Introduction  Humans are indirectly affecting forest trees by changing the Earth’s atmosphere. Increasing concentrations of atmospheric CO\textsubscript{2} have a beneficial effect on tree growth whereas increase in tropospheric O\textsubscript{3} is a health risk for trees. Both gases also have effect on the metabolism of phenolic compounds, which are considered to be defences against herbivores. On the other hand, phenolics are also used as host recognition cues by herbivores. Taken together, atmospheric changes may affect the performance and preference of herbivorous insects and detritivorous soil animals by changing the quality of their host plant. The aims of this thesis were to study how atmospheric change affects phenolic metabolism in the leaves of silver birch over time and whether these changes have any effect on insects or soil macrofauna.

Materials and methods  Seven years old saplings of two silver birch (Betula pendula Roth) clones were exposed to elevated concentrations of CO\textsubscript{2} and O\textsubscript{3}, singly and in combination, for three years in an open-top chamber experiment. The effects of CO\textsubscript{2} and O\textsubscript{3} on the growth and phenolic accumulation of short- and long-shoot leaves, leaf litter and buds, and on the growth and food consumption and preference of insects and soil macrofauna were studied.

Results  Elevated CO\textsubscript{2} increased the mass of short- and long-shoot leaves, irrespective of O\textsubscript{3}, while the mass of leaf litter was increased in clone 4 only. Elevated O\textsubscript{3} decreased the mass and size of short-shoot leaves while long-shoot leaves were not affected by O\textsubscript{3}. By contrast, both CO\textsubscript{2} and O\textsubscript{3} reduced the mass of buds, but the effects were prevented in the combined treatment. Elevated CO\textsubscript{2} increased the accumulation of intracellular phenolic compounds in leaves, while epicuticular compounds in leaves and buds were not affected. The effects of elevated O\textsubscript{3} on the accumulation of phenolic compounds were minor: fewer compounds were affected than by CO\textsubscript{2}, and the effects were altered over time. The growth of larvae of two geometrid moth species was not affected by elevated CO\textsubscript{2}, although their food consumption rate was increased. On the other hand, elevated O\textsubscript{3} reduced the growth of geometrid moth larvae fed with short-shoot leaves in spring. Food and oviposition preference was increased by elevated CO\textsubscript{2}, but the effect was dependent on tree clone and insect species. Elevated O\textsubscript{3} decreased the growth of young earthworms fed with clone 80 litter, but otherwise the effects of CO\textsubscript{2} and O\textsubscript{3} on soil animal performance were minor. The measured plant quality related variables explained only up to half of the variation in CO\textsubscript{2}- and O\textsubscript{3}-mediated effects on insect performance, and the contribution of litter quality to soil animal performance was also low.

Conclusions  The analysis of phenolic content values (i.e. the total amount per plant part) revealed that elevated CO\textsubscript{2} in general and elevated O\textsubscript{3} to some extent increase carbon allocation to certain phenolic compounds in birch leaves and that there are trade-offs among phenolic pathways between the studied tree clones. In silver birch, phenolic compounds may
serve as insect feeding stimulants, but the hypothesis of phenolics as silver birch defences against insect herbivores is questioned, since the growth of insects was not affected despite the observed CO$_2$ effects on phenolic compounds. It is obvious that O$_3$ is also detrimental to plant-feeding animals, but more research is needed to reveal the underlying factors. In addition, the effects of litter phenolics on soil animals may by nature be only indirect, and they should be investigated.

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ACKNOWLEDGEMENTS

The present study was carried out at the Finnish Forest Research Institute, Suonenjoki and Vantaa Research Units, at the Department of Ecology and Environmental Sciences, University of Kuopio and at the Department of Biology, University of Joensuu. I gratefully acknowledge the facilities and support provided by these institutions. My work was financially supported by the Academy of Finland, the European Commission, the Graduate School of Forest Sciences, the Universities of Kuopio and Joensuu, the Finnish Cultural Foundation, the Emil Aaltonen Foundation, the Finnish Society of Forest Science, the Kuopio Naturalists’ Society and the Niemi Foundation.

I express my deepest gratitude to my three supervisors. Docent Elina Vapaavuori, Professor Jarmo Holopainen and Professor Riitta Julkunen-Tiitto kindly guided me over the pitfalls of science with professional aplomb, and I highly appreciate their expertise in their specific fields of science. I sincerely thank both reviewers, Emeritus Professor John P. Bryant and Docent Juha-Pekka Salminen, for their helpful comments and constructive criticism on this thesis. I thank all co-authors for their contribution and I’m grateful for Jaakko Heinonen and Anne Kasurinen for our statistical discussions. Rosemary Mackenzie kindly checked the language of this thesis.

