten Dichte von 0,5g cm^{-3} und einem C-Gehalt von 50% einem Vorrat von ca. 69t ha^{-1}. Entsprechend enthält die gesamte Derbholzmasse der deutschen Wälder eine Kohlenstoffmenge von 702 Mio. t C. Diesem Betrag sind noch die Massen der Wurzeln, Zweige, Äste und Rinden hinzuzufügen, die nach Burschel (1993) etwa 50% des Derbholzes (Expansionsfaktor 1,5), also ungefähr 34,5t ha^{-1} bzw. 350 Mio. t C ausmachen. Demnach betragen die Gesamtvorräte an Baumkohlenstoff 103,5t ha^{-1} oder 1050 Mio. t (Beese et al. 2001).

In den Walböden sind nach Baritz (1998) in den oberen 90cm 1,17 Mrd. t C gespeichert. Davon ist ein erheblicher Teil (18%) in den Humusauflagen gespeichert, also in einem Pool, der bei Eingriffen des Menschen in das Ökosystem rasch mineralisiert werden kann. Insgesamt sind somit in den Waldökosystemen 2220 Mio. t C gespeichert, wobei ungefähr gleiche Anteile auf die Baumbiomasse, inklusive Wurzelbiomasse und auf die Böden entfallen.

Für bestehende und heranwachsende Waldökosysteme lässt sich mit Hilfe von Inventuren und Wuchsmodellen der Zuwachs und somit die C-Festlegung in der Biomasse herleiten. Die Entwicklung der C-Speicherung im Boden von Neuaufforstungen ist bisher wenig untersucht worden. In der hier vorliegenden Arbeit wird die C-Speicherung in Böden von Aufforstungsflächen zum einen bei unterschiedlicher Bestockung verglichen, zum anderen wird die Entwicklung mit fortschreitender Dauer der Bestockung mittels falscher Zeitreihen verfolgt.

Material & Methoden

Für die Untersuchung wurden Bestände in Niedersachsen zur Beprobung ausgewählt, die aus Aufforstungen ehemals landwirtschaftlich genutzter Flächen hervorgegangen sind. Die Flächen wurden so gewählt, dass die Proben einen repräsentativen Querschnitt der in Niedersachsen vorherrschenden Bodensubstrate widerspiegeln, und dass Bestände verschiedenen Alters und unterschiedlicher Bestockung in die Untersuchung Eingang finden. Neben den Waldböden wurden jeweils angrenzende Agrarflächen (Äcker und Wiesen) beprobt, um den direkten Vergleich zwischen diesen beiden Landnutzungsformen zu ermöglichen. Die Probennahme erfolgte im Oberweserbergland, im Südniedersächsischen Bergland, im Solling, im Niedersächsischen Harz, in der Heide und auf Röt- und Muschelkalkstandorten im Göttinger Wald. Insgesamt wurden 18 Wald- und 10 Agrarböden beprobt.

Um die Entwicklung der Kohlenstoffvorräte in Abhängigkeit vom Bestandesalter (Jahre nach der Aufforstung) darstellen zu können wurden folgende Altersklassen ausgeschieden: AKL 0 (Agrarflächen), AKL I (1-25 Jahre), AKL II (25-55 Jahre) und AKL III (>55 Jahre).

Mit dreifacher Wiederholung pro Bestand bzw. Agrarfläche wurden Bodensäulen bis zu einer Tiefe von 70cm entnommen und in folgende Tiefenstufen fraktioniert: org. Auflage, 0-10cm, 10-30cm und 30-70cm. Die Proben der org. Auflage und des Mineralbodens bis 10cm Tiefe wurden mit Hilfe eines „Boku-Rohres“ volumengerecht entnommen so, dass für diese Tiefenstufe die für die Vorratsberechnung benötigte Trockendichte direkt bestimmt werden konnte. Für die Tiefenstufen 10-30cm und 30-70cm wurden die Trockendichten des Oberbodens verwendet.

An den Proben wurde der Gesamt-Kohlenstoffgehalt (C_t) mit Hilfe eines Elementaranalysators bestimmt. Für die Proben, die nicht auf den Muschelkalkstandorten entnommen wurden, konnte der gemessene C_t-Gehalt mit dem Gehalt der Probe an organischem Kohlenstoff (C_{org}) gleichgesetzt werden. Für die auf Muschelkalk gewonnenen Bodenproben wurde der karbonatbürtige Kohlenstoffgehalt vom gemessenen C_t-Gehalt der Probe abgezogen. Aus den C_{org}-Gehalten der Proben wurden die C_{org}-Vorräte mittels der bestimmten Trockenraumdichten errechnet.

