CLIMATE CHANGE RESEARCH
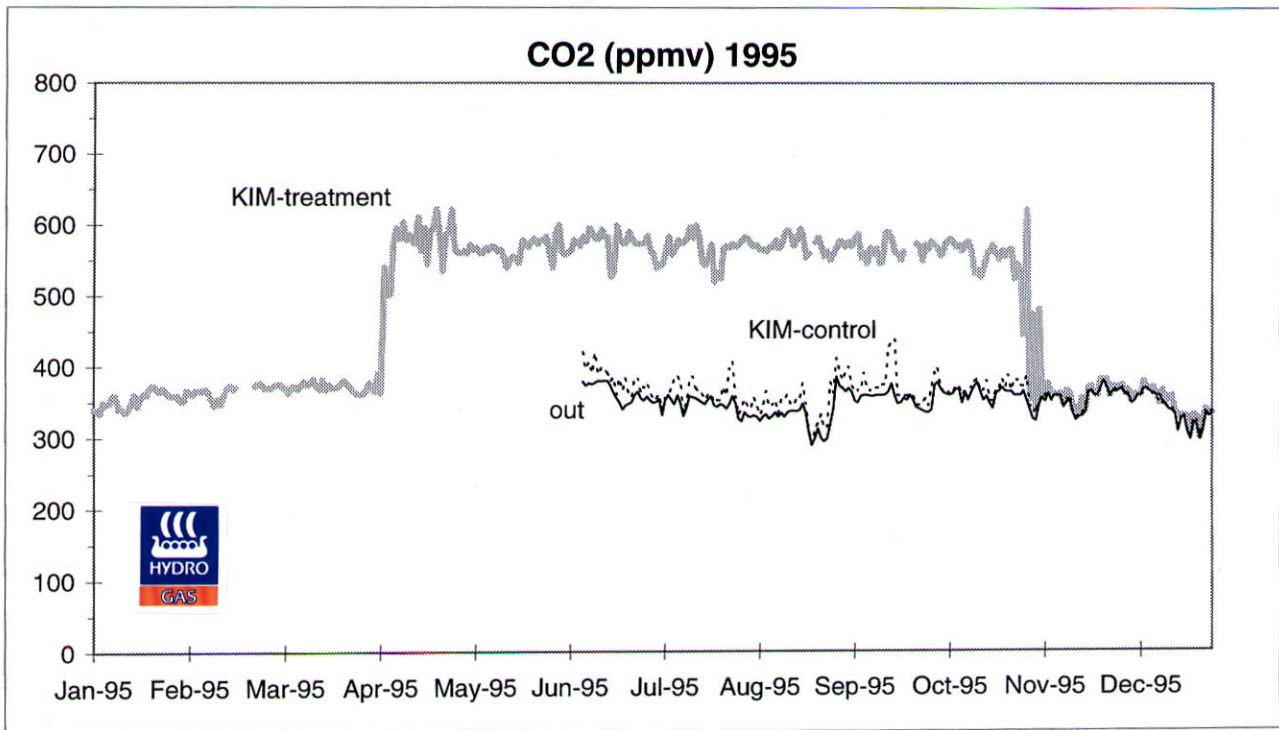
REPORT 6/1996

CLIMEX project: Results from the second year of treatment

May 1995 - December 1995

CO2 (ppmv) 1995

NIVA

HYDRO GAS

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NIVA - REPORT

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Abstract:

CLIMEX is an integrated, whole-ecosystem research project studying the response of entire forested catchments to increased CO_2 and temperature. These whole-catchment manipulation experiments are designed to allow quantification of the links between terrestrial and aquatic ecosystems and provide direct experimental evidence on the effect of climate change on natural forests, terrestrial ecosystems and water resources. The site is located at Risdalsheia, southernmost Norway. Treatment began in April 1994. This report describes results obtained during 1995, the second year of treatment. Significant responses in vegetation, soils and waters were measured.
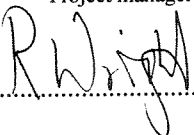
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Project manager

Richard F. Wright

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For the Administration

Bjørn Olav Rosseland

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Report on the second year of treatment

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Contents

Summary

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Acknowledgements

Affiliations

A. Jenkins & R. Collins (IH)

Institute of Hydrology, Wallingford, OXON OX10 8BB, UK.

R.F. Wright & A. Lukewille (NIVA)

Norwegian Institute for Water Research, P.O.Box 173 Kjelsaas, N-0411 Oslo, Norway.

P.S.J. Verburg & N. van Breemen (WAU-SSG)

Department of Soil Science and Geology, Agricultural University Wageningen, P.O.Box 37, 6700 AA Wageningen, The Netherlands.

W. Arp, F. Berendse & J. Frissel (WAU-TENC)

Department of Terrestrial Ecology and Nature Conservation, Agricultural University Wageningen, Bornsesteeg 69, 6708 PD Wageningen, The Netherlands.

D.Beerling (US)

Department of Animal and Plant Sciences, School of Biological Sciences, University of Sheffield, Sheffield S10 2TN, UK.

M.J. Vreeken-Buijs & L. Brussaard (WAU-TENC)

Department of Terrestrial Ecology and Nature Conservation, Agricultural University Wageningen, Bornsesteeg, 69 6708 PD Wageningen, The Netherlands.

1. The CLIMEX Project

Alan Jenkins (IH)

CLIMEX (Climate change experiment) is an international, cooperative research project studying the response of entire catchments to increased CO_2 and temperature. The CLIMEX project is located at Risdalsheia, southern-most Norway (Dise and Jenkins 1995). The project involves five catchments and employs multiple treatments and controls (Table 1.1). CLIMEX focuses on the *whole ecosystem* response to climate change, in particular plant-soil-water linkages and processes.

Table 1.1. *Overview of the 5 catchments at Risdalsheia included in the CLIMEX project.*

Catchment	Area (m^2)	Enclosure	Rain quality	Climate treatment	Start of monitoring
KIM	690	greenhouse	clean	CO_2 + air warming	June 1983
(Control)	170	greenhouse	clean	none	June 1993
EGIL	320	roof	acid	soil warming	June 1983
(Control)	80	roof	acid	none	June 1993
ROLF	220	no roof	acid	none	June 1983
METTE	650	no roof	acid	none	June 1993
CECILIE	380	no roof	acid	none	June 1993

The objectives and hypotheses of CLIMEX are detailed in Dise and Jenkins (1995). Briefly, they are, by enriching CO_2 and elevating temperature to boreal forest catchments:

-- to measure changes in plant CO_2 uptake, gas exchange and community phenology.
-- to measure changes in forest growth and nutrient status.
-- to measure changes in ground vegetation and nutrients.
-- to determine changes in mineralization of soil organic matter.
-- to determine changes in soil fauna and biologically-mediated processes.
-- to measure the effects on runoff water quality and quantity.

CLIMEX began in 1993 with installation of the greenhouse facilities. Background (pre-treatment) data were collected during the period April 1993 through March 1994. Treatment began in April 1994 and has continued for a second year.

The changes observed in the catchment ecosystem in response to the first year of treatment were entirely expected: increased CO_2 and temperature caused an increase in rates of photosynthesis, a decrease in stomatal densities and an increase in mean water use efficiency in several of the major plant species present at Risdalsheia (Jenkins 1995). An increase in biomass in Calluna is

also apparent. No change was detected in the soil and soil solution chemistry, tree nutrition or runoff water. It is anticipated that with continued treatment, the observed changes will lead to further changes in soil and plant chemistry, ecology and output chemical fluxes with time delays at each step. This report documents the changes observed during the second year of treatment (May 1995 - April 1996).

Funding for CLIMEX under the initial contract from the EU Commission of the European Communities ceased in May 1995 and began again under a new contract in Febraury 1996. The project was maintained during much of 1995, the second year of treatment, by funding from Norway (Research Council of Norway, Department of the Environment and Norwegian Institute for Water Research), the Netherlands (National Research Programme on Global Climate Change), and UK (Institute of Hydrology). Funding could not be found to maintain the involvement of the University of Bayreuth in the project but the tree growth and nutrient status responsibilities were taken up by the Danish Forest and Landscape Research Institute (PI's Lennart Rasmussen and Claus Beier). Their funding was not sufficient to contribute to this report. Continued work in this area is now the responsibility of RISØ National Laboratory, Environmental Science and Technology Department, Roskilde, Denmark.

2. Site Operation.

Richard F. Wright and Anke Lükewille (NIVA)

At KIM catchment (CO_2 and heating) the systems generally functioned satisfactorily during 1995. The CO_2 was turned on to the target level of 560 ppmv from 5 April 1995 through 31 October 1995 (Figure 2.1). This level was generally achieved except during summer days when overheating required high rates of ventilation. CO_2 consumption was 101 tons in 1994 and 70 tons in 1995; the difference is due to the filling of the tanks at the end of 1994 in preparation for the spring of 1995.

At KIM catchment the air heating system worked satisfactorily during 1995 except for a 3-week period in September when the circulation pump failed and the system had to be switched off during repairs (Figures 2.2a and 2.2b). The target values were generally met although the increase was somewhat lower than targeted during the coldest periods of the winter, and somewhat higher during mid-summer due to insufficient cooling. These deviations from the targets reflect inherent limitations in the design of the heating and ventilation systems for the greenhouse.

At KIM catchment relative humidity was somewhat higher in the treated section than in the control section (Figure 2.3); this also was the case in 1994.

Measurements of light beneath the roofs at both KIM and EGIL catchments shows that the roof structures block about 50% of the incoming light (Figure 2.4).

At EGIL catchment (soil heating) the heating cables functioned throughout 1995 with the exception of short periods due to power supply failures or where data were lost due to problems with the data loggers (Figure 2.5a and 2.5b). During the late autumn and winter months the systems cannot achieve the target temperature increase completely.

The sprinkling systems for precipitation functioned satisfactorily at both KIM and EGIL catchments during 1995. The lack of snow beneath the roofs was as usual compensated by adding extra water (ion-exchanged with appropriate additions of seasalts and acid) during the spring.

CO2 (ppmv) 1995
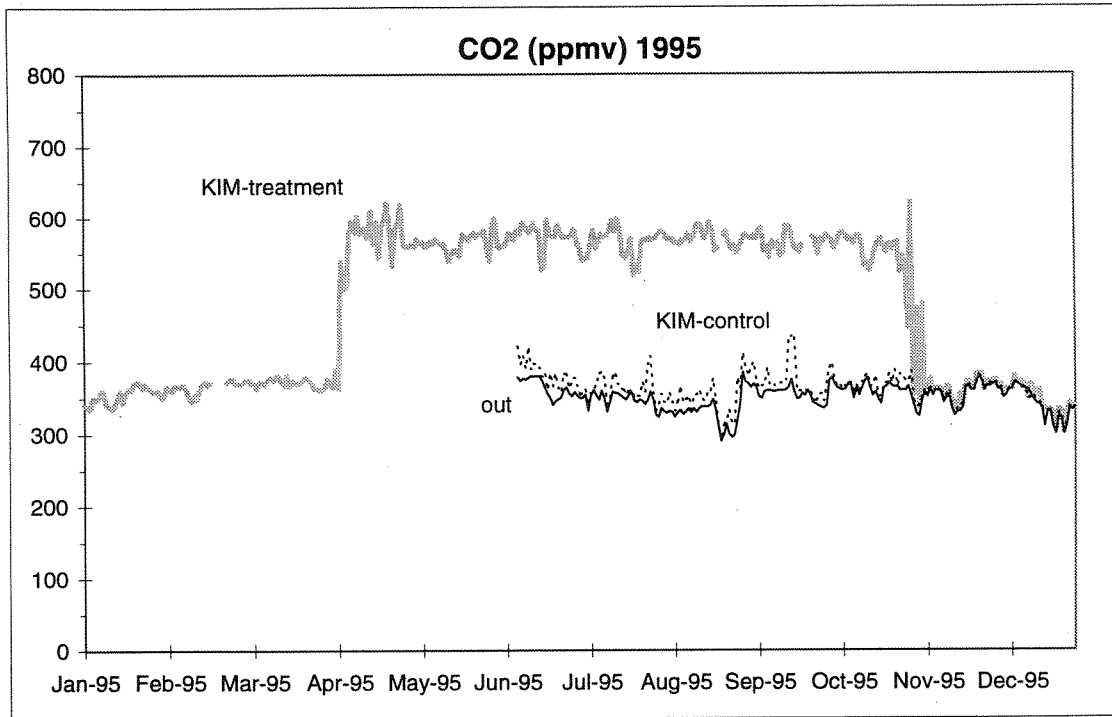
Figure 2.1 CO$_2$ concentrations (ppmv) in air at KIM treatment, KIM control and outside during 1995, the second year of treatment.
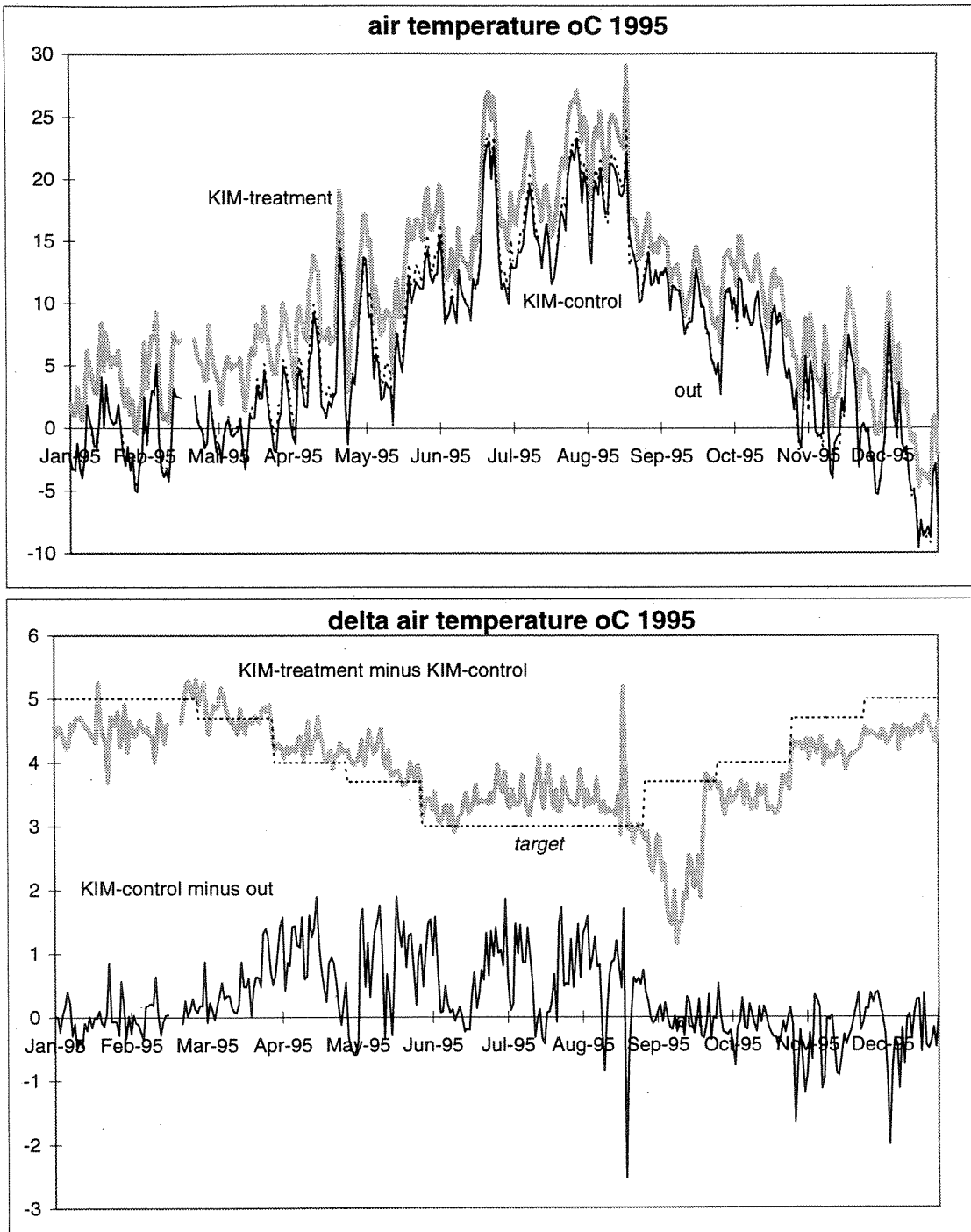
Figure 2.2 Air temperatures (°C) at KIM treatment, KIM control and outside during 1995, the second year of treatment. (a) measured temperature. (b) temperature differences and target levels.

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relative humidity (%) 1995

Figure 2.3 Relative humidity (%) at KIM treatment and KIM control during 1995, the second year of treatment.

light W/m2 1995

Figure 2.4 Light (W/m^2) at KIM treatment, KIM control and outside during 1995, the second year of treatment.

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Figure 2.5 Soil temperatures (°C) at the soil surface at EGIL treatment and EGIL control during 1995, the second year of treatment. (a) measured temperature. (b) temperature differences and target levels.

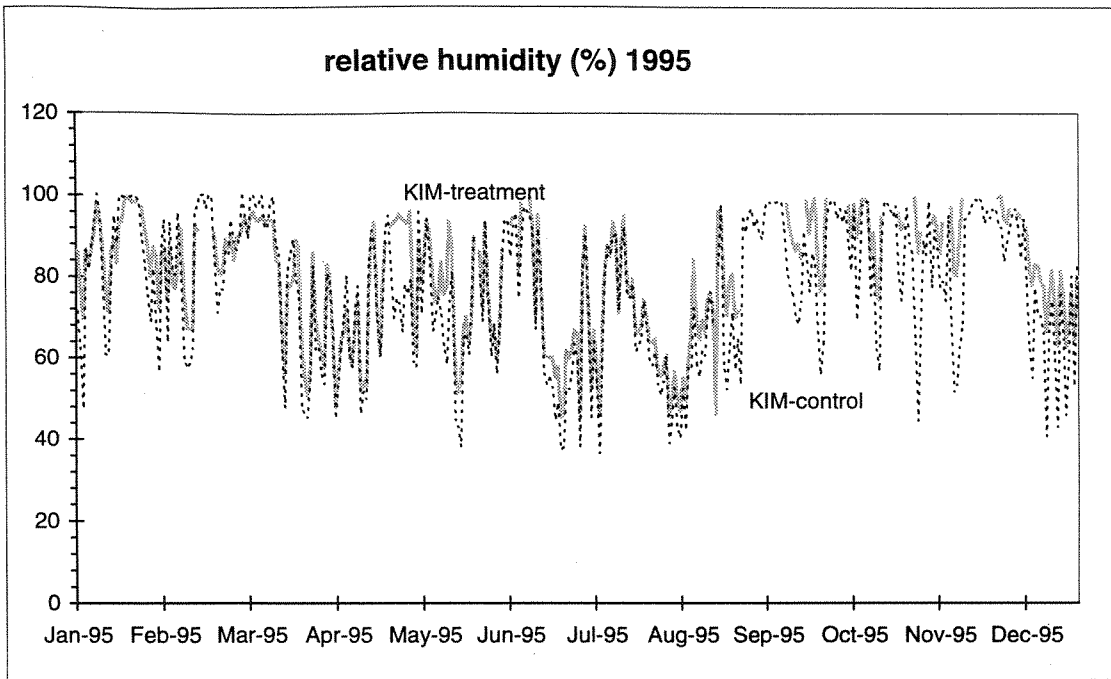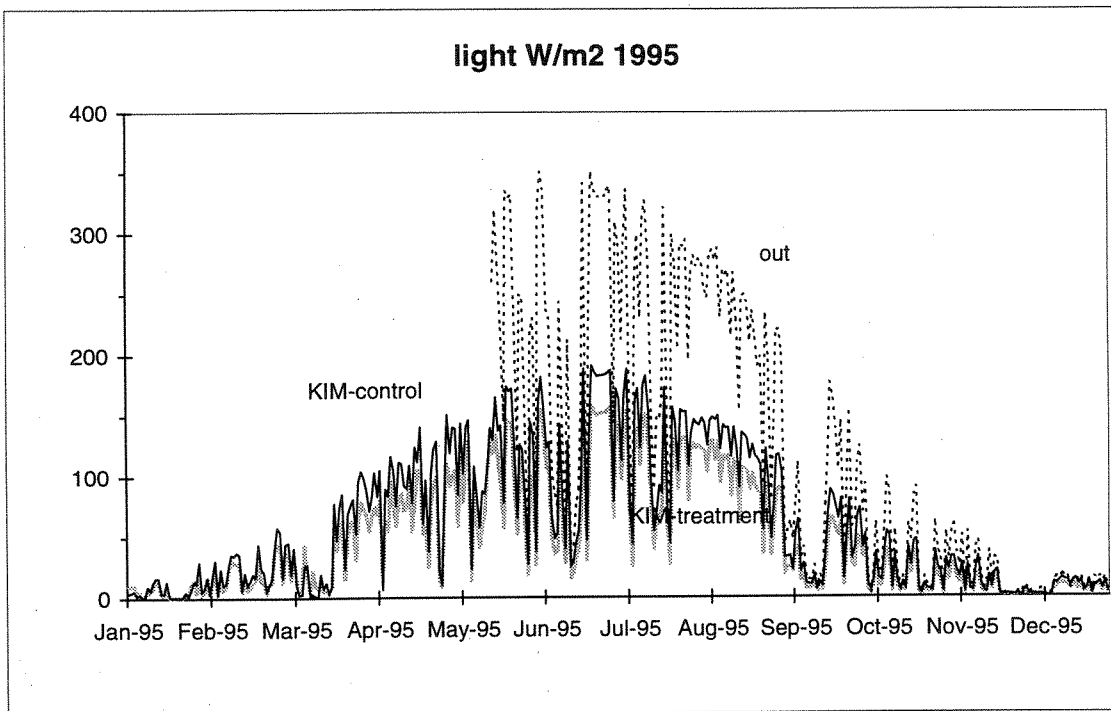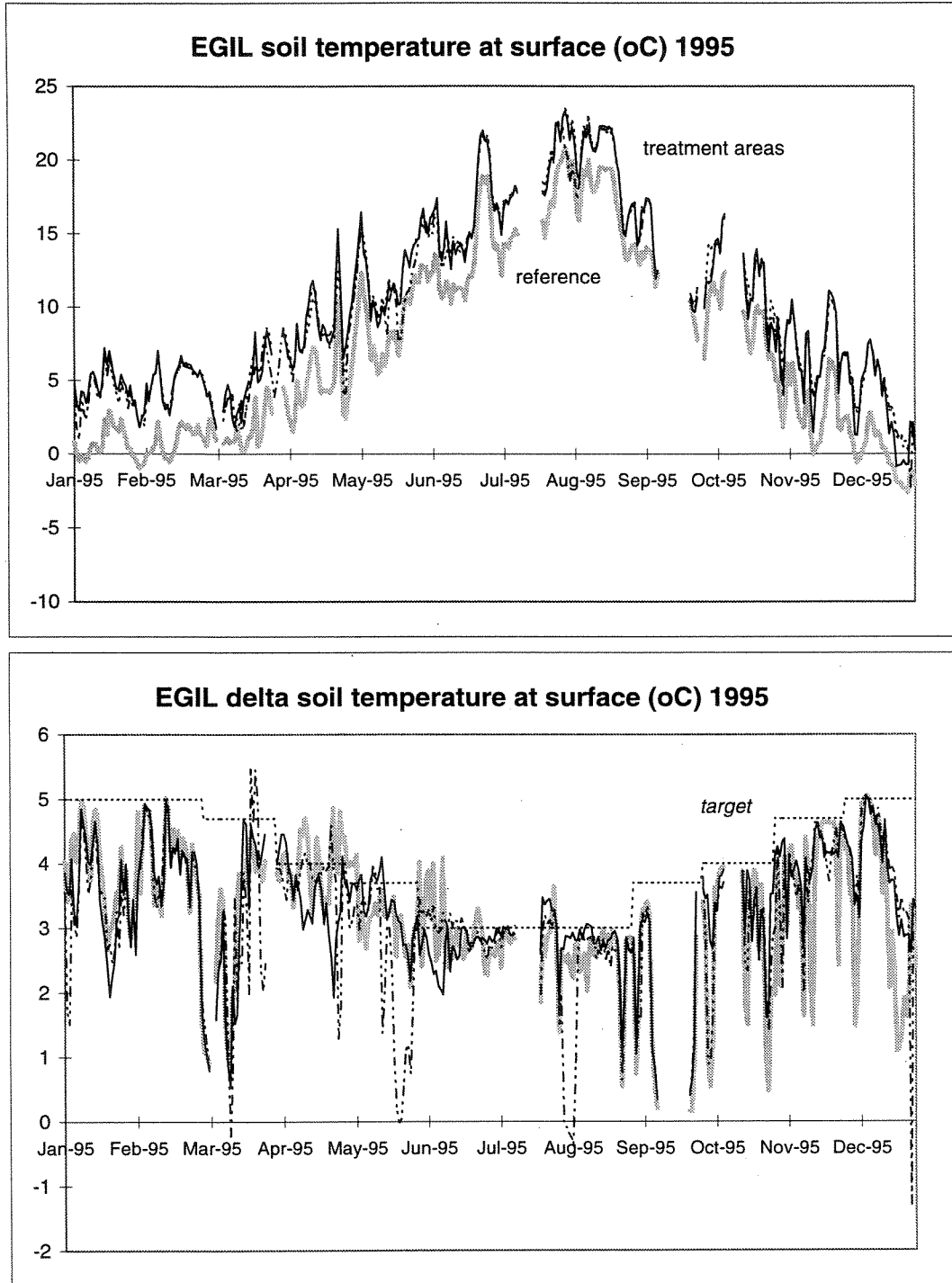# 3. Water Chemistry

Richard F. Wright and Anke Lükewille (NIVA)

Runoff samples from the 5 CLIMEX catchments were taken weekly during 1995, and water was analysed for H^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Al, NH_4^+, NO_3^-, Cl^-, SO_4^{2-}, TOC (total organic carbon), SiO_2, Tot-N (total nitrogen) and Tot-P (total phosphorous). Sampling and analysis methods are described by Wright (1995). In July and August 1995 only 1-2 samples were collected due to lack of runoff.

Nitrate and Ammonium Concentrations

At the reference catchments CECILIE, METTE and ROLF the concentrations of NO_3 and NH_4 were very similar and showed a strong seasonality (Figures 3.1 and 3.2). In January, at temperatures well above 0 °C, high NO_3 concentrations occurred related to high discharge in response to precipitation and snowmelt (Figure 3.3). Concentrations decreased during February and early March when temperatures fell below freezing, and then again increased from mid-March onwards at the start of spring snowmelt. There was another NO_3 peak corresponding to high precipitation in early May. NO_3 and NH_4 concentrations were very low at beginning of the vegetation period at the end of May and in June, even with high precipitation. High NO_3 and NH_4 values were found during the dry months July and August. High rainfall in September seems to dilute NO_3 in runoff. Peak values at high rainfall from October to December (at T > 0 °C) indicate that this "dilution" may also be caused by higher NO_3 and NH_4 availability and increased uptake by plants (and micro-organisms) until the end of the vegetation period.

At KIM-T with one exception (in February 1995), air temperatures did not drop below 0 °C (Figure 2.2), and there was no snow inside the greenhouse. In early 1995 (January to March) precipitation and runoff at KIM were lower than outside (Figure 3.4). This is because only rain (at T > 0 °C) and no snow (at T < 0 °C) was applied by means of the sprinkling system. In mid-April an amount of water equivalent to that falling as snow during the winter outside was added in the form of rain under the roof over a period of 4 weeks. During 1995 NO_3 concentrations at KIM-T did not exceed 20 mmol m^{-3}, except in July/August when they increased to about 70 mmol m^{-3} (Figure 3.1). No such peak was observed at KIM-C where the NO_3 values were in general lower than at KIM-T. NH_4 concentrations showed the same patterns (Figure 3.2).