My work would have been impossible without the skillful assistance of the staff of the institutions I’ve worked in. I thank the senior and junior scientists from the University of Tartu for friendship, cultural exchange and collaboration. I’m very glad that I had the opportunity and privilege to work and to share thoughts and joy with so many friends and colleagues in Suonenjoki, Kuopio, Joensuu and Vantaa. I’m deeply indebted to Jaana, Sarita and Timo for their hospitality. I sincerely thank all Quidditch players for pretending to play floorball with me during the past six years, and Teemu, Ari, Aki, Jarkko and Heikki for the longest and most memorable RPG-campaign I’ve ever taken part in.

I dedicate this thesis to my family. My parents took me to see the wonders of the woods, and dad taught me to hunt game and catch fish. I heartily thank Päivi for her support, encouragement and enthusiasm to my work and for sharing with me the spectacular sights of the Earth.
ABBREVIATIONS

CC  chamber control
CO₂  carbon dioxide
DHPPG  3,4’-dihydroxypioiophenone 3-β-D-glucoside
EC  elevated CO₂
EC+EO  elevated CO₂ and O₃ in combination
EO  elevated O₃
FACE  free-air CO₂ enrichment
HPLC  high-performance liquid chromatography
LMWP  low-molecular-weight phenolic compound
MS  mass spectrometry
O₃  ozone
OC  outside control
OTC  open-top chamber
PCA  principal component analysis
PGG  pentagalloylglucose
PLS  partial least squares
ppb  parts per billion, e.g. nl l⁻¹
ppm  parts per million, e.g. µl l⁻¹
RGR  relative growth rate
RCR  relative consumption rate
SI  simple interaction
SME  simple main effect
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by chapter numbers:


**Chapter 3**  Peltonen PA, Vapaavuori E, Heinonen J, Julkunen-Tiitto R, Holopainen JK. Food quality mediated interactive effects of CO$_2$ and O$_3$ concentrations on folivorous insects in birch (Betula pendula). Submitted for publication.

**Chapter 4**  Kasurinen A, Peltonen PA, Julkunen-Tiitto R, Vapaavuori E, Nuutinen V, Holopainen T, Holopainen JK. Effects of elevated CO$_2$ and O$_3$ on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. Submitted for publication.


I participated in the planning of tests and collecting of plant samples, executed the tests, analysed the phenolic compounds from plant samples, carried out the statistical analyses and was the main author of Chapters 2, 3 and 5. In Chapter 4, I was responsible for the section that dealt with phenolic compounds and was co-author of this publication.
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CHAPTER 1

GENERAL INTRODUCTION
GENERAL INTRODUCTION

1.1 Atmospheric change affects forest trees

Human activities began to change the Earth’s atmosphere dramatically during the final quarter of the last millennium. The burning of fossil fuels and changes in land use, particularly deforestation, have increased concentrations of the major greenhouse gas, carbon dioxide (CO$_2$) from 280 ppm in 1750 to 367 ppm in 1999 (IPCC 2001). It is estimated that the concentration of CO$_2$ could be 540 to 970 ppm by 2100. At the same time, the increased emissions of volatile organic compounds and reactive nitrogen species (i.e. NO$_x$) from anthropogenic sources have increased concentrations of photochemical air pollutant ozone (O$_3$) in the troposphere (Fowler et al. 1998, IPCC 2001, Vingarzan, 2004). Long-range (regional, continental or inter-continental) transport from distant sources, e.g. metropolitan areas, extends O$_3$ influence to remote, rural areas (Vingarzan, 2004). In fact, global background O$_3$ concentrations have increased from 10–15 ppb to the present-day level of 30–40 ppb within less than two centuries (Finlayson-Pitts & Pitts, 1997, Percy et al. 2003, Laurila et al. 2004). It is estimated that background O$_3$ concentrations will double by 2100, although there may be regional differences in this trend (Vingarzan, 2004). Consequently, it is estimated that by 2100, 60% of temperate and subpolar forests and almost half of all forests will be exposed to potentially phytotoxic O$_3$ concentrations (>60 ppb) (Fowler et al. 1999).

Numerous studies employing various enclosure techniques (growth chambers, open-top chambers, branch bags) and open field or free-air carbon dioxide exposure (FACE) facilities have shown that CO$_2$ enrichment has beneficial effects on forest trees at seedling stage. Elevated CO$_2$ increases carbon assimilation, and increases above-ground growth and litter production (Saxe et al. 1998, Norby et al. 1999, Pritchard et al. 1999, Karnosky et al. 2003, Ainsworth & Long 2005). By contrast, elevated O$_3$ decreases carbon assimilation and transport, increases carbon allocation to antioxidant defences and respiration, causes premature senescence of leaves and reduces growth cumulatively over time (Skärby et al. 1998, Wustman et al. 2001, Oksanen 2003). Because the concentration of both gases is increasing in the atmosphere, there has been discussion on whether CO$_2$ mitigates or ameliorates the deleterious effects of O$_3$ on trees. It has been found that elevated CO$_2$ may not protect trees from harmful O$_3$ effects (Karnosky et al. 2003), but may even exacerbate them (Wustman et al. 2001, Karnosky et al. 2005). However, making predictions on the responses of mature trees to CO$_2$ and O$_3$ on the basis of results obtained from seedling studies is not straightforward (Skärby et al. 1998, Norby et al. 1999, Kolb & Matyssek 2001), and therefore studies on older trees are needed.