Ergebnisse

Beim Vergleich der C-Gehalte und C-Vorräte in Böden mit forstwirtschaftlicher Nutzung zu Böden mit landwirtschaftlichen Nutzungsformen zeigen sich deutliche Unterschiede (vgl. Abb. 1). In den organischen Auflagen findet sich ein durchschnittlicher C-Gehalt von 240 g C/kg. Dagegen liegen die Kohlenstoffkonzentrationen der Mineralböden bis 30cm Tiefe zwischen 40g C/kg (Waldböden) und 25g C/kg (Agrarböden). In der Tiefenstufe 30-70cm liegen die C-Gehalte beider Landnutzungssysteme bei jeweils rund 7,0g C/kg. In den Humusauflagen sind im Durchschnitt 30t C/ha gespeichert, so dass bei einem Kohlenstoffvorrat von 153t C/ha bis 70cm Bodentiefe in Waldböden 20% des Gesamtvorrats in der organischen Auflage zu finden sind. Im Mineralboden unter Wald ist mit 123t C/ha etwa gleich viel Kohlenstoff gespeichert wie in landwirtschaftlich genutzten Böden (119t C/ha). Ohne die Berücksichtigung verschiedener Bestockungen (Nadel- oder Laubwald) bzw. Nutzungsarten (Wiese oder Acker), ist bei den untersuchten Böden der Mehrvorrat an Boden-Kohlenstoff unter Wald im Wesentlichen durch die C-Akkumulation in der Humusauflage begründet.

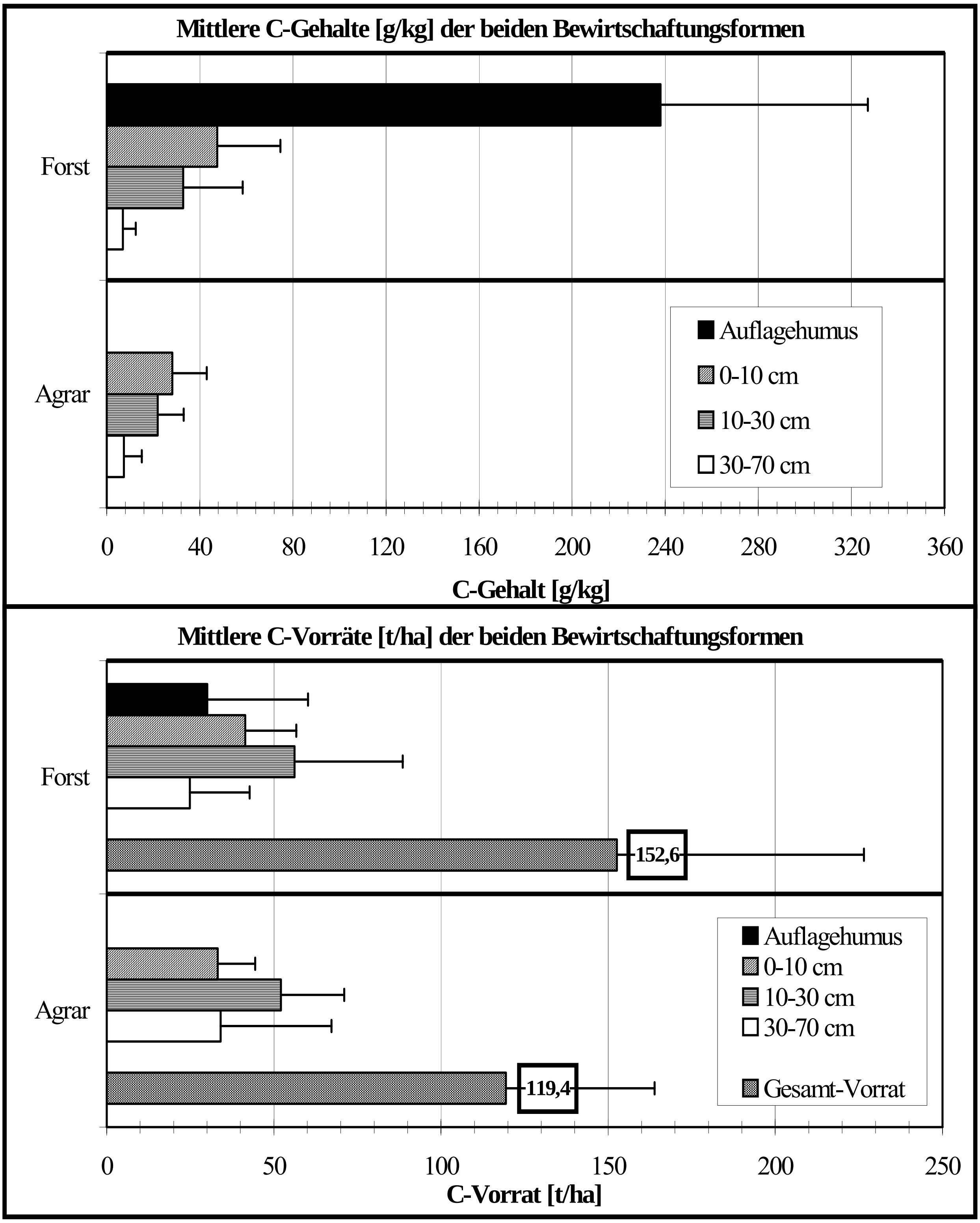

Abb. 1: *C-Gehalte [g/kg] und C-Vorräte [t/ha] in Böden mit forst- und landwirtschaftlicher Nutzung. Werte angegeben als Mittelwerte mit Standardabweichung (Forst n=18; Agrar n=10).*

