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Effects of climate change and simulated herbivory on growth responses and leaf characteristics of silver birch (*Betula pendula*) seedlings

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Academic dissertation

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ABSTRACT

The main objective of this thesis was to examine the effects of climate change and simulated herbivory (artificial foliage damage) on growth responses and leaf characteristics (chemical and morphological structure and consequent palatability) of silver birch (*Betula pendula*) seedlings. In order to achieve this target, seedlings were grown in climate controlled closed-top chambers under ambient and elevated levels of temperature and CO_2 with different nutrient regimens. Four degrees (0, 25, 50 or 75%) of defoliation was conducted on the seedlings. Additionally, the effects of climate change and nutrient availability on chemical composition and subsequent decay process of the abscised leaves were studied by leaving litter in bags in the field to decompose.

The results showed that height and biomass growth were stimulated in the same season of tissue loss especially in the fertilized seedlings defoliated to 25% and subjected to elevated temperature and CO_2. In comparison, the growth was slowed down in the seedlings defoliated to 50%. In the following season, compared to the intact controls, the corresponding growth was less in the seedlings defoliated to 25%, but higher in the seedlings defoliated to 50% and 75%. This phenomenon was evident particularly under elevated temperature.

In the same season of tissue loss, the concentrations of different phenolic compounds were lower and the leaves were smaller in defoliated seedlings than those in intact controls. Fertilization also lowered phenolic compounds, but increased leaf sizes. Leaves developed under elevated temperature were less palatable for blue alder leaf beetles (*Agelastica alni*). Similarly, the decay process of leaf litter produced under elevated temperature was slowed down. To summarize, climate change and increased nutrient availability may intensify the growth of defoliated seedlings, but also alter the quality of leaves or leaf litter so that they may repel herbivores and slow down the activity of soil microorganisms.

Keywords: deciduous trees, elevated CO_2, elevated temperature, folivory, growth over-compensation, leaf litter decomposition

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Joensuu, November 2008

Liisa Huttunen

LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles, which are referred to in the text by their Roman numerals I-IV.

I Huttunen, L., Niemelä, P., Peltola, H., Heiska, S., Rousi, M., Kellomäki, S. 2007. Is a defoliated silver birch seedling able to overcompensate the growth under changing climate? Environmental and Experimental Botany 60: 227-238. doi:10.1016/j.envexpbot.2006.10.010

II Huttunen, L., Niemelä, P., Julkunen-Tiitto, R., Heiska, S., Tegelberg, R., Rousi, M., Kellomäki. S. 2008. Does defoliation induce chemical and morphological defenses in the leaves of silver birch seedlings under changing climate? Chemoecology 18: 85-98. doi:10.1007/s00049-007-0397-5

III Huttunen, L., Ayres, M.P., Niemelä, P., Heiska, S., Tegelberg, R., Rousi, M., Kellomäki S. 2008. Consequences of climate change for compensatory growth in silver birch that experience defoliation. Submitted manuscript.

IV Huttunen, L., Aphalo, P. J., Lehto, T., Niemelä, P., Kuokkanen, K., Kellomäki S. 2008. Effects of elevated temperature, elevated CO_2 and fertilization on quality and subsequent decomposition of silver birch leaf litter. Manuscript.

In regard to the entire thesis, most of the work done in articles I-IV was carried out by Liisa Huttunen with a notation that in articles I-III, Dr. Susanne Heiska provided assistance in developing the statistical models, and in article IV, Docent Pedro J. Aphalo contributed to the data analysis. The co-authors of separate articles (I-IV) have participated in the writing by commenting on the text and thereby improving the manuscripts.

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1 INTRODUCTION

1.1 Background

In recent years, more attention has been drawn to the wide-ranging impacts of increasing atmospheric carbon dioxide concentration and globally rising temperatures on terrestrial ecosystems. These effects may especially be substantial in the northern hemisphere (IPCC 2007). For example, in the boreal forests, climate change is predicted to favor trees with continuous growth and thereby potentially increase the distribution and number of deciduous tree species, including birches (e.g. *Betula pendula* and *B. pubescens*) (Kellomäki et al. 2005). At the same time, global warming and rising winter temperatures may increase the abundance and dispersion of insect herbivores in mid and high latitude deciduous forests (Ayres and Lombardero 2000, Dale et al. 2001, Wolf et al. 2008). In Fennoscandinavia, warmer winters may improve the survival of eggs or individuals, for example, among Lepidoptera species, and consequently increase the risk of widespread foliage damage and tree deaths in the boreal birch forests (Virtanen and Neuvonen 1999, Niemelä et al. 2001, Niemistö et al. 2004).

The net consequences of herbivory are dependent on the extent to which trees are able to compensate for the lost foliage. These responses are driven by the evolutionary history and growth form of particular tree species (fast growing pioneer species, such as silver birch, *B. pendula* vs. slow growing late successional species, such as European beech, *Fagus sylvatica*), but also by physiological state, environmental conditions (e.g. temperature) and resource availability (e.g. CO_2, nutrients and water) of particular individuals (Belsky 1986, 1987, Maschinski and Whitham 1989, Simons and Johnston 1999, Puettmann and Saunders 2001, Rees et al. 2001, Dittmar et al. 2003, Prittinen et al. 2003, Kunstler et al. 2005). Several previous studies on trees have demonstrated partial compensation of shoot height and biomass growth for insect feeding damage (e.g. Hjältén et al. 1993, Kaitaniemi et al. 1999, Mattson et al. 2004, Osier and Lindroth 2004). As a comparison, in some perennial plants (grasses and forbs), tissue removal often leads to higher re-growth or seed production, which is classified as over-compensation (Agrawal 2000, Huhta et al. 2000). In some cases, this over-compensative growth in herbaceous plants may have resulted from conditions that favored their performance (e.g. Paige and Whitham 1987).

It is reasonable to assume that the ability of mid to high latitude deciduous tree species to recover from foliage damage may be improved by climate change. Generally, elevation in atmospheric CO_2 and temperature will stimulate photosynthetic activity and, accordingly, height and biomass growth in C_3 plants (Fetcher et al. 1988, Bazzaz 1990, Long 1991, Farrar and Williams 1991, Lee et al. 1998), such as in silver birch (Rey and Jarvis 1997, Kellomäki and Wang 2001, Riikonen et al. 2005). It is also expected, that long-term exposure to elevated CO_2 restores the photosynthetic capacity of defoliated trees to the equivalent of that of intact counterparts. This kind of result has been attained in herbaceous plants and in some coniferous and deciduous tree species when subjected to elevated CO_2 for couple of seasons (Bryant et al. 1998, Handa et al. 2005).

Changing climate may also have indirect effects on trees, for example in boreal ecosystems. Mutikainen et al. (2000) reported that the ability of defoliated silver birch seedlings to recover from and compensate for the loss of leaf biomass was enhanced by fertilization. This is essential since warming climate may accelerate mineralization rates of organic matter in soil and thus increase plant nutrient availability (Hättenschwiler et al.

1996, Mäkipää et al. 1999). At the same time, human activities increase atmospheric deposition of nitrogen increasing the soil N pools (Galloway et al. 2004). On the other hand, fertilization may magnify the long-term effect of defoliation on fortifying the resource partitioning in above-ground parts of plants (McNaughton et al. 1983). This may weaken the ability of roots to grow and store resources, and consequently reduce the efficiency of leaf production and stem growth during growing seasons after tissue loss (see Kozlowski 1992, Landhäusser and Lieffers 2002, 2003).

Beyond stimulated primary metabolism (photosynthesis and growth), the change in climate also stimulates the synthesis of carbon based secondary compounds in forest trees. For example, concentrations of various phenolic compounds in the leaves of trees are found to be increased with increasing CO_2 (e.g. Lavola and Julkunen-Tiitto 1994, Kuokkanen et al. 2001, 2003). On the other hand, if the rising temperature is accompanied by increasing plant nutrient availability by accelerating mineralization rates of organic matter in soil, this increment may decrease the phenolic concentrations in the leaves (Lavola and Julkunen-Tiitto 1994, Keinänen et al. 1999, Laitinen et al. 2005). Earlier these phenolic substances were thought to be metabolic waste products, but nowadays they are known to exhibit many biological functions, such as providing protection against insect feeding (Feeny 1976, Hartley and Lawton 1991, Ayres et al. 1997, Keinänen et al. 1999). Phenolics and tannins are known to be bitter tasting and thus they may repel herbivores (Bryant et al. 1992). In fact, the concentrations of phenolic compounds are reported to be increased in leaves of mountain birches (*B. pubescens* subsp. *czerepanovii*) after insect feeding damage (Haukioja and Niemelä 1979, Hanhimäki 1989, Haukioja and Neuvonen 1985, Kaitaniemi and Ruohomäki 2001, Lempa et al. 2004).

Besides physiology, the structure of leaves in trees is found to be affected by the patterns of climate change (Lindroth et al. 1993, Veteli et al. 2002). Elevation in temperature has been reported to increase the size and number of developing leaves (Ackerly et al. 1992, Gunn and Farrar 1999, Xiao et al. 2003). Elevation in CO_2 is also known to increase the content of structural carbon (such as lignin) in the leaves of silver birch (Cotrufo and Ineson 1996), which may increase the specific leaf weight (SLW; dry weight per unit leaf area, mg mm^{-2}) (Campbell et al. 1988, Lincoln et al. 1993, Veteli et al. 2002). According to Lincoln et al. (1993), the specific leaf weight is strongly linked to leaf density and toughness. Finally, increase in nutrient availability has been shown to enhance the size growth of individual leaves in different birch species (Niinemets et al. 2002).

In trees, insect feeding damage may also alter the leaf morphology. Under ambient climate, the leaves produced in downy birches (*B. pubescens*) after defoliation have been discovered to be smaller than those in their intact counterparts (Rautio et al. 2002). Furthermore, defoliation has increased the SLW of leaves in herbaceous plants and trees (Bassman and Zwier 1993, Páez et al. 1995). The alteration in leaf size and SLW may be linked to the plant morphological or mechanical defense (Karban and Baldwin 1997). For example, trees may escape from repetitive defoliation during the growing season, since smaller leaf size may prevent gregarious insects for visually selecting oviposition or mating sites on their host plants (Pilson and Rausher 1988, Kagata and Ohgushi 2001). Moreover, high SLW may in fact have a greater negative influence on insect feeding behavior than high content of chemical substances in leaves (Coley 1983, Choong 1996).

The changes in the structure and phytology of green leaves produced under elevated temperature, elevated CO_2 and increased nutrient availability may affect the subsequent decomposition process of the abscised leaves. In the leaf litter, the structural compounds (such as lignin and insoluble tannins) and proteins that are not degraded and transported from leaves during their senescence are known to affect the activity of microorganisms and their abundance on the forest floor in various soil types (Hättenschwiler and Bretscher 2001). The decomposition has been reported to be especially fast in litters with high N content, but slow in the litters with high tannin and lignin contents, and, accordingly, high C: N ratio (Harrison 1971, Hunt et al. 1988, Aerts and de Caluwe 1997).

At present, little is known about the combined effects of elevated temperature, elevated CO_2 and increased nutrient availability on plant-herbivore, and plant-soil interactions. However, it is likely that under the global patterns of climate change (concurrent elevations in temperature and CO_2, and increased nutrient availability), the foliage damage directly, or the quality of litter indirectly (alteration in rate of decomposition and nutrient cycle) may affect the net primary productivity in high latitude birch forests.

1.2 Aims of this thesis

The main objective of this thesis was to examine the effects of climate change (elevated temperature and/or CO_2, and consequent increasing nutrient availability) and simulated herbivory (artificial foliage damage, i.e. defoliation) on growth responses (articles I and III) and leaf characteristics (chemical and morphological structure and consequent palatability; article II) of silver birch (*Betula pendula*) seedlings. In order to achieve this target, seedlings were grown in climate controlled closed-top chambers under ambient and elevated levels of temperature and CO_2 at different amounts of fertilization. Four degrees (0, 25, 50 or 75%) of defoliation was conducted on the seedlings (articles I, II and III). In addition, the effects of elevated temperature, elevated CO_2 and increased nutrient availability on the chemical composition and subsequent decay process of silver birch leaf litter were studied (article IV).