1.2 Will climate change modify the fate of silver birch in the future?

It has been suggested that climate change, i.e. an increase in atmospheric CO$_2$ concentration and air temperature, may increase the competitive advantage of tree species with an indeterminate, continuous growth pattern, such as silver birch (Betula pendula Roth), over species with predetermined growth (e.g. conifers) (Kellomäki et al. 1996), and may thus increase the abundance of birches in Finland. However, the sensitivity of silver birch to ozone (Skärby et al. 1998, Oksanen & Rouxi 2001, Oksanen 2003) confounds this prediction. Moreover, the fate of the birch may be complicated
even further because not only plants but also animals feeding on these plants will be affected by atmospheric change.

Besides its historical and economic importance for humans as firewood, building material, raw material for sawmills, the pulp and paper industry and even as a source of artistic inspiration, birch is an important food plant for several insect and mammalian herbivores. For example, silver birch and downy birch (B. pubescens) are hosts for approximately over 200, mostly Lepidopteran insect species in Finland (Saalas 1949, Annila 1987). Birch also improves soils by cycling nutrients (Peralta & Alm 1990), and one important factor in this process is litter comminution by soil macrofauna (Swift et al. 1979), such as earthworms (Hendriksen 1990, Edwards & Bohlen 1996) and terrestrial isopods (woodlice) (Hassall et al. 1987).

To reduce herbivore damage, plants resort to mechanical and chemical traits that reduce herbivore performance (antibiosis) or preference (antixenosis) (Leimu & Koricheva 2006, Morris 2006). The nutritive quality of tissues plays a dominant role in the suitability of plants for plant-feeding animals and is generally determined by the concentration of nitrogen and secondary compounds (Scriber & Slansky 1981, Slansky 1992, Awmack & Leather 2002). Plants produce numerous secondary compounds that are harmful to herbivores or deter their feeding (Rosenthal & Berenbaum 1991). Of the non-nitrogenous secondary compounds, phenolic compounds occur universally in vascular plants (Harborne 1997). Although these compounds occur in all plant tissues, most studies have concentrated mainly on leaves and on high molecular weight oligomeric or polymeric compounds, such as condensed tannins, which occur in ferns, gymnosperms and woody angiosperms. It has been shown that hydrolysable and condensed tannins bind proteins and other substances, decreasing the palatability of food for herbivores, and affecting their food preference and growth (Hagerman & Butler 1991, Harborne 1997). In addition, phenolic compounds, including low molecular weight monomeric (LMWP) compounds, serve as chemical cues for feeding and oviposition, and are used in host selection by phytophagous insects (Bernays & Chapman 1994, Simmonds 2001, Städler 2002). In silver birch, condensed tannins and LMWP compounds seem to play a defensive role against insects (Mutikainen et al. 2000). However, in some cases herbivores have overcome the defences of birches and have defoliated vast areas of birch forests in different regions of Scandinavia and North America (Tenow 1972, Werner & Baker 1977, Annila 1979).

Food quality is also an important factor for litter feeding soil animals. Studies indicate that the concentration of minerals, proteins, sugars and phenolics in litter determine the suitability of food for earthworms (Edwards & Bohlen 1996). Food selection and consumption by earthworms seems to be influenced by phenolic compounds (Swift et al. 1979, Hendriksen 1990, Slapokas & Granhall 1991), which affect the palatability of litter at least in the early stage of decomposition (Edwards & Bohlen 1996, Schönholzer et al. 1998). Although earthworms are able to digest tannin-protein complexes (Kuiters 1990, Wardle & Lavelle 1997), this ability can be inhibited by the accumulation of other secondary metabolites in litter (Wardle & Lavelle 1997). Moreover, nitrogen concentration has been shown to affect earthworm growth and fecundity (Edwards & Bohlen 1996). These findings are supported by the observation that abundance of earthworms correlates negatively with litter C:N, lignin:N and...
concentration of phenolic compounds (Edwards & Bohlen 1996).