Tabelle 1 zeigt, dass die für Niedersachsen ermittelten C-Vorräte in Waldböden unterschiedlicher Bestockung mit den Ergebnissen anderer Untersuchungen weitgehend übereinstimmen. Größere Unterschiede treten dagegen beim Vergleich mit den Daten aus dem Deutschen Waldbodenbericht (BMELF 1997) auf, die sich beim Vergleich der Mediane allerdings reduzieren.

***Tab. 1**: Übersicht über die minimalen und maximalen sowohl mittleren C-Vorräte [t/ha] in Waldböden aus verschiedenen Studien; arithmetische Mittelwerte in eckigen [], Mediane in runden () Klammern.*

	Beese, Berthold (2001); bis 70 cm Tiefe	Büttner / BZE-Nds. (1997); bis 60 cm Tiefe	BMELF/Wald-Bodenber. (1997); bis 60 cm Tiefe	Egenolf/BZE (1995); bis 90 cm Tiefe	Ziegler (1991)
Auflage	2-94 [30,1]/(16,0)	2-128 [31,0]	0-466 (18,0)	7-56 [29,8]	2-212 [39,1]
Mineral-Boden	46-271 [122,5]/(112,0)	80-210 [105,0]	14-562 (91,0)	107-132 [107,5]	9-280 [117,5]
Gesamt	58-342 [152,6]/(128,0)	[136,0]	(109,0)	133-171 [137,3]	35-362 [156,6]

In Abbildung 2 sind die C-Vorräte in Wald- und Agrarböden in Abhängigkeit von der forstlichen Bestockung bzw. der landwirtschaftlichen Nutzungsform dargestellt. Von den vier untersuchten Varianten weisen die Nadelholzbestände mit durchschnittlich 171t C/ha den mit Abstand größten Gesamt-C-Vorrat auf. Dieser sehr hohe Vorrat stimmt mit den von Büttner (1997) unter Fichte gefundenen Gesamt-Kohlenstoffvorräten (Humusauflage bis 60 cm Bodentiefe) von 150-170t C/ha überein, wobei hier v.a. die Höhenlage der Bestände die C-Menge in Humusauflage und Mineralboden bestimmt. Dagegen ist in den Böden unter Laubholz und in den Agrarböden mit etwa 115-120t C/ha ungefähr gleich viel Kohlenstoff gespeichert. Vergleicht man allerdings diese drei Varianten hinsichtlich ihres im Mineralboden gespeicherten C-Vorrats, so ist in den mit Laubholz bestockten Böden mit rd. 104t/ha ein um ca. 15t C/ha geringerer Vorrat als in den landwirtschaftlich genutzten Böden zu finden.