At EGIL from January to early April the NO_3 concentrations generally followed those of the 3 reference catchments, but at a higher level (Figure 3.1). Although there was no snowmelt at EGIL (no snowcover during winter), concentrations increased during the first two weeks in March, at very low discharge values (Figure 3.4). NO_3 values were much higher until mid-May. They then decreased until June to reach the concentrations measured at the control catchments. Besides higher uptake at start of vegetation period, this decrease was certainly also a dilution effect due to additional water added at EGIL in the second half of May to

compensate for the lack of snow during the winter. Especially during the dry period in July but also in late August the NO_3 concentrations at EGIL were much higher than at the control catchments. From September to December 1995 the concentrations were similar to those at CECILIE, METTE and ROLF.

Compared to the control catchments NH_4 concentrations at EGIL were higher only in May (Figure 3.2). These high values may be caused by a concentration effect because discharge at EGIL was very low.

Organic Nitrogen and Total Carbon Concentrations

Organic N as well as total C concentrations at the control catchments followed the air (and soil) temperature curves (Figures 3.5 and 3.6). They increased with rising temperatures in spring and decreased during autumn and winter. Extreme high values at METTE and CECILIE were measured during August (almost no discharge). During the winter months Org-N and TOC concentrations are higher at KIM-T than at the controls (and KIM-C). At EGIL TOC and Org-N concentrations drop at the end of May when extra precipitation was applied (Figures 3.5 and 3.6).

Randomised Intervention Analysis

To test the significance of changes in concentrations of major elements and compounds measured in runoff, Randomised Intervention Analysis (RIA) was used. RIA compares paired, chronologically-ordered samples for pre- and post-manipulation periods from treated (EGIL, KIM-T) and untreated (ROLF, METTE, CECILIE) catchments. The changes in mean inter-catchment differences of major parameters were computed for each of 1000 random assignments. The actual mean difference between pre- and post-treatment periods was compared to the frequency distribution of mean differences obtained from the 1000 randomly ordered assignments. The catchments were paired as follows:

KIM-T	----	ROLF; METTE; CECILIE
EGIL	----	ROLF; METTE; CECILIE
EGIL	----	KIM(-T)
ROLF	----	METTE; CECILIE

Pre-treatment samples analysed within the RAIN project were paired for the period June 1991 to July 1994 (n = up to 60). 1991 was chosen because at that time a new equilibrium between clean rain input and output was established, i.e., no trends in SO_4, NO_3, ANC concentrations were observed any longer. The post-treatment period was end of July 1994 to November 1995.

RIA analysis indicates that after 19 months of treatment soil warming at EGIL catchment caused a significant increase in NO_3 and NH_4 concentrations in runoff (Figures 3.7 and 3.8).

Increases in inorganic N concentrations at EGIL were the only significant changes in runoff chemistry at the CLIMEX catchments up through the end of 1995.

Although air warming at KIM-T greenhouse leads also to higher soil temperatures, RIA does not indicate significant changes in N concentrations in runoff. At KIM catchment, however, RIA is not a very strong statistical text because the absolute concentrations at KIM are very low compared with the reference catchments; this is due to the clean rain treatment at KIM and not at the other catchments. A large change in N concentrations at KIM comprises a relatively small change in the difference between KIM and the reference catchments.

Due to enhanced CO_2 and temperature treatment at KIM-T, photosynthetic activity and plant productivity have increased (see chapters 5 and 6). At the ecosystem level this has probably been accompanied by higher N uptake during the prolonged vegetation period at KIM-T. Growth of *Vaccinium* plants, for example, has increased at KIM-T after start of treatment and the N concentrations in stems and leaves have not changed (see chapter 6).

Total N input was reduced by about 90% in 1984 when the clean precipitation treatment at KIM started (RAIN project). The catchment thus went from N-saturated (about 30% of input N was lost in runoff prior to 1994) to N-limited in 1994 when the CLIMEX manipulation began. At all other experimental catchments at Risdalsheia a significant fraction of the N input is lost in runoff as NO_3 and NH_4.

Runoff from EGIL-C alone (upper 20% of the catchment) is not sampled due to lack of suitable sampling point. At KIM-C sampling began only in May 1994; there are no pre-treatment values available. Thus, RIA analysis cannot be applied at KIM-C. Whether the observed differences in NO_3, Org-N and TOC between KIM-T and KIM-C are due to temperature and CO_2 manipulation will be tested by trend analyses (e.g., non-parametric Seasonal Kendall test) as soon as sufficient post-treatment data are available.

References

Wright, R.F. 1995. Input-output budgets. In: Dise, N.B. and Jenkins, A. (Eds) 1995. The CLIMEX Project: Whole Catchment Manipulation of CO_2 and Temperature. Climate Change Research Report 3/95, Norwegian Institute for Water Research, Oslo, 130 pp.
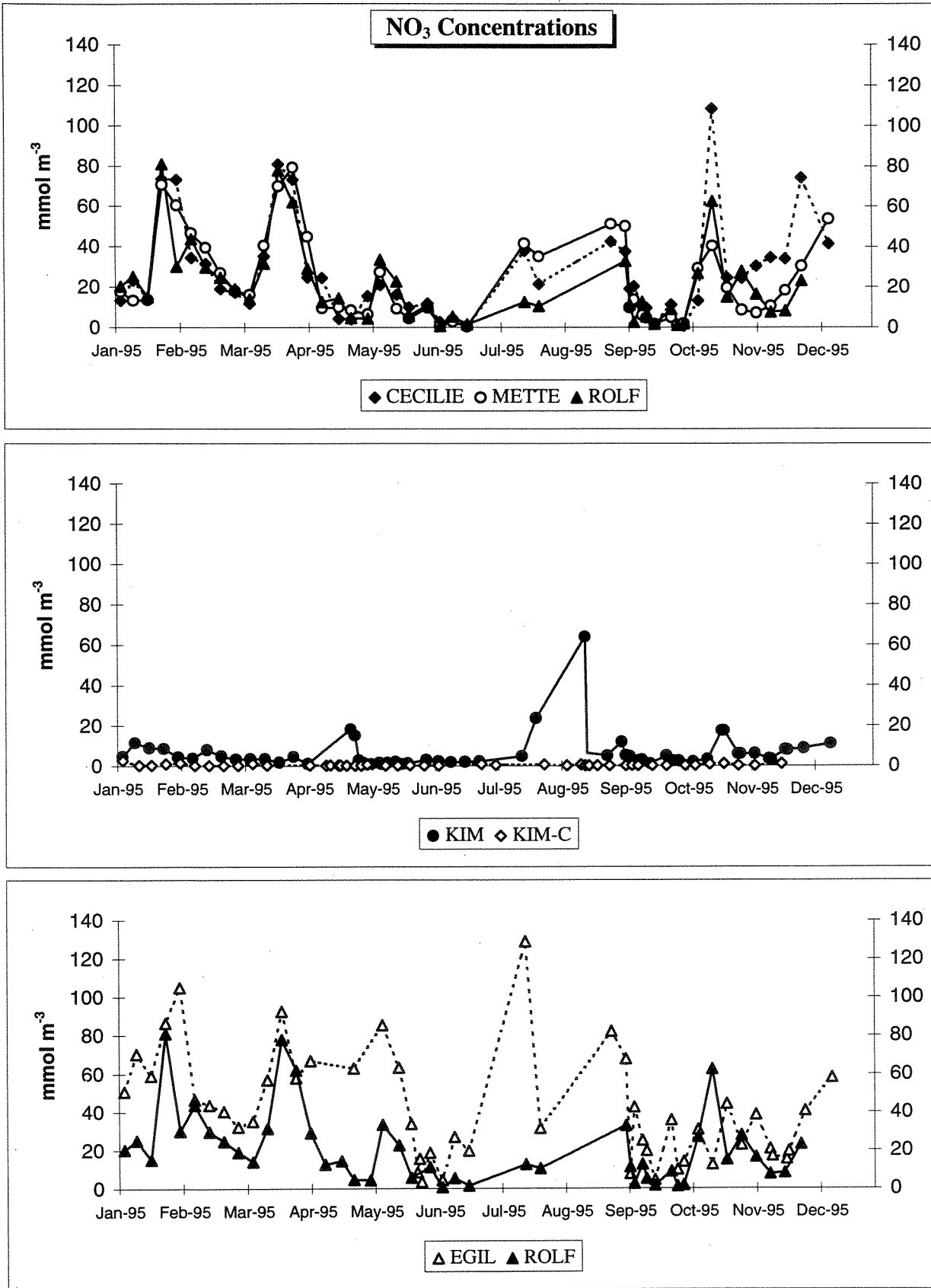
Figure 3.1 Nitrate concentrations at control and treated catchments
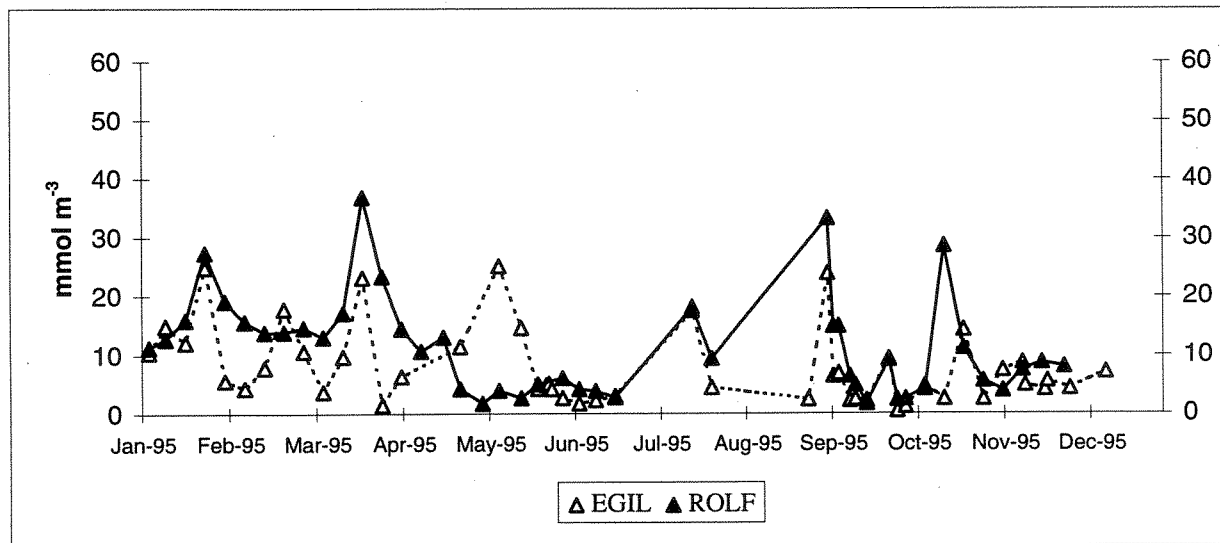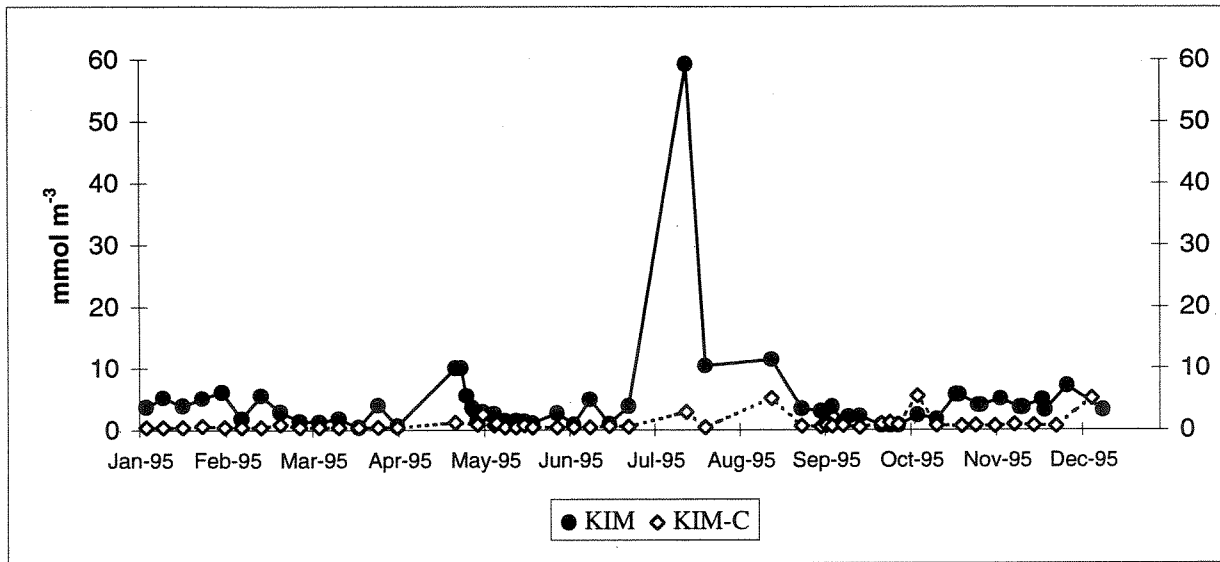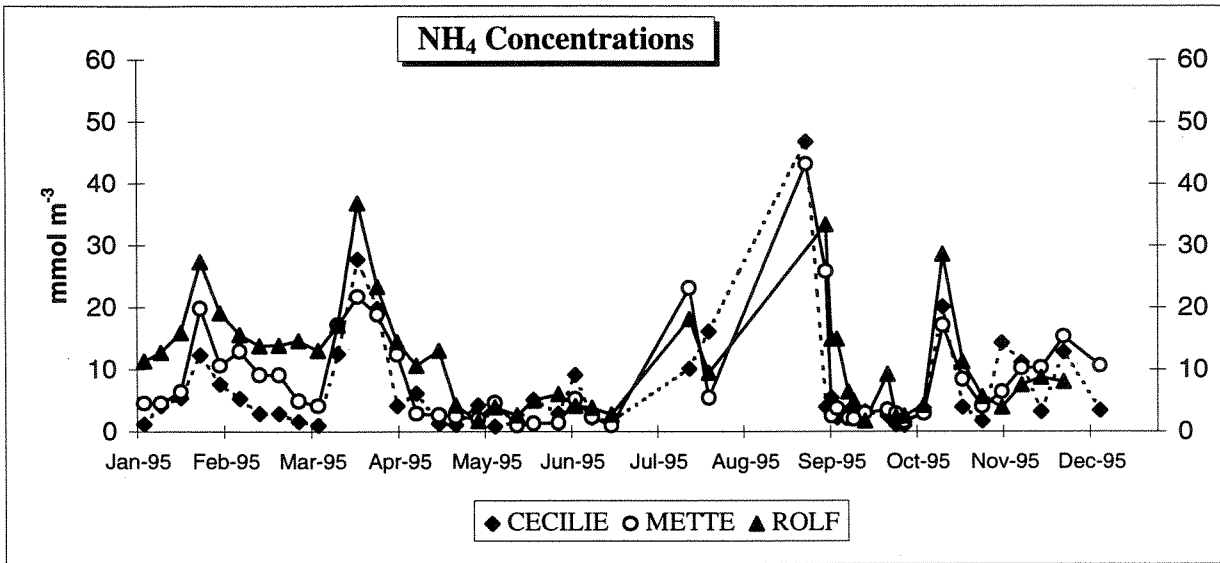
Figure 3.2   Ammonium concentrations at control and treated catchments
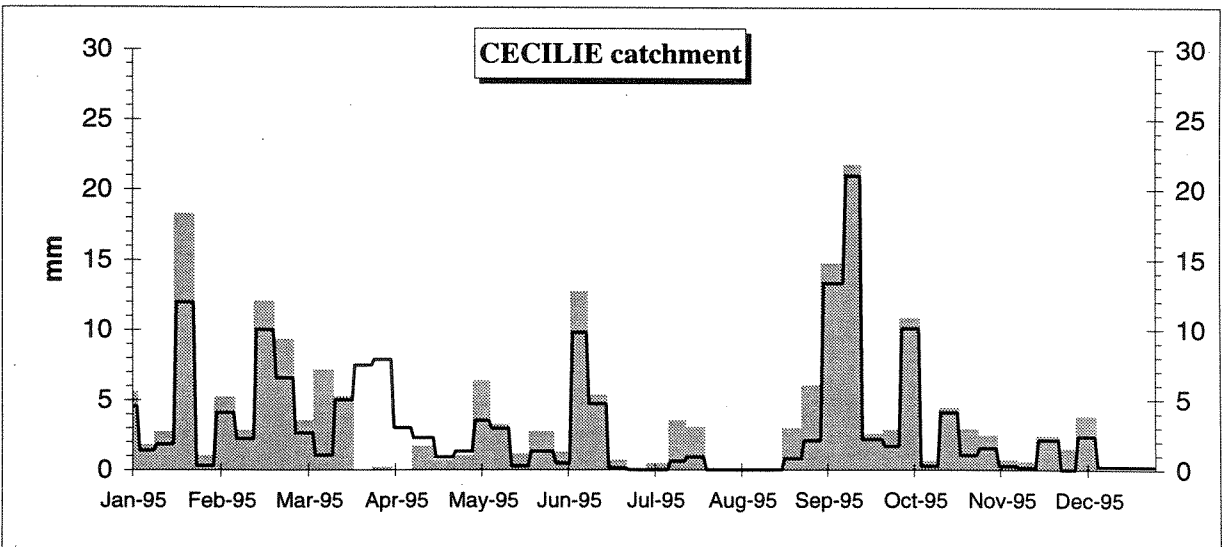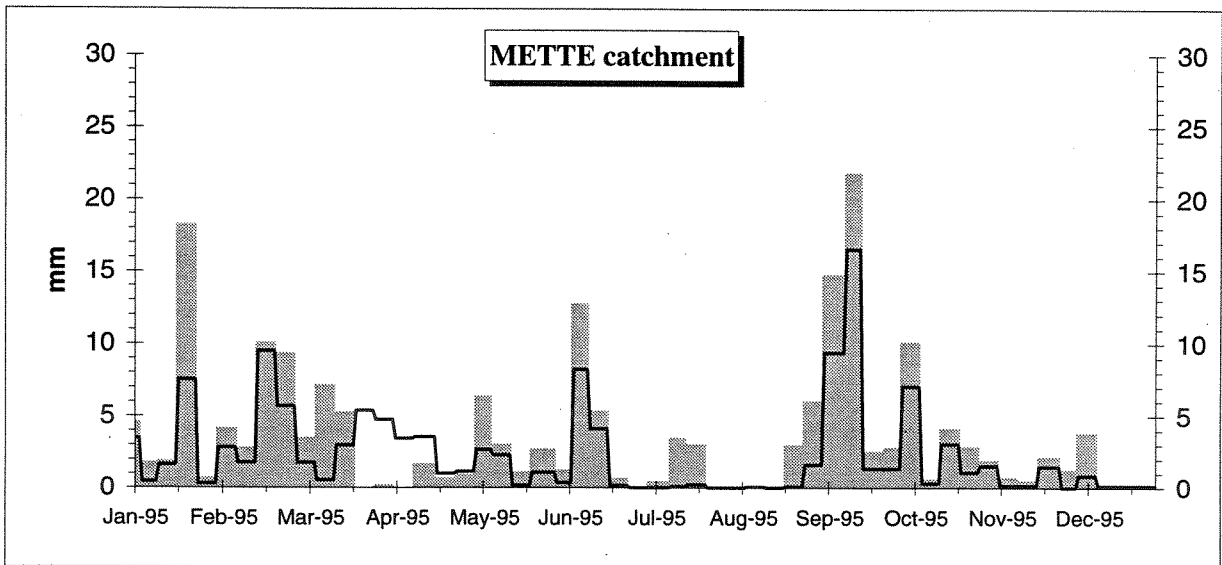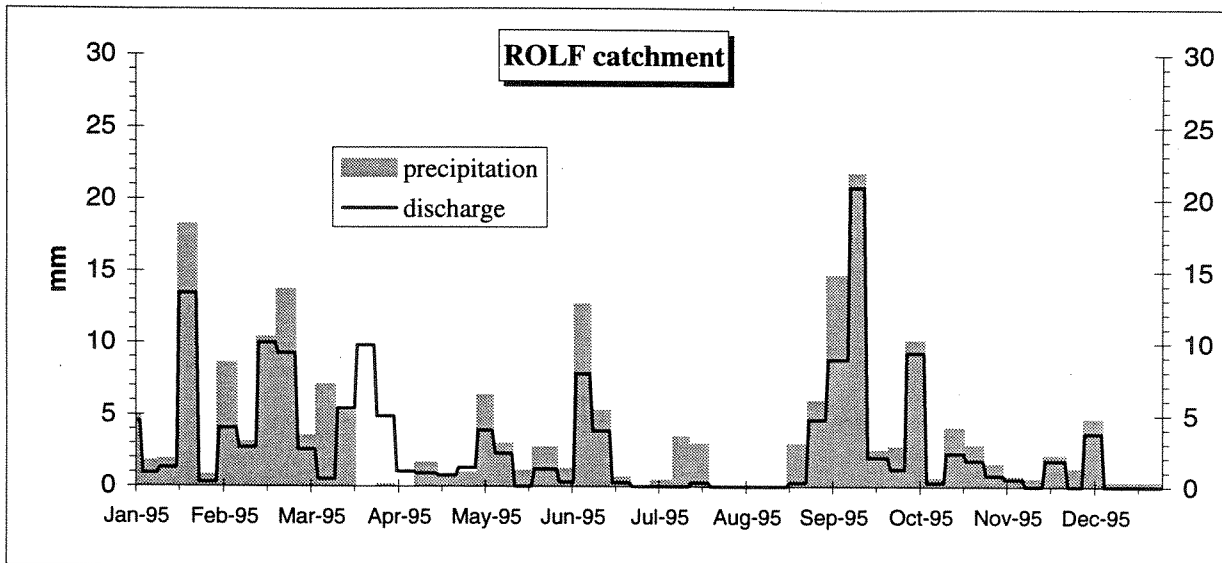
Figure 3.3   Daily precipitation and discharge at the CLIMEX control catchments during 1995

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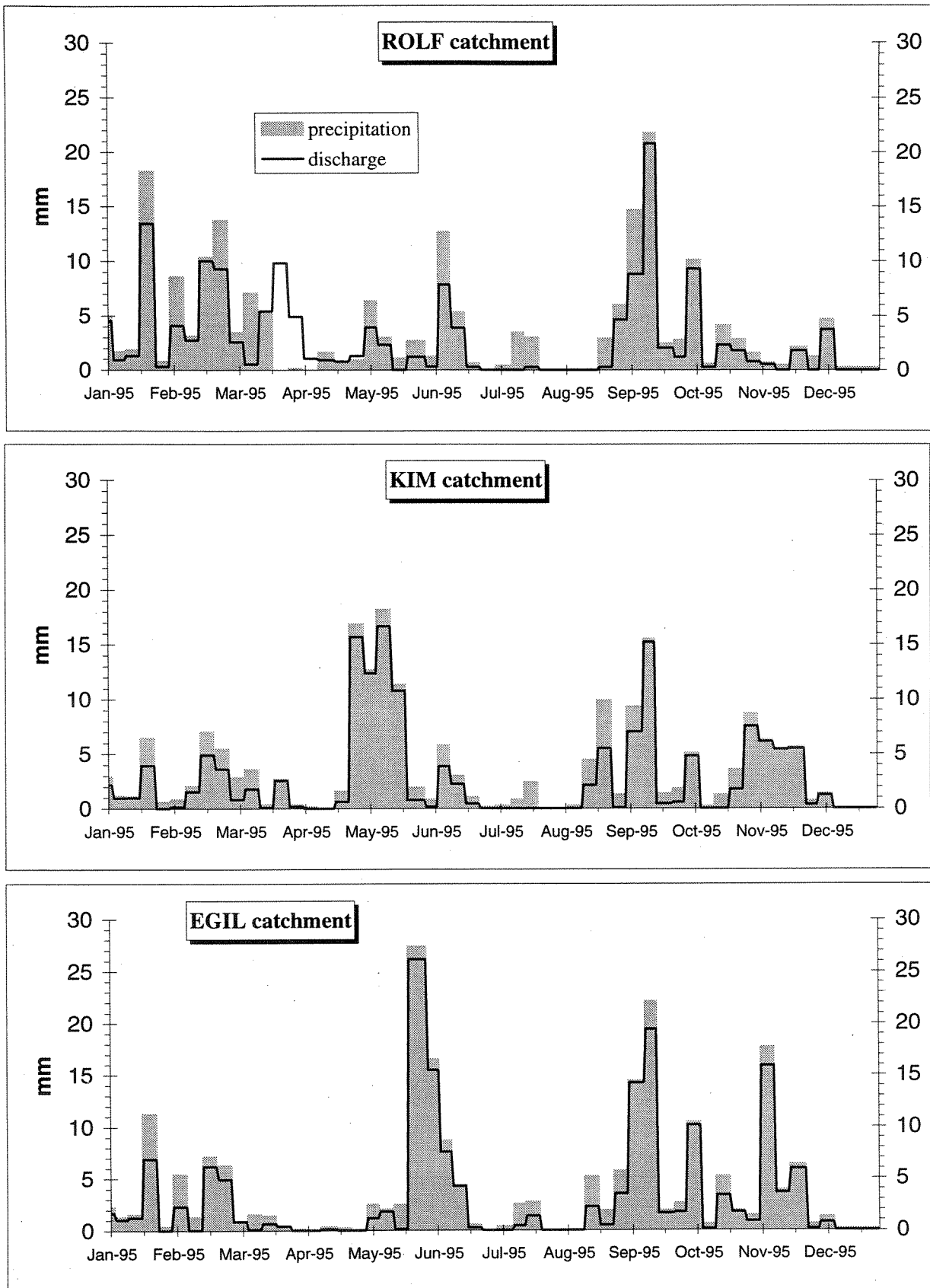
Fig. 3.4 Daily precipitation and discharge at ROLF, KIM and EGIL during 1995.