Although some studies have shown that the consumption of terrestrial isopods is reduced by a high concentration of phenolic compounds, such as tannins (Cameron & LaPoint 1978, Zimmer 1999) and is increased by a high concentration of minerals (Ca, Mg, Na, K) in litter (Nicolai 1988), and that phenolics may affect the fecundity of isopods (Zimmer & Topp 2000), evidence of the effect of litter quality on them is controversial. For example, some studies show that litter with high concentrations of phenolic compounds are less preferred by isopods (Nicolai 1988), while others show absolutely no correlation between phenolics and isopod food preference (Neuhauser & Hartenstein 1978). Nevertheless, there is evidence that birch leaf litter with high tannin concentration is less preferred by isopods (Hassall & Rushton 1984, Hassall et al. 1987). Because phenolic compounds are abundant in birches (e.g. Bryant et al. 1993, Mutikainen et al. 1999, Ossipov et al. 2001), any change induced in the accumulation of these compounds in trees could thus affect not only leaf-feeding insects but also litter-feeding soil animals.

1.3 Atmospheric change affects plant quality and plant-herbivore interactions

1.3.1 Current knowledge on the effects of CO\textsubscript{2}

Increasing atmospheric CO\textsubscript{2} concentrations may change plant-herbivore interactions by altering plant tissue quality (Bezemer & Jones 1998, Peñuela & Estiarte 1998, Saxe et al. 1998, Norby et al. 1999). In fact, Körner (2000) has stated that “the rate by which the CO\textsubscript{2} concentration is doubling and the dietary change that it causes, are unprecedented for the present biota”.

There has been intensive experimentation on this subject during recent decades, and information on the effects of elevated CO\textsubscript{2} on plant nutrients and secondary compounds has increased considerably. Studies have shown that CO\textsubscript{2} enrichment generally leads to a decrease in foliar nitrogen concentration (Bezemer & Jones 1998, Cotrufo et al. 1998a, Curtis & Wang 1998, Norby et al. 1999, Ainsworth & Long 2005), which may be caused by a dilution effect (Norby et al. 1999), because CO\textsubscript{2} increases leaf mass (Saxe et al. 1998), or by a decrease in the amount of RuBisCO (Ainsworth & Long 2005).

In contrast to nitrogen, elevated CO\textsubscript{2} usually increases the concentration of phenolic compounds (Bezemer & Jones 1998, Koricheva et al. 1998). Source-sink balance hypotheses, such as the carbon-nutrient balance hypothesis (Bryant et al. 1983) and the extended growth-differentiation balance hypothesis (Herms & Mattson 1992), have been formulated to explain carbon allocation to plant growth and secondary metabolism, but studies on the hypotheses have given variable results (Peñuelas & Estiarte 1998). One explanation for this variability could be unsuitability of the chosen variable. Because “analysis of ratios is based on the assumption of an isomeric relationship between denominator and numerator variables” (Raubenheimer & Simpson 1992), the use of concentration as a measure in carbon allocation studies may have led to misinterpretation of the results. In other words, ratio does not tell which variable has changed – the denominator or the numerator. To solve this problem, it has been suggested that the actual content or amount per plant or plant part be used instead (Koricheva 1999, Gianoli 2000).

Nevertheless, changes in food quality related variables measured as
Concentrations appear to correlate with herbivore performance. CO\textsubscript{2} driven reductions in food quality have been shown to reduce the digestibility of food, and to reduce growth and increase consumption rates, development times and mortality of herbivores (Peñuelas & Estiarte 1998). However, the effects seem to be species-specific and should be examined in detail for each insect-plant system. So far, only relatively few species have been investigated (Coviella & Trumble 1999).

In accordance with the observed effect of elevated CO\textsubscript{2} on herbivores, litter quality is expected to influence the rate of litter decomposition by soil heterotrophs (Lambers 1993), especially during the initial stages of litter decomposition when the concentration of phenolic compounds is still high (Coûteaux & Bolger 2000). Studies have shown that CO\textsubscript{2} enrichment has indeed increased condensed tannins in the leaf litter of, for example, *Betula papyrifera* (Parsons et al. 2004), but more information is needed on the effects on soluble phenolics and tannins (Peñuelas & Estiarte 1998, Hättenschwiler & Vitousek 2000). In spite of evidence that the complexity of the soil fauna community is an important factor in the decomposition process (Coûteaux et al. 1991, Tian et al. 1997), especially macrofauna having a major effect during the early stages (Swift et al. 1979), the effects of CO\textsubscript{2} on soil macrofauna are underrepresented in the literature (Coûteaux & Bolger 2000). The few available studies have shown that elevated CO\textsubscript{2} can decrease preference (Cotrufo et al. 1998b) and increase consumption in isopods (Hättenschwiler et al. 1999, Hättenschwiler and Bretschger 2001), but contrary to observations in insects, nitrogen concentration does not appear to mediate isopod consumption (David et al. 2001, Hättenschwiler and Bretschger 2001). Moreover, the role of phenolic compounds (lignin excluded) in this interaction is still unexplored, and there are no published reports on the effects of phenolic compound mediated responses of earthworms to elevated CO\textsubscript{2}.