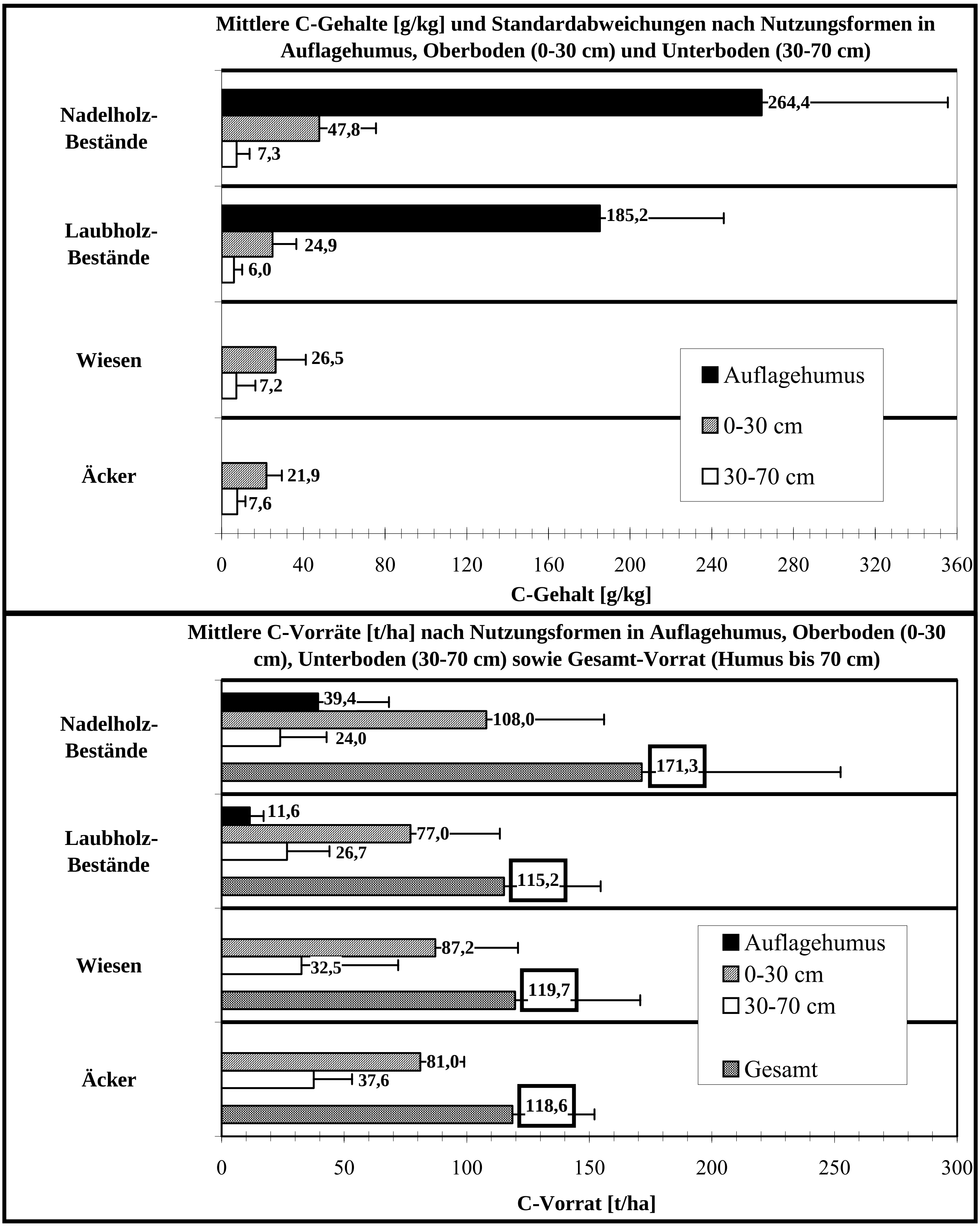

Abb. 2: *C-Gehalte [g/kg] und C-Vorräte [t/ha] der verschiedenen Nutzungsformen; getrennt nach Auflage, Oberboden [0-30cm] und Unterboden [30-70cm] sowie Gesamt-C-Vorrat. Werte angegeben als Mittelwerte mit Standardabweichung (Nadelholzbestände n=12; Laubholzbestände n=6; Wiesen n=7; Äcker n=3).*

Obwohl die C-Konzentrationen im Mineralboden unter Laubholz und landwirtschaftlichem Bewuchs keine großen Differenzen aufweisen, sind in den Agrarböden im Oberboden 5-10t

C/ha und im Unterboden 6-10t C/ha mehr gespeichert. Dieser Mehrvorrat resultiert aus den für die jeweilige Nutzungsform ermittelten Trockendichten bzw. Feinerdemassen, die für die Vorratsberechnung maßgebend sind. Aufgrund der häufigen und flächigen Befahrung der landwirtschaftlichen Flächen weisen diese höhere Dichten und Feinerdemassen auf, weshalb aus geringen C-Konzentrationen verhältnismäßig hohe C-Vorräte resultieren. Der Mehrvorrat von insgesamt 50t C/ha in den Böden unter Nadelwald resultiert überwiegend aus dem Kohlenstoff, der in der organischen Auflage gespeichert ist, aber auch aus höheren C-Konzentrationen und C-Vorräten des Oberboden. Durchschnittlich sind in der Humusauflage der Nadelwaldstandorte 40t C/ha akkumuliert. Dagegen sind in der Humusauflage der Laubwaldstandorte im Mittel nur rd. 12t C/ha gespeichert. In den oberen 30cm des Mineralbodens ist die C-Konzentration unter Nadelwald mit durchschnittlich 48g C/kg fast doppelt so hoch wie unter den drei anderen Nutzungsformen. Der daraus resultierende Vorrat von 108t C/ha ist um etwa 25t C/ha höher als in den Oberböden der drei anderen Alternativen. Unter Nadelwald ist im Vergleich zu den Oberböden der Laubholzstandorte etwa 40% mehr und im Vergleich zu den oberen 30cm der Agrarstandorte im Mittel 30% mehr in dieser Bodentiefe gespeichert.

Im Unterboden (30-70cm) liegt die C-Konzentration für alle vier Nutzungsarten zwischen 6,0 und 7,5g C/kg, d.h. bezüglich der Stoffkonzentration ist hier kein Effekt der jeweiligen Nutzungsform zu erkennen. Auch hinsichtlich des C-Vorrates im Unterboden ist zwischen den beiden untersuchten forstlichen Varianten kein Unterschied zu finden. Dagegen tritt im Vergleich der C-Vorräte der forst- und landwirtschaftlichen Flächen in 30 bis 70cm Bodentiefe ein Mehrvorrat von etwa 10t C/ha in den Agrarböden auf, der aus höheren Lagerungsdichten in landwirtschaftlichen Flächen resultiert.