The specific targets regarding the seedlings exposed to the different climatic and fertilization treatment combinations were to investigate:

1) whether defoliation induces over-compensation in height and biomass growth during the same (article I) and in the following growing season after tissue loss (article III),

2) whether defoliation alters the synthesis of variety of phenolic compounds (such as flavonol glycosides, flavonoid aglycones and tannins) and morphological structure (SLW and individual size) of leaves that develop after damage (article II),

3) whether the palatability of leaves to generalist insect herbivore, adult blue alder leaf beetle (*Agelastica alni*), is changed by defoliation (article II), and

4) whether the contents of insoluble condensed tannins and total carbon and nitrogen are altered in naturally abscised leaves, and whether this alteration affects the decay process of litter on the forest floor (article IV).

2 MATERIAL AND METHODS

2.1 The seedling material

The seedling material used in articles I-III were derived from seeds produced in a naturally regenerated silver birch stand of the Finnish Forest Research Institute, Punkaharju Research Unit (61º 48´N, 29º 18´E), Finland. The seeds were collected from 25 randomly selected mother trees in 1998 and 2000, thoroughly mixed and sowed in April 2002. The total number of resulting seedlings was 3456, and they grew in trays holding 24 plants (individual cell volume 1.2 dm^3) throughout the 2002 growing season (articles I and II, Table 1).

To investigate the growth responses of the seedlings in the forthcoming season 2003 (article III), the plant material was randomly selected from the same seedlings as employed in articles I and II. The selection was performed in October 2002, with the seedlings being stored in an open shelter to over-winter. Prior to the 2003 growing season, mortality and stem breakage was considerable among the selected plants; the number of remaining individuals was only 166 seedlings (Table 1). In mid May 2003, these seedlings were transferred to Joensuu (62°40' N, 29°45' E), Finland, and individually transplanted into plastic pots (vol. 2 dm^3) for the experiment reported in article III.

The seedling material utilized in article IV (total 768 plants, Table 1) originated from seeds produced in another naturally regenerated silver birch stand in Lieksa (63º 36´N, 29º 43´E), in eastern Finland. The seeds were sown in trays (individual cell volume 0.3 dm^3) in early June 1999. The development of resulting seedlings was monitored throughout the 1999 and 2000 growing seasons. The results relating to growth of the seedlings are reported in Mattson et al. (2004). In this thesis (article IV), the focus was on the chemical composition of naturally abscised leaves and subsequent decay process of leaf litter.

Table 1. General description of the experimental periods and sites, seedling material and treatments, and measured characteristics in the seedlings employed in the different articles of this thesis.

Article	**Exp. period**	**Exp. site**	**Seedlings**		**Treatment**	**Measured characteristics**
			Age	***Total no.***		
I	June - Oct 2002	Field-built, climatic controlled closed-top chambers, Mekrijärvi Research Station	One growing season (2002)	3456	Temperature CO_2 Fertilization Defoliation	1. Carbon uptake 2. Total leaf area 3. Height and biomass growth
II	June - Oct 2002	Field-built, climatic controlled closed-top chambers, Mekrijärvi Research Station	One growing season (2002)	3456	Temperature CO_2 Fertilization Defoliation	1. Area of individual leaves 2. Specific leaf weight 3. Chemical composition of green leaves 4. Feeding experiment with adult blue alder leaf beetles
III	June - Sept 2003	Laboratory-built climatic controlled Conviron chambers, University of Joensuu	Two growing seasons (2002-2003) (a	166	Temperature CO_2 Fertilization Defoliation	1. Total leaf area 2. Height and biomass growth
IV	Oct 2000	Field-built, climatic controlled closed-top chambers, Mekrijärvi Research Station	Two growing seasons (1999-2000)	768	Temperature CO_2 Fertilization	1. Chemical composition of naturally abscised leaves
	Oct 2000 - May 2004	Field near Mekrijärvi Research Station			Naturally changing climatic conditions	2. Decomposition of leaf litter

(a The seedlings were grown in climate controlled closed-top chambers at Mekrijärvi Research Station of the University of Joensuu during the 2002 season (see articles I and II) and at the University of Joensuu during the 2003 season.

2.2 Experiments in Mekrijärvi field chamber system (articles I, II and IV)

In articles I, II and IV, the seedling material was placed in 16 climatic controlled closed-top chambers located in a field at the Mekrijärvi Research Station (62°47' N, 30°58' E), University of Joensuu, Finland (Table 1, Fig. 1). The seedlings were grown in the chambers from mid June to October 2002 (articles I and II), and from mid-June 1999 to the end of the 2000 growing season (article IV). The seedling trays were positioned on metal mesh footboards bolted onto the chamber walls at 50 cm above the ground level. The location of the trays was changed during the experiments in order to reduce the effect of within-chamber variation.

The climatic treatments applied in the chambers were designed to correspond to the climate scenario (e.g. Carter et al. 2002) in situ, where atmospheric CO_2 concentration was predicted to double by the year 2080 from the present level. The treatments included combinations of ambient or elevated temperature with ambient or elevated CO_2. The ambient CO_2 concentration was set at a value of ambient average (360 μmol mol^{-1}), and the elevated average was double the ambient (720 μmol mol^{-1}). The ambient temperature was set to track the natural temperatures outside the chambers. The elevated temperature was set at 2°C above the ambient temperature from June to early September, at 4ºC above the ambient from September to November and from March to May, and at 6ºC above the ambient from December to February. Each of the four climatic treatments was replicated in four chambers. A more detailed description of the chamber system has been provided by Kellomäki et al. (2000).

Figure 1. Climate controlled closed-top chambers in the field at the Mekrijärvi Research Station, University of Joensuu. Photo by Topi Ylä-Mononen.

In each climatic treatment, the seedlings were divided between different nutrition regimens. Some of the seedlings were not receiving any fertilizer (unfertilized seedlings), and some of them were fertilized according to the nutrient regimen. The moderate fertilizer level (in articles I and II; 130 kg and in article IV; 150 kg N ha^{-1}) was set to correspond to the average amount of nitrogen used in Finnish forest nurseries for birch seedlings (see Juntunen and Rikala 2001). The high fertilizer level (in articles I and II; 270 kg, and in article IV; 500 kg N ha^{-1}) was set to simulate the conditions prevailing in reforested arable lands under Finnish conditions after intensive practice of agriculture (see Wall and Hytönen 2005). The fertilizers used were dissolved in water and applied to the seedlings once a week in order to maintain the prescribed levels of nutrition without the risk of metabolic over-stressing. An equal dose of fertilizer was applied to the same numbers of seedlings within a climatic treatment (in articles I and II, to 288 seedlings, and in article IV, to 64 seedlings). Throughout the experiments (I, II and IV), all the seedlings were irrigated regularly.

In articles I and II, and in article III, the seedlings within each climatic and fertilizer treatment combination were randomly allocated to one of four levels of defoliation (0, 25, 50 or 75% of the total leaf area; initial n = 84, 96, 96, or 12 seedlings per climate, fertilizer and defoliation level). The seedlings employed in article IV were not defoliated. On July 1 and 29 in 2002, defoliation to 25 and 50% of total leaf area was performed by tearing that amount from the apical portion of each leaf. During the second defoliation, only the leaves that developed along with height growth after the first defoliation were torn. Defoliation to 75% was conducted on July 29 to investigate the impact of simulated late-seasonal insect outbreak on plant growth and the ability to store resources for the next growing season (see article III).

Defoliation to 75% was carried out by treating all the leaves of the seedlings that were randomly selected among the intact seedlings (three plants per fertilizer treatment per chamber). Because of heterogeneous practices in the performance of defoliation between the levels of 0%-75%, the seedlings defoliated to 75% were excluded in all measurements in articles I and II. The impacts of this highest defoliation level were examined and included in the article III, with the notation that during winter 2002-2003, none of the seedlings that were unfertilized, defoliated to 75%, and grown under ambient climate, survived. This treatment combination was excluded from the analyses.

2.3 Experiment in laboratory built Conviron chamber system (article III)

The experiment in article III was carried out in environmental chambers (Conviron, Controlled Environments Ltd, Winnipeg, Canada) built in a laboratory at the University of Joensuu, Finland (Table 1, Fig. 2 and 3). In early June 2003, all the seedlings were placed in randomized positions in the chambers, each plant in similar kind of climatic treatment (combinations of elevated or ambient temperature and CO_2) as it had acclimatized in the Mekrijärvi chamber system. The conditions applied in the chambers were set at a constant level throughout the experiment in summer 2003. The treatments included a combination of ambient temperature (during day and night, 20ºC) and ambient CO_2 (360 µmol mol^{-1}), an ambient temperature and elevated CO_2 (720 µmol mol^{-1}), an elevated temperature (during day and night, 25ºC) and ambient CO_2, and a combination of elevated temperature and CO_2. Each climatic treatment existed in one chamber without any replications.

Figure 2. A laboratory-built climate controlled Conviron chamber at the Faculty of Forest Sciences, University of Joensuu. Photos by Liisa Huttunen.

Light conditions within the chambers were adjusted with glow lamps (100 W, 230 V) to correspond to the average conditions prevailing in the latitude of 62 ºN during summer time, and the relative humidity of the air was set to correspond to a long-term average prevailing at Mekrijärvi during June through September from 1998 to 2002. Fertilizer treatments to seedlings continued as described in articles I and II with the exceptions that the dose of fertilizer was reduced, and the highest fertilizer treatment level (270 kg N ha^{-1}; see articles I and II) was excluded in the experiment during the second growing season. This was because most of the highly fertilized seedlings died during the winter 2002-2003. Therefore the fertilizer treatment levels were 0 and 100 kg N ha^{-1} (see article III). The quantity of similarly fertilized seedlings ranged from 11 to 29 in the different climatic treatments. This included an average of five seedlings (a range from 3 to 12 plants) per defoliation treatment within a fertilizer level. All the seedlings were irrigated daily throughout the experiment.

2.4 Measurements

Net photosynthesis rate, height and biomass growth

In article I, the net photosynthesis rates of the differently treated seedlings were analyzed on June 27, July 22, and August 12 in 2002. The measurements were recorded on the third, fully expanded and intact leaf from the apex of the shoot (leaf area ≥ 6.25 cm^2) in three randomly selected seedlings per treatment per chamber. The measurements were taken using a LCA-4 portable gas analysis system (Analytical Development Co. Ltd., Hoddesdon, Hertfordshire, UK) at a stabile leaf temperature (25 °C) between 0900 and 1500 h.

The height growth of the seedlings was recorded weekly, starting in June, until the seedlings had formed apical buds in the autumn (articles I and III). The dry biomass of different plant parts (stem with branches, leaves and roots) was analyzed from the randomly selected seedlings within each treatment combination in the middle (article I) and at the end of the growing season (articles I and III). The selected seedlings were cut from the root collar, and divided into roots, stem and leaves. The total leaf area of each harvested plant was also determined by sampling five fully grown leaves of each leaf pool and measuring their individual area using a portable meter (LI-COR LI-3000, Lincoln, Nebr.) (articles I and III). Subsequently the sample leaves were dried along with other plant parts (leaves, stems and roots) at 105°C for 24 hours. Thereafter the dry weight (g dry weight, DW) of each plant part or individual leaf sample was determined. The specific leaf weight, SLW (dry weight per unit leaf area, mg mm^{-2}) of the individual leaf samples and the total dry mass of all the leaves were used to estimate the total leaf area per plant.

Figure 3. The two-year old intact silver birch (*Betula pendula*) seedlings. The seedlings in the figure are fertilized (100 kg N ha^{-1}) and grown under different climatic treatments (from left to right: elev. T and CO_2, elev. T, elev. CO_2 and amb. T and CO_2). Photo by Liisa Huttunen.