Figure 3.5   Organic nitrogen concentrations at control and treated catchments

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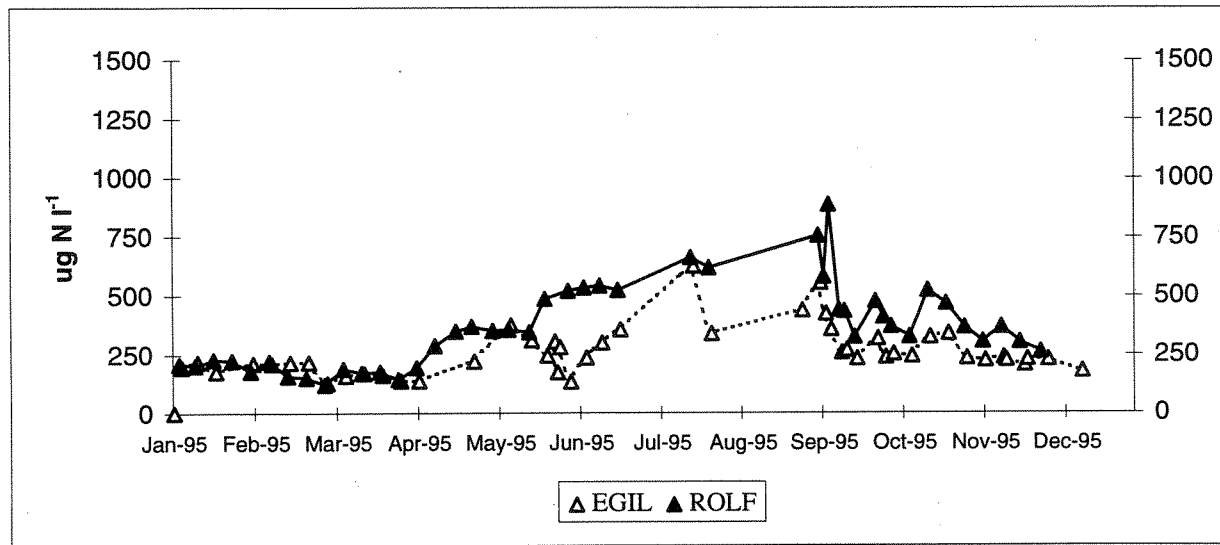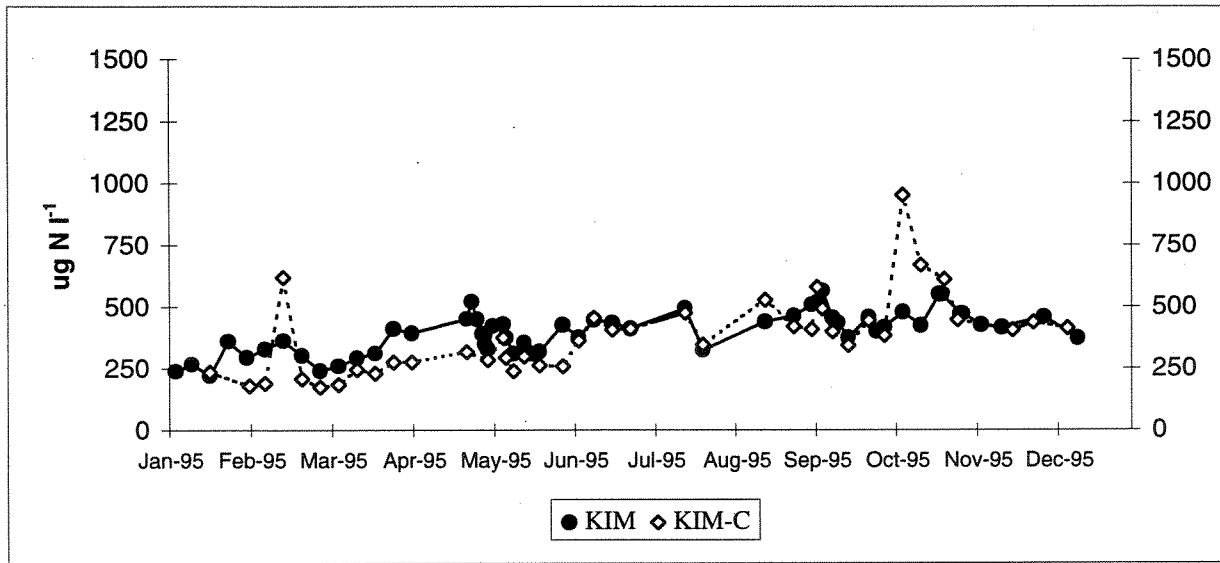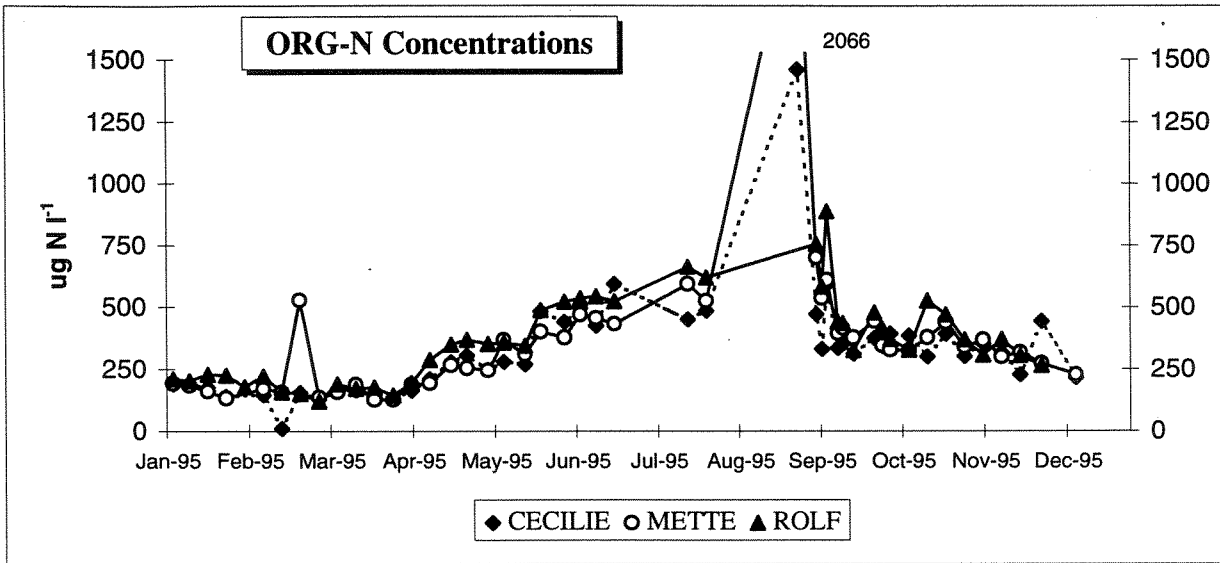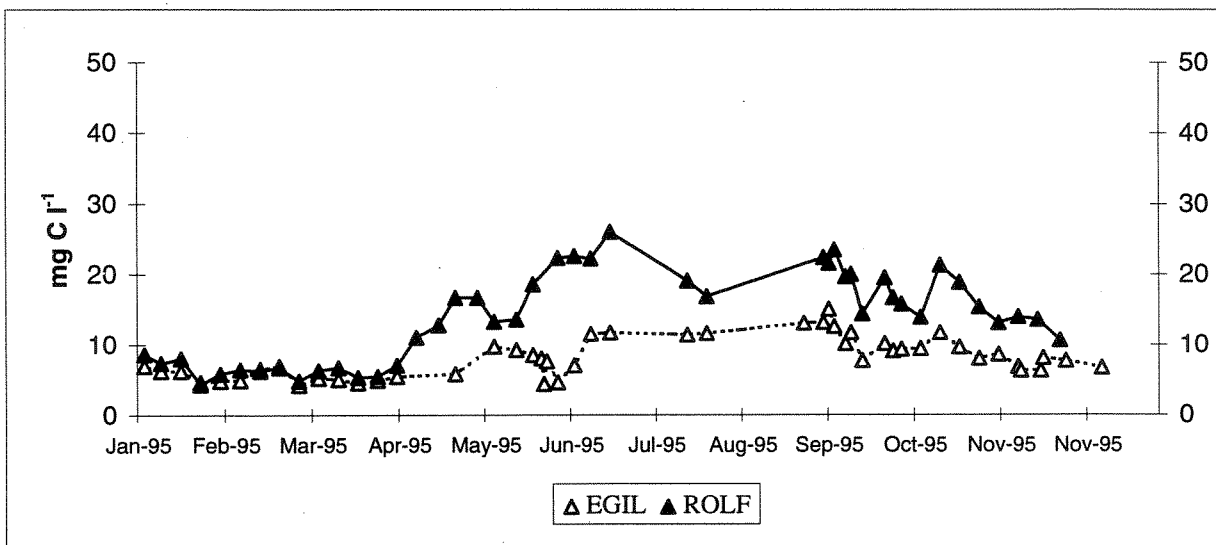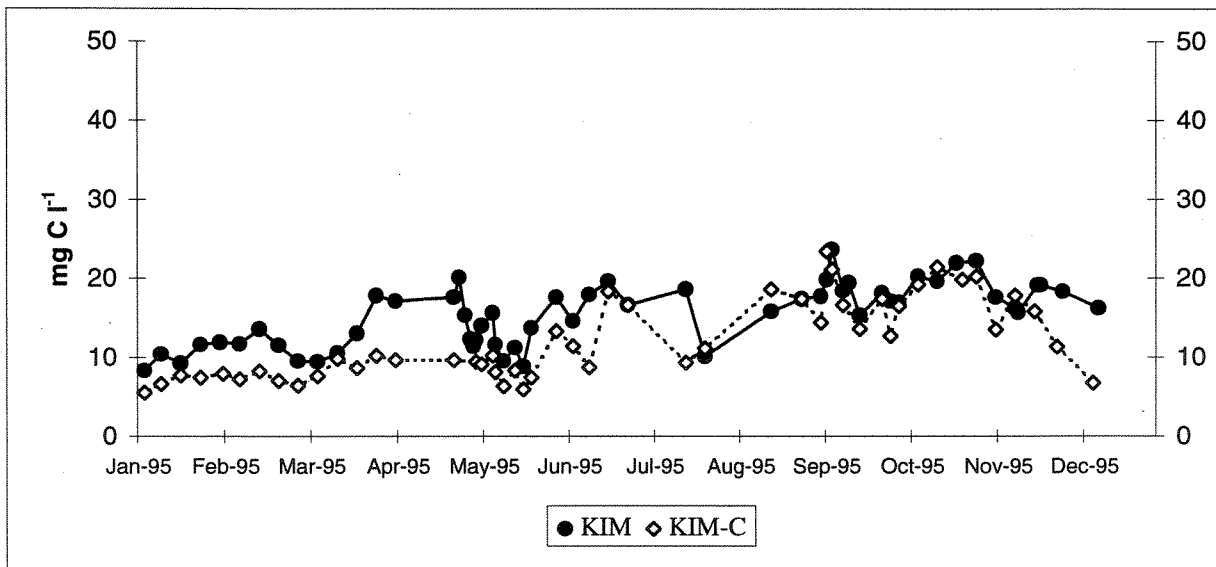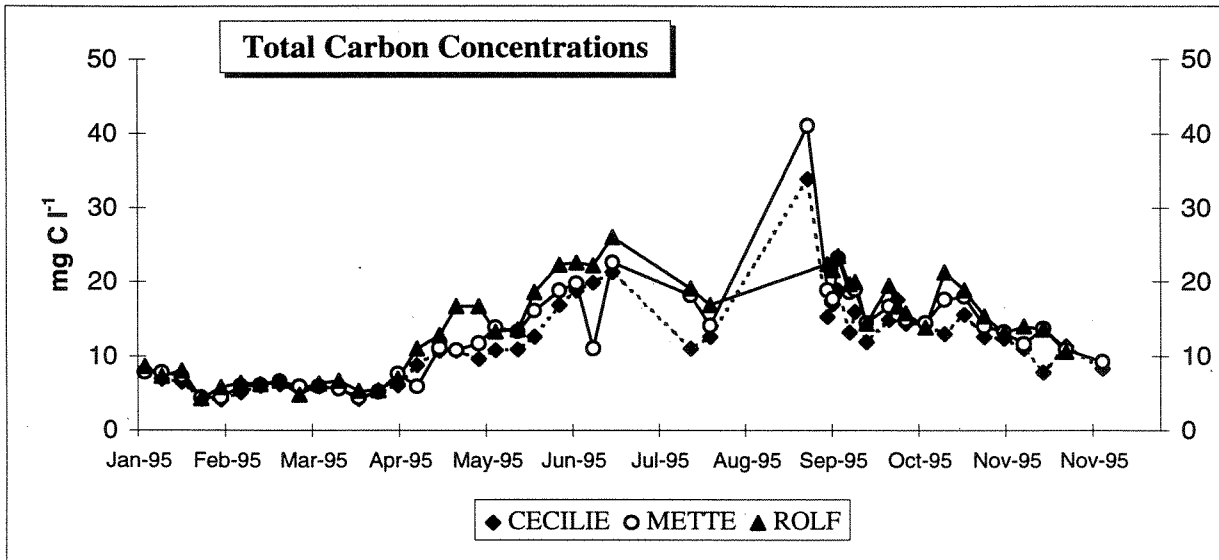**Figure 3.6** Total carbon concentrations at control and treated catchments
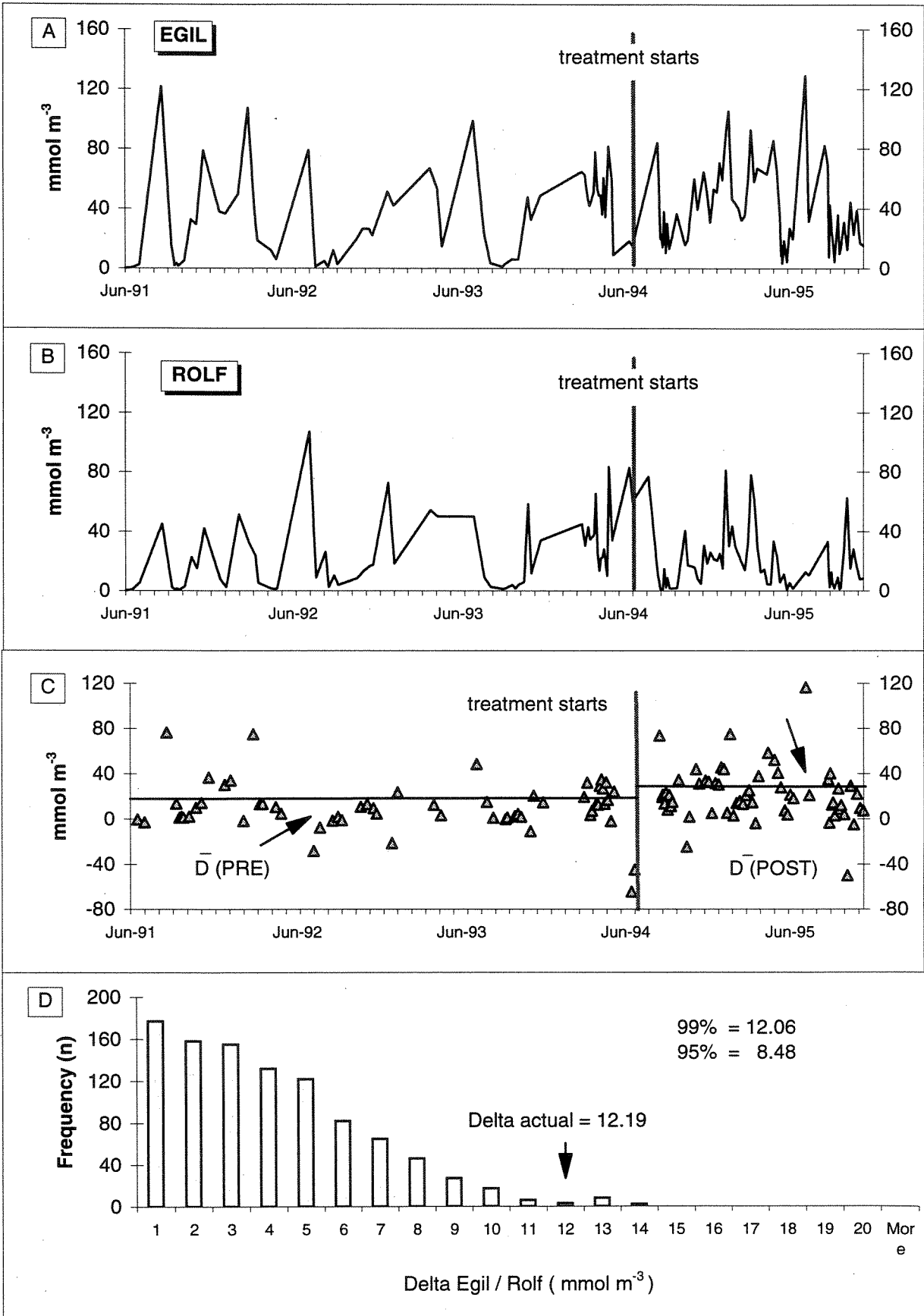
Figure 3.7 Randomised intervention analysis: nitrate concentrations at EGIL and ROLF
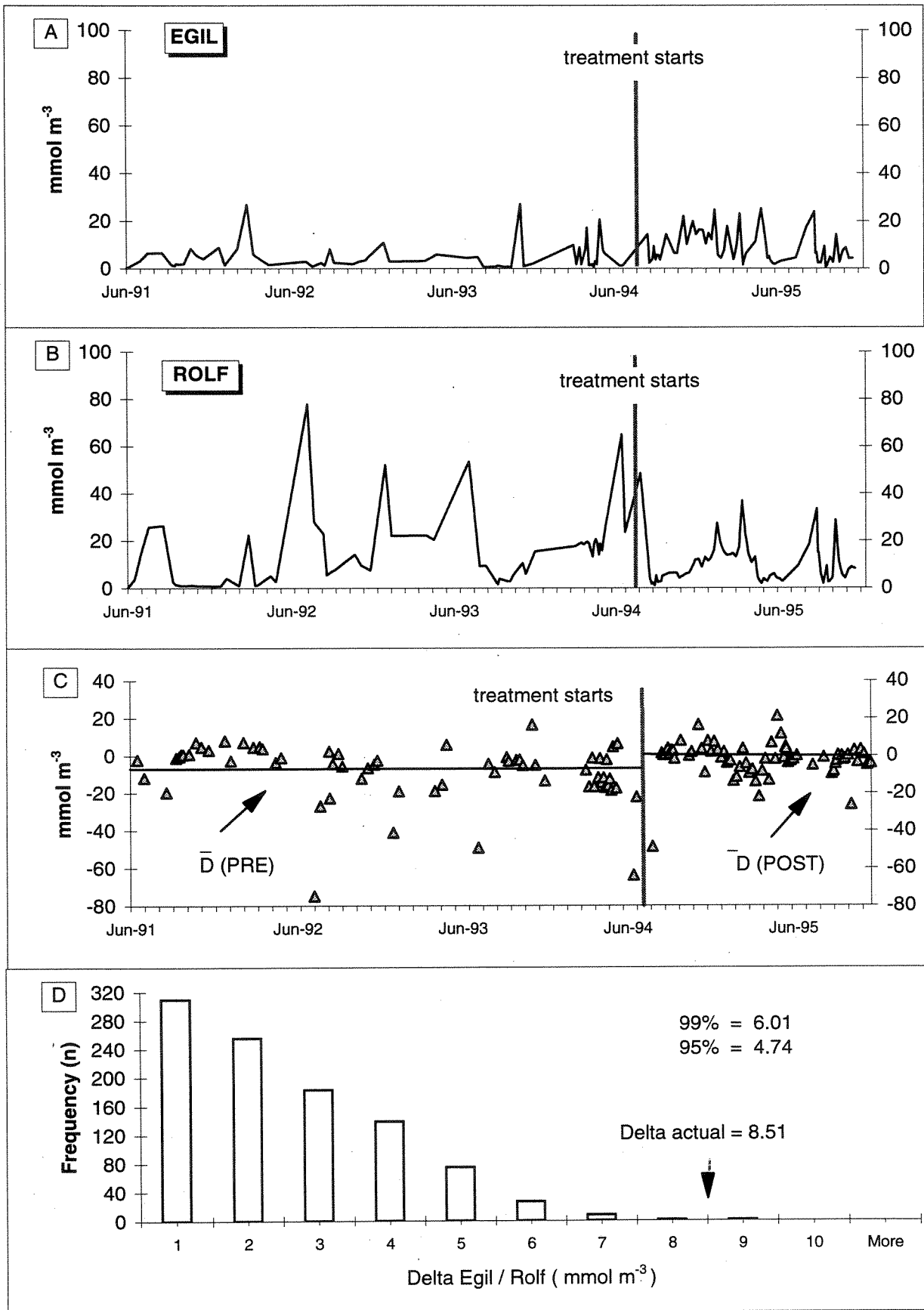
Figure 3.8 Randomised intervention analysis: ammonium concentrations at EGIL and ROLF

4. Soil Water Responses

Rob Collins and Alan Jenkins (IH)

Tdr time series data for 1995 (Figure 4.1) indicate that a relatively high soil moisture level was maintained in KIM until late June. This was followed by a rapid drying resulting in extremely low volumetric water contents, less than 0.03 m^3 m^{-3}, during early August. Soil moisture contents then rose again following the onset of rainfall in late summer. This behaviour is supported by data derived from the pressure transducer (Figure 4.2), which indicates that the head of water above the bedrock, in the proximity of the instrument, fell to zero in early July. The period following this, indicated by a steady reading of c.70mm, reflects instrument malfunction caused by the the extremely low moisture content of the soil. The head of water during these weeks is likely to have been negligible. Fluctuations of the water table in response to rainfall in late summer and autumn are evident.

Twenty four probes were installed in EGIL during August 1995, located predominantly in the deeper pockets of soil (Figure 4.3). Tdr measurements were undertaken for 5 months to determine if soil moisture differs significantly in comparison to KIM and to provide data for hydrological modelling purposes.

Table 4.1 summarises the data available from the soil moisture monitoring programme in KIM and EGIL for 1995.

In August 1995, a further tracer experiment was undertaken in KIM. Rainfall dosed with a known concentration of Lithium Bromide was sprayed onto the catchment in hydrological steady steady state with respect to inputs and outputs. The recovery of bromide in runoff and soil solution enabled assessment of the flow contribution from pre event (old) soil water and rainfall (new) water. In addition, dominant flowpathways were identified and soil water residence times were determined. Initially runoff is dominated by old water, (Figure 4.4) but mixing of new and old water in the soil surface layers results in an increasing new water contribution with time, such that after 15 hours of tracer injection, new water contributes c.60% to the flow. Analysis of soil water recovered from lysimeters at varying depths indicates that the organic soil surface layers act as the dominant flowpathway for rainwater through the catchment but that a significant pathway also exists at the soil-bedrock interface. The dominant flowpathways and runoff mechanisms in KIM are illustrated in Figure 4.5.

Table 4.1 Soil Moisture data availability (PT indicates Pressure Transducer)

Catchment & (Instrument)	Period	Temporal Resolution	Notes
Kim (Tdr)	09/05/95 to 31/08/95	12 hours	sensors 19-29 no data
Egil (Tdr)	01/09/95 to 31/01/95	4 hours	sensor 2 no data
Kim (PT)	02/06/95 to 22/10/95	Hourly	Error at low soil moisture
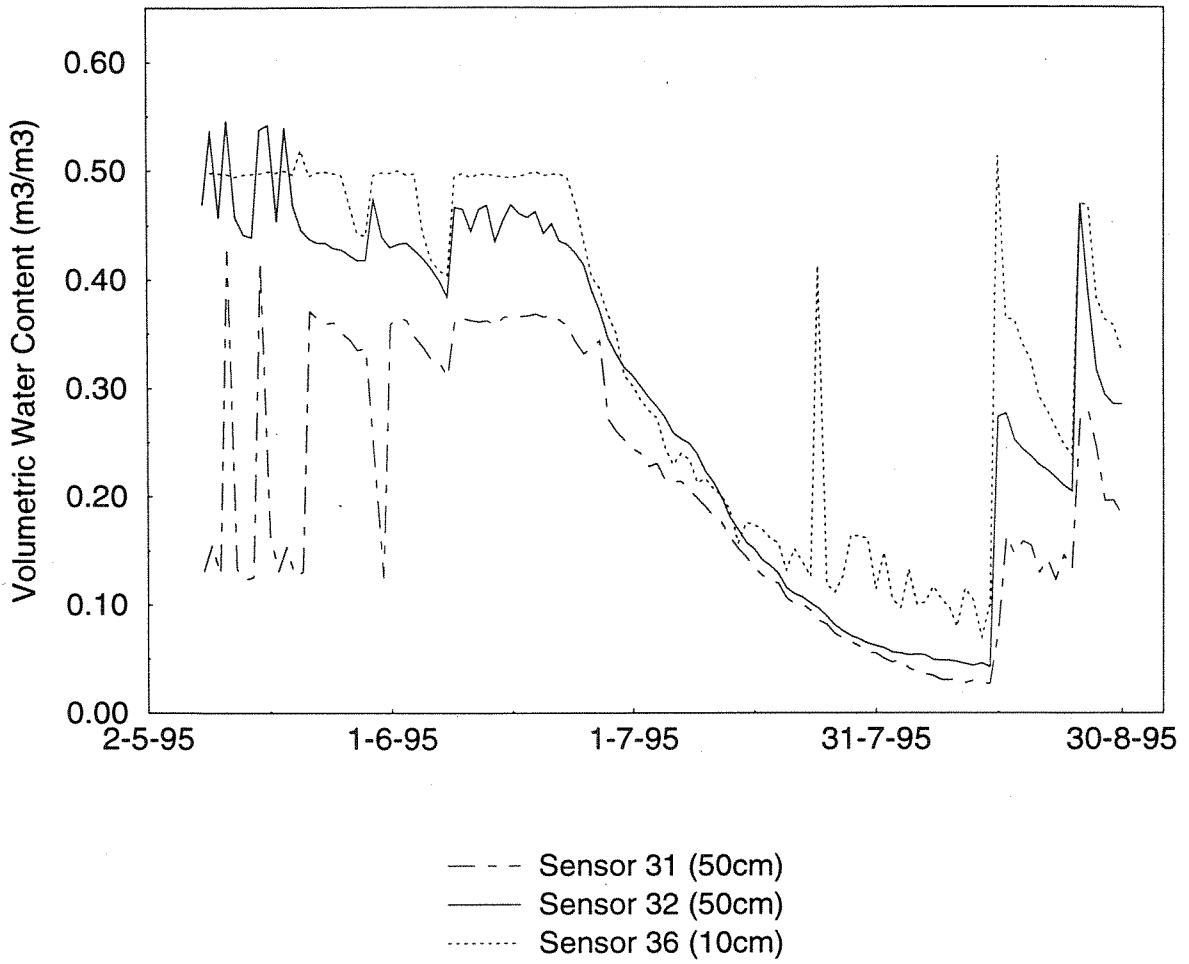
Figure 4.1 Daily Tdr data
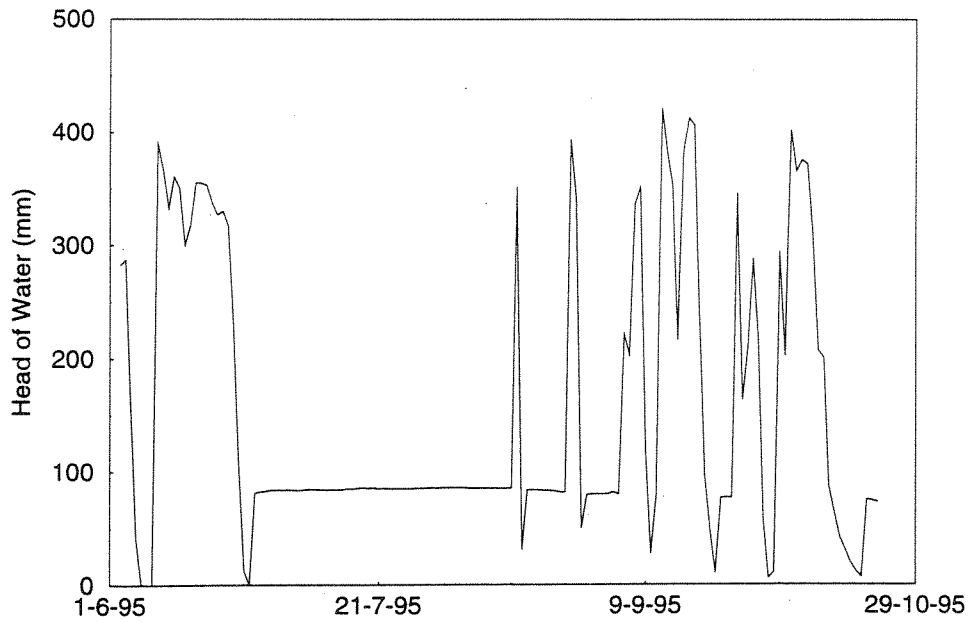
Figure 4.2 Daily Pressure Transducer data
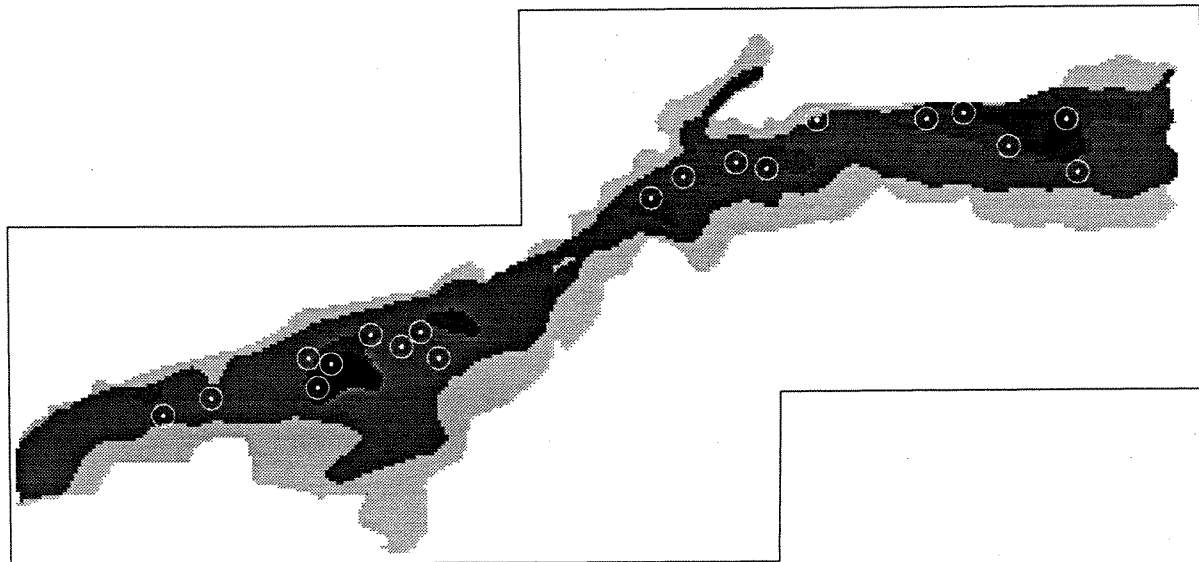
Figure 4.3 Soil Depth and Tdr probe location