Neben dem Einfluß der Nutzungsart (dadurch indirekt auch des Klimas und des Bodensubstrats), ist auch die zeitliche Entwicklung der C-Akkumulation ein wichtiger Aspekt. Abbildung 3 zeigt die C-Gehalte und C-Vorräte der untersuchten Böden abhängig von der Dauer der forstlichen Bestockung. Dabei stellen die als Altersklasse 0 (AKL 0) ausgewiesenen Agrarflächen die Ausgangssituation dar. Die Entwicklung der C-Festlegung zeigt sich in den für die jeweiligen Altersspannen gemittelten Werte. Die Humusauflage ist in den älteren Beständen erwartungsgemäß deutlich stärker ausgeprägt als in den Jungbeständen. Mit zunehmendem Bestandesalter erfolgt in der Humusauflage eine massive Akkumulation organischer Substanz (7,4kg/m^2 in AKL I auf 18,3kg/m^2 in AKL III). Vergleicht man die C-

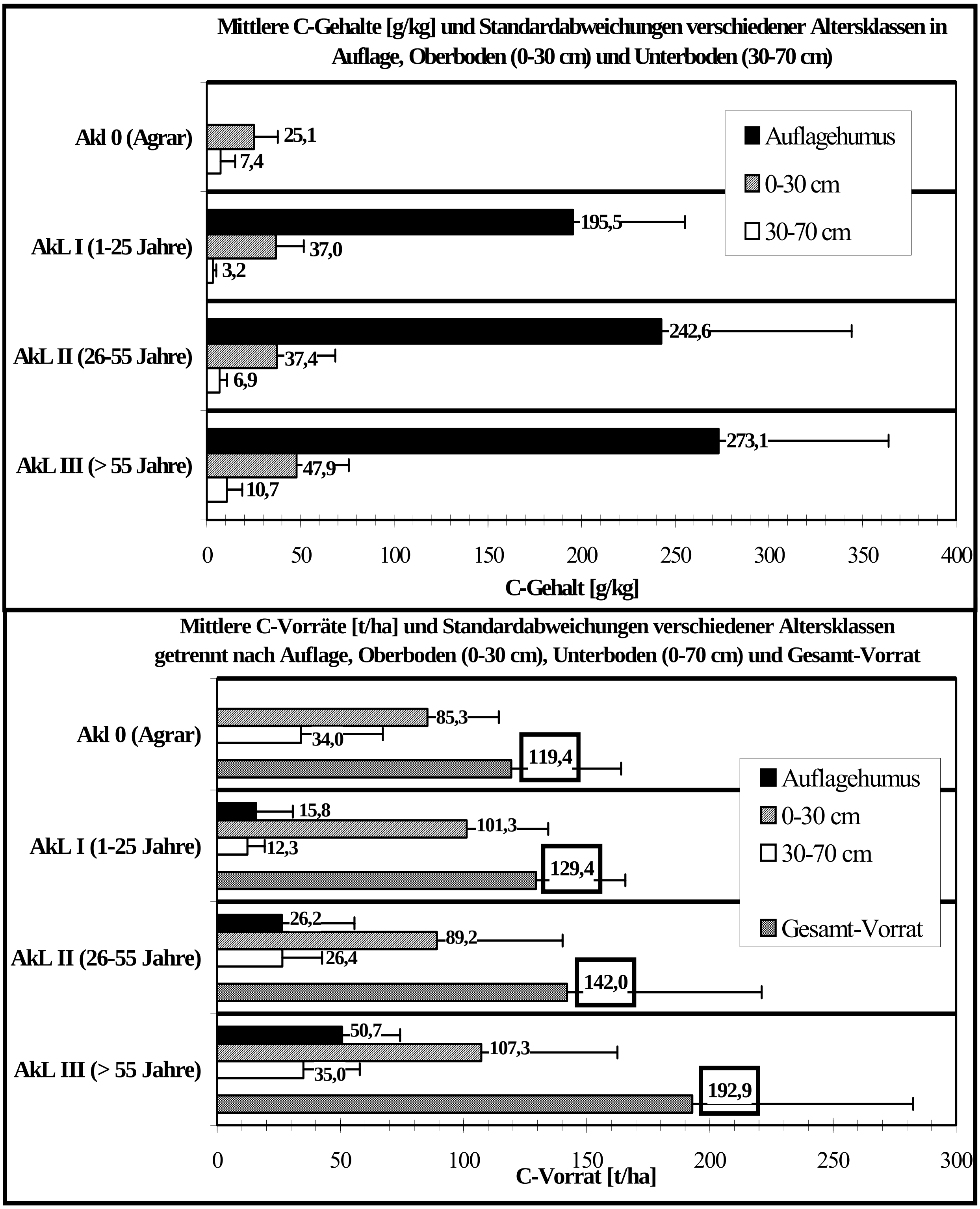

Abb. 3: *Mittlere C-Gehalte [g/kg] und C-Vorräte [t/ha] verschiedener Altersklassen (Jahre nach Aufforstung); getrennt nach Auflage, Oberboden [0-30cm] und Unterboden [30-70cm] sowie Gesamt-C-Vorrat (AKL 0 n=10; AKL I n=5; AKL II n=8; AKL III n=5).*