In article III, the relative height growth was measured comparing the absolute height growth during the growing season 2003 to the height at the beginning of the season (relative seasonal height growth = absolute seasonal height growth, cm, in proportion to the height at the beginning of the season, cm).

Chemical and morphological analysis of green leaves and leaf litter

Leaf samples were collected from three different seedlings per treatment combination per chamber two weeks after defoliation (July 17 and August 16, 2002) for extraction and determination of phenolic compounds and for morphological analyses (article II). For the assay of soluble phenolics, the dried extracts were dissolved in a methanol: water solution (300 µl:300 µl) and analyzed as described by Julkunen-Tiitto and Sorsa (2001) using high-performance liquid chromatography (HPLC).

The detected phenolic compounds were quantified based on their UV-spectra and retention times. In the leaf samples that were different than those used in HPLC analysis the area and dry biomass were analyzed. The insoluble condensed tannins were determined using the acid-butanol assay as described by Porter et al. (1986) from the dried extract residue of leaf samples collected in August 2002 and used in the HPLC analysis (article II), or from air dried and powdered leaf litter samples collected in September 2000 (article IV).

In article II, the total nitrogen was analyzed from leaf samples using standard Kjeldahl method, and in article IV, the contents of total carbon and nitrogen were analyzed from leaf litter using a Leco CHN-1000 elemental analyzer (LECO Corporation, St. Joseph, MI, USA).

2.5 Feeding experiment in birch leaves with adult blue alder leaf beetles

A feeding experiment was conducted with adult blue alder leaf beetles (*Agelastica alni*) to examine the palatability of late seasonal leaves produced under different defoliation, fertilization and climatic treatment combinations (article II, Fig. 4). The sample leaves were collected from three different seedlings per treatment combination per chamber. All the leaves used in the experiment were fully grown and intact, and taken from the apex of the shoots in mid August 2002. The beetles were allowed to feed on the leaves in Petri dishes under laboratory conditions for 24 hours. The area of each leaf was measured before and after, and the dry mass after the experiment for the determination of the absolute eaten leaf biomass (mg DW). This was calculated by multiplying the SLW (mg mm^{-2}) of the remaining leaf by the eaten area (mm^2) of the leaf.

Figure 4. On left, larvae of blue alder leaf beetle (*Agelastica alni*) on the leaf of grey alder (*Alnus incana*). On right, an adult blue alder leaf beetle. Photos by Liisa Huttunen (left) and Heikki Roininen (right).

2.6 Leaf litter decomposition experiment

The decomposition of leaf litter was examined in article IV (Table 1). In the end of September 2000, the naturally abscised leaves of similarly treated seedlings within the different fertilization and climatic treatment combinations were collected and pooled as leaf sample units. The litter was air dried, and samples (whole leaves) from the pooled units were weighed and put in permeable bags made of terylene gauze. The bags were strung together side-by-side in a random order. A string consisted altogether of 48 bags, each of them representing a fertilizer and climatic treatment combination within each chamber. The length of a string was 4.8 m. These strings were laid on top of the mineral soil nearby the Mekrijärvi Research Station in October 2000. The bag sets were left to be buried under detritus detached from the vegetation in the experimental site. The litter bags were lifted one string set at a time in May and October in 2001, 2002 and 2003, and in May 2004. The remaining content of each bag was air dried and weighed for the mass loss determination in each time of harvests.

2.7 Statistical analyses

In articles I, II and IV, the experiments were implemented using split-plot design with a completely randomized main plot structure. Climate conditions (different combinations of temperature and CO_2 treatments) composed the main plot factor and fertilization within a climatic treatment a sub-plot factor. In articles I and II, defoliation was employed as a split sub-plot factor. The differences in the measured characteristics between the intact and defoliated plants under different treatment combinations in articles I and II were tested using the mixed model analysis of variance (ANOVA for split-split-plot design, SPSS for Windows, 13.0-15.0, Mixed models, Chicago, IL). Temperature, CO_2, fertilization, defoliation, and their interactions were included in the model as fixed terms, and chamber as a random term.

In article III, the differences in the measured characteristics were tested using analysis of variance (ANOVA, SPSS for Windows, 14.0, Mixed models, Chicago, IL) for completely randomized design (CRD). Climate (consisting of the combination of temperature and CO$_2$), fertilization, defoliation, and their interactions were included in the model as fixed terms. In article IV, the statistical testing was executed using linear mixed effects model from NLME package (R version 2.6.0, The R Foundation for Statistical Computing). Temperature and CO$_2$, and their interaction were included in the model as fixed terms. Since the fertilizer was applied to individual plants inside each chamber, it was nested within the chambers, and included in the model as fixed covariance term.

The number of replicates within each treatment combination in articles I, II and IV was four ($n = 4$), which derived from chamber-specific means of the seedlings. In the articles I-III, the interaction terms found in ANOVA were complex, so the effect of the highest interaction was analyzed using Bonferroni adjusted pairwise comparisons of estimated marginal means (EM means) in data split by the lower interaction term. In article II, the effects of the factors on the palatability of leaves (feeding trial with blue alder leaf beetles) were tested using linear mixed model analysis of variance (ANOVA) for completely randomized design, and the relationship between the total soluble phenolics and the total plant biomass using the bivariate Spearman rank correlation test. In article IV, a following model was employed predicting remaining litter over time:

$$y = a - b \times x \tag{1}$$

where the term “a” is the intercept at day = 1 (log(day)=0), “b” the slope, and “x” is the log-transformed time of incubation in days from the initiation of the experiment.

3 RESULTS

3.1 Growth responses of defoliated and intact silver birch seedlings under different climatic and fertilization treatments

Carbon uptake (article I)

After the foliage damage in mid growing season 2002, the mean rates of net carbon uptake within the different defoliation, fertilization and climatic treatment combinations ranged from 2.5 to 11.3 μmol CO$_2$ m^2 s^{-1} (article I). The carbon uptake was higher in defoliated than in intact seedlings (Table 2; Carbon uptake, mid 2002, late 2002; Def. (D) = ↑). This was especially pronounced in fertilized seedlings under ambient temperature with ambient CO$_2$ and elevated temperature with elevated CO$_2$. Under ambient climate, the carbon uptake was as much as 75% higher in the seedlings defoliated to 25% compared to that of their intact controls. In late season, the net carbon uptake was significantly reduced in the seedlings defoliated to 50% and subjected to high fertilization under elevated temperature with ambient CO$_2$ or ambient temperature with elevated CO$_2$ (Table 2; Carbon uptake, mid 2002, late 2002; CO$_2$ × T × F × D = ↕).

Table 2. Summary of the effect of the factors and their interactions on the measured characteristics (critical alpha in articles I-III; *p*<0.05, and in article IV; *p*<0.1). Abbreviations: **T** = temperature, **Fert. (F)** = fertilization, **Def. (D)** = defoliation, ↑ = statistically significant increasing effect, ↓ = statistically significant decreasing effect, ↕ = statistically significant non-linear effect; the trend of the effect is dependent on the main factor or the level of the sub factor, **ns** = statistically non-significant effect. Concerning the data and results of the article IV, all the seedlings were intact. Thus the effect of defoliation is absent in the table.

Measured characteristics	**T**	**CO_2**	**CO_2 × T**	**Fert. (F)**	**T × F**	**CO_2 × F**	**CO_2 × T × F**	**Def. (D)**	**T × D**	**CO_2 × D**	**CO_2 × T × D**	**F × D**	**T × F × D**	**CO_2 × F × D**	**CO_2 × T × F × D**
Carbon uptake															
mid 2002	**ns**	**ns**	**ns**	↑	**ns**	**ns**	↑	↑	↓	↑	**ns**	↕	↕	↕	↕
late 2002	**ns**	**ns**	**ns**	↑	**ns**	↑	**ns**	↑	**ns**	**ns**	↕	**ns**	**ns**	**ns**	↕
Individual leaf area															
mid 2002	**ns**	**ns**	**ns**	↓	↑	**ns**	**ns**	↓	**ns**	**ns**	**ns**	**ns**	**ns**	↓	**ns**
late 2002	**ns**	↑	**ns**	↑	**ns**	**ns**	**ns**	↓	**ns**	**ns**	**ns**	↓	**ns**	**ns**	**ns**
Specific leaf weight (SLW)															
mid 2002	↓	↑	**ns**	↑	↕	**ns**	↕	**ns**	↓	**ns**	↕	↕	**ns**	↕	↕
late 2002	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
Total leaf area															
mid 2002	**ns**	**ns**	**ns**	↑	**ns**	**ns**	**ns**	↑	↑	**ns**	**ns**	**ns**	↕	↕	**ns**
late 2002	**ns**	**ns**	**ns**	↑	**ns**	**ns**	**ns**	↕	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
late 2003	↑	**ns**	↕	↑	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
Height growth															
2002, absolute	↑	↑	**ns**	↑	**ns**	↑	**ns**	↕	**ns**	↕	**ns**	**ns**	**ns**	**ns**	**ns**
2003, absolute	↑	↑	↑	↑	↑	↑	↑	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
2003, relative	↓	↑	↕	↑	↑	↑	↑	**ns**	**ns**	**ns**	**ns**	↕	**ns**	**ns**	**ns**
Total biomass															
2002	**ns**	↑	**ns**	↑	**ns**	↑	**ns**	↕	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**
2003	↑	↑	↑	↑	**ns**	**ns**	**ns**	↕	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**	**ns**

Table 2. Continued.

Measured characteristics	**T**	**CO_2**	**CO_2 × T**	**Fert. (F)**	**T × F**	**CO_2 × F**	**CO_2 × T × F**	**Def. (D)**	**T × D**	**CO_2 × D**	**CO_2 × T × D**	**F × D**	**T × F × D**	**CO_2 × F × D**	**CO_2 × T × F × D**
Chemical composition; green leaves															
Total phenolics															
mid 2002	ns	ns	ns	↓	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
late 2002	ns	ns	ns	↓	ns	ns	ns	↕	ns	ns	↕	ns	ns	ns	ns
Tannins															
late 2002	↓	ns	ns	↓	↓	ns	ns	ns	ns	ns	↕	↓	ns	ns	↕
Nitrogen															
late 2002	ns	ns	ns	↑	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Leaf palatability; feeding experiment with blue alder leaf beetle (intake, mg DW)															
late 2002	↓	ns	↕	ns	↕	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Chemical composition; abscised leaves															
Tannins															
late 2000	ns	↑	ns	↓	↓	ns	ns								
Total nitrogen															
late 2000	↑	↓	ns	ns	ns	ns	ns								
Total carbon															
late 2000	ns	ns	ns	↓	ns	ns	↑								
C:N ratio															
late 2000	ns	↑	ns	↓	ns	↓	ns								
Decomposition experiment (mass loss rate of remaining mass, % DW)															
first harvest	ns	ns	↓	ns	ns	ns	ns								
final harvest	↓	ns	ns	ns	ns	ns	ns								

Individual leaf area and specific leaf weight (SLW, mg mm^{-2} DW) (article II)

After defoliation in mid and late season 2002, the mean areas of fully grown leaves under the different treatment combinations ranged between 18.4 and 46.5 cm^2. The leaves produced in mid season were slightly greater than those in late season. In fertilized seedlings, defoliation decreased the leaf size especially in late season; the fully grown leaves were about 30% smaller than those in their intact controls (Table 2; Individual leaf area, mid 2002; F × D = ↓).

Within the different treatment combinations, the mean specific leaf weight (SLW) varied between 0.018 and 0.045 mg mm^{-2} during the growing season 2002. Regardless of treatment, the SLW was slightly less in mid than in late season. In mid season, the SLW was higher in the leaves developed under elevated CO_2 and less under elevated temperature compared to that under ambient climatic treatment. Especially under elevated temperature, the SLW decreased as a result of the concurrent effect of defoliation and fertilization, but under elevated CO_2 this effect was reversed (Table 2; Specific leaf weight, mid 2002; CO_2 × T × F × D = ↕).