Bromide Trace
Kim catchment - August 1995

$$C = -1.01 \times 10^{-4} + 1.06 \times 10^{-5}\, Q - 2.04 \times 10^{-10}\, Q^2 + 9.78 \times 10^{-16}\, Q^3$$
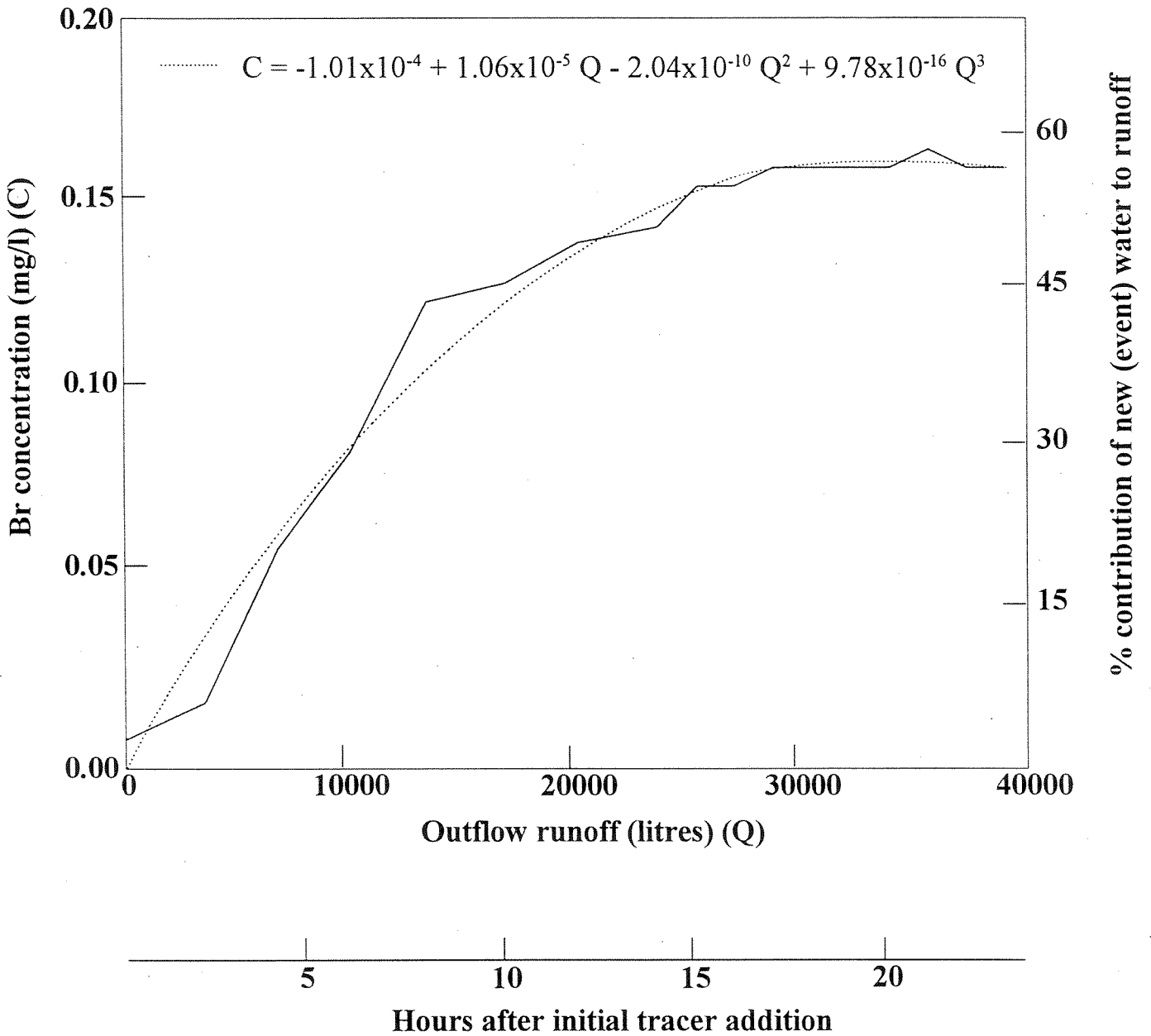
Figure 4.4 Bromide trace, Kim Catchment, August 1995

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Kim catchment - Runoff mechanisms

60% new water contribution to runoff at steady state

Surface layers (0-15cm) 70% new water rapid flow

Zone of mixing
Deep water (predominantly old) mixes with surface flow

Soil-rock interface flow

Slow flow down through soil matrix
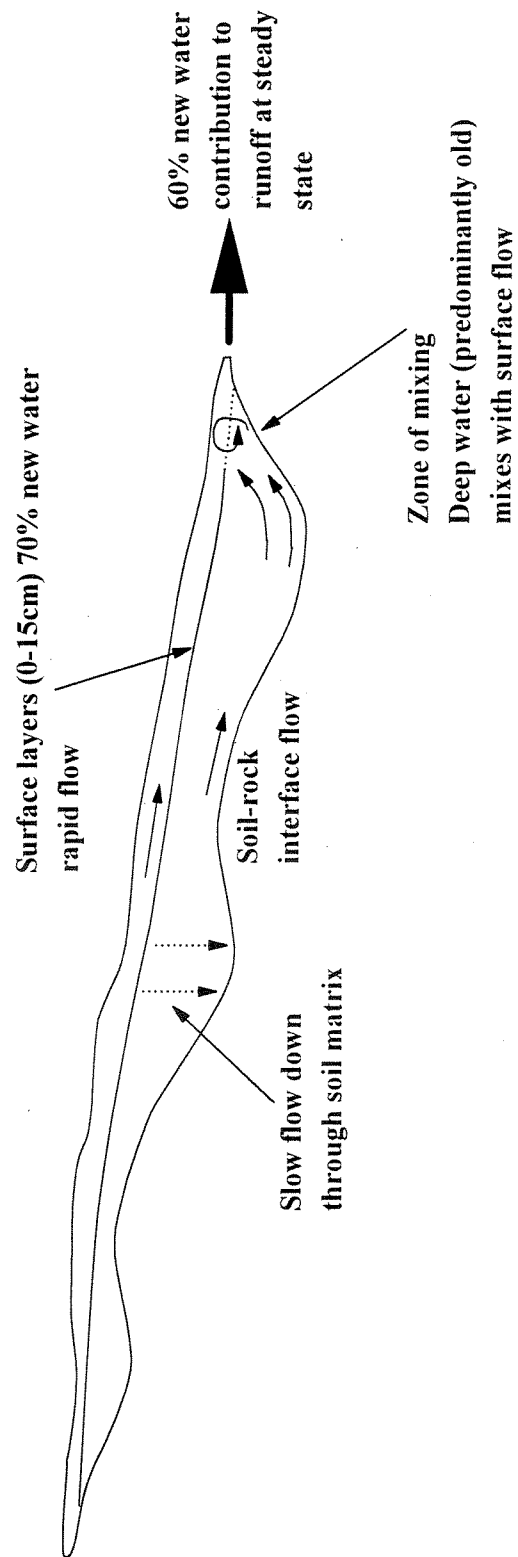
Figure 4.5 Kim Catchment Runoff Mechanisms

5. Decomposition of Soil Organic Matter

Paul Verburg and Nico van Breemen (WAU-SSG)

INTRODUCTION

The amount of C present in soils is estimated to be 1580 Gt. The net uptake of C by vegetation is 61.4 Gt yr^{-1} whereas the release of C through respiration is 60 Gt C yr^{-1} (Schimel, 1995). Changes in decomposition rate of Soil Organic Matter (SOM) due to climate change might significantly influence the net exchange of CO_2 between the atmosphere and the land surface which now involves a sequestration of 1.4 Gt C yr^{-1}. An increase in temperature could affect decomposition by stimulating microbial activity (Swift et al., 1979) and by changing soil moisture levels due to increased evapotranspiration.

An increase in atmospheric CO_2 could affect decomposition by increasing litter production due to increased Net Primary Production particularly of C_3 plants (Mooney et al., 1991). Elevated CO_2 may also cause changes in composition of the litter formed although the effect appears to depend on nutrient status of the soil as well as plant species. It is, however, likely that if CO_2 influences the chemical composition of litter, decomposition rates will also be affected (Coûteaux et al., 1992). Elevated CO_2 causes a decrease in the amount of water transpired per unit biomass. Depending on the absolute increase in biomass, moisture conditions in the soil may change.

In many temperate ecosystems, plant growth is limited by the availability of N. Due to increased decomposition more N could be mineralized and consequently stimulate plant growth. Nitrogen that is not taken up by plants may nitrify to nitrate which can leach in runoff and cause eutrophication and acidification of surface waters.

The different hypotheses discussed above will be tested using field and complementary laboratory studies.

FIELD STUDIES

Decomposition of fresh litter

We investigated the influence of temperature and substrate quality on the decomposition rate of fresh litter under field conditions by incubating litter in litterbags. In 1993, young birch trees (*Betula pubescens*) were grown at either 360 or 700 ppmv CO_2. Litter produced by these trees was collected and analyzed for C, N and lignin. The high-CO_2 litter had an average C/N ratio of 88 whereas the C/N ratio of the low-CO_2 litter was 67. The lignin content was 157 and 164 mg/g dry matter for the high- and low-CO_2 litter respectively. In April 1994, we incubated both high-CO_2 and low-CO_2 litter in the treated and control parts of KIM and EGIL. Unfortunately

in 1993 not enough litter was produced to incubate both low- and high-CO_2 litter in all catchment sections. Mesh size of the litterbags was 1.5 mm. We incubated the litterbags 2 years. A first pilot experiment with litter from *Pinus sylvestris* suggested that decomposition rates were significantly higher in KIM and EGIL than outside the roofed catchments. No difference in decomposition rate was found between litter incubated under *Vaccinium myrtillus* or *Calluna vulgaris*. A second pilot experiment showed that before the start of the treatments, the decomposition rate was higher in the treatment section of KIM (KIM-T) than in the control section (KIM-C) although the difference was not statistically significant. No distinction was made between the control and treatment section of EGIL.

Both after one and two years of incubation, we found no statistical difference in mass loss between the high- and low-CO_2 litter (Table 5.1). In EGIL, mass loss in the soil-heated part (EGIL-T) was lower than in the control part (EGIL-C). The most likely cause is that the litter dried out due to contact with the heating cables causing a decrease in decomposition rate. At the time of sampling, litterbags from the soil heated part usually had the lowest moisture content. In KIM, mass loss after two years appeared to be higher in the treatment part although the difference was not significant.

From 1995 to 1996, a second experiment was executed with litter from the same birches after two years of exposure to elevated CO_2. The C/N ratio of the low- and high-CO_2 litter was 90 and 79 respectively. Both in KIM and METTE the high-CO_2 litter appeared to decompose fastest although differences are not always statistically significant (Table 5.1). Again, in EGIL-T decomposition rates are lower than in EGIL-C. In this experiment, also in KIM-T mass loss is smaller than in KIM-C although not statistically significant.

The experiments with birch litter show no clear effect of CO_2 treatment on decomposability of the litter. Coûteaux et al (1991) found increased decomposition for litter produced at elevated CO_2 when a complete food web was added. In this case the difference in C/N ratio between low- and high-CO_2 litter was much larger than in our experiment both after one year and two years exposure to elevated CO_2. Cotrufo et al. (1994) found a reduced decomposition of birch litter produced at high CO_2. However, they found a highest C/N ratio of 53 under elevated CO_2 which is still much lower than the C/N ratio found in any of our treatments.

N mineralization

Increased decomposition might result in increased mineralization of N depending on C/N ratio of the substrate. We measure N mineralization by incubating undisturbed soil cores in the field (Raison et al., 1987). Soil cores are incubated in plots dominated by either *Calluna vulgaris* or *Vaccinium myrtillus* in both treatment and control sections of KIM and EGIL as well as in the reference catchment METTE. The measurements are done over four periods: May-June, June-August, August-October and October-May.

Under *Calluna vulgaris*, the annual N mineralization in KIM-T increased whereas no increase was measured in KIM-C (Table 5.2) in the treatment year. Both in EGIL-T and EGIL-C mineralization remained unchanged. In the pretreatment year, mineralization was already much

higher in EGIL-T compared to EGIL-C. The most likely cause for this difference is the larger soil depth and consequently higher moisture level in EGIL-T. The lack of a clear treatment effect is in contrast with data showing an increase in NO_3 in runoff (Lükewille and Wright, in press). However, the mineralization studies suggest that nitrification has increased in the treatment section eventhough net mineralization remained the same. Under *Vaccinium myrtillus,* mineralization in KIM-T increased during the treatment period. In KIM-C, however, mineralization almost doubled from 1993 to 1994. Measurements in the outside reference catchment METTE suggest that mineralization did not increase between the two consecutive years. Therefore, the observed increase in mineralization in KIM-C is probably by chance. Measurements will continue until August 1996 in order to have two full treatment years.

Table 5.1. Mass loss of birch litter

Site	Litter type	Mass loss (mg/g)[1]		
		1 year exposure	2 year exposure	
		1 year[2] 2 years[2]	1 year[2]	
EGIL-C	low CO_2	370 (100) a,b[3] 450 (60) a[3]	430 (40) a	
	high CO_2	380 (90) a	470 (90) a	390 (90) a,b
EGIL-T	low CO_2	350 (110) a,b[3] 330 (90) b[3]	380 (80) a,b	
	high CO_2	290 (80) b	330 (60) b	350 (80) b
KIM-C	low CO_2	520 (80) a[3] 500 (90) a[3]	450 (70) a,b	
	high CO_2	500 (90) a	520 (90) a	490 (50) a
KIM-T	low CO_2		350 (170) b	
	high CO_2	490 (90) a[3]	580 (50) a[3]	460 (100) a,b
METTE	low CO_2		390 (60) a	
	high CO_2			470 (30) b

[1] Standard deviation in parentheses (n=10). Different letters indicate significant differences for each incubation time within each catchment at the 0.1 level of significance.
[2] Incubation time
[3] n=6

Soil solution chemistry

From 1992 to 1995, we have taken soil solution samples in all catchments. In KIM and EGIL, samples were taken at five locations and in METTE at three locations at four to five times each year. At each location, lysimeters were installed at 5, 15 and 25 cm depth. No clear treatment effects could be deduced from the lysimeter data. In EGIL, already before the start of the treatment NH_4 or NO_3 concentrations were higher in the treatment section than in the control part (Table 5.3) which is consistent with the N mineralization data. In addition, two tracer experiments conducted in October 1994 and August 1995 showed that concentrations of

elements change rapidly during rain events (Collins et al., in prep). Therefore, comparison of samples taken in different years becomes difficult since hydrological conditions may vary between years. In combination with the data obtained from the tracer experiment, lysimeter data will be used to evaluate the regulation of Al release from mineral and organic phases.

Table 5.2. Annual N mineralization in pretreatment (1993-1994) and treatment (1994-1995) year.

Catchment	Section	Vegetation	N mineralization (g m^{-2})	
			1993-1994	1994-1995
KIM	control	*Calluna*	4.0 (0.8)[1]	4.2 (0.6)
	treatment		2.9 (0.7) 4.4 (0.7)	
	control	*Vaccinium*	2.1 (0.4) 3.9 (0.6)	
	treatment		4.1 (0.8) 5.8 (0.6)	
EGIL	control	*Calluna*	3.6 (0.4) 4.5 (0.6)	
	treatment		6.8 (1.0) 6.5 (1.3)	
METTE		Calluna	4.4 (0.7) 4.4 (0.5)	
		Vaccinium	6.2 (0.9) 6.2 (0.7)	

[1] Standard error in parentheses (n=10).

Table 5.3. Nitrogen concentrations in soil solution in EGIL catchment in two pretreatment (1993 and 1994) and one treatment period (1995) (concentrations in µeq l^{-1})

Site	Depth (cm)	April 1993			April 1994			April 1995		
		NH$_4$	NO$_3$	Cl	NH$_4$	NO$_3$	Cl	NH$_4$	NO$_3$	Cl
	5	4	0	472	8	13	191	0	0	295
EGIL-C	15	1	0	531	0	0	237	0	0	340
	25	3	1	507	1	0	249	1	0	326
	5	34	85	491	19	54	126	28	88	213
EGIL-T	15	9	40	484	8	47	134	2	65	220
	25	6	25	422	2	32	131	1	57	234

Organic matter turnover

Turnover times of organic matter can be calculated using the stable isotope ^{13}C when ^{13}C content of the litter changes for instance due to a change from forest (C$_3$ species) to grass (C$_4$ species) (Balesdent et al., 1987). In KIM we use the isotope signal from ^{13}C depleted CO$_2$ added in the high CO$_2$ treatments to trace the fate of plant litter in the soil. Each autumn, samples of litter (L) and fermentation (F) layer in the soil as well as fresh litter were taken in all

catchments in the different plots for ^{13}C analysis. Indeed for most species the fresh litter samples show a lower ^{13}C content in the treatment section (Table 5.4, a decrease in δ^{13}C means a decrease in ^{13}C content). For *Pinus sylvestris* the difference between control and treatment section are smaller since litter has partly been formed in the years before the start of the treatment.

Table 5.4. Δ^{13}C values of fresh litter in 1995 (‰)

Vegetation		KIM-C	KIM T	METTE
Calluna vulgaris		-28.7	-35.1	-26.4
Betula pubescens	-28.2	-34.6	-28.2	
Pinus sylvestris		-25.2	-27.4	-25.4
Vaccinium myrtillus		-25.2	-36.3	-28.7

In the soil, only in the litter layer under *Vaccinium*, a decrease in δ^{13}C could be detected (Table 5.5). One more sampling will be carried out in the autumn of 1996.

Table 5.5. Δ^{13}C values of L and F layer in 1995 (‰)

Vegetation	Layer	KIM-C	KIM-T	METTE
Calluna vulgaris	L	-26.7 (0.5)[1]	-26.0 (0.6)	-27.2 (0.7)
	F	-25.9 (0.3)	-25.5 (0.3)	-27.1 (0.6)
Vaccinium myrtillus	L	-25.8 (0.4)	-27.2 (1.0)	-27.4 (0.4)
	F	-26.5 (1.1)	-26.0 (0.3)	-27.4 (0.3)

[1] standard deviation in parentheses (n=5)

LABORATORY STUDIES

C mineralization in isolated soil columns

Most of the C fixed by plants is ultimately released to the atmosphere as CO_2 due to decomposition. In the field, the contribution of CO_2 coming from decomposition is difficult to estimate due to interference by CO_2 from root respiration. CO_2 produced by decomposition alone can be estimated by using soil columns without vegetation. Undisturbed soil columns taken from outside the catchment under *Calluna vulgaris* have been incubated at 5, 10 and 17°C to look at the effect of temperature on C mineralization. Five replicates were used at each temperature. The columns are 60 cm long and 16 cm wide. The CO_2 emission was measured

continuously by capturing CO_2 with soda-lime (Edwards, 1982). Soil solution and outflow chemistry as well as soil moisture were measured weekly.

The results point to increased C loss at increased temperature both as CO_2 and Total Organic Carbon (TOC) in drainage water due to increased decomposition (Table 5.6). At the same time more nitrate occurs in the leachate resulting in increased acidification of the drainage water. At low pH, inorganic Al is mobilized. The C and N data will be used to validate the SOILN model (Johnsson et al., 1987) in order to link the C and N turnover to hydrology and temperature.

Table 5.6. Average values for selected parameters in soil column experiment.

Parameter	Unit	Temperature		
		5°C	10°C	15°C
CO_2 emission	$gCO_2 column^{-1} week^{-1}$ 0.85	1.18	1.69	
pH outflow	-	4.52	4.53	3.96
TOC outflow	$MG\ l^{-1}$	3.7	3.7	6.0
Al^{n+} outflow	$\mu eq\,l^{-1}$	143	242	484
NO_3 outflow	$\mu eq\,l$	120	288	344

Gross N fluxes in organic surface horizons.

An increase in N mineralization is likely to favour plant growth. It is not clear how the microbial population will respond to an increase in temperature in these N limited systems. In soils, both plants and microbes compete for N. We hypothesized that an increase in temperature would result in an increase in both gross mineralization and immobilization of N in the absence of plants. This hypothesis was tested by adding ^{15}N in combination with a numerical algorithm to calculate gross mineralization and immobilization from data on microbial biomass, respiration, extractable inorganic N and $^{15}N/^{14}N$ ratios. The calculations showed that in the litter layer both gross mineralization as well as immobilization increased at elevated temperature. However, net mineralization (=gross mineralization - immobilization) did not increase. In the fermentation layer, gross mineralization and immobilization did not increase at elevated temperature (Figure 5.1). Based on C/N ratio of the microbial biomass we concluded that the microbial population in the L layer was dominated by fungi whereas bacteria were dominant in the F layer.
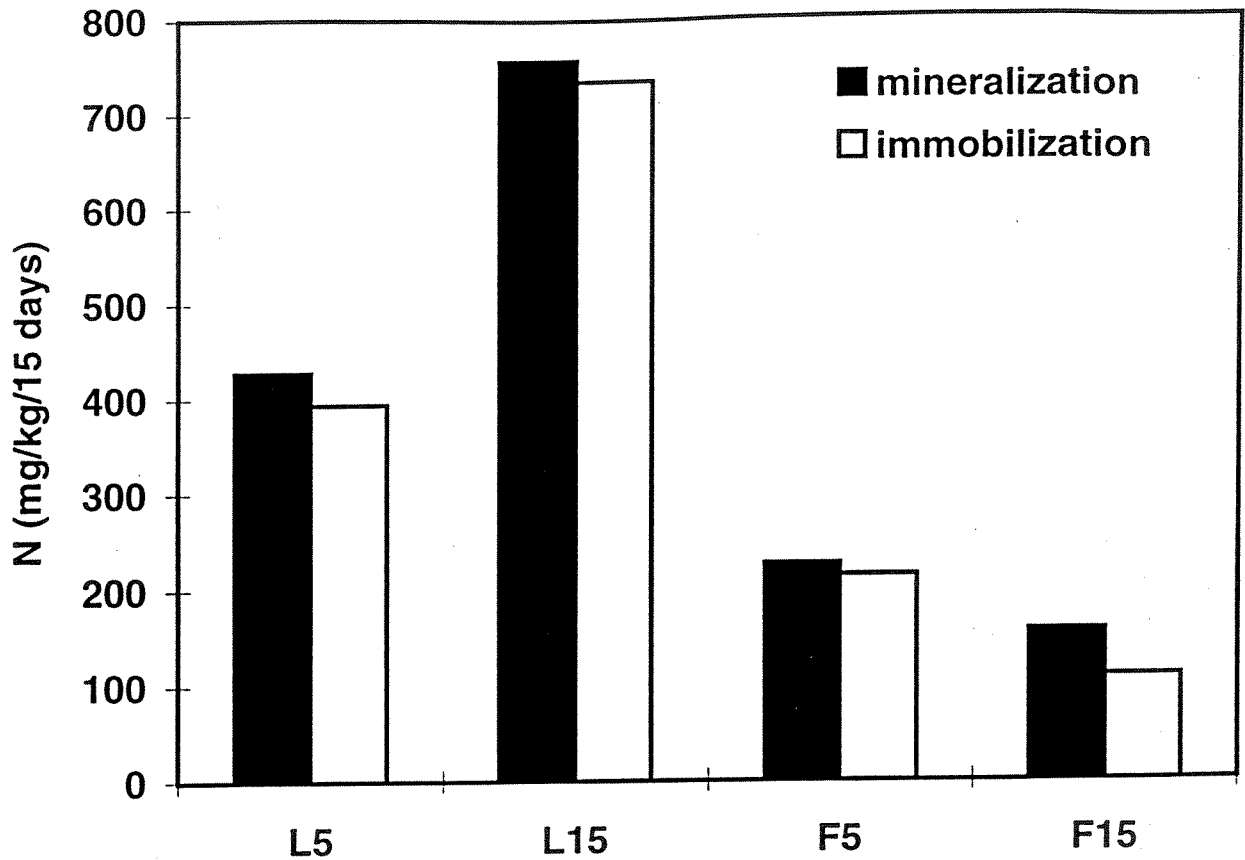
Figure 5.1. Calculated gross $NH4^+$ fluxes in litter (L) and fermentation (F) layer at 5 and 15 degrees Celsius.

Below ground C inputs

In a pot experiment we will use the pulse labelling technique with ^{14}C (Keith et al., 1986) to quantify the change in belowground C inputs for *Calluna vulgaris* as a function of CO_2 and N. Young seedlings will be grown at ambient and elevated CO_2. In each CO_2 treatment also a low and high N treatment will be included. In the same experiment we will investigate competition for N between microbes and plants by injecting ^{14}C labelled glucose in the rhizosphere. We expect the fastest glucose decomposition where demand for N by plants is lowest; i.e. at low CO_2 and high N levels. Slowest glucose decomposition will occur at high CO_2 and low N.

DATA EVALUATION

The data obtained from the different experiments will be used to evaluate the possible long term effects of climate change on SOM. Various models have been developed to describe SOM dynamics. The CENTURY model (Parton et al., 1987) is probably the most well-known example. Due to a poor description of the relationships between biological processes and substrate quality these models are probably too rigid to describe long term effects of climate change (Van Breemen and Van Dam, 1993). The NICCCE model (Van Dam and Van Breemen, 1995) might provide the necessary refinement to describe the effects of climate change. In this model composition of plant litter is considered in terms of carbohydrates, proteins, hemicellulose and lignin contents. Added is the explicit simulation of microbial biomass with a variable C/N ratio. SOM is divided in three pools having different turnover times as often used in other models (Van Veen et al., 1985, Parton et al., 1988, Hsieh, 1992). NICCCE also allows for description of ^{13}C and ^{15}N isotope concentration in different compartments within an ecosystem.

REFERENCES

Balesdent, J., Mariotti, A., and Guillet, B. 1987. Natural ^{13}C abundance as a tracer for studies of soil organic matter dynamics. *Soil Biol. Biochem.* **19**: 25-35

Collins, R., Verburg, P.S.J., and Jenkins, A. (In prep.) The contribution of 'old' and 'new' water to a storm hydrograph determined by tracer addition to a whole catchment.