Konzentrationen in den Auflagen der Altersklassen I bis III, so zeigt sich ein kontinuierlicher Anstieg von durchschnittlich 196g C/kg (AKL I) über 243g C/kg (AKL II) auf 273g C/kg

(AKL III). Offensichtlich steigt neben der Mächtigkeit der organischen Auflage auch deren Gehalt an Kohlenstoff mit dem Alter an.

In der AKL I sind ca. 16t C/ha, in der AKL II 26t C/ha und in der AKL III 51t C/ha gespeichert. Gemessen am Gesamtkohlenstoffvorrat entspricht dies einem jeweiligen Anteil von 12, 18 bzw. 24%. Eine Entwicklung über die Zeit läßt sich auch in den oberen 30cm des Mineralbodens erkennen. Im Oberboden der landwirtschaftlichen Flächen (AKL 0) findet sich eine durchschnittliche C-Konzentration von 25g C/kg und ein Vorrat von 85t C/ha. In den Böden der Altersklassen I und II ist der C-Gehalt mit rd. 37g C/kg um etwa 50% (12g/kg) höher. Auch der C-Vorrat ist in den Böden dieser Jungbestände mit Werten zwischen 89 und 101t C/ha höher. In den Böden, die vor mehr als 55 Jahren aufgeforstet wurden (AKL III) ist im Oberboden die C-Konzentration mit durchschnittlich 48g C/kg fast doppelt so hoch wie auf den Agrarflächen und auch der Vorrat ist um mehr als 20t erhöht. Im Unterboden ist dagegen kein ausgeprägter Trend hinsichtlich der C-Festlegung in Abhängigkeit vom Bestandesalter zu erkennnen. Auffällig ist beim Vergleich der C-Gehalte jedoch, dass die mittlere Kohlenstoffkonzentration im Unterboden der Bestände der AKL I deutlich geringer ist als in den Agrarböden und erst in der folgenden Altersklasse mit zunehmender Bewaldungsdauer ansteigt (vgl. Abb. 3, obere Grafik). Dieser kurzfristige Konzentrationsabfall in 30-70cm Bodentiefe läßt sich dadurch erklären, dass das Wurzelwerk der Neuaufforstung in der ersten Phase nach der Etablierung noch nicht so stark entwickelt und tief verzweigt ist, dass über die Wurzelstreu organisches Material in größerem Umfang in diese Tiefenstufe gelangen kann. In der ersten Phase der Aufforstung scheint also zunächst ein Abbau der zersetzbaren organischen C-Verbindungen im Unterboden zu erfolgen, der noch nicht durch den Einbau organischen Materials aus der neuen Bestockung kompensiert werden kann. Erst in der AKL II ist das Ausgangsniveau der C-Konzentration in dieser Bodentiefe wieder erreicht und wird schließlich auf den Aufforstungsflächen, die vor mehr als 55 Jahren entstanden sind, übertroffen. Ein ähnliches Muster konnte Heinsdorf (1990) für die Kahlschlagswirtschaft von Kiefernbeständen im nordostdeutschen Tiefland beobachten. Zwar überwiegt nach Kahlschlägen der C-Abbau in der Auflage, aber auch im Mineralboden kommt es bei ausbleibender Nachlieferung organischen Materials zu einem Kohlenstoffabbau, der nach 30-35 Jahren wieder ausgeglichen wird.

Betrachtet man die zeitliche Entwicklung des Gesamtkohlenstoffvorrates (Humusauflage & Mineralboden bis 70cm) der vier Altersstufen, so läßt sich eine deutliche Zunahme der C-Speicherung mit zunehmender Bestockungsdauer beobachten. Diese Entwicklung läßt sich Abbildung 4 entnehmen.