Total leaf area (articles I and III)

Within the different treatment combinations, the mean total leaf area of the seedlings ranged between 0.2 and 1.2 m^2 in 2002 and 2003 (articles I and III). In mid season 2002, defoliation to 25% or to 50% increased the total leaf area compared to that of their intact controls (article I, Table 2; Total leaf area, mid 2002; Def. (D) = ↑). This was noticeable especially under elevated temperature at moderate fertilization, but at high fertilization, the result was the reverse (Table 2; Total leaf area, mid 2002; T × F × D = ↕). In late season 2002, averaged across the climatic and fertilizer treatments, defoliation to 25% increased, and defoliation to 50% reduced the total leaf area (Table 2; Total leaf area, late 2002; Def. (D) = ↕). At the end of the 2003 growing season, the previous year defoliation had no effect on total leaf area of the seedlings, whereas the area was greatly increased by fertilization or by elevated temperature with ambient or elevated CO_2 (article III).

Height growth (articles I and III)

During the 2002 growing season, defoliation to 25% stimulated the height growth in the seedlings, especially under the combination of elevated temperature and CO_2 with moderate fertilization (article I). Within the treatment, the seedlings were the tallest of all with the height of 75 cm. This height was about 5% greater compared to the height of intact controls, and over 11% greater compared to the height of 50% defoliated seedlings. Across the different climatic and fertilizer treatments, the increase in height of 25% defoliated seedlings ranged between 3 and 9%. Instead, defoliation to 50% tended to slow down the height growth; the final height of the seedlings was about 1-5 cm less than in their intact controls (Table 2; Height growth, 2002, absolute; Def. (D) = ↕).

At the end of the 2003 growing season, the mean final height was the greatest (88.3 cm) in the seedlings that were previously defoliated to 50%, fertilized and subjected to temperature elevation (article III). In these seedlings, the absolute height growth was, on average, 50 cm during the second year (see Fig. 5). Although the effect of previous year’s defoliation was not significant on the absolute height growth, the relative height growth was increased in the most severely defoliated and fertilized seedlings. These plants nearly tripled their height in the second year. In contrast, the relative height growth of the unfertilized seedlings was decreased by defoliation (Table 2; Height growth, 2003, relative; F × D = ↕). During the 2003 growing season, these unfertilized and defoliated seedlings reached only about 60% of their final height in the 2002 growing season.

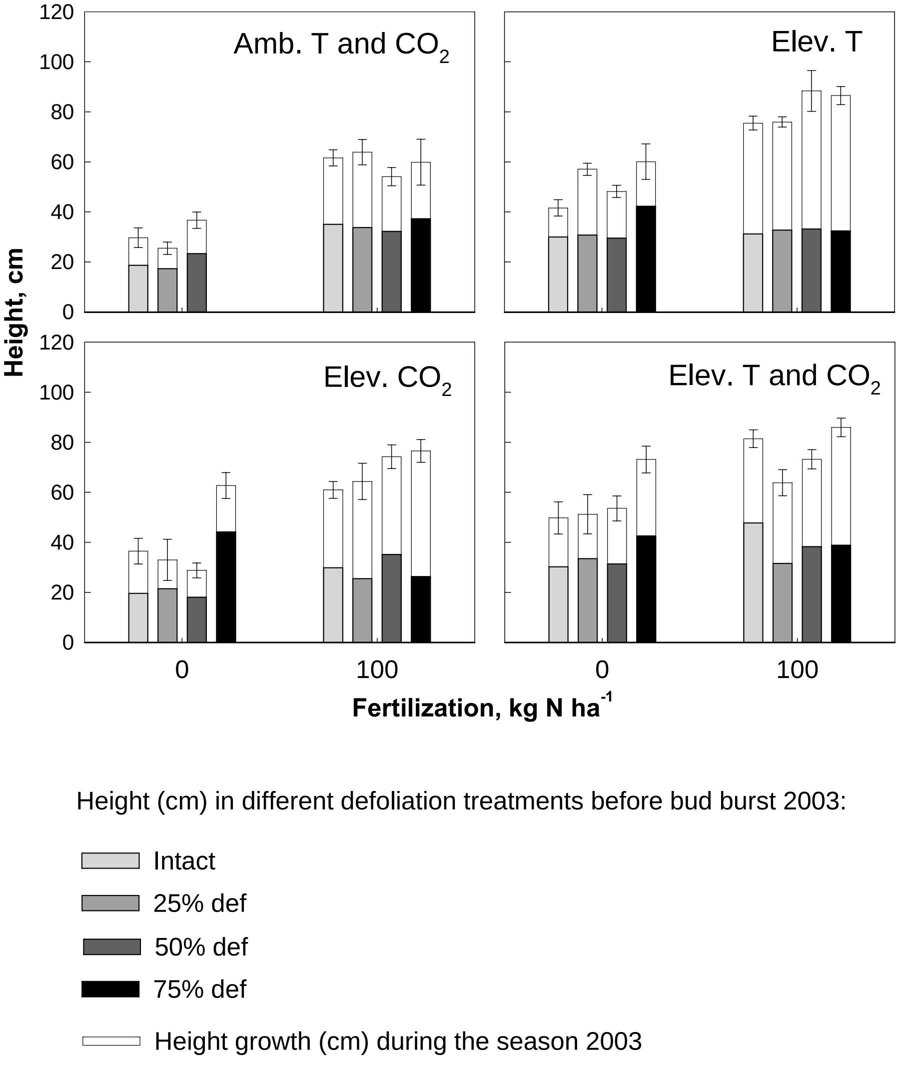

Figure 5. Total height (cm, ± SE bars) of birch seedlings consisting of the height after the season 2002 (measured before bud burst in spring 2003), and during the season 2003.

Biomass growth (articles I and III)

After the first growing season, the total mean dry biomass (g DW) of the seedlings within the different treatment combinations ranged from 2.8 to 7.0 g. Across the climatic treatments, the biomass was higher in the 25% defoliated, but less in the 50% defoliated seedlings compared to that of their intact counterparts (article I, Table 2; Total biomass 2002; Def. (D) = ↕). The mass gain was noticeable in the fertilized seedlings subjected to elevated CO_2 (Table 2; Total biomass 2002; CO_2 × F = ↑). Within this treatment combination, the biomass was nearly 40% higher than that produced under ambient climate. The root: shoot ratio was increased in the fertilized seedlings that were defoliated to 50% and grown under elevated CO_2, although fertilization decreased the ratio.

At the end of the 2003 season, the total dry biomass of the seedlings ranged from 3.2 to 26.3 g. The greatest biomass was observed in the seedlings that were defoliated to 75%, fertilized and grown under elevated temperature with ambient CO_2 (article III). The effect of last year defoliation appeared to be nonlinear, and it caused an inverse response to the seedlings in the second year compared to that in the first year (Table 2; Total biomass 2003; Def. (D) = ↕). Averaged across the climatic and fertilizer treatments, the mean total biomass was the lowest in the seedlings defoliated to 25%. However, the shares of stem and leaves (% of total plant biomass) were increased, but the share of roots was decreased in the seedlings defoliated in the previous year.

3.2 Leaf characteristics of defoliated and intact silver birch seedlings under different climatic and fertilization treatments

Chemical composition (article II)

During the 2002 growing season, the mean concentrations of total soluble phenolics in the green leaves under the different treatment combinations ranged between 4.5 and 29.8 mg g^{-1} (article II). In the late season, under ambient temperature with ambient CO_2, the total soluble phenolics were noticeably decreased in the leaves of defoliated seedlings compared to those of intact controls. However, the concentrations were slightly increasing as a response to defoliation under elevated temperature with elevated CO_2 (Table 2; Total phenolics, late 2002; CO_2 × T × D = ↕).

Defoliation to 25% increased the concentrations of kaempferol-3-rhamnoside and 3.4'-dihydroxypropiophenone-3-β-D-glucopyranoside (DHPPG) in the leaves. This increase in DHPPG was especially pronounced under the concurrent elevation of temperature and CO_2, being 9% higher than that in the leaves of intact controls. Fertilization reduced the concentrations of specific phenolic acids and flavonoids and, accordingly, the total phenolics in the leaves in mid and late season 2002 (Table 2; Total phenolics, mid 2002, late 2002; Fert. (F) = ↓).

The contents of insoluble condensed tannins also decreased in the leaves with increased nutrient availability (Table 2; Fert. (F) = ↓). In late season 2002, the mean contents of tannins under the different treatment combinations varied between 13.9 and 43.4 mg g^{-1}. Averaged across the fertilizer treatments, the tannin contents were less in the leaves produced in defoliated than in intact seedlings under elevated temperature with elevated CO_2, but the response to defoliation was nonlinear under other climatic treatments (Table 2; CO_2 × T × D = ↕). The content of total nitrogen in the green leaves increased by fertilization, ranging from 1.9% DW (no fertilizer application) to 4.8% DW (high level of fertilizer application) (article II; Table 2; Fert. (F) = ↑).

Palatability to adult blue alder leaf beetles (article II)

The average eaten leaf mass by the beetles within the different defoliation, fertilization and climatic treatment combinations varied from 0.01 up to 3.17 mg DW. Averaged across the fertilizer and defoliation treatments, the intake was least (0.30 mg) on the leaves produced under elevated temperature (Table 2; T = ↓), whereas the intake was highest on the leaves produced under ambient temperature (0.68 mg). The most eaten leaves were developed in the fertilized seedlings under ambient climate. The eaten amount was 28% greater than in the leaves of unfertilized seedlings. This result was opposite to that observed under elevated temperature, where the eaten amount of leaves in fertilized seedlings was approximately 41% less than in the leaves of seedlings with no fertilization (Table 2; T × F = ↕).

3.3 Chemical composition and decay process of naturally abscised leaves produced under different climatic and fertilization treatments (article IV)

At the end of the 2000 growing season, within the different climatic and fertilizer treatment combinations, the mean content of insoluble condensed tannins in the abscised leaves varied between 41.8 and 119.5 mg g^{-1}. Averaged across the climatic treatments, the content decreased along with increasing nutrients (Table 2; Fert. (F) = ↓). This response was similar to that of the green leaves (article II). However, the effect of fertilization on the content of tannins in the leaf litter produced under elevated temperature was less pronounced than that under ambient temperature (Table 2; T × F = ↓).

Increased nutrient availability as a result of fertilization also decreased the mean content of total carbon in the abscised leaves (Table 2; Fert. (F) = ↓). The carbon content within different treatment combinations varied between 41.6 and 43.8% DW. On average, the highest carbon content was observed in the litter produced under elevated temperature with no fertilization (43.5% DW) and under the combination of elevated temperature and CO_2 with moderate fertilization (43.8% DW) (Table 2; CO_2 × T × F = ↑).

The mean content of total nitrogen within the different climatic and fertilizer treatment combinations varied from 0.7 to 1.6% DW. The content was significantly decreased by elevated CO_2 (Table 2; CO_2 = ↓), and increased by elevated temperature (Table 2; T = ↑). The mean values of C:N ratio in the different treatment combinations varied between 28 and 63. The highest value of the ratio was observed in the leaves subjected to elevated CO_2, under which the ratio decreased significantly along with increased nutrient availability (Table 2; CO_2 × F = ↓).

At the time of the first harvest (after 214 days of incubation) of the decomposition experiment, the average remaining mass of litter produced under the different climatic treatments varied between 56 and 65% DW. The mass loss was low in the litter produced under ambient temperature with elevated CO_2 (Table 2; CO_2 × T = ↓). At the end of the experiment (after 1357 days of incubation), between the different climatic treatments, the remaining mass of litter varied from 38 to 47% DW. During the four-year experiment, the fastest rate of mass loss was observed in the litter produced under ambient temperature with elevated CO_2, whereas the rate was the slowest in the litter produced under elevated temperature with ambient or elevated CO_2 (Table 2; T = ↓).