### 1.3.2 Interactive effects of CO\textsubscript{2} and O\textsubscript{3} seem to be complex and unpredictable

In field conditions, the effects of elevated CO\textsubscript{2} may not be so straightforward due to other interacting environmental factors, such as light, temperature, soil nitrogen availability and air pollution. Tropospheric O\textsubscript{3} may also influence plant-herbivore interactions by affecting plant quality, but interaction with elevated CO\textsubscript{2} may produce unpredictable results. For example, nitrogen concentration has been shown to decrease in leaves of deciduous trees under O\textsubscript{3} exposure (Lindroth et al. 2001, Oksanen and Rousi 2001, Holton et al. 2003, Yamaji et al. 2003), but the effect was enhanced, or only manifested, under simultaneous exposure to elevated CO\textsubscript{2} and O\textsubscript{3} (Kopper et al. 2001, Kopper and Lindroth 2003b, Agrell et al. 2005). Originally it was suspected that O\textsubscript{3} might hamper carbon assimilation and thus decreases the available precursors for the production of phenolic compounds in plants, but studies have shown that elevated O\textsubscript{3} actually increases the concentration of phenolic compounds (Koricheva et al. 1998, Yamaji et al. 2003). However, the interactive effects of CO\textsubscript{2} and O\textsubscript{3} on phenolics may be antagonistic (e.g. Percy et al. 2002) or synergistic (e.g. Kopper and Lindroth 2003a), a result that was not predictable from the results of the individual effects of gases.

In the literature, the number of papers dealing with the effects of O\textsubscript{3} on herbivores and detritivores is low in comparison to those describing the effects of CO\textsubscript{2}. Nevertheless, it has been shown that O\textsubscript{3} reduces food palatability,
increases or decreases performance, and increases the food preference and consumption of herbivores (Docherty et al. 1997, Flückiger et al. 2001). However, recent studies with FACE facilities have shown that CO\textsubscript{2} and O\textsubscript{3} may have variable interactive effects on herbivores, even within the same plant-insect system (Percy et al. 2002, Holton et al. 2003, Kopper and Lindroth 2003b). On the other hand, not all plant-insect systems are affected by elevated CO\textsubscript{2} and O\textsubscript{3} applied either singly or in combination (Kopper et al. 2001).

It is reasonable to expect that O\textsubscript{3} induced alterations in leaf quality will be reflected subsequently in litter quality and decomposition. Indications of altered litter quality (Findlay et al. 1996, Liu et al. 2005) and reduced decomposition (Findlay et al. 1996, Kim et al. 1998) under O\textsubscript{3} enrichment has in fact been found, but contradictory results have also been reported (e.g. Boerner and Rebbeck 1995, Scherzer et al. 1998, Kainulainen et al. 2003, Parsons et al. 2004). Moreover, Loranger et al. (2004) have demonstrated that elevated CO\textsubscript{2} and O\textsubscript{3} as individual treatments can decrease the abundance of soil fauna (Acari, Collembola) in temperate forest ecosystems, although the effects are negated under the combined treatment. So far, the interactive, food quality mediated effects of CO\textsubscript{2} and O\textsubscript{3} on soil macrofauna have not been studied.

1.4 Aims of the study

In this thesis my aim was to analyse individual phenolic compounds in green leaves (Chapters 2 and 3), leaf litter (Chapter 4) and bud exudates (Chapter 5) of 7-year-old silver birch saplings, and to measure how atmospheric change affects carbon allocation to these compounds. Particular emphasis was placed on temporal changes in responses. I also wanted to find out how atmospheric change affects the growth, food consumption and food or oviposition preference of herbivorous and detritivorous invertebrates, and to assess which phenolic compounds, if any, contribute to the observed effects.

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CHAPTER 6

GENERAL DISCUSSION
GENERAL DISCUSSION

6.1 Methodological considerations

Classic OTCs have advantages over other enclosure techniques (branch bags, growth chambers, glasshouses) in that they enable plant growth under natural light and soil conditions, but they do, however, suffer from altered microclimatic conditions (i.e. the “chamber effect”) such as higher temperature and relative humidity, and lower wind speed and reduced light (Ceulemans & Mousseau 1994, Karnosky et al. 2005). Although OTCs are good for studying individual level effects, free-air exposure facilities, such as FACE, enable community level studies and are not hampered by the chamber effect (Karnosky et al. 2001). Nevertheless, OTCs are applicable, since for example interactions with temperature are difficult to study using free-air exposure facilities. Moreover, with regard to CO\textsubscript{2} effects on boreal forest trees, we have to rely on OTC studies because no FACE studies have yet been carried out on boreal forest ecosystems (Ainsworth & Long 2005). Furthermore, the results obtained from trees grown in OTCs and free-air fields have been similar for CO\textsubscript{2} (Ainsworth & Long 2005) and O\textsubscript{3} (Grantz et al. 2006, and references therein).