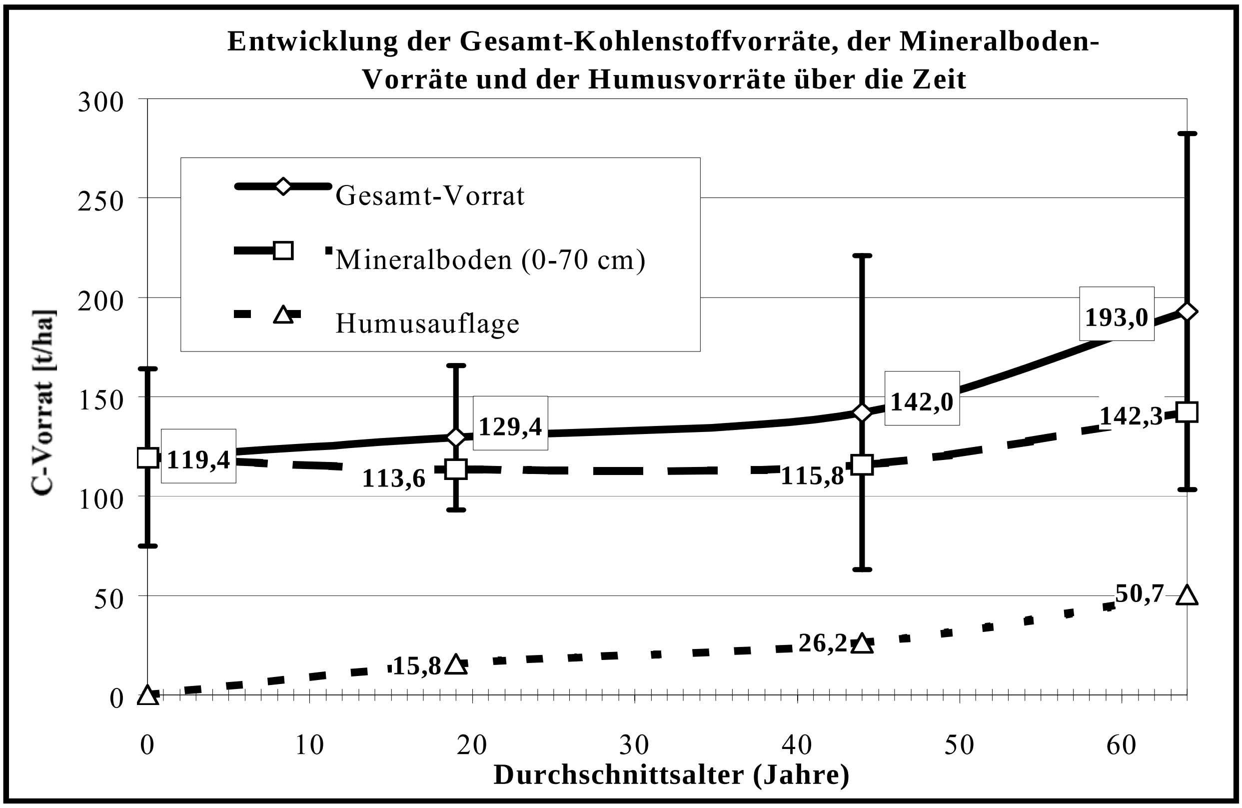

***Abb. 4:** Zeitliche Entwicklung der Kohlenstoffvorräte in Humusauflagen und Mineralböden [t/ha]. Datenpunkte angegeben als Mittelwerte; für den Gesmat-Kohlenstoffvorrat mit Standardabweichung (AKL 0 n=10; AKL I n=5; AKL II n=8; AKL III n=5).*

Durch die Bildung der Durchschnittsalter der zur jeweiligen Altersklasse gehörenden Bestände, ist der Vorrat in der AKL I (1-25 Jahre) für das Alter 19 Jahre, in der AKL II (25-55 Jahre) für das Alter 44 Jahre und in der AKL III (> 55 Jahre) für das Alter 64 Jahre aufgetragen. Es fällt auf, dass der Gesamtvorrat an Kohlenstoff mit dem Alter der Bestände kontinuierlich aber nicht gleichmäßig zunimmt. Der Gesamtkohlenstoffvorrat steigt vom Zeitpunkt 0 (Agrarflächen) von etwa 119t C/ha auf 193t C/ha in der AKL III bzw. bei einem Durschnittsalter von 64 Jahren, d.h. um 74t/ha. 19 Jahre nach der Aufforstung sind rd. 16t C/ha in der Humusauflage gespeichert, bei einem mittleren Alter von 44 Jahren sind es 26t C/ha und im Durchschnittsalter von 64 Jahren weist die organische Auflage einen Vorrat von 51t C/ha auf. Im Mineralboden bis 70cm Tiefe ist eine Erhöhung des C-Vorrats im Vergleich zum landwirtschaftlichen Ausgangsniveau erst unter den 50 Jahre alten Beständen zu beobachten. Es zeigt sich, dass der Anstieg des Gesamt-Kohlenstoffvorrats in den Jungbeständen (bis durchschnittlich 44 Jahre) gegenüber dem Ausgangsniveau von rd. 10 bzw. 20t C/ha allein aus dem Aufbau der organischen Auflage resultiert, wohingegen in den älteren Beständen der insgesamt um 74t C/ha höhere C-Vorrat sowohl aus der Festlegung in der Humusauflage als auch aus einer erhöhten C-Akkumulation im Mineralboden hervorgeht. Dies steht im Widerspruch zu der allgemeinen Aussage, dass der C-Anstieg fast ausschließlich im Auflagehumus erfolgt. Die organische Auflage ist jedoch das Speicherkompartiment, das primär für die erhöhte C-Festlegung unter Wald verantwortlich ist, da 70% dieses Mehrvorrates in der Humusauflage lokalisiert sind.