4 DISCUSSION AND CONCLUSIONS

4.1 Net carbon uptake, individual and total leaf area and specific leaf weight

In the present thesis, the rate of photosynthesis was examined in defoliated and intact silver birch seedlings under different climatic and fertilizer treatment combinations (article I). In mid season 2002, the net carbon uptake was especially stimulated in seedlings experienced tissue loss to 25% and grown under ambient temperature with ambient CO_2 and elevated temperature with elevated CO_2 at increased nutrient availability. In the fully grown leaves produced after damage, the carbon uptake was higher compared to that in intact controls. Similarly, the rate of photosynthesis has been previously reported to be accelerated in the remaining leaves of mountain birch after defoliation (Prudhomme 1982).

The increase in the carbon uptake (photosynthesis) of new leaves in silver birch seedlings may have come from the existence of increased sink capacity (see van den Boogaard et al. 2001, Thomson et al. 2003, Boege 2005). Those birch seedlings defoliated to 25% prioritized direct compensation for lost photosynthetically active tissues by partitioning assimilates to the production of new leaves. In addition, the accelerated carbon uptake in the seedlings particularly defoliated to 25% may be explained by the altered synthesis of hormones (e.g. methyl jasmonate and abscisic acid, cytokinins, gibberellins and auxins) that regulate for example photosynthesis (Jordi et al. 1994, 2000, Wingler et al. 1998). The production of these substances in plants has been previously reported to be pronounced, for example, by herbivore feeding damage (Morvan-Bertrand et al. 2001, Khan 2005).

In mid August 2002, the net carbon uptake was significantly reduced in the damaged seedlings especially when they had experienced tissue loss to 50%. The lowered photosynthesis rates may result from decreased carbon demand in the shoot sinks (stem and leaves). This may be associated with declining growth rate caused by the natural changes in the photoperiod towards the end of the season (Mølmann et al. 2003). The decreased shoot sinks especially in 50% defoliated seedlings may have caused accumulation of assimilates (such as starch) in chloroplasts, which may have inhibited the net carbon uptake in the late-seasonal leaves (Scheidegger and Nösberger 1984, Sasek et al. 1985, Paul and Foyer 2001, Iglesias et al. 2002, Pimenta-Barrios and Ramírez-Hernández 2002).

After defoliation in mid and late season 2002, the sizes of individual leaves in the damaged seedlings were smaller than those in the intact controls (article II). Rautio et al. (2002) have observed the same phenomenon in downy birch after defoliation. This may relate to the occurrence of morphological or mechanical defense; through evolutionary history, it is optimal for a deciduous tree to reduce the area of an individual leaf where the insect herbivores most likely graze, oviposite or mate (Prokopy and Owens 1983, Pilson and Rausher 1988, Kagata and Ohgushi 2001). An alternative possibility is that the seedlings after tissue loss simply had low energy reserves to produce larger leaves and consequently greater total leaf mass and area (Ruohomäki et al. 1997). This explanation seems more likely relating to the seedlings defoliated to 50% than to those defoliated to 25%.

Regardless of the smaller size of the individual leaves, defoliation to 25% tended to magnify the leaf production in the silver birch seedlings grown under elevated temperature at moderate fertilization in 2002 (see articles I and II). It has been previously reported that elevation in temperature or increasing nutrient availability enhances the number of developing leaves in plants (Ackerly et al. 1992, Gunn and Farrar 1999, Houle 1999, Müller et al. 2000, Xiao et al. 2003), thus fueling the increase of the total leaf area. On the other hand, when compared to the intact counterparts, the total leaf area was reduced in those silver birch seedlings that had experienced tissue loss to 50%, fertilized at the highest level and subjected to elevated CO_2 (article I). Earlier reports have highlighted the fact that defoliated trees have not been able to fully compensate for the lost foliage (O'Neil 1962, Kulman 1971, Albaugh et al. 1998). This is because of a rapid decrease in their photosynthetically active tissue, which has altered the resource partitioning (Anten and Hirose 1998) and the net primary productivity by reducing the capacity of total assimilation and synthesis of carbohydrates for production of new tissues (Little et al. 2003). As a result of reduced foliage to different degrees, the later performance (height and biomass growth, see articles I and III) of the defoliated birch seedlings was affected, and appeared to be nonlinear (either increasing or decreasing). This response in plant growth is discussed in more detail later on. However, previous tissue loss seemed not to have any effect on total leaf area in the 2003 growing season (article III).

In relation to the possible occurrence of morphological or mechanical defense, the specific leaf weight (SLW) had changed in the individual leaves produced after tissue loss in mid July 2002 (article II). The increase in SLW was noticeable in the leaves of defoliated seedlings grown under elevated CO_2 with increasing nutrient availability. This kind of response in SLW may have provided morphological protection against insect herbivores by reducing the palatability of leaves; the force needed to bite a leaf may be increased or the digestibility of the leaf material reduced by high SLW (Gras et al. 2005). The increase in SLW in birch seedlings is also in line with the result by Campbell et al. (1988), who showed that the elevation in atmospheric CO_2 increases the structural dry matter and protein per unit leaf area.

On the other hand, defoliation decreased the SLW under elevated temperature. Earlier, high temperatures have been found to lower the specific leaf weight in different plant species (Chabot and Chabot 1977, Khattak and Pearson 2005, Wassner and Ravetta 2005). This may be associated with the increased stem growth and, consequently, stem-leaf ratio (Wassner and Ravetta 2005), which were magnified in defoliated birch seedlings under elevated temperature. In the case of stimulated sink strength, assimilates are partitioned on the production of new leaves rather than supporting tissue in cell walls of individual leaves (Chapin III 1980, Atkin et al. 1996). In addition, the low SLW may have provided a highly cost-efficient photosynthetic system in defoliated silver birch seedlings subjected to elevated temperature, which is in line with the results of Brown and Byrd (1997).

In mid August 2002, defoliation, fertilization, elevation in temperature or in CO_2 did not affect the SLW. One possible explanation for this unresponsiveness may be that damage to the mid summer leaves is more detrimental to growth and survival of the seedlings than the damage to the late summer leaves (Mendoza et al. 1987, Maschinski and Whitham 1989, Whitham et al. 1991). Therefore, the effect of foliage damage in mid season may have had more influence on the structure of birch leaves than that in late season.

4.2 Height and biomass growth of the seedlings

Changes in the quality and quantity of photosynthetic units, leaves, through environmental and resource amelioration (elevation in CO_2, temperature and fertilization) enhanced carbon uptake and increased both the height and biomass growth, especially in the seedlings that had experienced tissue loss (articles I and III). Vigorous growth in the above-ground parts (stem, branches and leaves) of the defoliated seedlings may have resulted from stimulated synthesis of growth regulators, i.e. plant hormones (such as gibberellic acids), fuelling leaf production and cell expansion and division in the apical meristems (e.g. Bostrack and Struckmyer 1967, Elliott et al. 2001, Morvan-Bertrand et al. 2001). These substances have a central role in coordinating plant stress responses (Smith and Boyko 2007, Wu et al. 2007). However, defoliation induced impact on the seedlings differed between the years and the severity of damage; the seedlings defoliated to 25% over-compensated their growth in 2002 (article I), but in 2003, the effect was completely reversed (article III). Overshooting the optimum during the same season of foliage damage seemed to produce extremely low root: shoot ratios (see also McNaughton et al. 1983, Markkola et al. 2004), especially in the seedlings subjected to elevated temperatures. This increased strength of shoot sinks over the root sink was detrimental for the later survival of the seedlings. In the year following defoliation, primarily those seedlings that were damaged to 25%, fertilized and subjected to the combination of elevated temperature and CO_2 could not reach the same height and biomass growth as their intact controls (article III). The reason for this kind of response to damage may be in limited energy reserves in roots that also function as carbohydrate storage organs (see Chapin III et al. 1990, Abod and Webster 1991, Gansert and Sprick 1998, Landhäusser and Lieffers 2002, 2003, Kabeya and Sakai 2003).

During the growing seasons 2002 and 2003, the seedlings defoliated to 50% attained entirely opposite results in total height and biomass gain compared to those defoliated to 25%. The 50% defoliated seedlings could not reach the same final height as their intact controls in 2002 (article I). Several studies with trees have demonstrated only weak re-growth directly after insect leaf-feeding damage (e.g. Mattson et al. 2004, Osier and Lindroth 2004) regardless of stimulated carbon uptake in the remaining foliage (Prudhomme 1982). On the other hand, this kind of deceleration in height growth may be beneficial for the seedlings. Namely, Price (1991) has hypothesized that many herbivorous insects feed preferentially on vigorously growing plants or plant parts, in which case over-compensation in sprouting units may attract even more insects and worsen the damage in the prevailing year of defoliation.

Surprisingly, in 2003, the final heights of the 50% defoliated seedlings were greater than those in their intact counterparts under the different combinations of fertilizer and climatic treatments (article III). The reason for strengthened growth in height and biomass during the 2003 season may be that stress reaction immediately after tissue loss was stabilized and the delayed synthesis of plant hormones was stimulated (see Fig. 5). The production of these substances may have carried across the years by a memory effect in molecular level of primordial cells of the seedlings (Bruce et al. 2007). However, in 2003, the plant hormones may have had a greater influence on growth of severely defoliated (50% and 75%) than that of mildly defoliated (25%) seedlings. Compared to the intact controls, the total biomass at the end of the 2003 season was increased by 50% especially in the seedlings defoliated to 75% and grown under elevated temperature at high nutrient availability. This may be due to their higher energy reserves stored in roots during the 2002 growing season, which gives support to the suggestion that re-growth after tissue loss greatly relies on stored carbohydrate reserves (see Kozlowski 1992, von Fircks and Sennerby-Forsse 1998, Bollmark et al. 1999, Landhausser and Lieffers 2002, 2003).

4.3 Chemical composition and palatability of leaves

According to the theories of plant resource allocation between synthesis of primary metabolites and secondary substances (Herms and Mattson 1992, Jones and Hartley 1999), enhanced growth in defoliated silver birch seedlings in 2002 predicted lowered production of deterrent compounds. The concentrations of these chemicals (total soluble phenolics and insoluble condensed tannins) were actually decreased in the leaves of defoliated seedlings under the different climatic and fertilizer treatment combinations (article II). These results are different than those observed in the leaves of mountain birches after defoliation (e.g. Lempa et al. 2004). Reduced production of the phenolic compounds may be related to the fast growth of silver birch at the juvenile stage, in which case the resources are allocated to the production of cell structures as a response to damage rather than to secondary metabolites (see Maschinski and Whitham 1989). Conversely, reduced growth rate by the late season or the natural aging of the leaves (see Mauffettee and Oechel 1989, Kudo 2003) may explain the increased concentrations of phenolics observed in the leaves of silver birch seedlings towards the end of the 2002 growing season (article II).

Although defoliation decreased the total soluble phenolics in the leaves, it increased the contents of kaempferol-3-rhamnoside and 3.4’-dihydroxypropiophenone-3-β-D-glucopyranoside (DHPPG), especially under both elevated temperature and CO_2 in mid August 2002 (article II). In earlier studies, Mori et al. (1992) have found similar responses in DHPPG in the leaves of defoliated Japanese white birch (*B. platyphylla* var. *japonica*), and Keinänen et al. (1999) in silver birch. The primary function of DHPPG may be that of defense against herbivores (Keinänen et al. 1999, Mutikainen et al. 2000), in which case the result of stimulated synthesis of the compound by defoliation is reasonable.

Even if the concentrations of kaempferol-3-rhamnoside and DHPPG were increased by foliage damage, this did not affect the absolute intake (mg DW) of the adult blue alder leaf beetles (*A. alni*) on the leaves of the seedlings under any of the combinations of climatic and fertilizer treatments (article II). In general, the overall feeding of the beetles was highest on the leaves produced under ambient temperature with ambient CO_2 at increased nutrient availability. The content of nitrogen in the leaves was increased by fertilization, which may partially explain the feeding behavior of the beetles. Many herbivorous insects tend to maximize their nitrogen accumulation from the host plants to maintain or stimulate their own protein synthesis (Slansky and Feeney 1977, Lindroth et al. 1997). However, the total intake of the beetles on the leaves produced under elevated temperature was the least regardless of the amount of nitrogen. This behavior may be related to the leaf water content (see Scriber and Slansky 1981). As suggested by Williams et al. (2000, 2003), it is possible that under elevated temperature the leaves contained low amounts of water, which most affected the feeding behavior of the beetles.