In our three-year long silver birch experiment, fumigations were successful and gas concentrations were close to target values most of the time (Vapaavuo et al. 2002, Chapter 2). However, the chamber effect was evident and affected the morphology, phytochemistry, physiology and growth of the trees (Eichelmann et al. 2004, Riikonen et al. 2004, Oksanen et al. 2005, Chapters 2 and 3). It appears that a chamber environment was less stressful than the ambient environment especially for clone 4 (Eichelmann et al. 2004, Riikonen et al. 2004) and may have diminished the effects of CO\textsubscript{2} and O\textsubscript{3} by reducing the stomatal conductance of trees (Riikonen et al. 2005). Hence, the results might have been higher under free-air conditions. On the other hand, the temperature inside the chambers is in accordance with that expected to prevail by the end of the century in Finland (Jylhä et al. 2004), and our OTCs may thus have given realistic results in this respect.

The sample drying and extraction methods and HPLC method that were used are good for analysing flavonoids in silver birch (Keinänen 1993, Keinänen & Julkunen-Tiitto 1996), but are less well suited for the analysis of hydrolysable tannins like ellagitannins (Salminen 2003). On the other hand, the accumulation of hydrolysable tannins is low in silver birch (Salminen et al. 2002). The accumulation of PGG, an ellagitannin precursor (Salminen et al. 2001), was not affected by elevated CO\textsubscript{2} or O\textsubscript{3} in silver birch (Chapters 2 and 3) indicating that ellagitannins were not affected either.

Plant-animal bioassays can be done either in the field or in the laboratory. In the field it is possible to study changes in free populations or individuals or groups of individuals confined in branch bags or clip cages etc. on their host plants. The OTC technology used did not allow population level studies, but provided plant material for laboratory bioassays. Although it was easier to study specific animal performance and preference, and food quality related hypotheses with bioassays in the laboratory, this methodology has some limitations. For instance, there are ecological factors that may modify animal responses to plant chemistry in the field (e.g. competitive ability, dispersal potential), but cannot be easily modeled in the laboratory (Berenbaum 1986). In addition, the controlled environment chambers and microcosms used in the bioassays of
insects and soil animals, respectively, are simplifications of their complex natural environments (Opp & Prokopy 1986), even though climatic conditions were set as close as possible to those prevailing in the field. On the other hand, complex climatic interactions, which were not the focus of the studies, such as increased temperature inside the chambers, did not confound the results obtained from laboratory tests.

The quality of test animals in laboratory-reared populations may change fast due to inbreeding, and genotypic alterations may influence animal behaviour (Kogan 1986, Opp & Prokopy 1986, Tingey 1986). The larvae of E. autumnata were reared under laboratory conditions for three years and may thus have suffered from some level of inbreeding, despite progeny control. Some of the larvae used in the experiments in 2001 were malformed prior to pupation and did not pupate normally. However, none of the larvae showed any such symptoms or abnormal behavior during the experiments. It is possible that subtle changes in food quality had caused this effect. Habituation or conditioning of larvae to food before the experiments (Berenbaum 1986) was avoided by keeping separate populations of moths for experiments and breeding.

Though laboratory-reared animals may have problems, animals collected from the field are not without problems either, because they can be infested with pathogens or parasites that change their behaviour (Tingey 1986). The larvae of R. hastata were obtained from eggs laid by captive wild adults under controlled conditions, whereas O. antiqua eggs were collected from the field. All eggs were stored under controlled conditions. Leaf weevil adults were collected from the field as well, but none of these wild individuals showed any indication of parasitism or disease during or after the experiments.

The great variation in the results of choice feeding tests (Chapter 3) shows that a higher number of replicates would have been needed (Opp & Prokopy 1986). In fact, low replication may have reduced the power of the statistical tests in all experiments (Quinn & Keough 2005), despite the fact that the range of statistical significance was broadened to 0.1 in order to minimize type II decision errors (Chapters 2-5). This meant, however, that inferences made from results within the 0.05-0.1 range were considered with caution, due to the increased probability of their being affected by chance.