Cotrufo, M.F., Ineson, P., and Rowland, A.P. (1994) Decomposition of tree leaf litters grown under elevated CO_2: Effect of litter quality. *Plant and Soil.* **163**: 121-130.

Coûteaux, M.M., Bottner, P., Rouhier, H., and Billes, G. 1992. Atmospheric CO_2 increase and plant material quality: production, nitrogen allocation and litter decomposition of sweet chestnut. p. 429-436. In: A. Teller, P. Mathy and J.N.R. Jeffers (eds.) Responses of forest ecosystems to environmental changes. Proc. First European Symposium on Terrestrial Ecosystems. Elsevier Applied Science, London & N.Y., 1009 pp.

Edwards, N.T. 1982. The use of soda-lime for measuring respiration rates in terrestrial systems. *Pedobiologia.* **23**: 321-330

Hsieh, Y.-P. 1992. Pool size and mean age of stable soil organic carbon in cropland. *Soil Sci. Soc. Am. J.* **56**: 460-464

Johnsson, H., Bergstrom, L., Jansson, P.-E., and Paustian, K. 1987. Simulated nitrogen dynamics and losses in a layered agricultural soil. *Agric. Ecosystems. Environ.* **18**: 333-356

Keith, H., Oades, J.M., and Martin, J.K. (1986) Input of Carbon to soil from wheat plants. *Soil Biol. Biochem.* **18**:445-449

Lükewille, A., and Wright, R.F. in press. *Global Change Biology*

Mooney, H.A., Drake, B.G., Luxmoore, R.J., Oechel, W.G., and Pitelka, L.F. 1991. Predicting ecosystem responses to elevated CO_2 concentrations. *BioScience* **41**: 96-104

Parton, W.J., Schimel, D.S., Cole, C.V., and Ojima, D.S. 1987. Analysis of factors controlling soil organic matter levels in Great Plains grasslands. *Soil Sci. Soc. Am. J.* **51**: 1173-1179

Parton, W.J., Stewart, J.W.B., and Cole, C.V. 1988. Dynamics of C, N, P, and S in grassland soils: a model. *Biogeochem.* **5**: 109-132

Raison, R.J., Connell, M.J., and Khanna, P.K. 1987. Methodology for studying fluxes of soil mineral-N in situ. *Soil Biol. Biochem.* **19**: 521-530

Schimel, D.S. (1995) Terrestrial ecosystems and the carbon cycle. *Global Change Biology* **1**: 77-91

Swift, M.J., Heal, O.W., and Anderson, J.M. 1979. Decomposition in terrestrial ecosystems. *Studies in Ecology*. **Vol. 5**, Blackwell Scientific Publications, Oxford etc., 372 pp.

Van Breemen, N., and Van Dam, D. 1993. Studying effects of elevated CO_2 on decomposition of soil organic matter. In: Methodologies to assess CO_2 effects on terrestrial ecosystems. Ecosystem Research Report 6.

Van Dam, D., and Van Breemen, N. 1995. NICCCE: A model for cycling of Nitrogen and Carbon isotopes in coniferous forest ecosystems. *Ecological Modelling* **79**: 255-275

Van Veen, J.A., Ladd, J.N., and Amato, M. 1985. Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)]glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. *Soil Biol. Biochem.* **17**: 747-756

6. Plant Productivity and Turnover

Wim Arp, Frank Berendse and Joep Frissel (WAU-TENC)

Introduction

During 1995 growth measurements and harvests of plant material from Calluna vulgaris and *Vaccinium myrtillus* were continued. The same methods were used as in 1993 and 1994 and which are described in earlier reports. A summary of these methods:

Calluna vegetation is divided into five plots in each of the five treatment areas (KIM-elevated; KIM-ambient; METTE-control; EGIL-elevated and EGIL ambient). The *Vaccinium* vegetation is only present in KIM and METTE catchments, and is in these three areas divided into five plots.

Four times per growing season two shoots (approximately four year old) were harvested from each *Calluna* or *Vaccinium* plot. This material is analysed for carbon and nitrogen content, and the relationship between biomass and other parameters (stem diameter, shoot length) is determined.

During these four visits per year plants are also measured non-destructively in the field. The stem diameter, shoot length, number of branches and leaves, and status of flowering is recorded for five plants in each plot. When possible measurements are made on the same plants each period, only dead or dying plants are replaced by healthy plants. The non-destructive growth measurements are converted to estimated biomass using the regressions calculated using the harvested plants.

Vaccinium carbon and nitrogen content

The analysis of the nitrogen and carbon content of the *Vaccinium* plant material has been completed for selected harvests of 1993, 1994 and 1995. The aboveground material harvested was separated into leaves, young stems (1 and 2 year old, green), and older stems (3 to 5 year old, partly woody). Leaf material was analysed for the second harvest of each year at the end of June when the new leaves had completely expanded. C and N of stem material was determined for the second and the fourth harvest of each year.

The only significant differences in leaf nitrogen content was between the outside control and the ambient treatment in 1994 (Figure 6.1, Table 6.1). The CO_2 and temperature treatment lowered the N content in 1995, but this was only marginally significant (p=0.052). Leaves grown in KIM had a lower carbon content than plants growing outside. The carbon content of plants in KIM elevated was slightly higher than for plants in KIM ambient. The combination of a higher C content and a lower percentage N resulted in a significantly higher leaf C/N ratio in 1995 for

plants in elevated CO_2 and temperature. The outside control plants had the lowest leaf C/N ratio in 1994.

The nitrogen content of young (green) stems (Figure 6.1B) and older stems (Figure 6.1C) was not consistently affected by elevated CO_2 and temperature. While young stems had the the lowest nitrogen content in the elevated treatment at most harvests, this difference was already apparent in 1993 and indicates site differences rather than a treatment effect. This also applies to the higher C-N ratio in KIM elevated (Table 6.1). Both young and old stems from the outside control tend to have a higher carbon content than plants growing in KIM. This is significant for the old stems for the last harvest in all three years, and agrees with the higher carbon content of the control leaves. Except for a *reduced* carbon content of old stems in KIM elevated (1994, harvest 2), there is no effect of treatment on C and N content of old stems.

While the nitrogen content of leaves declines during the growing season (data not shown), the percentage N in stems increased from harvest 2 (June) to harvest 4 (October). This increase can be the result of reallocation of nitrogen from senescing leaves, and may optimise conditions for photosynthesis in the green stems during the part of the year when *Vaccinium* has no leaves.

Although the nitrogen content of *Vaccinium* has not decreased significantly as a result of high CO_2 and temperature, growth of *Vaccinium* appears to be enhanced. The total amount of nitrogen in the *Vaccinium* vegetation, and the availability of nitrogen in the soil must therefore be increased. Because this system is still nitrogen limited, any available nitrogen is used to increase growth, and the nitrogen content is kept at a minimal level. The potential effect of elevated CO_2 to lower the nitrogen content of leaf tissue will therefore be very small.

Growth measurements

Both in *Calluna* and in *Vaccinium* a number of branches and plants marked for growth measurements died between harvests. Sometimes this was due to drought stress (*Calluna*), insect damage (*Calluna* in KIM elevated) or physical damage (snow). Most times the reason for reduced growth and eventually death of branches appear to be caused by old age of the plants. Most of the plants which were originally marked in 1993, when healthy and fast growing plants were selected, are now showing a much slower growth rate, and many have died. They are replaced by younger plants, and in the case of *Vaccinium*, by new shoots emerging from the rhizome or from older stems. Marked plants which stopped growing or died were replaced by newly selected healthy plants. Because the growth rate differs between the surviving 'originally' marked plants and the 'new' marked plants, data are presented separately for each group.

Vaccinium

The stem diameter growth of *Vaccinium* during the last 3 years is shown in Figure 6.2. It is apparent from this figure that the diameter of *Vaccinium* shoots nearly does not increase during this period. Plants grow by yearly forming new branches, which are smaller and thinner than the previous year's branches, and which have fewer leaves. Plants completely stop growing and start to die when the new branches do not grow more than a few centimeters and do not form any leaves. This stage appears to reached when the plant is 4-7 years old, although when new shoots sprout from older stems plants may live longer.

In 1993 and 1994 there is very little difference between plants from the two parts of KIM, but in 1995 stem diameter growth appears to be higher in KIM-elevated for all but the oldest cohort. The length of the first year cohort of plants marked in 1993 also strongly decreased during these years (Figure 6.3). While this decrease is continued from 1994 to 1995 for plants in KIM-ambient, first year shoots in KIM-elevated showed a small increase in length.

Data on the number of leaves per first-year branch is presented in Figure 6.4. The number of leaves in KIM-elevated was lower in 1993 and 1994, but in 1995 first year branches had on average one more leaf in KIM elevated. Because the number of leaves is determined during the time the buds are formed, any effect of the treatment on number of leaves could at the earliest be seen in 1995, one year after the start of the treatment. The average area per leaf was determined for plants harvested in June 1995. An image analysis program was used to measure the size of over 1300 individual leaves. Area per leaf varied greatly between plants, but there was no significant difference between treatments.

Apart from number of leaves remaining, leaf senescence can also be expressed as the fraction of green leaf area of the leaves which are still attached. In Figure 6.5 can be seen that leaf senescence was delayed in KIM-elevated in 1994, but that in 1995 leaves senesced earlier. The reason for this effect may be the start of the growing season as determined by temperature. In 1994 the treatment started in spring when plants in both compartments were starting to grow, while in 1995 plant growing in KIM-elevated were three weeks ahead of plants in KIM-ambient in leaf emergence. In both cases the length of the growing season appears to be extended in KIM-elevated for *Vaccinium*.

For *Vaccinium* plants which were harvested no difference could be determined for relationship between stem diameter and biomass. Therefore any change in stem diameter would signify a change in biomass.

Calluna

Stem diameter growth of *Calluna* shoots growing in both KIM compartments and in METTE control is shown in Figure 6.6. The stem diameter increase shown in this graph is calculated as the difference between the mean stem diameter of the first two harvests of the season and the mean stem diameter of the last two harvests of the season. This increase is calculated for all individual branches, and the mean value for each of the years, treatments and cohorts is shown in the figure. This stem diameter increase represents approximately the growth over the months June, July and August.

In 1993 the stem diameter increase did not differ between the three treatments, and also the diameter growth for all cohorts except the youngest was comparable. In 1994 growth in KIM-ambient was reduced compared to the previous year and to the other treatments. The diameter growth of KIM-elevated and METTE-control plants was higher for the older cohorts. In 1995 there was no difference in growth between treatments for the younger cohorts, but the older cohorts still grew more in KIM-elevated.

The decrease in diameter-growth from 1993 to 1995 is, comparable to the results found for *Vaccinium,* a result of the ageing of the plants. Although *Calluna* stems do show growth, the stem diameter of each consecutive cohort is smaller than the previous, and when the plants have reached a certain age the growth stops completely.

Calluna plants harvested in 1995 have not yet been analysed, but data for 1994 show that the relationship between stem diameter and biomass has changed in KIM elevated, resulting in more biomass per unit stem diameter. This effect amplifies the increase in stem diameter growth in KIM-elevated, resulting in a large increase in *Calluna* biomass.
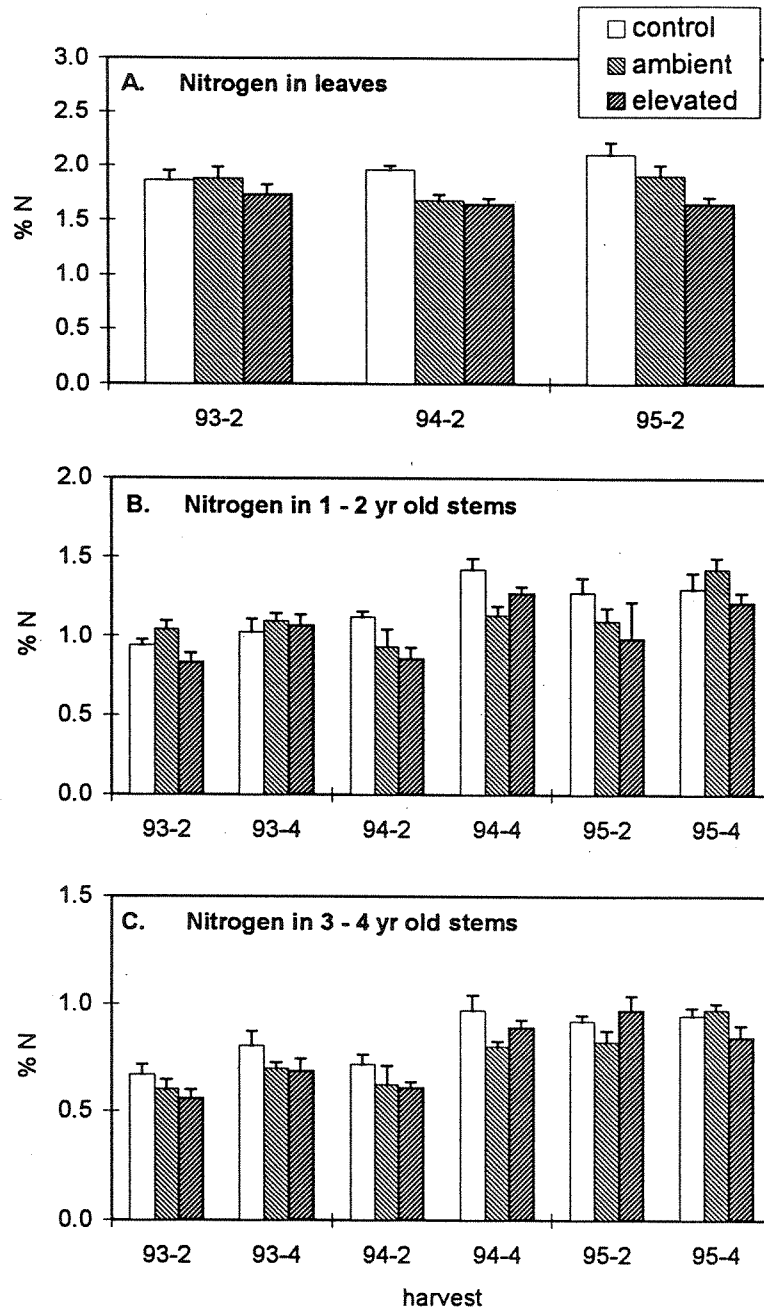
Figure 6.1. Nitrogen content of *Vaccinium* leaves (A), young stems (B) and old stems (C) harvested in June of 1993, 1994 and 1995 in KIM-elevated, KIM-ambient and METTE-control
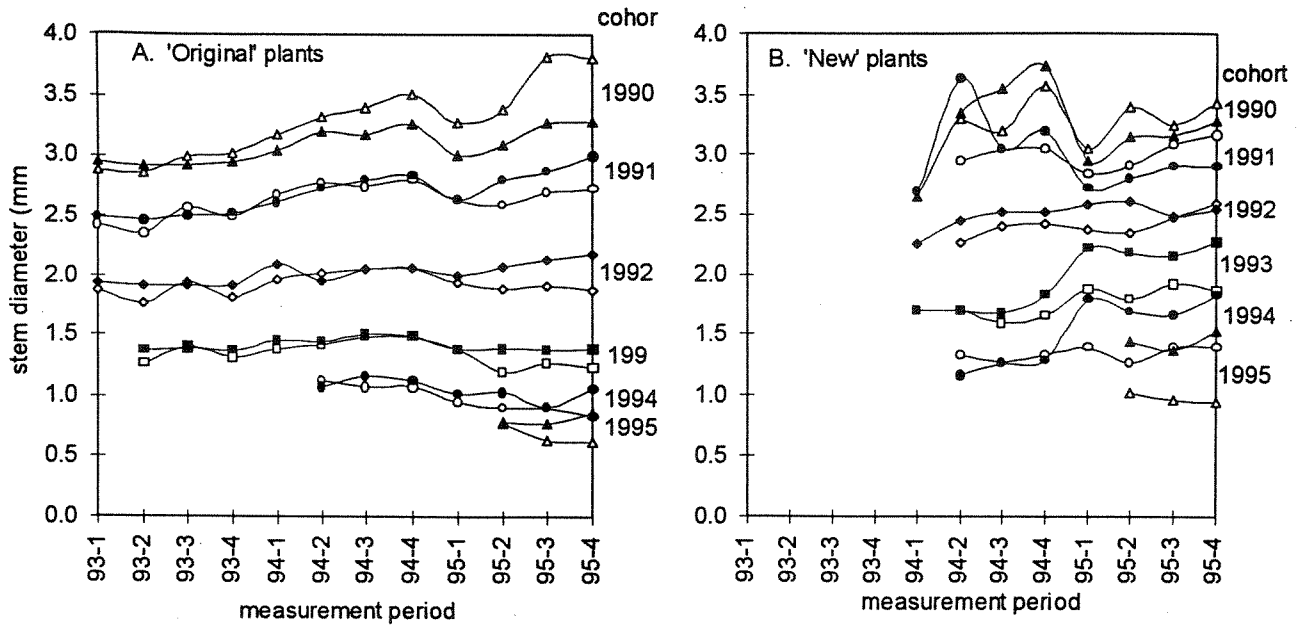
Figure 6.2. Stem diameter of *Vaccinium* at all four measurement periods in 1993, 1994 and 1995, for all marked branch cohorts. Open symbols: KIM-ambient; closed symbols: KIM-elevated. A: Plants originally marked in 1993; B: New plants (replacements) marked in 1994 and later. The total number of plants ('original' and 'new') for each treatment is 25.
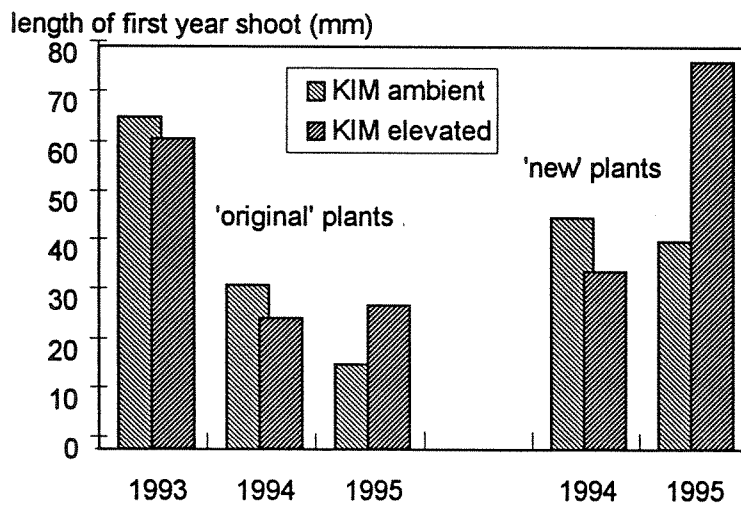
Figure 6.3. Average length of the first year branch in KIM-elevated and KIM-ambient for 1993, 1994 and 1995.
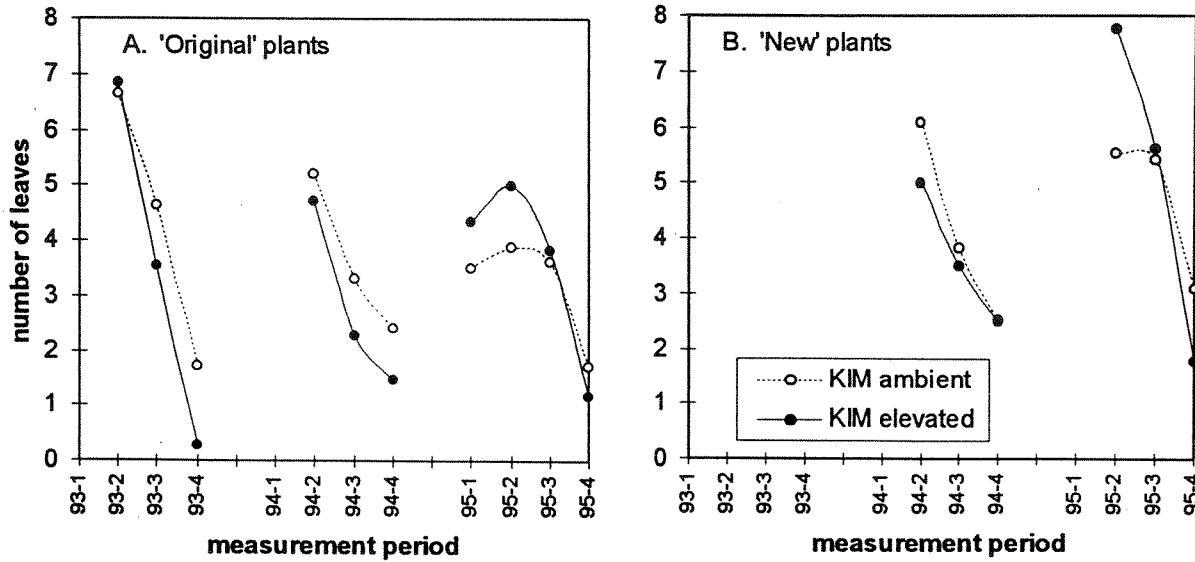
Figure 6.4. Average number of leaves on the first year branch of *Vaccinium*. See legend of figure 2 for explantion of symbols.
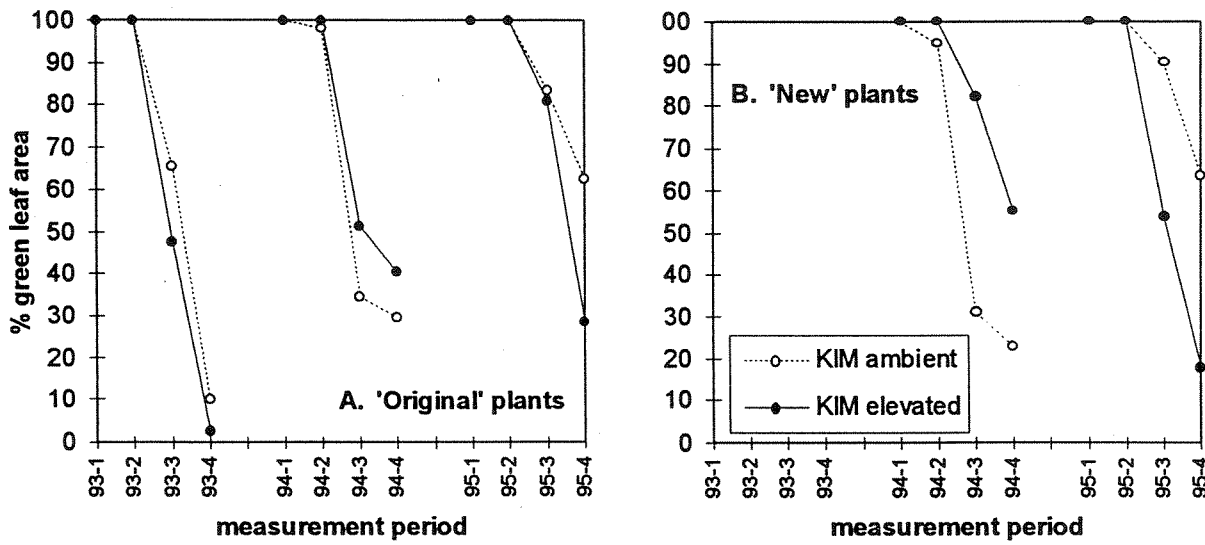
Figure 6.5. Percent green leaf area of remaining leaves of *Vaccinium*. See legend of figure 2 for explantion of symbols.
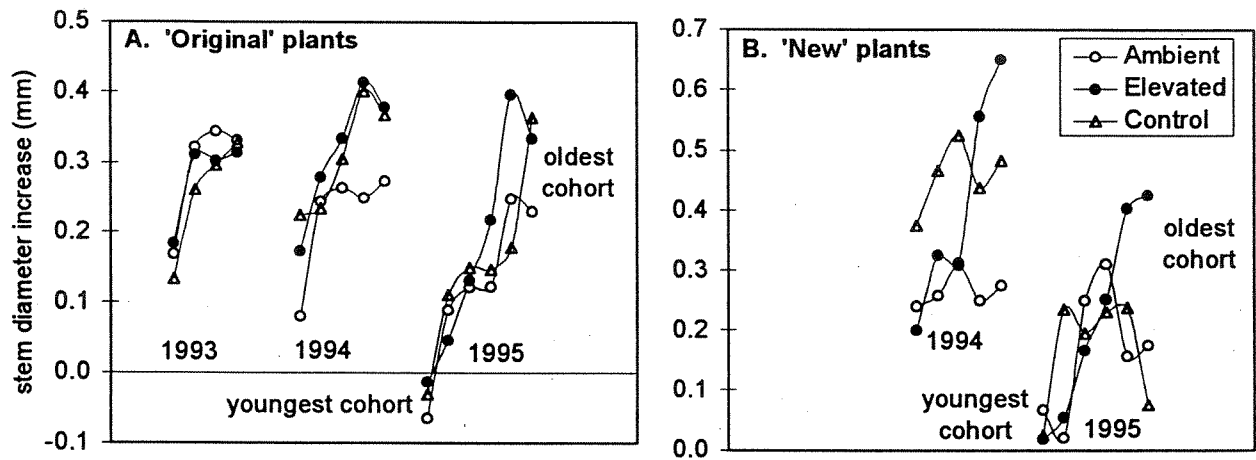
Figure 6.6. Stem diameter increase of *Calluna* in 1993, 1994 and 1995, for all marked branch cohorts. The increase is calculated as the difference between the average stem diameter measured in May and June and the average stem diameter of the August and October measurements. Open circles: KIM-ambient; closed circles: KIM-elevated; open triangles: METTE-outside control. A: Plants originally marked in 1993; B: New plants (replacements) marked in 1994 and later. The total number of plants ('original' and 'new') for each treatment is 25.