4.4 Decomposition rate of abscised leaves

Based on the curves as output from the model predicting remaining litter over time (article IV), the decay process of silver birch litter was decelerated on the abscised leaves produced under elevated temperature. This was similar to the feeding behavior of blue alder leaf beetles, which also was reduced on leaves developed under elevated temperature (article II). In general, the decomposition rate is explained by the initial chemical composition of abscised leaves, which either accelerates or slows down the activity of microorganisms on the forest floor (Hättenschwiler and Bretscher 2001). For example, high content of nitrogen may make the litter more degradable for the microbial and invertebrate soil organisms (Hunt et al. 1988, Aerts and de Caluwe 1997). As herbivorous insects, the litter feeders similarly need this element for their own protein synthesis (e.g. Rosswall 1982, Hodge et al. 2000). Nevertheless, the initial amount of nitrogen did not directly explain the mass loss of silver birch litter (article IV). Although nitrogen content was relatively high in the abscised birch leaves produced under elevated temperature with ambient or elevated CO_2, the decomposition rate was slow. Instead, the initial concentration of total carbon in the abscised leaves was high under the combination of elevated temperature and CO_2 with increased nutrition.

Earlier, elevations in temperature or in CO_2 have been shown to increase the structural carbon, such as lignin, hemicellulose and cellulose, in the leaves of some temperate grasses (Ford et al. 1979, Ziska et al. 2005). In general, high concentrations of structural carbon or carbon based polyphenols and tannins in the decaying leaf material have reduced the consumption by the litter-feeders (Harrison 1971, Suberkropp et al. 1976, Gallardo and Merino 1993, Wardle et al. 1998, Pérez-Harguindeguy et al. 2000, Fioretto et al. 2005). However, tannins leach quite quickly from the decaying leaves (Schofield et al. 1998). Therefore, it is unlikely that these compounds would have significantly slowed the decomposition of silver birch leaves over time. On the other hand, lignin, a highly abundant carbon based substance in the leaves, is known to degrade slowly in litter (Fioretto et al. 2005). Moreover, lignin interferes with the enzymatic degradation of carbohydrates (such as cellulose and hemicellulose) and proteins (Alexander 1977, Cooke and Whipps 1993) further slowing the decomposition rates (Melillo et al. 1982). The increased carbon content in the leaves produced under elevated temperature with ambient or elevated CO_2 may indicate lignified cell structures and thus ultimately explain the decelerated rate of decomposition in the silver birch litter.

4.5 Conclusions

The results obtained in this thesis illustrate that even mild tissue loss under elevated temperature and CO_2 at increased nutrient availability may cause lowered net primary productivity in silver birch seedlings within a few years after defoliation (articles I and III). The seedlings seemed to recover well from foliage damage via increased growth in the above-ground parts during the same season when the defoliation occurred (article I). However, in those seedlings that over-compensated the height and leaf mass growth in the first year, the total biomass was less than in their intact counterparts after the second year (article III). This kind of response in resource allocation towards shoot growth lowered the root growth and also the ability of birch seedlings to store resources in the roots. In addition, the production of deterring carbon based substances was lowered in the leaves of growth over-compensating seedlings directly after tissue loss (article II).

Under changing climate, if the above-ground growth tends to substantially increase in birch seedlings within the season when the defoliation occurs, and, consequently, if the total concentrations of deterrent compounds of new-born leaves tend to be less, it is possible that the damaged plants attract even more defoliators in the future. Repeated foliage loss within the same season, or season after damage may cause serious reduction in growth or, at worst, widespread tree deaths. The insect feeding may be detrimental to seedlings that have low energy reserves in their smaller storage organs, roots. Therefore, the later survival of silver birch seedlings from repeated damage may not be dependent on the production of deterrent compounds, but on the plants' success in competition and ability to store resources under changing climate.

The results in this thesis suggest that elevation in temperature drive the leaf mechanical structure and physiology altering the subsequent feeding behavior and the decomposition process on leaves or leaf litter by insects or microbial organisms (articles II and IV). This may be due to decreased water content in the leaves (article II), or increased content of total carbon in litter (article IV), which both are associated to lignified cell structures and high content of fiber. This implies that the changes in leaves may reduce their palatability or slow their decomposition rate on the forest floor. The slowed decay process may inform on slowed nutrient cycle in boreal forests under changing climate. However, although the leaves produced under elevated temperature may be less edible for herbivorous insects, the total amount of decaying leaf matter will decrease in deciduous forests after insect outbreaks. The decreased amount of litter and stimulated microbial activity in the soil may compensate the decelerated decay process of leaves under the climate change maintaining the carbon cycle from soil to plant and back to the soil in the boreal birch forests.

REFERENCES

Abod, S.A. & Webster, A.D. 1991. Carbohydrates and their effects on growth and establishment of *Tilia* and *Betula*: I. Seasonal changes in soluble and insoluble carbohydrates. Journal of Horticultural Science 66: 235–246.

Ackerly, D.D., Coleman, J.S., Morse, S.R. & Bazzaz, F.A. 1992. CO_2 and temperature effects on leaf area production in two annual plant species. Ecology 73:1260–1269.

Aerts, R. & de Caluwe, H. 1997. Nutritional and plant-mediated controls on leaf litter decomposition of *Carex* species. Ecology 78: 244-260.

Agrawal, A.A. 2000. Overcompensation of plants in response to herbivory and the by-product benefits of mutualism. Trends in Plant Science 5: 309-313.

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Kress, L.W. & King, J.S. 1998. Leaf area and above- and belowground growth responses of loblolly pine to nutrient and water additions. Forest Science 44: 317–328.

Alexander, M. 1977. Introduction to soil microbiology. Second edition. Wiley and Sons, New York. 467 p.

Anten, N.P.R. & Hirose, T. 1998. Biomass allocation and light partitioning among dominant and subordinate individuals in *Xanthium canadense* stands. Annals of Botany 82: 665–673.

Atkin, O.K., Botman, B. & Lambers, H. 1996. The causes of inherently slow growth in alpine plants: an analysis based on the underlying carbon economies of alpine and lowland *Poa* species. Functional Ecology 10: 698-707.

Ayres, M.P., Clausen, T.P., MacLean, S.F. Jr., Redman, A.M. & Reichardt, P.B. 1997. Diversity of structure and antiherbivore activity in condensed tannins. Ecology 78: 1696-1712.

— & Lombardero, M.J. 2000. Assessing the consequences of global change for forest disturbance from herbivores and pathogens. Science of the Total Environment 262: 263-286.

Bassman, J.H. & Zwier, J.C. 1993. Effect of partial defoliation on growth and carbon exchange of two clones of young *Populus trichocarpa* torr. & gray. Forest Science 39: 419-431.

Bazzaz, F.A. 1990. The response of natural ecosystems to the rising global CO_2 levels. Annual Review of Ecology and Systematics 21: 167-196.

Belsky, A.J. 1986. Does herbivory benefit plants? A review of the evidence. The American Naturalist 127: 870–892.

— 1987. The effects of grazing: confounding of ecosystem, community and organism scales. The American Naturalist 129: 777–783.

Boege, K. 2005. Influence of plant ontogeny on compensation to leaf damage. American Journal of Botany 92: 1632–1640.

Bollmark, L., Sennerby-Forsse, L. & Ericsson, T. 1999. Seasonal dynamics and effects of nitrogen supply rate on nitrogen and carbohydrate reserves in cutting-derived *Salix viminalis* plants. Canadian Journal of Forest Research 29: 85–94.

Bostrack, J.M. & Struckmyer, B.E. 1967. Effect of gibberellic acid on the growth and anatomy of *Coleus blumei*, *Antirrhinum majus* and *Salvia splendens*. New Phytologist 66: 539-544.

Brown, R.H. & Byrd, G.T. 1997. Transpiration efficiency, specific leaf weight, and mineral concentration in peanut and pearl millet. Crop Science 37: 475-480.

Bruce, T.J.A., Matthes, M.C., Napier, J.A. & Pickett, J.A. 2007. Stressful "memories" of plants: Evidence and possible mechanisms. Plant Science 173: 603-608.

Bryant, J.P., Reichardt, P.B. & Clausen, T.P. 1992. Chemically mediated interactions between woody plants and browsing mammals. Journal of Range Management 45: 18-24.

—, Taylor, G. & Frehner, M., 1998. Photosynthetic acclimation to elevated CO_2 is modified by source: sink balance in three component species of chalk grassland swards grown in a free air carbon dioxide enrichment (FACE) experiment. Plant, Cell and Environment 21 : 159-168.

Campbell, W.J., Allen, L.H. Jr. & Bowes, G. 1988. Effects of CO_2 concentration on rubisco activity, amount, and photosynthesis in soybean leaves. Plant Physiology 88: 1310-1316.

Carter, T., Bärlund, I., Fronzek, S., Kankaanpää, S., Kaivo-oja, J., Luukkanen, J., Wilenius, M., Tuomenvirta, H., Jylhä, K., Kahma, K., Johansson, M., Boman, H., Launiainen, J., Laurila, T., Lindfors, V., Tuovinen, J.-P., Aurela, M., Syri, S., Forsius, M. & Karvosenoja, N. 2002. The FINSKEN global change scenarios. In: Käyhkö, J. & Talve, L. (eds). Understanding the global system. The Finnish perspective. Painosalama, Turku. p. 27-40.

Chabot, B.F. & Chabot, J.F. 1977. Effects of light and temperature on leaf anatomy and photosynthesis in *Fragaria vesca*. Oecologia 26: 363-377.

Chapin III, F.S. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11: 233-260.

—, Schulze, E.-D. & Mooney, M.A. 1990. The ecology and economics of storage in plants. Annual Review of Ecology and Systematics 21:423-447.

Choong, M.F. 1996. What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. Functional Ecology 10: 668–674.

Coley, P.D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecological Monograms 53: 209-233.

Cooke, R.C. & Whipps, J.M. 1993. Ecophysiology of fungi. Blackwell Scientific, Oxford. 337 p.

Cotrufo, M.F. & Ineson, P. 1996. Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. Oecologia 106: 525-530.

Dale, V. H., Joyce, L. A., McNulty, S., Neilson, R. P., Ayres, M. P., Flannigan, M. D., Hanson, P. J., Irland, L. C., Lugo, A. E., Peterson, C. J., Simberloff, D., Swanson, F. J., Stocks, B. J. & Wotton, B. M. 2001. Climate change and forest disturbances. Bioscience 51: 723-734.

Dittmar, C., Zech, W. & Elling, W. 2003. Growth variations of Common beech (*Fagus sylvatica* L.) under different climatic and environmental conditions in Europe - a dendroecological study. Forest Ecology and Management 173: 63-78.

Elliott, R.C., Ross, J.J., Smith, J.J., Lester, D.R. & Reid, J.B. 2001. Feed-forward regulation of gibberellin deactivation in pea. Journal of Plant Growth Regulation 20: 87–94.

Farrar, J.F. & Williams, M.L. 1991. The effects of increased atmospheric carbon dioxide and temperature on carbon partitioning source-sink relations and respiration. Plant, Cell and Environment 14: 819–830.

Feeny, N.M. 1976. Plant apparency and chemical defence. In: Wallace, J. & Mansel, R. (eds). Biochemical interaction between plants and insects. Plenum Press, New York. p. 1-40.

Fetcher, N., Jaeger, C.H., Strain, B.R. & Sionit, N. 1988. Long-term elevation of atmospheric CO_2 concentration and the carbon exchange rates of saplings of *Pinus taeda* L. and *Liquidamber styraciflua* L. Tree Physiology 4: 255-262.