6.2 Carbon allocation to growth and phenolic compounds in silver birch under elevated CO$_2$ and O$_3$ concentrations

6.2.1 Effects on growth

The results of studies conducted with these silver birch trees show unequivocally that CO$_2$ enrichment has beneficial effects on the growth of trees even after the seedling stage (Eichelmann et al. 2004, Kull et al. 2003, Riikonen et al. 2004, 2005), which is in accordance with recent studies (e.g. Ainsworth & Long 2005). At the plant part level, elevated CO$_2$ increased leaf dry weight markedly (Eichelmann et al. 2004, Chapters 2 and 4), whereas it had only a minor effect on bud dry weight (Chapter 5) and did not affect the size of these plant parts (Chapters 2 and 5). By contrast to CO$_2$, elevated O$_3$ reduced whole-tree growth (Kull et al. 2003, Riikonen et al. 2004) as was expected according to previous studies (e.g. Skärby et al. 1998), but the effects were less pronounced and often statistically insignificant at the plant part level.
Contrary to findings in temperate deciduous forest tree species (Karnosky et al. 2003), the growth reductions observed in silver birch were ameliorated by elevated CO$_2$ (Kull et al. 2003, Riikonen et al. 2004, Chapters 2, 4 and 5). Differences in the elevation of CO$_2$ gas concentrations between the studies with temperate and boreal deciduous trees (+200 ppm vs. +360 ppm, respectively) may have affected the results, but they may also have been influenced by differences in other climatic factors. For example, it has been shown that springtime frost can predispose trees to ozone damage in northern boreal forest zone (Oksanen et al. 2006). Environmental conditions inside the OTCs may have been more benign and thus may have mitigated the effects of otherwise harsh northern conditions, especially in clone 4 (Eichelmann et al. 2004). On the other hand, high air temperatures in autumn may increase the deleterious effects of O$_3$ (Kubiske et al. 2006). Given the predictions of future climatic conditions in Finland, for example a 2–12°C increase in springtime temperatures and 2–7°C increase in autumn temperatures (Jylhä et al. 2004), the deleterious effects of O$_3$ on birch growth are difficult to predict. The wide genetic variation in the sensitivity of birches to O$_3$ (Pääkkönen et al. 1997, Yamaji et al. 2003) adds even more uncertainty to the predictions.

6.2.2 Effects on phenolics

The results demonstrate the applicability of graphical vector analysis and show that the content value is a good indicator of carbon allocation to phenolic compounds (Chapters 2, 4 and 5). The observed differences in phenolic composition between green and abscised leaves (Chapters 2, 3 and 4) indicate that some compounds are actively produced in green leaves, and may be subject to turnover, or that they are degraded or leached more rapidly during or after leaf abscission. Nevertheless, elevated CO$_2$ was found generally to increase carbon allocation to phenolic compounds in silver birch leaves and leaf litter, although not all individual compounds or compounds groups were affected. For example, the content of DHPPG and flavonoid aglycones was unresponsive to CO$_2$ enrichment, whereas the contents of phenolic acids, flavonol glucosides and condensed tannins were markedly increased (Chapters 2 and 4). On the other hand, the effects of O$_3$ on phenolics showed temporal variation in green and abscised leaves, indicating that O$_3$ was able to alter the carbon allocation patterns over time, but not to the same degree as did CO$_2$.

Interestingly, in parallel to leaf epicuticular compounds, the content of flavonoid aglycones found in bud exudates was also unresponsive to CO$_2$ (Chapter 5). These results imply that the accumulation of epicuticular compounds in leaf and bud glandular trichomes may be independent of the accumulation of intracellular compounds in leaves, as suggested by Cuadra et al. (1997) and Onyilagha and Grotewold (2004). Lavola and Jukunen-Tiitto (1994) and Lavola et al. (1994) have reported similar differences in responses to CO$_2$ enrichment among leaf and stem phenolics of birch. This indicates that phenolic pathways are also regulated independently in different plant parts.

6.3 Phenolic compounds as silver birch defences under changing atmospheric conditions

6.3.1 Implications for insect herbivores

Bioassays showed intriguing variation in the responses of herbivorous insects and
detritivorous soil macrofauna to CO$_2$- and O$_3$-mediated effects in birch quality. Despite clear changes in phenolic compounds under elevated CO$_2$ and O$_3$, the growth of juvenile insects was affected only by elevated O$_3$ (Chapter 3). The results contradict the hypothesis that phenolic compounds are defences against insect herbivores in silver birch. Similarly, information on the vast structural diversity of ubiquitous condensed tannins (Ayres et al. 1997) and their variable effects on insect herbivores (Ayres et al. 1997, Forkner et al. 2004) cast doubt on the importance of phenolics as defences against insects and thus on the relevance of current, herbivory-oriented plant defence hypotheses (reviewed by Stamp 2003).