Table 6.1. Percent nitrogen, percent carbon and C-N ratio of *Vaccinium* leaves, young stems (1 and 2 year old), and old stems (3 to 5 year old) growing in KIM elevated and ambient areas and in the outside control area. Data are given for the second and fourth harvest of 1993, 1994 and 1995. The mean value is the average of 5 replications. The column p (probability) indicates significant differences between control and ambient treatment (roof effect), and between ambient and elevated treatment (CO_2 and temperature effect):

$0.05 < (*) < 0.1; 0.01 < * < 0.05; ** < 0.01$

Vaccinium %N

part	treatment	mean 93-2	p	mean 93-4	p	mean 94-2	p	mean 94-4	p	mean 95-2	p	mean 95-4	p
leaf	control	1.87				1.96	**			2.11			
leaf	ambient	1.89				1.68				1.91			
leaf	elevated	1.74				1.65				1.65	(*)		
stem1	control	0.94		1.02		1.12		1.42	*	1.28		1.30	
stem1	ambient	1.04		1.10		0.93		1.13		1.09		1.43	
stem1	elevated	0.83	*	1.07		0.86		1.27		0.98		1.22	(*)
stem3	control	0.67		0.81		0.72		0.97	(*)	0.92		0.94	
stem3	ambient	0.60		0.70		0.62		0.80		0.82		0.97	
stem3	elevated	0.56		0.69		0.61		0.89	(*)	0.97		0.84	(*)

Vaccinium %C

part	treatment	mean 93-2	p	mean 93-4	p	mean 94-2	p	mean 94-4	p	mean 95-2	p	mean 95-4	p
leaf	control	55.12				54.41	**			54.28	*		
leaf	ambient	54.92				53.48				53.32			
leaf	elevated	54.54				53.29				53.92	(*)		
stem	control	52.53		53.12		52.98		53.69	(*)	53.96	(*)	53.70	
stem	ambient	53.19		52.84		52.98		52.40		52.62		53.14	
stem	elevated	52.16	(*)	53.62		51.70		52.08		52.92		53.46	
stem3	control	52.06		52.65	*	51.85		52.30	*	53.55		53.22	*
stem3	ambient	51.99		51.99		52.39		51.42		52.84		52.35	
stem3	elevated	53.32		52.53		51.22	*	51.76		52.63		52.63	

Vaccinium C/N ratio

part	treatment	mean 93-2	p	mean 93-4	p	mean 94-2	p	mean 94-4	p	mean 95-2	p	mean 95-4	p
leaf	control	29.71				27.79	**			26.03			
leaf	ambient	29.46				31.90				28.20			
leaf	elevated	31.80				32.43				32.77	*		
stem1	control	55.97		53.41		47.44	(*)	38.03	*	43.22		42.73	
stem1	ambient	51.31		48.60		59.10		46.72		49.46		37.67	
stem1	elevated	64.00	*	51.15		62.13		41.23	(*)	54.32		44.28	(*)
stem3	control	79.03		67.04		73.36		55.01	*	58.30	(*)	56.92	
stem3	ambient	87.35		74.43		89.71		64.31		64.99		53.99	
stem3	elevated	95.75		78.01		84.96		58.60	(*)	54.86		63.53	(*)

7. Gas Exchange Responses

David Beerling (US)

Introduction

A preliminary account of the CLIMEX system and the gas exchange responses of the plants during first season of treatment to elevated CO_2 and temperature was given by Beerling & Woodward (1996). This report provides an analysis of the performance of the CLIMEX greenhouse in the second year of operation by comparing the manipulated climate with that monitored in the control section of greenhouse and outside. In particular, the effects of the temperature manipulations on the relative humidity and vapour pressure deficit (VPD) of the air are considered - both critical climatic features controlling the stomatal responses of plants. The ecophysiological responses of the plants (short- and long-term leaf gas exchange responses, stomatal density and leaf nutrient status) to elevated CO_2 and temperature during the second year of continuous exposure are reported for the two dominant tree species (*Betula pubescens* and *Pinus sylvestris*) and the ground shrub *Vaccinium myrtillus* within the CLIMEX enclosure between June and September 1995.

Materials and methods

The relative humidity in the three areas was monitored and recorded using DGT dataloggers and a comparison of the relative humidity values inside the treated and control sections of the greenhouse with those outside shows closely matched values (Figure 7.1a) with mean values for each section of 77.6 % (treated) , 71.3 % (control) and 72.9 % (outside) respectively. Some large fluctuations are apparent but these tended to be rather short-lived (Figure 7.1a).

The vapour pressure deficit of the air has been calculated for the treated and control sections of the greenhouse, and outside, using the monitored relative humidity and temperature values as follows. Saturated vapour pressure, es, (Pa) was calculated from air temperature, t $^{\circ}C$, with the following temperature dependence :

$$e_s = 613.75 \times \exp\left(\frac{17.456 \times t}{240.97 + t}\right)$$

where h is the relative humidity (%) of the air. VPDs calculated for the CLIMEX greenhouse in both the treated (mean 0.51 kPa) and control (mean 0.5 kPa) sections were generally similar (Figure 7.1b), with relatively small fluctuations throughout the growing season.

Leaf gas exchange measurements

Gas exchange measurements (photosynthesis, stomatal conductance and intercellular CO_2 concentrations) were made on leaves of *B. pubescens, P. sylvestris* and *V. myrtillus* in June, July, August and September 1995. In each case measurements were made at 2-hourly intervals over a two day period between 9.00 hrs and 15.00 hrs. All measurements of were made using an infra red gas analyzer (PP. Systems, Herts. U.K.), at ambient CO_2 concentrations, temperatures, VPDs and irradiance levels to estimate the performance of the plants under ambient conditions. Individual trees were selected for gas exchange measurements which had not reach the ceiling of the greenhouse and which had not taken on a krummholz appearance. These individuals were tagged and subsequent measurements made on the same individuals throughout the season. For each species, gas exchange measurements were replicated in the outside catchment, greenhouse control and greenhouse treated areas (given in brackets) as follows : *B. pubescens* (n = 10, 5, 14), *P. sylvestris* (n = 10, 5, 14) and *V. myrtillus* (n = 10, 10 ,10).

Maximum and average rates of carboxylation and electron transport of leaves were calculated using the approach of Beerling and Quick (1995). Maximum rates were calculated from an isotopically-derived value of ci (see next section), the highest measured photosynthetic rate recorded over the sampling period (June-July), and leaf temperature and PAR data. Average rates were calculated using the mean photosynthetic rate, leaf temperature and PAR of all measurements on each species over June and July and the isotopically-derived ci value. These values of A, ci, PAR and temperature were then used with the equations of Beerling and Quick (1995) to calculate Vmax and Jmax. Values were calculated for leaves of *B. pubescens, P. sylvestris* and *V. myrtillus* growing in the three areas of the experiment.

Stable carbon isotope analyses

Leaves of *B. pubescens, P. sylvestris* and *V. myrtillus* from 5 - 10 tagged individuals were collected in late-July from several localities within the control and treated sections of the greenhouse and from an outside reference catchment. Leaves were oven dried at 80 $^\circ$C for 24 h, and then ground to a homogenised fine powder for isotopic analysis. Isotope determinations were made on a 2-3mg sub-sample for each species collected from each CO_2 concentration using VG-Isogas triple beam stable isotope-ratio mass spectrometer combined with an autoanalysis unit; measurements were reproducible to 0.05 %. The isotopic composition of a sample is calculated as the ratio of a sample to a standard (%) :

$$\delta^{13}Cp = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000$$

where Rsample and Rstandard are the 13C : 12C ratios of the sample and the universally accepted PDB standard respectively. As plants have a lower 13C : 12C ratio than the standard, 13Cp is negative, and carbon isotope discrimination calculated as :

$$\Delta = \frac{(\delta^{13}Ca - \delta^{13}Cp)}{\left(1 + \frac{\delta^{13}Cp}{1000}\right)}$$

where 13Ca is the average isotopic composition of the atmospheric CO_2, recorded biologically using the C4 species *Zea mays* grown over the same period in the three different areas (greenhouse treatment, greenhouse control and outside). Farquhar (1983) developed a model relating the carbon isotope composition of C4 plants to that of the atmospheric CO_2 :

$$\delta^{13}C_p = \delta^{13}C_a - a - (b_4 + b_3 \times \phi - a) \times \frac{c_i}{c_a}$$

where e is the leaf to air vapour pressure deficit taken as the mean of measurements between June and July.

Stomatal density measurements

The stomatal densities of the trees and the ground shrubs were made using direct microscopic observations on leaves stained with a 1% w/v solution of diphenylboric acid 2-aminoethylester (Sigma chemicals) in methanol (Schnitzler et al. 1996). Leaves were stained for 5 mins and then rinsed in methanol, mounted under coverslips in distilled water and observed under epifluorescence light at x 40 giving a final field of view of 0.13 mm-2. 10 counts were made on 5 leaves per species from the treatment and control sections of the greenhouse and from plants growing in the outside reference catchment.

Results

Leaf gas exchange

The seasonal course of photosynthesis and stomatal conductance of *P. sylvestris*, *B. pubescens* and *V. myrtillus* in the treated and control sections of the greenhouse and, for comparison, in an outside catchment, is depicted in Figsures 7.2, 7.3 and 7.4, respectively. *P. sylvestris* displayed generally increased photosynthetic rates (Figure 7.2a) and lower rates of stomatal conductance (Figure 7.2b) throughout the growing season in elevated CO_2 and temperature, compared with

counterparts growing in the control section of the greenhouse and outside. *B. pubescens* showed minimal responses to the treatments (Figure7.3), with no discernible effects of elevated CO_2 and temperature. The ground shrub *V. myrtillus* had significantly higher mid-season rates of photosynthetic (p < 0.05) and generally lower stomatal conductances (Figure 7.4b) under elevated CO_2 and temperature.

Maximum and average rates of carboxylation and electron transport of leaves of each species calculated for the period June - July show some differences due to the greenhouse (Table 1). *B. pubescens* and *P. sylvestris* inside the greenhouse had reduced maximum rates compared with their counterparts growing outside, largely governed by differences in PAR, not observed in the understorey shade-tolerant shrub *V. myrtillus* (Table 1). Average values however showed no effects of the greenhouse-reduced PAR. No major effects of growth at elevated CO_2 and temperature were found (Table 1) on Vmax and Jmax (Table 1) in any of the species studied.

Stable carbon isotope responses

Discrimination against 13C decreased with elevated CO_2 and temperature in *B. pubescens* and *V. myrtillus* (Figure 7.5a), as would be expected from theory, since plants in the control and treated sections of the greenhouse tended to have lower conductances than those growing outside. In *P. sylvestris* however, high stomatal conductances and low photosynthetic rates in the control section of the greenhouse led to a small increases in 13C discrimination (Figure 7.5a). Isotopically-derived ci / ca ratios showed near-constant values in each species irrespective of where the species grew (Figure 7.5b) whereas leaf WUE (eq. 7) increased markedly for each species growing in elevated CO_2 and temperature (Figure 7.5c).

Stomatal density responses

Stomatal densities of leaves developing within the CLIMEX greenhouse were reduced relative to the values of plants growing outside (Figure 7.6) in all the species studied, most probably due to the decreased greenhouse irradiance. A further and significant (P < 0.05) elevated CO_2 and temperature effect was observed (Figure 7.7) in *B. pubescens, V. uliginosum,* and *V. myrtillus*.

Discussion

Analyses of the climatic data indicate that the construction of the greenhouse over the existing vegetation exerted minor effects on the relative humidity and vapour pressure deficit of the air (Figures 7.1 and 7.2), even with increased air temperatures. This insensitivity can be most readily attributed to the continuous opening of large-scale vents in the greenhouse by the climate computer to regulate temperature and CO_2, and to the large vegetated surface area within the greenhouse supplying additional moisture to the atmosphere via evapotranspiration.

The dominant tree (*P. sylvestris* and *B. pubescens*) and shrub (*V. myrtillus*) species exhibited increased leaf water-use efficiencies under elevated CO_2 and temperature (Figure 7.?c), and near-constant ci / ca ratios, consistent with responses shown by herbaceous taxa grown at elevated CO_2 (Beerling and Woodward 1995; Jackson et al. 1994) and analyses of historical sequences of leaves (Beerling 1996) and tree rings (Marshall and Monserud 1996) of woody taxa spanning the past 200 years of CO_2 increase. Gas exchange measurements indicate that these two results were most likely brought about through a reduction in stomatal conductance, stomatal density and/or an increase in leaf photosynthesis. The reduction in stomatal density of each species is consistent with responses observed in the first year of treatment (Beerling and Woodward 1996), and provides experimental evidence for a continued density reduction in mature trees and shrubs grown continuously at elevated CO_2.

Mature trees of *P. sylvestris* and the dominant ground shrub *V. myrtillus* both exhibited increased rates of photosynthesis in the elevated CO_2 and temperature section of the greenhouse, not observed in plants growing in the control section, indicating a clear treatment effect, and in accordance with other experimental (Calloway et al. 1994; Delgado et al. 1994; Wang et al. 1995) and modelling (Long 1991; Beerling and Woodward 1994) studies. Associated with this increase in photosynthesis was an increase in soil mineralisation rates from 25 to ca. 38 kg N ha^{-1} year^{-1} and a decrease in leaf C:N ratios (Table 2) of both species, suggesting alterations to N-partitioning between plant components. These observations indicate increased deployment of available N to the photosynthetic enzymes and, together with the calculated carboxylation rates (Table 1), suggest increased amounts of Rubisco in the leaves of both species but with an unchanged activation state - photosynthetic features also reported for winter wheat grown in elevated CO_2 and temperature (Delgado et al. 1994). Decreasing leaf C:N ratios in the needles and leaves of *P. sylvestris* and *V. myrtillus* grown at elevated CO_2 and temperature supports the suggestion that higher levels of soluble carbohydrates in leaves increase nitrate reduction and its incorporation into amino acids (Stitt and Schulze 1994).

There was no change in the leaf C : N ratios of *B. pubescens* directly attributable to elevated CO_2 and temperature (Table 2), in contrast to results obtained for *B. pubescens* leaves developing in elevated CO_2 in the Solardomes at Lancaster (Cotrufo et al. 1994) showing markedly higher C:N ratios than their ambient grown counterparts. This difference is frequently observed in field studies (O'Neill and Norby, 1996) and suggests either a strong interactive effect of temperature on the response or differences in the nutritional status of the soils in the two systems, with strong N-limitation in situ but not necessarily to the same extent in pot-based experiments. The lack of response of the photosynthetic system of birch is consistent with reports for small birch trees grown at elevated CO_2 in controlled environments (Pettersson and McDonald 1992). Despite increased soil N-availability in the treated catchment, *B. pubescens* appeared unable to utilize it in competition with the other species present possibly because it tends, rather unusually, to maintain a constant root : shoot ratio in elevated CO_2 (Pettersson et al. 1993).

Measurements of stomatal conductance of leaves of field-grown plants exposed to CO_2 enrichment are increasingly showing the degree of stomatal closure to be limited or absent (Beerling et al. 1996) despite strong reductions being commonly reported in laboratory based experiments. The data for *B. pubescens* support this trend and show the low sensitivity of stomatal closure over the entire growing season to elevated CO_2 and temperature with little or no increase in photosynthetic rates, as observed in small saplings of B. pendula grown in elevated CO_2 only (Pettersson and McDonald 1992). The mechanisms of differential stomatal sensitivity remain unclear (Mansfield et al. 1990) but may relate to differences in microclimate experienced by plants in field versus controlled environment studies.

Gas exchange measurements made over the course of the day provide only a short-term picture of the dynamics of leaf CO_2 and H_2O exchange rates, whereas leaf carbon isotope ratios reflect a signal of CO_2 and H_2O exchange integrated over the lifetime of the tissue. The gas exchange data can be compared with the isotope data by inserting the mean ci/ca ratio of the gas exchange measurements made between June - July for *P. sylvestris, B. pubescens* and *V. myrtillus* into the equation (Farquhar et al. 1982):

$$\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \times \frac{c_i}{c_a}$$

using values of values ^{13}C reported earlier. A comparison of the observed and predicted values for each species shows close correspondence between the two, indicating that the gas exchange measurements satisfactorily described the long-term leaf gas exchange behaviour for the June-June sampling period. For *P. sylvestris* leaf ^{13}C is consistently underpredicted by 1 - 2% because the ci/ca ratio of the gas exchange measurements over estimated the isotopically-derived values. This may indicate greater limitation of CO_2 fixation by stomatal conductances of trees growing in each area (greenhouse control, greenhouse treated and outside) in the June-July interval than suggested by spot gas exchange measurements and/or difficulties associated with making accurate photosynthetic measurements on conifers.

Overall, the dominant conifer and ground shrub species of the boreal forest-tundra transition responded to elevated CO_2 and temperature with consistently increased rates of CO_2 fixation and lower rates of H_2O loss over the second continuous year of treatment. Measurements on plant biomass responses are required to determine whether these altered gas exchange processes are translated into increased dry matter production and catchment run off. Preliminary data for the shrubs *Calluna vulgaris* and *V. myrtillus* suggest increased biomass accumulation in the treated catchment but no effects on *B. pubescens*. Whole ecosystem CO_2 flux measurements are next required to identify whether the system is a source or sink for atmospheric CO_2. Measurements of net ecosystem CO_2 fluxes on boreal woodlands indicate their carbon balance is strongly sensitive to temperature - a feature making their response to combined CO_2 and temperature increases difficult to forecast. These complexities are borne out in the current uncertainties regarding the role of boreal ecosystems in the contemporary carbon cycle. Double

convolution of the global flask network of atmospheric CO_2 $^{13}C/^{12}C$ data indicate the temperate/boreal zone of the Northern hemisphere was a net sink for 3.5 Gt of carbon in 1992 (Ciais et al. 1995) whereas ecological modelling of the atmosphere-plant-soil system suggests this is unlikely to be the case (Lloyd and Farquhar 1996). In situ experimentation on the boreal forest ecosystem, of the sort described here will be critical for resolving these uncertainties (Körner 1995).

Acknowledgements

References

Beerling DJ (1996) Ecophysiological responses of woody plants to past CO2 concentrations. Tree Physiol. 16: 389-396.

Beerling DJ Quick WP (1995) A new technique for estimating rates of carboxylation and electron transport in leaves of C3 plants. Global Change Biol 1: 289-294.

Beerling DJ, Woodward FI (1994) The climate change experiment (CLIMEX) : phenology and gas exchange responses of boreal vegetation to global change. Global Ecol Biogeog Lett 4: 17-26.

Beerling DJ, Woodward FI (1995) Leaf stable carbon isotope composition records increased leaf water-use efficiency of C3 plants in response to atmospheric CO_2 enrichment. Funct Ecol 9: 394-401.

Beerling DJ, Woodward FI (1996) In situ responses of boreal vegetation to elevated CO_2 and temperature : first season results. Global Ecol Biogeog Lett in press.

Beerling DJ, Heath J, Woodward FI, Mansfield TA (1996) CO_2 and drought interactions in trees: observations and mechanisms. New Phytol 134; in press.

Calloway RM, DeLucia EH, Thomas EM, Schlesinger WH (1994) Compensatory responses of CO_2 exchange and biomass allocation and their effects on the relative growth rate of ponderosa pine in different CO2 and temperature regimes. Oecologia 98: 159-166.

Ciais P, Tans PP, Trolier M, White JWC, Francey RJ (1995) A large northern hemipshere terrestrial CO2 sink indicated by 13C/12C ratio of atmospheric CO_2. Science 269: 1098-1102.

Cotrufo MF, Ineson P, Rowland AP (1994) Decomposition and tree litters grown under elevated CO2 : effect of litter quality. Plant and Soil 163: 121-130.

Delgado E, Mitchell RAC, Parry MAJ, Driscoll SP, Mitchell VJ, Lawlor DW (1994) Interacting effects of CO_2 concentration, temperature and nitrogen supply on the photosynthesis and composition of winter wheat leaves. Plant Cell Environ 17: 1205-1213.

Farquhar GD (1983) On the nature of carbon isotope discrimination in C4 species. Aust J Plant Physiol 10: 205-226.

Jackson RB, Sala OE, Field CB, Mooney HA (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. Oecologia 98: 257-262.

Körner Ch (1995) Towards a better experimental basis for upscaling plant responses to elevated CO2 and climatic warming. Plant Cell Environ 18: 1101-1110.

Lloyd J, Farquhar GD (1996) The CO_2 dependence of photosynthesis, plant growth and responses to elevated atmospheric CO_2 concentrations and their interaction with soil nutrient status. I. General principles and forest ecosystems. Funct Ecol 10: 4-32.

Long SP (1991) Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO2 concentrations : has its importance been underestimated ? Plant Cell Environ 14: 729-739.

Mansfield TA, Hetherington AM, Atkinson CJ (1990) Some aspects of stomatal physiology. Annu Rev Plant Physiol Plant Mol Biol 41: 55-75.

Marshall JD, Monserud RA (1996) Homeostatic gas-exchange parameters inferred from 13C/12C in tree rings in conifers. Oecologia 105: 13-21.

O'Neill EG, Norby RJ (1996) Litter quality and decomposition rates of foliar litter produced under CO_2 enrichment In Koch GW, Mooney HA (eds) Carbon Dioxide and Terrestrial Ecosystems. Academic Press, San Diego, pp. 87-103.

Pettersson R, McDonald AJS (1992) Effects of elevated carbon dioxide concentration on photosynthesis and growth of small birch plants (Betula pendula Roth.) at optimal nutrition. Plant Cell Environ 15: 911-919.

Pettersson R, McDonald AJS, Stadenberg I (1993) Response of small birch plants (Betula pendula Roth.) to elevated CO2 and nitrogen supply. Plant Cell Environ 16: 1115-1121.

Schnitzler JP, Jungblut TP, Heller W, Köfferlein M, Hutzler P, Heinzmann U, Schmelzer E, Ernst D, Langebartels C, Sanderman H (1996) Tissue localization of u.v. B-screening pigments and of chalcone synthase mRNA in needles of Scots pine seedlings. New Phytol 132: 247-258.

Stitt M, Schulze ED (1994) Plant growth, storage and resource allocation : from flux control in a metabolic chain to the whole plant level. In Schulze ED (ed) Flux Control in Biological Systems : from Enzymes to Populations and Ecosystems. Academic Press, San Diego, pp. 57-118.

Wang K, Kellomäki S, Laitinen K (1995) Effects of needle age, long-term temperature and CO_2 treatments on the photosynthesis of Scots pine. Tree Physiol 15: 211-218.
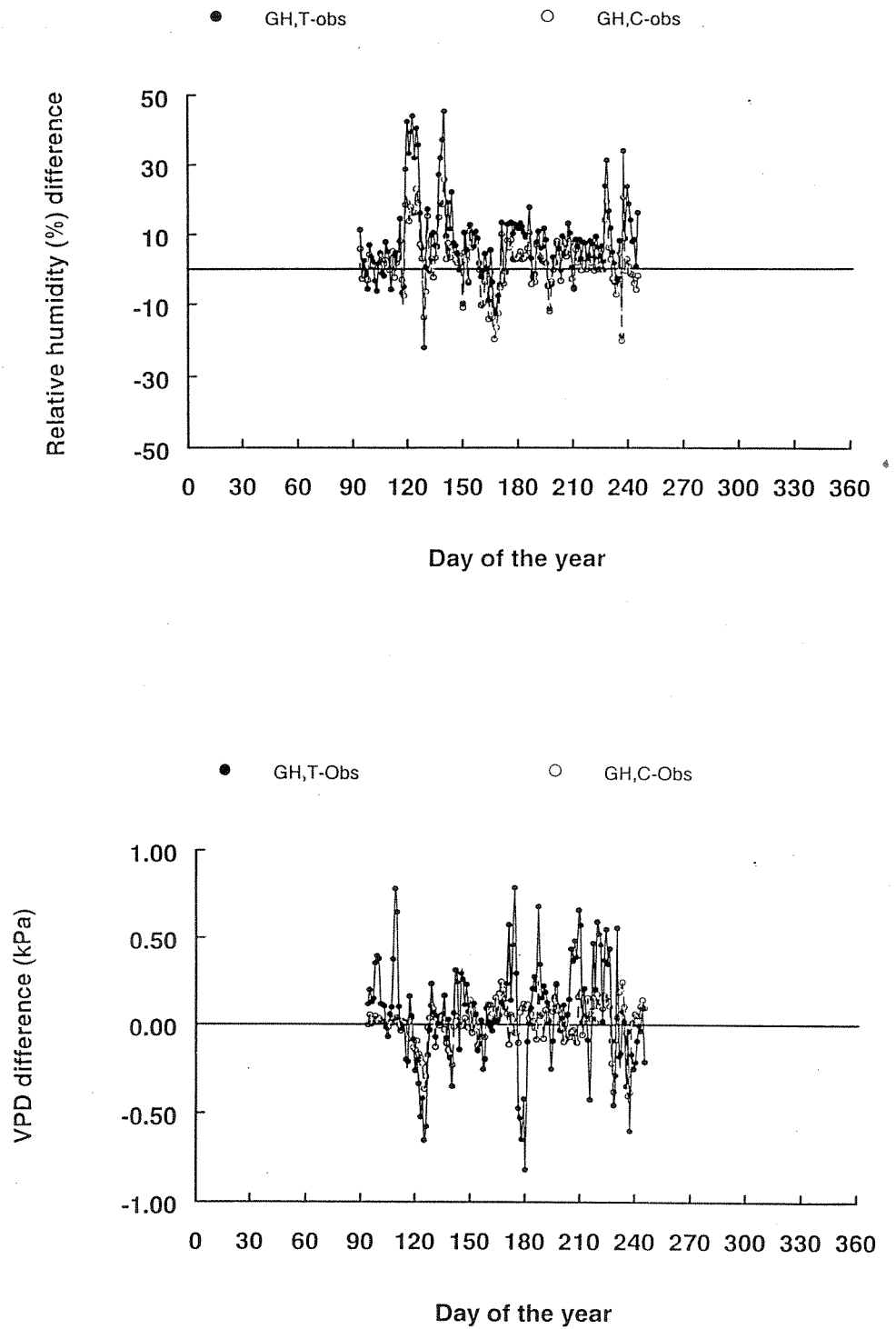
Figure 7.1. Differences in (a) the relative humidity of the air and b) the vapour pressure deficit of the air in the treated and control sections of the greenhouse compared with outside over the 1995 growing season.
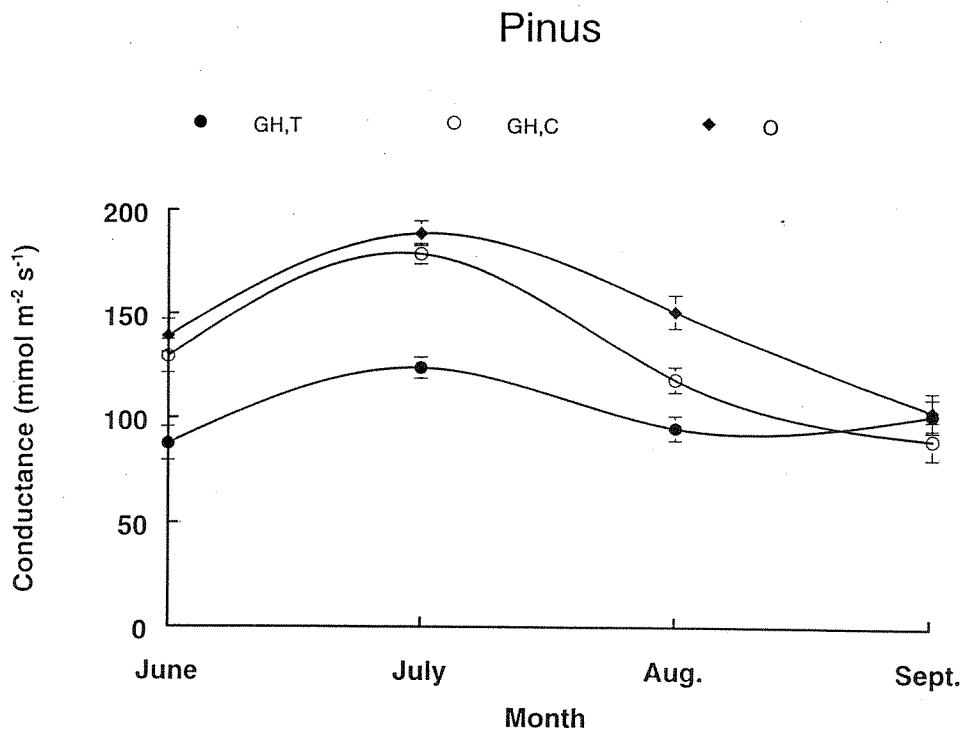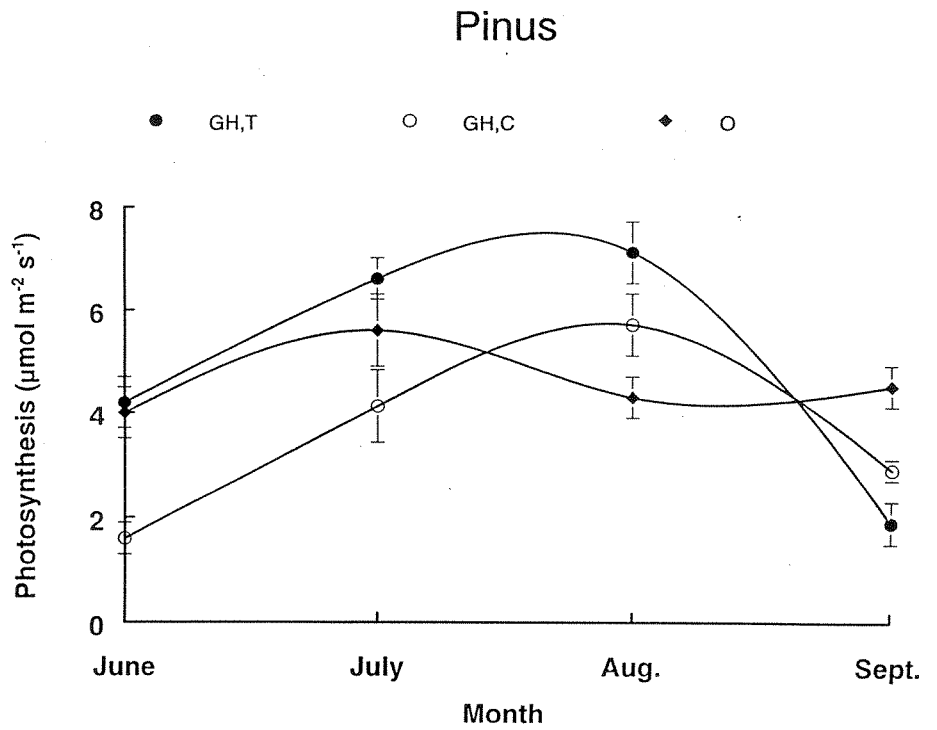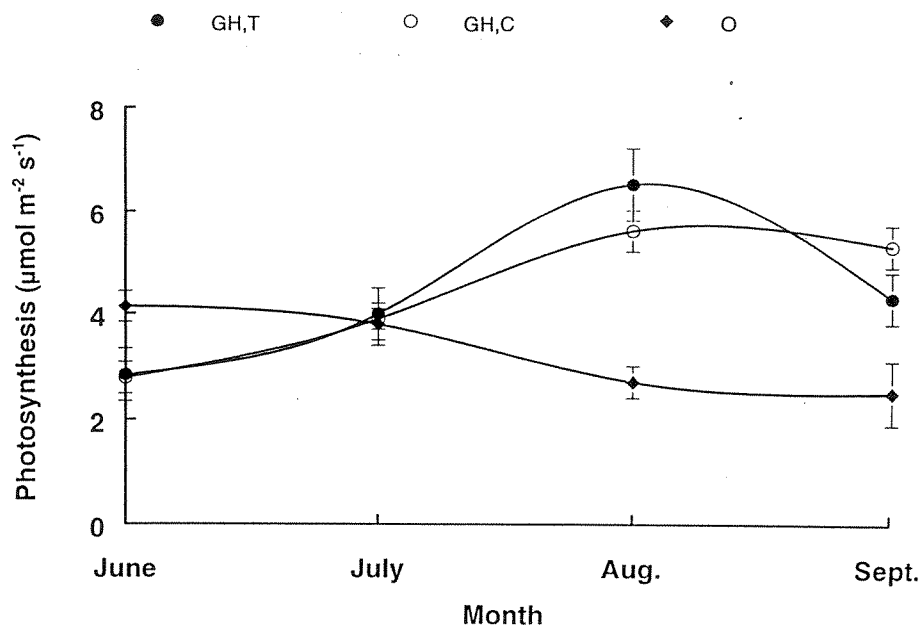
54

Figure 7.2. Seasonal course of (a) photosynthesis and (b) stomatal conductance in P. sylvestris trees growing in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O). Measurements are means 1 s.e. Statistical comparisons were made using a pooled variance t-test on the replicate greenhouse control and treated data (UNISTAT, 1994). This procedure identified significant (p < 0.05) differences between control and treated sections for (a) photosynthesis and (b) stomatal conductance in July, Aug. and Sept. and June, July, Aug. respectively.