Fioretto, A., Di Nardo, C., Papa, S. & Fuggi, A. 2005. Lignin and cellulose degradation and nitrogen dynamics during decomposition of three leaf litter species in a Mediterranean ecosystem. Soil Biology and Biochemistry 37: 1083–1091.

Ford, C.W., Morrison, I.M. & Wilson, J.R. 1979. Temperature effects on lignin, hemicellulose and cellulose in tropical and temperature grasses. Australian Journal of Agricultural Research 30: 621 – 633.

Gallardo, A. & Merino, J. 1993. Leaf decomposition in two Mediterranean ecosystems of Southwest Spain: influence of substrate quality. Ecology 74: 152–161.

Galloway, J.N., Dentener, F.J., Capone, D.G., Boyer, E.W., Howarth, R.W., Seitzinger, S.P., Asner, G.P., Cleveland, C.C., Green, P.A., Holland, E.A., Karl, D.M., Michaels, A.F., Porter, J.H., Townsend, A.R. & Vöosmarty, C.J. 2004. Nitrogen cycles: past, present, and future. Biogeochemistry 70: 153–226.

Gansert, D. & Sprick, W. 1998. Storage and mobilization of nonstructural carbohydrates and biomass development of beech seedlings (*Fagus sylvatica* L.) under different light regimes. Trees – Structure and Function 12: 247-257.

Gras, E.K., Read, J., Mach, C.T., Sanson, G.D. & Clissold, F.J. 2005. Herbivore damage, resource richness and putative defenses in juvenile versus adult *Eucalyptus* leaves. Australian Journal of Botany 53: 33-44.

Gunn, S. & Farrar, J.F. 1999. Effects of a 4 °C increase in temperature on partitioning of leaf area and dry mass, root respiration and carbohydrates. Functional Ecology 13:12-20.

Handa, T.I., Körner, C. & Hättenschwiler, S. 2005. A test of the treeline carbon limitation hypothesis by in situ CO_2 enrichment and defoliation. Ecology 86: 1288–1300.

Hanhimäki, S. 1989. Induced resistance in mountain birch: defence against leaf-chewing insect guild and herbivore competition. Oecologia 81: 242–248.

Harrison, A.F. 1971. The inhibitory effect of oak leaf litter tannins on the growth of fungi in relation to litter decomposition. Soil Biology and Biochemistry 3: 167–172.

Hartley, S.E. & Lawton, J.H. 1991. Biochemical aspects and significance of the rapidly induced accumulation of phenolics in birch foliage. In: Tallamy, D.W. & Raupp, M.J. (eds). Phytochemical induction by herbivores. John Wiley, New York, New York. p. 105-132.

Haukioja, E. & Niemelä, P. 1979. Birch leaves as a resource for herbivores: seasonal occurrence of increased resistance in foliage after mechanical damage. Oecologia 39: 151–159.

— & Neuvonen, S. 1985. Induced long-term resistance of birch foliage against defoliators: defensive or incidental? Ecology 66: 1303–1308.

Herms, D.A. & Mattson, W.J. 1992. The dilemma of plants: to grow or defend. The Quarterly Review of Biology 67: 283–335.

Hjältén, J., Danell, K. & Ericson, L. 1993. Effects of simulated herbivory and intraspecific competition on the compensatory ability of birches. Ecology 74: 1136–1142.

Hodge, A., Robinson, D. & Fitter, A. 2000. Are microorganisms more effective than plants at competing for nitrogen? Trends in Plant Science 5: 304-308.

Houle, G. 1999. Nutrient availability and plant gender influences on the short-term compensatory response of *Salix planifolia* ssp. *planifolia* to simulated leaf herbivory. Canadian Journal of Forest Research 29: 1841–1846.

Huhta, A.- P., Hellström, K., Rautio, P. & Tuomi, J. 2000. A test of the compensatory continuum: fertilization increases and below-ground competition decreases tolerance of tall wormseed mustard (*Erysimum strictum*). Evolutionary Ecology 14: 353-372.

Hunt, H.W., Ingham, E.R., Coleman, D.C., Elliott, E.T. & Reid, C.P.P. 1988. Nitrogen limitation of production and decomposition in prairie, mountain meadow, and pine forest. Ecology 69: 1009-1016.

Hättenschwiler, S., Schweingruber, F.H. & Körner, C. 1996. Tree ring responses to elevated CO_2 and increased N deposition in *Picea abies*. Plant, Cell and Environment 19: 1369-1378.

— & Bretscher, D. 2001. Isopod effects on decomposition of litter produced under elevated CO_2, N deposition and different soil types. Global Change Biology 7: 565-579.

Iglesias, D.J., Lliso, I., Tadeo, F.R. & Talon, M. 2002. Regulation of photosynthesis through source: sink imbalance in citrus is mediated by carbohydrate content in leaves. Physiologia Plantarum 116: 563-572.

IPCC (International Panel on Climate Change) 2007. Climate Change 2007: The Physical Science Basis. Regional Climate Projections. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Available at: http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr.pdf

Jones, C.G. & Hartley, S.E. 1999. A protein competition model of phenolic allocation. Oikos 86: 27-44.

Jordi, W., Pot, C.S., Stoopen, G.M. & Schapendonk, A.H.C.M. 1994. Effect of light and gibberellic acid on photosynthesis during leaf senescence of Alstroemeria cut flowers. Physiologia Plantarum 90: 293-298.

—, Schapendonk, A., Davelaar, E., Stoopen, G.M., Pot, C.S., de Visser, R., van Rhijn, J.H.A., Gan, S. & Amasino, R.M. 2000. Increased cytokinin levels in transgenic P(SAG12)-IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning. Plant, Cell and Environment 23: 279-89.

Julkunen-Tiitto, R. & Sorsa, S. 2001. Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. Journal of Chemical Ecology 27: 779-789.

Juntunen, M.-L. & Rikala, R. 2001. Fertilization practice in Finnish forest nurseries from the standpoint of environmental impact. New Forests 21: 141-158.

Kabeya, D. & Sakai, S. 2003. The role of roots and cotyledons as storage organs in early stages of establishment in *Quercus crispula*: a quantitative analysis of the nonstructural carbohydrate in cotyledons and roots. Annals of Botany 92: 537-545.

Kagata, H. & Ohgushi, T. 2001. Clutch size adjustment of a leaf-mining moth (Lyonetiidae: Lepidoptera) in response to resource availability. Annals of the Entomological Society of America 95: 213–217.

Kaitaniemi, P., Neuvonen, S. & Nyyssönen, T. 1999. Effects of cumulative defoliations on growth, reproduction, and insect resistance in mountain birch. Ecology 80: 524-532.

— & Ruohomäki, K. 2001. Sources of variability in plant resistance against insects: free caterpillars show strongest effects. Oikos 95: 461-470.

Karban, R. & Baldwin, I.T. 1997. Induced responses to herbivory. University of Chicago Press. 319 p.

Khan, N.A. 2005. The influence of exogenous ethylene on growth and photosynthesis of mustard (*Brassica juncea*) following defoliation. Scientia Horticulturae 105: 499–505.

Khattak, A.M. & Pearson, S. 2005. Light quality and temperature effects on antirrhinum growth and development. Journal of Zhejiang University Science B 6: 119–124.

Keinänen, M., Julkunen-Tiitto, R., Mutikainen, P., Walls, M., Ovaska, J. & Vapaavuori, E. 1999. Trade-offs in secondary metabolism: Effects of fertilization, defoliation, and genotype on birch leaf phenolics. Ecology 80: 1970-1986.

Kellomäki, S., Wang, K.-Y. & Lemettinen, M. 2000. Controlled environment chambers for investigating tree response to elevated CO_2 and temperature under boreal conditions. Photosynthetica 38: 69-81.

— & Wang, K.-Y. 2001. Growth and resource use of birch seedlings under elevated carbon dioxide and temperature. Annals of Botany 87: 669-682.

—, Strandman, H., Nuutinen, T., Peltola, H., Korhonen, K.T. & Väisänen, H. 2005. Adaptation of forest ecosystems, forests and forestry to climate change. FINADAPT Working Paper 4, Finnish Environment Institute Mimeographs 334. Helsinki. 44 p.

Kozlowski, T.T. 1992. Carbohydrate sources and sinks in woody plants. The Botanical Review 58: 107–222.

Kudo, G. 2003. Variations in leaf traits and susceptibility to insect herbivory within a *Salix miyabeana* population under field conditions. Plant Ecology 169: 61–69.

Kulman, J.H. 1971. Effects of insect defoliation on growth and mortality of trees. Annual Review of Entomology 16: 289-324.

Kunstler, G., Curt, T., Bouchaud, M. & Lepart, J. 2005. Growth, mortality, and morphological response of European beech and downy oak along a light gradient in sub-Mediterranean forest. Canadian Journal of Forest Research 35: 1657–1668.

Kuokkanen, K., Julkunen-Tiitto, R., Keinänen, M., Niemelä, P. & Tahvanainen, J. 2001. The effect of elevated CO_2 and temperature on the secondary chemistry of *Betula pendula* seedlings. Trees – Structure and Function 15: 378-384.

—, Yan, S.C. & Niemelä, P. 2003. Effects of elevated CO_2 and temperature on the leaf chemistry of birch *Betula pendula* (Roth) and the feeding behaviour of the weevil *Phyllobius maculicornis*. Agricultural and Forest Entomology 5: 209–217.

Laitinen, M.- L., Julkunen-Tiitto, R., Tahvanainen, J., Heinonen, J. & Rousi, M. 2005. Variation in birch (*Betula pendula*) shoot secondary chemistry due to genotype, environment and ontogeny. Journal of Chemical Ecology 31: 697-717.

Landhäusser, S.M. & Lieffers, V.J. 2002. Leaf area renewal, root retention and carbohydrate reserves in a clonal tree species following above-ground disturbance. The Journal of Ecology 90: 658-665.

— & Lieffers, V.J. 2003. Seasonal changes in carbohydrate reserves in mature northern *Populus tremuloides* clones. Trees - Structure and Function 17: 471-476.

Lavola, A. & Julkunen-Tiitto, R. 1994. The effect of elevated carbon dioxide and fertilization on primary and secondary metabolites in birch, *Betula pendula* (Roth). Oecologia 99: 315-321.

Lee, H., Overdieck, D. & Jarvis, P.G. 1998. Biomass, growth and carbon allocation. In: Jarvis, P.G. (ed.). European forests and global change: The effects of rising CO_2 and temperature. Chapter 5. Cambridge University Press. p. 126-191.

Lempa, K., Agrawal, A.A., Salminen, J.-P., Turunen, T., Ossipov, V., Ossipova, S., Haukioja, E. & Pihlaja, K. 2004. Rapid herbivore-induced changes in mountain birch phenolics and nutritive compounds and their effects on performance of the major defoliator, *Epirrita autumnata*. Journal of Chemical Ecology 30: 303-321.

Lincoln, D.E., Fajer, E.D. & Johnson, R.H. 1993. Plant-insect herbivore interactions in elevated CO_2 environments. Trends in Ecology and Evolution 8: 64–68.

Lindroth, R.L., Kinney, K.K. & Platz, C.L. 1993. Responses of deciduous trees to elevated atmospheric CO_2: productivity, phytochemistry and insect performance. Ecology 74: 763-777.

—, Klein, K.A., Hemming, J.D.C. & Feuker, A.M. 1997. Variation in temperature and dietary nitrogen affect performance of the gypsy moth (*Lymantria dispar* L). Physiological Entomology 22: 55–64.

Little, C.H.A., Lavigne, M.B. & Ostaff, D.P. 2003. Impact of old foliage removal, simulating defoliation by the balsam fir sawfly, on balsam fir tree growth and photosynthesis of current-year shoots. Forest Ecology and Management 186: 261–269.

Long, S.P. 1991. Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations. Has its importance been underestimated? - Opinion. Plant, Cell and Environment 14: 729-739.