Schlussfolgerungen

Der Vergleich der aufgeforsteten Waldböden mit den landwirtschaftlich genutzten Agrarböden zeigt, dass die im Boden gespeicherte C-Menge durch eine Bewaldung tendenziell erhöht wird. Die Höhe des im Boden fixierten Kohlenstoffs hängt von der Dauer der Bewaldung ab. Je nach nach Nutzungsform, Bestockung bzw. Bewuchs und Alter wurde ein um durchschnittlich 30 bis 70t C/ha höherer Vorrat gefunden. In jedem Fall war die Humusauflage das Speicherkompartiment, in dem der Hauptteil (70%) des Zuwachses an Kohlenstoff festgelegt wurde. Die organische Auflage der Waldböden stellt somit das Bodenkompartiment dar, in welchem am schnellsten eine spürbare Erhöhung der akkumulierten Kohlenstoffmenge im Boden erfolgt. Da es sich hierbei um einen labilen Vorrat handelt, hängt eine dauerhafte Speicherung dieses Vorrats entscheidend von Walderhaltungsmaßnahmen und ökologisch optimierten Bewirtschaftungsmethoden ab.

Die gefundenen Raten stehen im Einklang mit den Befunden von Sauerbeck (1990), der von einer jährlichen Einbindung von 1t C/ha im Boden ausgeht. Es muß allerdings festgestellt werden, dass die hier vorgestellten Resultate eine große Varianz aufweisen, so dass nur eine Trendaussage gemacht werden kann.

Würden z.B. 500.000 ha der insgesamt ca. 17,2 Mio. ha (MLUR, 2001) umfassenden landwirtschaftlichen Nutzflächen der BRD aufgeforstet, so könnten in Auflagen und Mineralböden dieser Flächen 500.000t C/ha und Jahr bzw. 32,5 Mio. t C/ha in 65 Jahren festgelegt werden. Bei einem jährlichen Gesamtausstoß von ca. 240 Mio. t C in Deutschland macht der im Boden festgelegte Kohlenstoff nur einen sehr geringen Anteil aus und ist fast zu vernachlässigen (0,2%).

In den Beständen und Böden unserer Waldökosysteme sind große Mengen an Kohlenstoff gespeichert, die durch weitere Aufforstungsmaßnahmen weiter erhöht werden können. Bei den geringen Flächen die zur Aufforstung zur Verfügung stehen, da rechtliche und ökonomische Hindernisse eine großflächige Aufforstung verhindern, kommt der Aufforstung als Kohlenstoffsenke nur geringe Bedeutung zu. Dies würde sich auch nicht wesentlich ändern, wenn durch eine Ausweitung der Datenbasis die Verlässlichkeit der Aussage verbessert würde.

Zusammenfassung

Als Folge des Kyoto-Protokolls von 1997 hat sich die Bundesrepublik Deutschland verpflichtet die Treibhausgasemissionen bis zum Jahre 2010 um mindestens 21% zu senken. Aus Sicht der Forstwirtschaft stellt sich die Frage, inwieweit Waldprojekte zur Erfüllung der Emissions-reduktionsverpflichtung herangezogen werden können. Fest steht jedoch, dass durch die Neuschaffung von Wald und die Vorratsansammlung in bestehenden Wäldern Kohlenstoffsenken geschaffen werden.

In dieser Arbeit wird die Kohlenstoffspeicherung in Böden verschiedener Aufforstungs-Standorte mit landwirtschaftlich genutzten Standorten in Niedersachsen verglichen. Durch eine Aufforstung wird die im Boden gespeicherte C-Menge tendenziell erhöht. Je nach Nutzungsform, Bestockung und Alter (Jahre nach der Aufforstung) ist im Vergleich zu den landwirtschaftlichen Standorten ein um durchschnittlich 30 bis 70t C/ha höherer Vorrat im Boden unter Neuaufforstungen zu finden. Dabei ist die Humusauflage das Speicherkompartiment, in dem jeweils der Hauptteil (70%) des Zuwachses an Kohlenstoff festgelegt wird.

Abstract

As a consequence of the Kyoto-Protocol the Federal Republic of Germany is committed to reduce greenhouse gas emissions by 21% from 1990 to 2010. This poses the question as to what extend forestry sector can contribute in emission reduction. It is now widely accepted that new afforestation and existing forests can act as major carbon sinks.

In this study carbon stored by soils under different forests are compared to those under agricultural use in Lower Saxony. Soils under forest stands showed higher carbon content than agricultural soils, depending on the factors such as land use management, forest crop and age (years after forest establishment). On an average forest soils had from 30 to 70t /ha higher amount of carbon than agricultural soils. Litter layer under forests is the main soil component of carbon increase (70%).

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