Betula

Betula

Figure 7.3. Seasonal course of (a) photosynthesis and (b) stomatal conductance in B. pubescens trees growing in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O). Measurements are means 1 s.e. Statistical comparisons were made as described in Fig. 5. Significant ($p < 0.05$) differences between control and treated sections for both photosynthesis and stomatal conductance were found in Sept.

Vaccinium

● GH,T ○ GH,C ◆ O

Photosynthesis (µmol m^{-2} s^{-1}) vs Month (June, July, Aug., Sept.)

Vaccinium

● GH,T ○ GH,C ◆ O

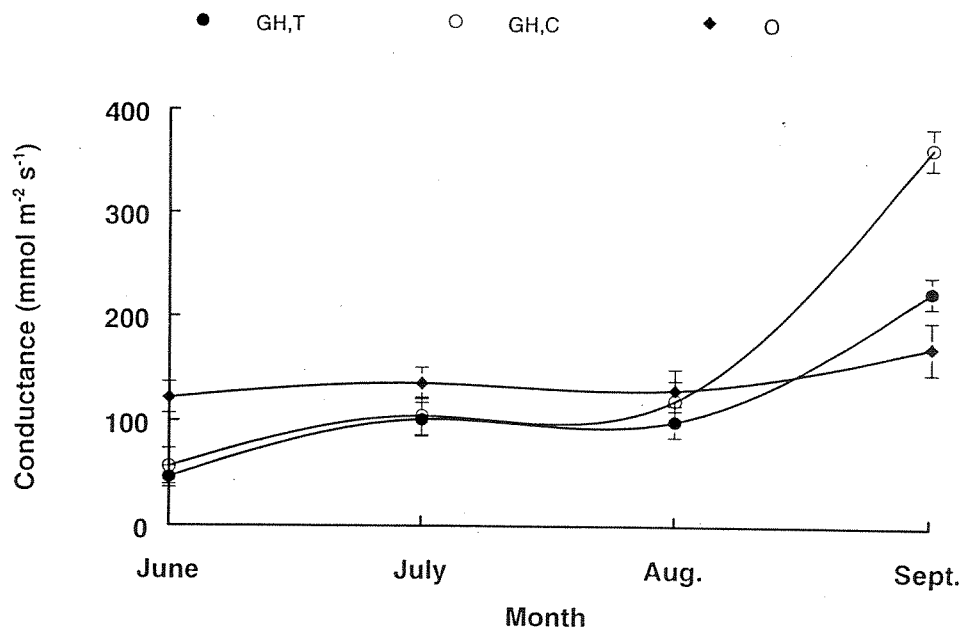Conductance (mmol m^{-2} s^{-1}) vs Month (June, July, Aug., Sept.)

Figure 7.4. Seasonal course of (a) photosynthesis and (b) stomatal conductance in V. myrtillus growing in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O). Measurements are means 1 s.e. Measurements are means 1 s.e. Statistical comparisons were made as described in Fig. 5. Significant ($p < 0.05$) differences between control and treated sections for (a) photosynthesis and (b) stomatal conductance were found in July and Aug. and July, Aug. and Sept. respectively.
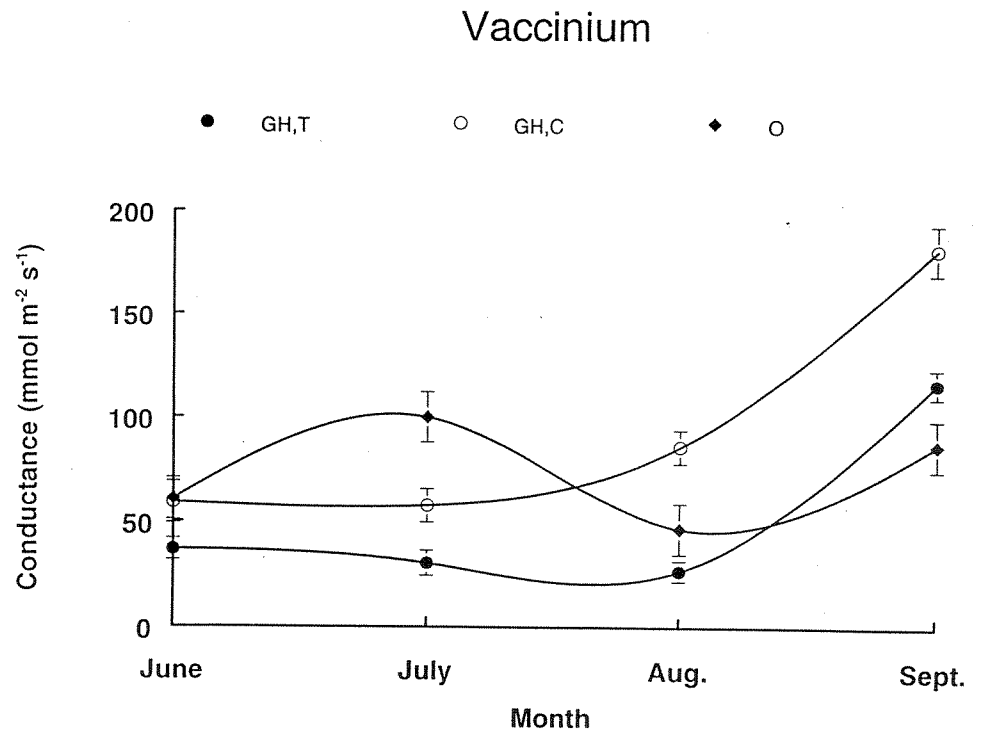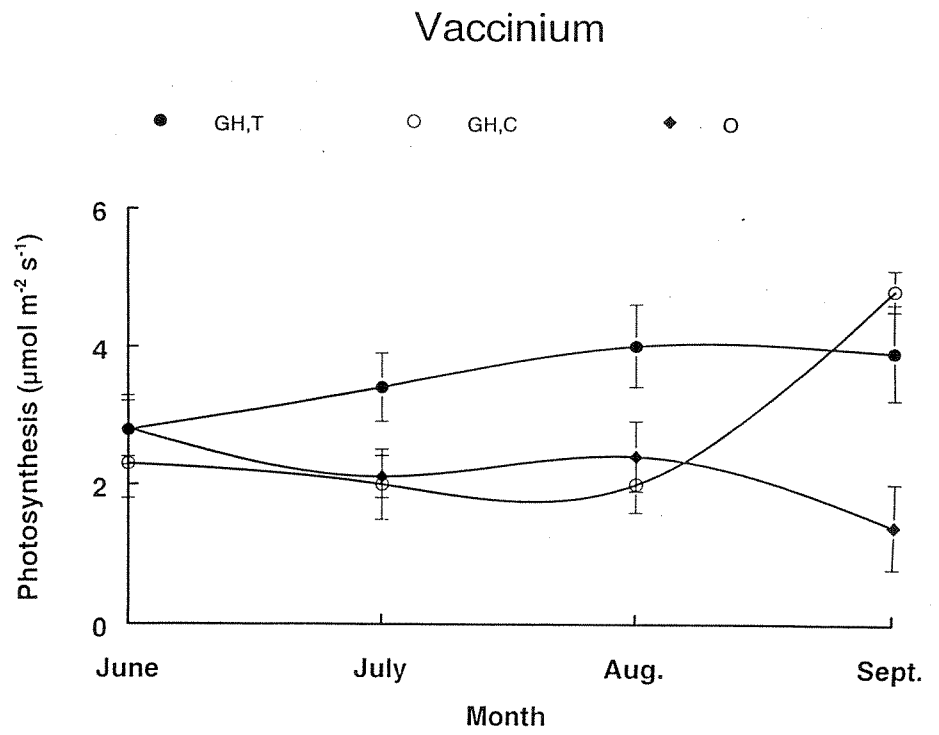
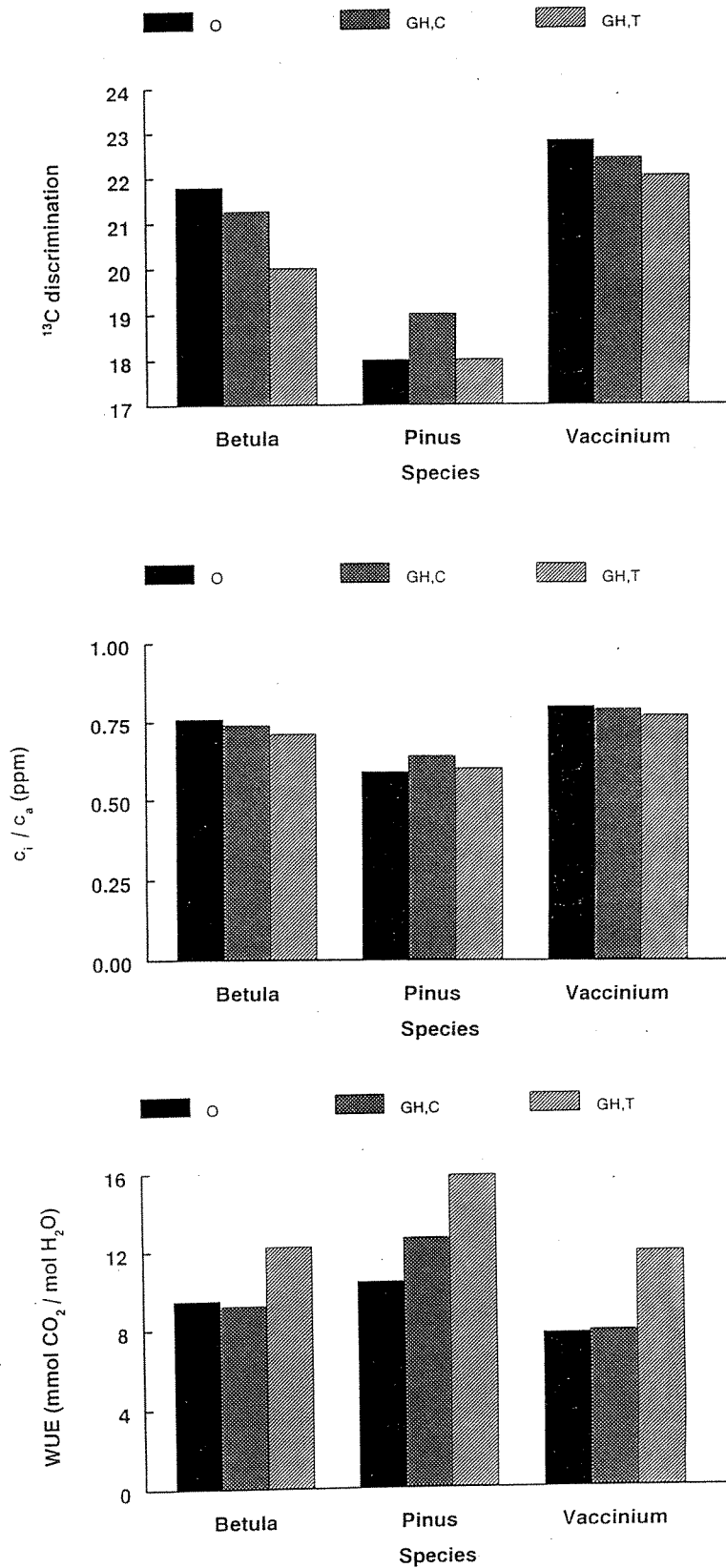**Figure 7.5.** Changes in (a) the extent of 13C discrimination, (b) isotopically-derived ratio of intercellular to ambient CO2 concentrations (ci / ca ratio) and (c) isotopically-derived leaf water use efficiencies of B. pubescens, P. sylvestris and V. myrtillus leaves/needles which developed in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O).

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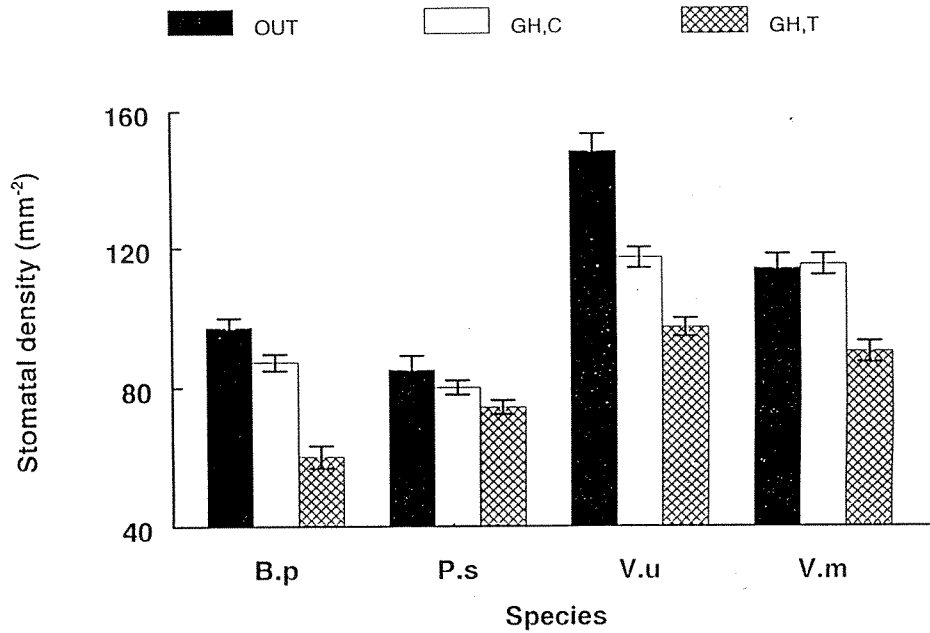
Figure 7.6. Stomatal density changes of leaves of B. pubescens (B.p), P. sylvestris (P.s), Vaccinium uliginosum (V.u) and V. myrtillus (V.m) developing in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O). Values are means 1 s.e. Pooled variance t-tests revealed that leaves developing in elevated CO_2 and temperature had significantly ($p < 0.05$) reduced stomatal densities for B. pubescens, V. uliginosum and V. myrtillus compared with the control section of the greenhouse.

Figure 7.7. Comparison of measured and predicted leaf 13C composition for B. pubescens, P. sylvestris and V. myrtillus in the treated (GH,T) and control (GH,C) sections of the CLIMEX greenhouse and outside (O). The solid line indicates the fit to the data (intercept = -4.44, slope = 0.889, p <0.01, r = 0.96), the broken line indicates the 1:1 slope.
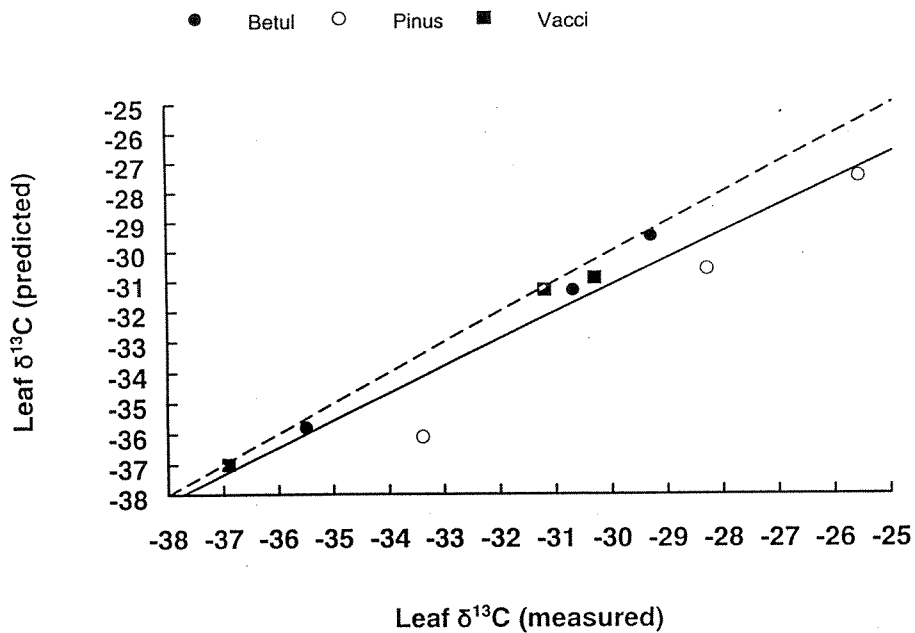
Table 1. Maximum and average (in brackets) rates of carboxylation (V_{max}) and electron transport (J_{max}) of leaves of plants investigated in the CLIMEX experiment

Species	Treatment					
	Ouside		Greenhouse, control		Greenhouse, treatment	
	V_{max}	J_{max}	V_{max}	J_{max}	V_{max}	J_{max}
			(μmol m^{-2} s^{-1})			
Betula pubescens	31.6(5.6)	73.6(18.5)	19.9(5.3)	42.0(16.6)	21.6(5.3)	52.3(15.5)
Pinus sylvestris	61.4(8.8)	119.9(27.9)	26.4(6.4)	59.2(19.7)	23.8(10.6)	52.0(30.3)
Vaccinium myrtillus	14.4(3.5)	36.4(11.7)	14.2(3.0)	28.0(9.2)	11.3(3.6)	28.2(11.0)

Table 2. Leaf C and N content (%) of plants investigated in the CLIMEX experiment (P. Verburg, pers. comm.)

Species	Treatment								
	Ouside			Greenhouse, control			Greenhouse, treatment		
	C	N	C/N	C	N	C/N	C	N	C/N
				(%)					
Betula pubescens	57.4	1.9	30.2	55.8	1.7	32.8	55.0	1.6	34.3
Pinus sylvestris	61.0	2.0	30.5	56.9	1.0	56.9	57.4	1.2	47.8
Vaccinium myrtillus	55.5	1.1	50.5	55.0	.90	61.1	54.8	1.2	45.6

8. Soil Ecology

Madelein Vreeken-Buijs and Lijbert Brussaard (WAU)

Introduction

The effect of soil fauna on the decomposition of litter and the mineralization of nutrients can vary greatly depending on, for example, the quality of the organic substrate (Verhoef and Brussaard, 1990). It is well documented that a raise in atmospheric CO_2 leads to a relative decrease in the nitrogen content of the litter (Eamus and Jarvis, 1989), to structural and density changes of the leaf tissue (Thomas & Harvey, 1983; Radoglov and Jarvis, 1990; Lemon, 1983) and to an increase in the level of phenolic substances in the leaves (Jonasson et al., 1986, Laine and Hettonen, 1987). Although they generally stimulate decomposition through their role in physically breaking down organic matter, soil mesofauna may also indirectly inhibit decomposition by excessive grazing on primary decomposers (fungi and bacteria) (Coleman et al., 1983). Such impacts of the soil fauna at the ecosystem level have received insufficient attention. The stimulating effect of the soil fauna community on the decomposition rate of chestnut and beech litter (Anderson, 1973) and of birch litter (Setälä et al., 1988; Huhta and Setälä, 1990; Setälä et al., 1990) has been shown, as well as a stimulatory effect on the uptake of nutrients by birch seedlings (Setälä and Huhta, 1991).

Such effects however, are modified as the quality of the litter changes as a result of changing atmospheric conditions. Coûteaux et al. (1991) studied the effects of different levels of complexity of the soil fauna community on the decomposition and mineralization of sweet chestnut litter, grown at two different CO_2 levels. It was found that the positive effect of the most complex faunal community as compared to the simplest system was much more pronounced in litter produced under high CO_2, with a relatively low initial nitrogen content. Only in the microcosms with the most complex faunal community did the decomposition rate of the "high CO_2" litter approximate the decomposition rate of the "ambient CO_2" litter. In the latter type of litter no significant effect of the complexity of the soil fauna on the decomposition rate was found. These results indicate an increasing importance of the soil fauna at higher atmospheric CO_2 levels. However, the Coûteaux et al. (1991) experiment did not include predatory species, and this may in longer lasting experiments lead to population explosions of collembola and microbivorous mites (Vreeken-Buijs, in prep.) and nematodes (Santos and Whitford, 1981; Setälä et al., 1991) which in turn may lead to overgrazing of the microbial population and subsequent inhibition of decomposition. Therefore, we chose to conduct a two-year litterbag field experiment and two laboratory experiments comparing a system without mites and collembola to a system with the microarthropod fauna, representing the complete microarthropod community of the Risdalsheia soil (including predatory microarthropods). In a mesocosm experiment the effects on N-mineralization and litter mass loss was monitored, while in the second experiment the effect on the uptake of nutrients by birch seedlings was studied.

In contrast with the gnotobiotic systems in glass house experiments by Coûteaux et al. (1991), we chose to work with systems, in which the species composition of the meso- and microfauna is not controlled, but maximal resemblance to the natural situation is pursued. The litterbag experiment is the link to the field situation.. In the spring and summer of 1994 the three main experiments were started and sampled every six months.

II.   Field experiment: Litterbag study

Aims

1. To study the differences in soil fauna between the different treatments.
2. To study the differences in litter decomposition rate between the treatments.
3. To study the effect of the soil mesofauna on the litter decomposition rate under the different treatments.
4. To study the changes in the chemical composition of the decomposing litter with and without soil mesofauna under the different treatments

Treatments

KIM-C:	roofed	ambient CO_2	ambient temperature	clean precipitation
KIM-T:	roofed	raised CO_2	raised temperature	clean precipitation
EGIL-C:	roofed	ambient CO_2	ambient temperature	ambient precipitation
EGIL-T:	roofed	ambient CO_2	raised temperature	ambient precipitation
METTE:	open	ambient CO_2	ambient temperature	ambient precipitation

Set-up

Litterbags (15 x 15 cm) were filled with 4 g (dry matter) birch litter, grown under conditions of ambient CO_2 and clean precipitation (KIM), raised CO_2 and clean precipitation (KIM), ambient CO_2 and ambient precipitation (EGIL, 2x). Half of the bags had a mesh size of 1.5 mm and half a mesh size of 40 μm to exclude soil meso- and macrofauna (Anderson, 1973). Ten replicates of each treatment were sampled at 6 sampling occasions.

All litterbags were defaunated by freezing (-40°C, 2x48 hr) in advance of the experiment. After sampling the weight loss, loss of nutrients (N and P), C/N ratio and lignin/N ratio of the litter was assessed and microarthropods, nematodes and enchytraeids were quantified, subdivided into functional groups. In January 1994 all litterbags were filled and sewn closed with polyester yarn and defaunated by freezing. Late April 1994 litterbags were placed in the different compartments of the two roofed catchments at Risdalsheia, Norway. Bags were grouped in patches of ten coarse- and ten fine-mesh bags, to facilitate retrieving. On day later the initial sapling took place, necessary to assess the mass loss due to handling and to quantify the initial C and N content of the different litter types. In September 1995 the second sampling took place.

In April 1995 the third sampling took place in the KIM and EGIL catchment, while in the METTE reference catchment litterbags were placed for the first time, due to a shortage of initial litter material in 1994. In September 1995 and May 1996 the last two samplings took place in all five catchments. Until now the chemical data set of the litter is not yet complete, but a short overview of the weight loss results and the biotic data is presented.

Results

1. All faunal data (microarthropod functional groups, nematodes and enchytraeids) showed a skew distribution and therefore were log transformed. After 6 months, colonization of both the coarse and the fine mesh litterbags had occurred, though in the fine mesh bags this was predominantly by mites, belonging to the group of the non-cryptostigmatids, formed mainly by very tiny, fluid feeding fungivores, that also in earlier experiments have managed to enter through meshes of 40 μm and less. Most other functional groups had successfully colonized the coarse mesh bags after six months. The nematophagous mites and the predatory collembola only occurred sporadically. Significantly more microarthropods are found in the coarse than in the fine mesh bags in all functional groups except the nematophagous mites and the predatory collembola, because of their sporadic occurrence and the non-cryptostigmatic mites, because of their contamination of the fine mesh litterbags. Higher numbers of nematodes were found in the fine mesh bags than in the coarse mesh bags, probably due to a decreased predation pressure. Differences in mesofauna composition between the catchments were analysed from coarse mesh litterbags only (table 1).