Markkola, A., Kuikka, K., Rautio, P., Härmä, E., Roitto, M. & Tuomi, J. 2004. Defoliation increases carbon limitation in ectomycorrhizal symbiosis of *Betula pubescens*. Oecologia 140: 234-240.

Maschinski, J. & Whitham, T.G. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. The American Naturalist 134: 1-19.

Mattson, W.J., Kuokkanen, K., Niemelä, P., Julkunen-Tiitto, R., Kellomäki, S. & Tahvanainen, J. 2004. Elevated CO_2 alters birch resistance to Lagomorpha herbivores. Global Change Biology 10: 1402–1413.

Mauffettee, Y. & Oechel, W.C. 1989. Seasonal variation in leaf chemistry of the coast live oak *Quercus agrifolia* and implications for the California oak moth *Phryganidia californica*. Oecologia 79: 439–445.

McNaughton, S.J., Wallace, L.L. & Coughenour, M.B. 1983. Plant adaptation in an ecosystem context: effects of defoliation, nitrogen, and water on growth of an African C4 sedge. Ecology 64: 307–318.

Melillo, J.M., Aber, J.D. & Muratore, J.F. 1982. Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63: 621-626.

Mendoza, A., Pinero, D. & Sarukhan, J. 1987. Effects of experimental defoliation on growth, reproduction and survival of *Astrocaryum mexicanum.* The Journal of Ecology 75: 545-554.

Mori, K., Qian, Z.-H. & Watanabe, S. 1992. Synthesis of 3, 4'dihydroxypropiophenone 3-β-D-glucopyranoside, a constituent of *Betula platyphylla* var. *japonica*, by enzymatic transglucosylation. Liebigs Annalen der Chemie 1992: 485-487.

Morvan-Bertrand, A., Ernstsen, A., Lindgård, B., Koshioka, M., Le Saos, J., Boucaud, J., Prud'homme, M.-P. & Junttila, O. 2001. Endogenous gibberellins in *Lolium perenne* and influence of defoliation on their contents in elongating leaf bases and in leaf sheaths. Physiologia Plantarum 111: 225-231.

Mutikainen, P., Walls, M., Ovaska, J., Keinänen, M., Julkunen-Tiitto, R. & Vapaavuori, E. 2000. Herbivore resistance in *Betula pendula*: effect of fertilization, defoliation, and plant genotype. Ecology 81: 49-65.

Müller, I., Schmid, B. & Weiner, J. 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. Perspectives in Plant Ecology, Evolution and Systematics 3: 115-127.

Mäkipää, R., Karjalainen, T., Pussinen, A. & Kellomäki, S. 1999. Effects of climate change and nitrogen deposition on the carbon sequestration of a forest ecosystem in the boreal zone. Canadian Journal of Forest Research 29: 1490 – 1501.

Mølmann, J.A., Berhanu, A.T., Stormo, S.K., Ernstsen, A., Junttila, O. & Olsen, J.E. 2003. Metabolism of gibberellin A19 is under photoperiodic control in *Populus, Salix* and *Betula*, but not in daylength-insensitive *Populus* overexpressing phytochrome A. Physiologia Plantarum 119: 278-286.

Niemelä, P., Chapin III, S., Danell, K. & Bryant, J.P. 2001. Herbivory-mediated responses of selected boreal forests to climate change. Climatic Change 48: 427–440.

Niemistö, P., Hokkanen, T. & Varama, M. 2004. Karikemäärän muutokset 1982–2001 ja puiden kunto lumi- ja hallamittariesiintymän vaivaamissa koivikoissa Noormarkussa. Metsätieteen aikakauskirja 1/2004: 21–41.

Niinemets, Ü., Portsmuth, A. & Truus, L. 2002. Leaf structural and photosynthetic characteristics, and biomass allocation to foliage in relation to foliar nitrogen content and tree size in three *Betula* species. Annals of Botany 89: 191-204.

O'Neil, L.C. 1962. Some effects of artificial defoliation on the growth of jack pine (*Pinus banksiana* Lamb.). Canadian Journal of Botany 40: 273–280.

Osier, T.L. & Lindroth, R.L. 2004. Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. Oecologia 139: 55–65.

Páez, A., González O., M. E., Yrausquin, X., Salazar, A. & Casanova, A. 1995. Water-stress and clipping management effects on guineagrass: I. Growth and biomass allocation. Agronomy Journal 87: 698-706.

Paige, K.N. & Whitham, T.G. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. The American Naturalist 129: 407-416.

Paul, M.J. & Foyer, C.H. 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52: 1383-1400.

Pérez-Harguindeguy, N., Diaz, S., Cornelissen, J.H.C., Vendramini, F., Cabido, M. & Castellanos, A. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil 218: 21–30.

Pilson, D. & Rausher, M.D. 1988. Clutch size adjustment by a swallowtail butterfly. Nature 333: 361–363.

Pimenta-Barrios, E. & Ramírez-Hernández, B.C. 2003. Phenology, growth, and response to light of ciruela mexicana (*Spondias Purpurea* L., Anacardiaceae). Economic Botany 57: 481–490.

Porter, L.J., Hrstich, L.N. & Chan, B.C. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25: 223– 230.

Price, P.W. 1991. The plant vigor hypothesis and herbivore attack. Oikos 62: 244-251.

Prittinen, K., Pusenius, J., Koivunoro, K., Rousi M., Roininen, H., 2003. Mortality in seedling populations of Silver Birch: genotypic variation and herbivore effects. Functional Ecology 17: 658-663.

Prokopy, R.J. & Owens, E.D. 1983. Visual detection of plants by herbivorous insects. Annual Review of Entomology 28: 337-364.

Prudhomme, T.I. 1982. The effect of defoliation history on photosynthetic rates in mountain birch. Reports from the Kevo Subarctic Research Station 18: 5-9.

Puettmann, K.J. & Saunders, M.R. 2001. Patterns of growth compensation in eastern white pine (*Pinus strobus* L.): the influence of herbivory intensity and competitive environments. Oecologia 129: 376–384.

Rautio, P., Markkola, A., Martel, J., Tuomi, J., Härmä, E., Kuikka, K., Siitonen, A., Leal Riesco, I. & Roitto, M. 2002. Developmental plasticity in birch leaves: defoliation causes a shift from glandular to nonglandular trichomes. Oikos 98: 437–446.

Rees, M., Condit, R., Crawley, M., Pacala, S. & Tilma, D. 2001. Long-term studies of vegetation dynamics. Science 293: 650-655.

Rey, A. & Jarvis, P.G. 1997. Growth response of young birch trees (*Betula pendula* Roth.) after four and a half years in elevated CO_2. Annals of Botany 80: 809-816.

Riikonen, J., Holopainen, T., Oksanen, E. & Vapaavuori, E. 2005. Leaf photosynthetic characteristics of silver birch during three years of exposure to elevated concentrations of CO_2 and O_3 in the field. Tree Physiology 25: 621–632.

Rosswall, T. 1982. Microbiological regulation of the biogeochemical nitrogen cycle. Plant and Soil 67: 15-34.

Ruohomäki, K., Haukioja, E., Repka, S. & Lehtilä, K. 1997. Leaf value: effect of damage to individual leaves on growth and reproduction of mountain birch shoots. Ecology 78: 2105-2117.

Sasek, T.W., De Lucía, E.H. & Strain, B.R. 1985. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO_2 concentrations. Plant Physiology 78: 612–622.

Scheidegger, U.C. & Nösberger, J. 1984. Influence of carbon dioxide concentration on growth, carbohydrate content, translocation and photosynthesis of white clover. Annals of Botany 54: 735-742.

Schofield, J.A., Hagerman, A.E. & Harold, A. 1998. Loss of tannins and other phenolics from willow leaf litter. Journal of Chemical Ecology 24: 1409-1421.

Scriber, J.M. & Slansky, F. 1981. The nutritional ecology of immature insects. Annual Review of Entomology 26:183-211.

Simons, A.M. & Johnston, M.O. 1999. The cost of compensation. The American Naturalist 153: 683-687.

Slansky, F. & Feeney, P. 1977. Stabilization of the rate of nitrogen accumulation by larvae of the cabbage butterfly on wild and cultivated food plants. Ecological Monographs 47: 209-228.

Smith, C.M. & Boyko, E.V. 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata, 122: 1-16.

Suberkropp, K., Godshalk, G.L. & Klug, M.J. 1976. Changes in the chemical composition of leaves during processing in a woodland stream. Ecology 57: 720-727.

Thomson, V.P., Cunningham, S.A., Ball, M.C. & Nicotra, A.B. 2003. Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. Oecologia 134: 167–175.

van den Boogaard, R., Grevsen, K. & Thorup-Kristensen, K. 2001. Effects of defoliation on growth of cauliflower. Scientia Horticulturae 90: 1-16.

Veteli, T.O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H. & Tahvanainen, J. 2002. Effects of elevated CO_2 and temperature on plant growth and herbivore defensive chemistry. Global Change Biology 8: 1240-1252.

Virtanen, T. & Neuvonen, S. 1999. Climate change and macrolepidopteran biodiversity in Finland. Chemosphere: Global Change Science 1: 439-448.

von Fircks, Y. & Sennerby-Forsse, L. 1998. Seasonal fluctuations of starch in root and stem tissues of coppiced *Salix viminalis* plants grown under two nitrogen regimes. Tree Physiology 18: 243–249.

Wall, A. & Hytönen, J. 2005. Soil fertility of afforested arable land compared to continuously forested sites. Plant and Soil 275: 247-260.

Wardle, D.A., Barker, G.M., Bonner, K.I. & Nicholson, K.S. 1998. Can comparative approaches based on plant ecophysiological traits predict the nature of biotic interactions and individual plant species effects in ecosystems? Journal of Ecology 86: 405-420.

Wassner, D.F. & Ravetta, D.A. 2005. Temperature effects on leaf properties, resin content, and composition in *Grindelia chiloensis* (Asteraceae). Industrial Crops and Products 21: 155–163.

Whitham, T.G., Maschinski, J., Larson, K.C. & Paige, K.N. 1991. Plant responses to herbivory: the continuum from negative to positive and underlying physiological mechanisms. In: Price, P.W., Lewinsohn, T.M., Fernandes, G.W. & Benson, W.W. (eds). Plant-animal Interactions: Evolutionary Ecology in Tropical and Temperature Regions. John Wiley and Sons, New York. p. 227-256.

Williams, R.S., Norby, R.J. & Lincoln, D.E. 2000. Effects of elevated CO_2 and temperature-grown red and sugar maple on gypsy moth performance. Global Change Biology 6: 685–696.

—, Lincoln, D.E. & Norby, R.J. 2003. Development of gypsy moth larvae feeding on red maple saplings at elevated CO_2 and temperature. Oecologia 137: 114–122.

Wingler, A., von Schaeven, A., Leegood, R.C., Lea, P.J. & Quick, W.P. 1998. Regulation of leaf senescence by cytokinin, sugars, and light. Effect on NADH-dependent hydroxypyruvate reductace. Plant Physiology 116: 329-335.

Wolf, A., Kozlov, M.V. & Callaghan, T.V. 2008. Impact of non-outbreak insect damage on vegetation in northern Europe will be greater than expected during a changing climate. Climatic Change 87: 91-106.

Wu, G., Shao, H.B., Chu, L.Y. & Cai, J.W. 2007. Insights into molecular mechanisms of mutual effect between plants and the environment. A review. Agronomy for Sustainable Development 27: 69-78.

Xiao, C.-W., Zhou, G.-S. & Ceulemans, R. 2003. Effects of elevated temperature on growth and gas exchange in dominant plant species from Maowusu sandland, China. Photosynthetica 41: 565-569.

Ziska, L.H., Reeves, J.B. & Blank, B. 2005. The impact of recent increases in atmospheric CO_2 on biomass production and vegetative retention of Cheatgrass (*Bromus tectorum*): implications for fire disturbance. Global Change Biology 11: 1325-1332.