Table 1. ANOVA on the 10log transformed biotic data of the course mesh litterbags: P values.

functional group	catchment		time	catchment*time	
predatory mites	0.214		< 0.001	0.067	
cryptostigmatic mites	0.587		< 0.001	0.023	*
astigmatic mites	0.344		< 0.001	0.804	
non-cryptostigmatic mites	0.236		< 0.001	0.181	
nematophagous mites	0.313		0.067	0.929	
omnivorous collembola	0.007	(highest in EGIL-T)	< 0.001	0.001	**
predatory collembola	0.888		0.075	0.965	
total microarthropods	0.486		< 0.001	0.110	
total nematodes	< 0.001	(highest in EGIL-C, lowest in KIM-T)	< 0.001	0.001	**
emchytraeids	0.584		0.017	0.780	

No effect of catchment was found on total microarthropod numbers, only the omnivorous collembola reacted to the soil heating in EGIL-T by increasing their numbers significantly. The difference in nematode numbers may be an effect of soil moisture differences. This can however

not be tested by the moisture content of the litterbags themselves, because these data represent only two moments in a whole year, and will not provide a proper view of soil moisture variation in the litter layer. In general, microarthropod numbers increased until the 18 month sampling and had decreased at the last sampling. Higher numbers were found in the September samplings, then in the April samplings, just after snow melt. 2/3. Litter weight loss was calculated as percentage dry mass remaining (Fig. 1A, table 2).

Table 2. ANOVA on the percentage dry weight remaining (n=7)

	catchment	time	catchment * time	mesh	catchment * mesh	time * mesh	catchment * time * mesh
%dry weight remaining	<0.001 (KIM<EGIL)	<0.001	<0.001	<0.001 (F < C)	0.040	0.053	0.107

Remarkably though, the average decomposition rate was higher in the fine mesh litterbags then in the coarse mesh bags. However, this effect varied among the different catchments, as was expected. Therefore it is useful to examine the mass loss results in more detail:

catchments compared	tested effects on remaining litter mass	main effect	mesh size	interaction with mesh size	interaction with time	mesh size * time
KIM-C/ KIM-T	CO$_2$ and temperature	-	-	*	-	-
EGIL-C/ KIM-C	precipitation	***	-	-	***	-
EGIL-C/ EGIL-T	soil heating	-	**	-	-	*
EGIL-C/ METTE	roofing	*	*	*	*	-

Comparing the two KIM catchments, we find an interaction between mesh size and the CO$_2$ + temperature treatment. There is significantly higher mass loss in the high CO$_2$ catchment than in the reference catchment in the fine mesh bags, that exclude (most of) the microarthropods, while no difference is found in the coarse mesh bags (P= 0.04).

- Comparing the EGIL-C to the KIM-C catchment, we find a higher decomposition rate in KIM (clean precipitation), than in EGIL (ambient precipitation) (P< 0.001), irrespective of mesh size.

- Comparing the two EGIL catchments, we see no effect of soil heating. The mesh size effect (higher decomposition in the fine mesh litterbags) was found in both catchments, but only after 1.5 year (mesh*time: $P=0.018$).

- Comparing EGIL-C to METTE (0, 6 and 12 months sampling only), we found a higher decomposition rate in the open air catchment, than in the roofed catchment ($P= 0.020$), though this was only a temporal effect, since no significant difference was found any more at the 12 month sampling. Since these samplings took place in different years, this could be an effect of different weather conditions (cover * mesh: $P=0.018$). The mesh size effect was only found in the open catchment (cover *mesh: $P=0.029$).

4. The chemical data set is not yet complete, so an analysis cannot yet be made.

Preliminary conclusions and discussion

1. During the two year incubation, the different climate treatments had in general no effect on the microarthropod densities in the coarse mesh litterbags. Since the increase in numbers of the omnivorous collembola was only found in the EGIL-T catchment and not in the KIM-T catchment, this could be a result of the on average drier conditions in the soil heating area, and not of the temperature increase itself. This has to be checked by soil moisture data (TDR).

2. Litter mass loss of the coarse mesh litterbags was higher in both KIM catchments, than in the EGIL catchments, the difference after 24 months being on average 15% of initial dry weight. An explanation to this difference has not yet been found, for the opposite effect would be expected, regarding the different nutrient status of the soil resulting from the different precipitation. Maybe the difference is caused by microclimatic differences between KIM and EGIL. No effect of the CO_2 -treatment on the decomposition rate was found during the two year incubation in the KIM catchment.

3. The effects of mesh size found, cannot be explained as an effect of microarthropod exclusion, but of a reduced mesofaunal diversity, since, although numbers were sometimes even higher than in the coarse mesh bags, in the fine mesh bags the numbers of all except one functional group were significantly reduced (fig. 1B). Infestation of the fine mesh litterbags was higher in KIM than in EGIL, maybe due to more suitable microclimatic conditions for the non-cryptostigmatic mites, especially in the first year of the treatment. The difference in microarthropod composition also caused an increase in nematode numbers in the fine mesh litterbags, since nematodes are predated not only by specialist nematophagous mites, but also by general predatory mites and cryptostigmatic mites.

Generally speaking, a reduced microarthropod population appears to correspond to an increased decomposition rate. This was most evident in the EGIL catchment, where the level of

contamination of the fine mesh bags with non-cryptostigmatic mites was low and the average remaining litter dry weight in the fine mesh bags was approximately 3.5 % lower than in the coarse mesh litterbags. This is contradictory to most of the cited literature. In KIM this effect was only found in the elevated treatment (3.2 % difference). Judas et al. (1996) reported a negative effect of mesofaunal grazing on microbial N-immobilization in a litterbag experiment. Therefore we may assume, that in a highly N-limited environment like the Risdalsheia soil, the threshold between grazing (generally considered to affect decomposition positively), and overgrazing (inhibiting decomposition) may be very low, with overgrazing of the fungal population, and so an impeded decomposition being the rule. Fungi are crucial to the N supply of the decomposer food web due to their ability to transfer nitrogen from other soil layers to the litter. The overgrazing may even be restricted to specific fungal species (selective grazing), resulting in decomposition effects, uncorrelated to the total fungal biomass (Faber, 1991). In the fine mesh bags the overgrazing pressure was lifted and decomposition increased.

In KIM-T high-CO_2 grown litter was incubated, with a slightly lower N-content, while in KIM-C ambient-CO_2 grown litter was used, like in both EGIL catchments. Zak et al. (1996) have assessed an increase in soil microbial biomass under elevated CO_2. Together this may explain why a reduced mesofauna in litterbags in the KIM-T catchment led to an increased decomposition rate, while no effect was found in the KIM-C catchment.

4. All above conclusions are anticipating on the C and N data not yet available.

General conclusions:

- Microarthropod functional group densities are not affected by elevated CO_2 and/or temperature.
- Acid rain has an inhibitory effect on litter decomposition rate.
- Reduced microarthropod numbers resulted in increased decomposition rates.
- New initiatives concerning field experiments studying the effect of climatic change on soil microarthropods are not recommended.

III. Laboratory experiments

The analysis of the results of the two laboratory experiments has not been completed yet.

References

Anderson, J. M. (1973) The breakdown and decomposition of Sweet Chestnut (*Castanea sativa* Mill.) and beech (*Fagus sylvatica* L.) leaf litter in two deciduous woodlands. I. Breakdown, leaching and decomposition. *Oecologia* **12**: 251-274.

Coleman, D. C., C. P. P. Reid and C.V. Cole (1983) Biological strategies of nutrient cycling in soil systems. *Adv. Ecol. Res.* **13**: 1-54.

Coûteaux, M.-M., M. Mousseau, M.-L. Célérier and P. Bottner (1991) Increased atmospheric CO_2 and litter quality: decomposition of sweet chestnut leaf litter with animal food webs of different complexities. *Oikos* **61**: 54-64

Eamus, D. and P. G. Jarvis (1989) The direct effects of increase in atmospheric CO_2 concentration on natural and commercial temperate trees and forests. *Adv. Ecol. Res.* **19**: 1-55.

Faber, J. H. (1991) Functional classification of soil fauna: a new approach. *Oikos* **62**: 110-117.

Huhta, V. and H. Setälä (1990) Laboratory design to simulate complexity of forest floor for studying the role of fauna in the soil processes. *Biol. Fertil. Soils* **10**: 155-162.

Jonasson, S., J. P. Bryant, F. S. Chapin and M. Anderson (1986). Plant phenols and nutrients in relation to variations in climate and rodent grazing. Am. Nat. 128: 394-408.

Judas, M., M. Gobel and J Schauermann (1996) Mass loss of *Urtica dioica* L. roots in a beech forest as affected by soil fauna and soil type. *Pedobiologia* **39**: 528-537.

Laine, K. M. and H. Hettonen (1997). Phenolics/nitrogen ratios in blueberry (*V. myrtillus*) in relation to temperature and microtine density in Finnish Lapland. *Oikos* **50**: 389-395.

Lemon, E. R. (1983) CO_2 and plants: The response of plants to rising levels of atmospheric carbon dioxide. Westview Press. Boulder CO.

Radoglov, K. M. and P. G. Jarvis (1990). Effects of CO_2 enrichment on four poplar clones. I. Growth and leaf anatomy *J. Exper. Bot.*(in press).

Santos, P. F. and W. G. Whitford (1981) The effects of microarthropods on litter decomposition in a Chihuahuan desert ecosystem. *Ecology*, **62**: 654-663

Setälä, H., J. Haimi and V. Huhta (1988) A microcosm study on the respiration and weight loss in birch litter and raw humus as influenced by soil fauna. *Biol. Fertil. Soils* **5**: 282-287.

Setälä, H. and V. Huhta (1991) Soil fauna increase *Betula pendula* growth: Laboratory experiments with coniferous forest floor. *Ecology* **72**: 665-671.

Setälä, H., E. Martikainen, M. Tyynismaa, and V. Huhta (1990) Effects of soil fauna on leaching of nitrogen and phosphorous from experimental systems simulating coniferous forest floor. *Biol. Fertil. Soil* **10**: 170-177

Setälä, H., M. Tyynismaa, E. Martikainen and V. Huhta (1991) Mineralisation of C, N and P in relation to decomposer community structure in coniferous forest soil. *Pedobiologia* **35**: 285-296.

Thomas, J. F. and C. N. Harvey (1983) Leaf anatomy of four species grown under continuous long-term CO_2 enrichment. *Bot. Gaz.* **144**: 303-309.

Verburg, P. S. J. and N. van Bremen (1994) Chapter 5: Soil chemistry and decomposition of organic matter. In: N.B. Dise and A. Jenkins (Eds.) The CLIMEX Project: Whole catchment Manipulation of CO2 and Temperature. Inst. of Hydrology, Wallingford, U. K. pp: 61-71.

Verhoef, H. A. and L. Brussaard (1990) Decomposition and nitrogen mineralisation in natural and agro-ecosystems; the contribution of soil animals. *Biogeochemistry* **11**: 175-211.

Zak, D. R., D. B. Ringelberg, K. S. Pregitzer, D. L. Randlett, D. C. White and P. S. Curtis (1996). Soil microbial communities beneath *Populus grandidentata* crown under elevated CO_2. *Ecol. Appl.* **6**: 257-262
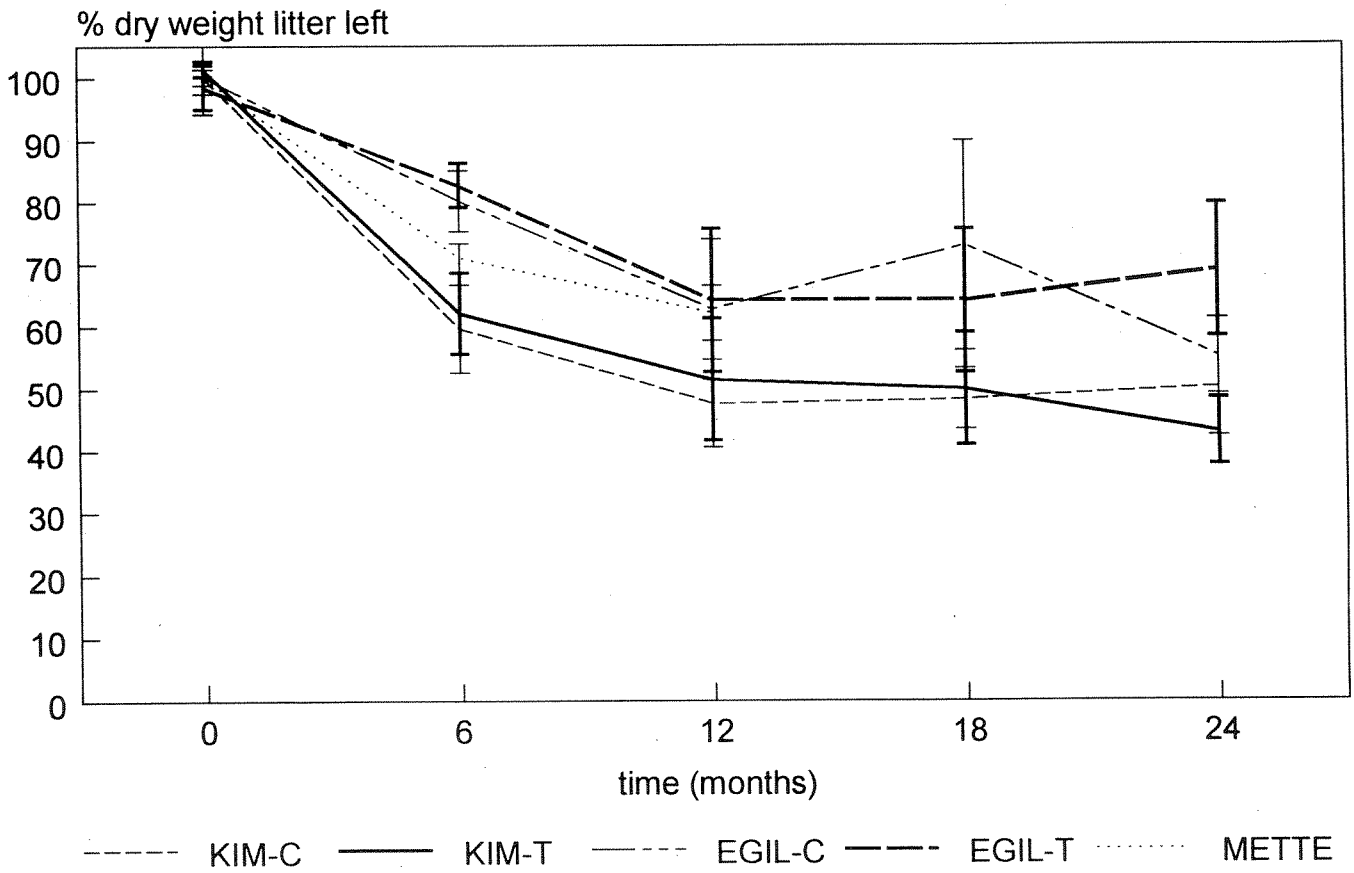
Figure 8.1A. Log-transformed average total microarthropod numbers (n=3), extracted from litterbags sampled from the five catchments.
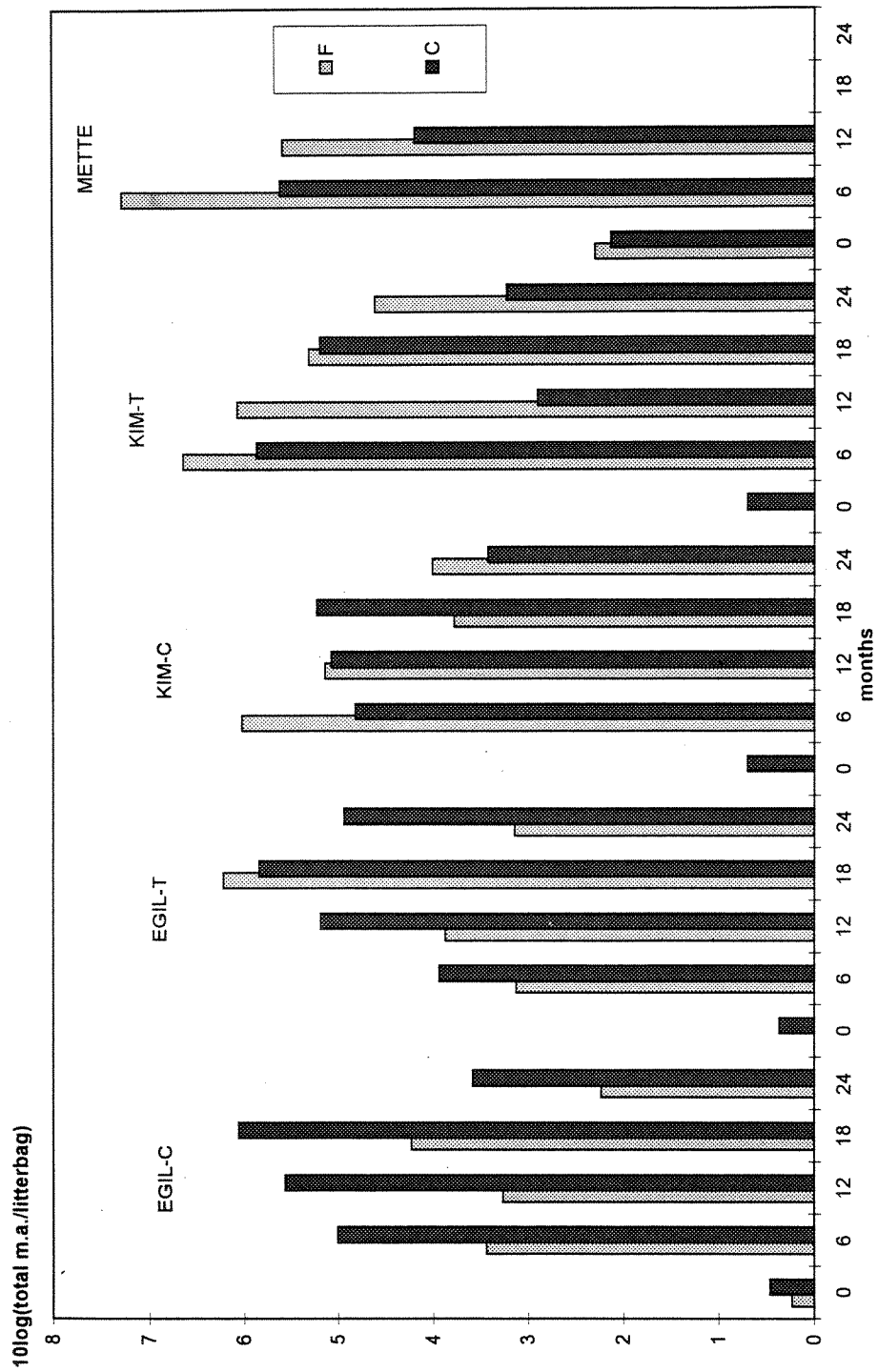
Figure 8.1B. Log-transformed average total microarthropod numbers, corrected for non-cryptostigmatic mite infestation (n=3).
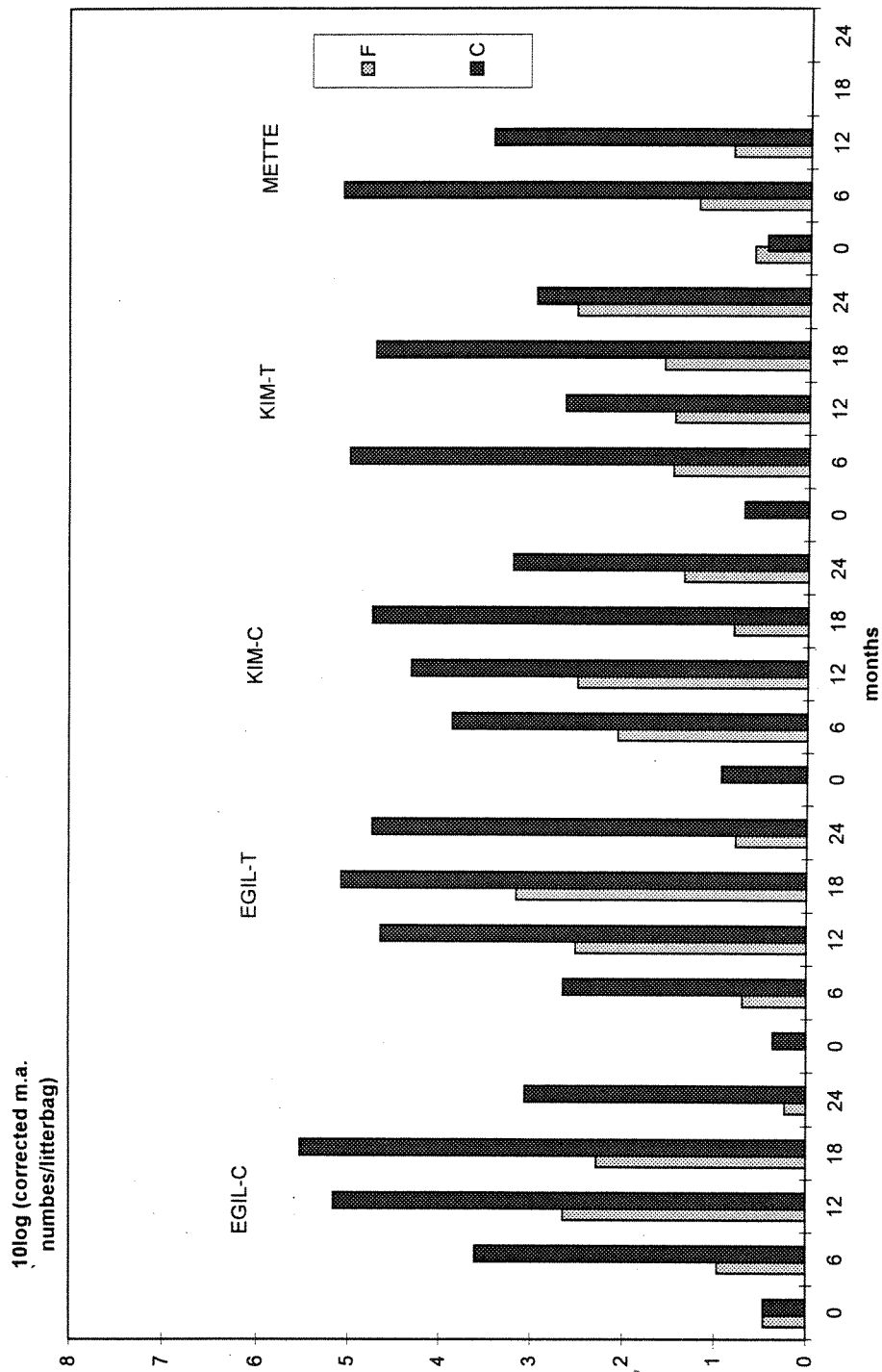
Figure 8.2. Percentage of dry weight left in the coarse mesh litterbags in the five catchments.

9. CLIMEX Publication List

Jenkins, A., Schulze, E.D., van Breemen, N., Woodward, F.I. and Wright, R.F. 1992. CLIMEX -climate change experiment. In, Teller, A., Mathy, P. and Jeffers, J.N.R. (Eds) Responses of Forest Ecosystems To Environmental Changes. Elsevier, London.

Arp, W. and Berendse, F. 1993. Plant growth and nutrient cycling in nutrient-poor ecosystems. In, Van de Geijn, S.C., Goudriaan, J. and Berendse, F. (Eds) Climate Change: Crops and Terrestrial Ecosystems. Agrobiologische Thema's 9, CABO-DLO, Wageningen, The Netherlands.

Jenkins, A., Wright, R.F., Berendse, F., van Breemen, N., Brussaard, L., Schulze, E.D. and Woodward, F.I. 1993. The CLIMEX project - climate change experiment. In, Rasmussen, L., Brydges, T. and Mathy, P. (eds) Experimental Manipulations of Biota and Biogeochemical Cycling in Ecosystems. Ecosystems Research Report 4, CEC, Brussels.

Jenkins, A. and Wright, R.F. 1993. The CLIMEX project - raising CO_2 and temperature to whole catchment ecosystems. In, Schulze, E.D. and Mooney, H.A. (Eds) Design and Execution of Experiments on CO_2 Enrichment. Ecosystems Research Report No. 6., CEC. Brussels.

Beerling, D. J. and Woodward, F.I. 1994. The climate change experiment (CLIMEX): Phenology and gas exchange responses of boreal vegetation to global change. Global Ecology and Biogeography Letters 4, 17 - 26.

Dise, N.B. and Jenkins, A. (Eds) 1995. The CLIMEX Project: Whole Catchment Manipulation of CO_2 and Temperature. Climate Change Research Report 3/95, NIVA, Oslo. 130pp.

Jenkins, A. (Ed) 1995a. CLIMEX - Climate Change Experiment: Progress Report December 1992 - June 1993. Climate Change Research Report 1/95. NIVA, Oslo. 12pp.

Jenkins, A. (Ed) 1995b. CLIMEX - Climate Change Experiment: Progress Report July 1993 - December 1993. Climate Change Research Report 2/95, NIVA, Oslo. 31pp.

Jenkins, A. (Ed) 1995c. CLIMEX - Climate Change Experiment: Final Report on Phase I, the first year of treatment May 1994 - December 1994. Climate Change ResearchReport 4/95, NIVA, Oslo. 47pp.

Wright, R.F., Indroy, A-S., Hogberget, R., Lukewille, A., Sorvag, T. and Willbergh, M. 1995. CLIMEX Project: climate data for the first year of treatment April 1994 - March 1995. Climate Change Research Report 5/95, NIVA, Oslo. 21 pp.

Jenkins, A. and Wright, R.F. 1995. The CLIMEX Project: Performance of the Experimental Facility During the First Year of Treatment. In, Jenkins, A., Ferrier, R.C. and Kirby, C. (Eds) Ecosystem Manipulation Experiments. Ecosystem Research Report No. 20, pp323 - 328. CEC, Brussels.

Lukewille, A., Arp, W., Verburg, P., Jenkins, A. and Wright, R.F. 1995. The CLIMEX soil heating experiment at Risdalsheia, Southern Norway. In, Jenkins, A., Ferrier, R.C. and Kirby, C. (Eds) Ecosystem Manipulation Experiments. Ecosystem Research Report No. 20, pp331 - 334. CEC, Brussels.

Beerling, D.J. and Woodward, F.I. 1996. *In situ* gas exchange responses of boreal vegetation to elevated CO2 and temperature: first season results. Global Ecology and Biogeography Letters 5, in press.

Beerling, D.J., Heath, J., Mansfield, T.A. and Woodward, F.I. 1996. Drought - CO_2 interactions in trees: mechanisms and observations. New Phytologist, in press.

Beerling, D.J. 1996. Ecophysiological responses of boreal forest-tundra transition to elevated CO_2 and temperature: treatment effects during the second season. Oecologia, submitted.

Lukewille, A. and Wright, R.F. 1996. Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. Global Change Biology 2, in press.

Jenkins, A. (Ed). 1996. CLIMEX project: Report on the second year of treatment May 1995 - December 1995. Climate Change Research Report 6/96. NIVA, Oslo.
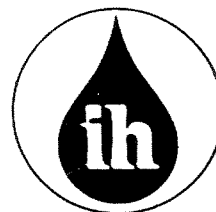
CLIMATE CHANGE RESEARCH REPORTS

1/1995   Jenkins, A. (ed). 1995. CLIMEX Climate Change Experiment: Progress Report December 1992 - June 1993. 12 pp.

2/1995. Jenkins, A. (ed). 1995. CLIMEX Climate Change Experiment: Progress Report July 1993 - December 1993. 31 pp.

3/1995   Dise, N.B., and Jenkins, A. (eds). 1995. The CLIMEX project: Whole Catchment Manipulation of CO_2 and Temperature. 130 pp.

4/1995   Jenkins A. (ed). 1995. CLIMEX Climate Change Experiment: Final Report on Phase I the first year of treatment May 1994 - December 1994. 47 pp.

5/1995   Wright, R.F., Indrøy, A-S., Høgberget, R., Lükewille, A., Sørvåg, T., and Willbergh, M. 1995. CLIMEX Project. Climate data for first year of treatment April 1994 - March 1995. 21 pp.

6/1996   Jenkins A. (ed). 1996. CLIMEX project: Report on the second year of treatment May 1995 - December 1995. 74 pp.