It is clear that resistance is comprised of many interacting factors in plants (Kause et al. 1999, Ossipov et al. 2001, Haukioja et al. 2002, Haukioja 2003) and phenolics may play only a partial role. The raison d’être of plant phenolic production is thus an interesting question. Harborne (1991) has suggested that flavonoids have evolved with a primary, physiological function (e.g. protection from UV-radiation), while a secondary, ecological function as a cue for feeding and oviposition has evolved thereafter from feeding-inhibitory activity against herbivores. It is possible that one compound may have several functions (Harborne 1991), but it has also been suggested that plants may produce a variety of compounds with no actual purpose; a certain reservoir or “screen” of compounds waiting for an opportunity, such as altered environmental conditions, to enhance the plant’s fitness (Firn & Jones 2003). However, more experiments using a wider range of species are needed before any firm inferences on the role and importance of phenolic compounds in plant resistance can be made.

Interestingly, Swihart and Bryant (2001) have shown that tree ontogeny (maturation) has an influence on tree resistance against mammalian herbivores in northern latitudes: juvenile-stage trees are less browsed than mature-stage trees. In other words, the resistance is higher in juvenile trees. Because the silver birch trees used in the experiments were 10 years old and were approaching maturity at the time when the bioassays were done (Riikonen et al. 2004), it is possible that the overall resistance of the trees against insect herbivores had declined. Moreover, although it is possible that phenolics serve merely as feeding and oviposition stimulants, i.e. as cues for host recognition, the food or oviposition preference was affected in birch by tree genotype and insect species (Chapters 3 and 5). Swihart and Bryant (2001) found that the density of herbivory diminishes with an increase in the latitudinal origin of conspecific plants. However, in insect herbivores, this correlation may be different (Swihart & Bryant 2001 and references therein), as was seen in the results for two tree genotypes in this thesis: southern clone 4 was preferred by birch aphids, whereas northern clone 80 was preferred by P. argentatus weevils and R. hastata moths.

Slansky (1992) suggested that allelochemicals (secondary compounds) may also be harmful to insect intestinal microbes or to the detoxication enzymes they produce, but the unresponsiveness of E. autumnata larvae to phenolics induced by CO$_2$ (Chapter 3) does not support this idea. The observed O$_3$ effect on insect growth may be better explained by O$_3$ induced foliar oxidative stress, which may be an important mechanism of plant resistance to insects (Appel 1993, Bi & Felton 1995). The observed increases in ascorbate (ASC) levels and ASC use rate by ascorbate oxidase (AO) in O$_3$-treated leaves of the same trees used in the experiments (Padu et al. 2005) may provide one explanation for the observed decline in E. autumnata
growth. Felton and Summers (1993) have proposed that AO, which is a ubiquitous plant enzyme, is able to reduce the bioavailability of ascorbic acid for insects that require this vitamin for growth, molting and reproduction. Although herbivores are generally able to detoxify pro-oxidant chemicals in their diet (Lindroth 1991) the detoxification capacity of the young E. autumnata larvae may have been overcome. Recently, Cornelissen et al. (2006) presented the novel idea of foliar pH as a determinant of leaf quality. These authors reported that an increase in pH, which correlates, for example, negatively with plant carbon and positively with plant nitrogen, increases digestion by ruminant herbivores. Provided this effect is same for folivorous insects, the observed increase of carbon in O$_3$-exposed birch leaves could have led to decreases in pH and digestibility, and consequently to a reduction in larval growth.

6.3.2 Implications for detritivorous soil animals

Both CO$_2$ and O$_3$ affected the growth of soil macrofauna, but the influence of phenolics on this interaction was uncertain (Chapter 4). Possibly the effect was indirect by nature. It has been shown that microorganism-mediated preprocessing of litter is important for earthworms (Wright 1972) and isopods (Zimmer and Topp 1997). In plants, O$_3$ induces mechanisms similar to the defence responses invoked by pathogens (Sandermann et al. 1998) including the synthesis of phenolic compounds, and at least some phenolics have been shown to possess both antimicrobial (Cowan 1999, Rauha et al. 2000) and antifungal (Alcerito et al. 2002) properties. Taken together, it is possible that soil macrofauna were affected via phenolic-mediated changes in microorganisms. There is in fact evidence that CO$_2$ and O$_3$ can alter the composition of microorganisms in soils (e.g. Coûteaux & Bolger 2000, Chung et al. 2006), but further investigations are needed to ascertain the validity of this hypothesis.

Elevated CO$_2$ has been shown to increase total leaf area per plant (Saxe et al. 1998, Norby et al. 1999, Pritchard et al. 1999), a result which was also observed in the trees used in this study (Riikonen et al. 2004). This means that the input of low quality litter will increase under CO$_2$ enrichment, which in turn may reduce the comminution and consumption of litter by soil macrofauna (Coûteaux & Bolger 2000). The effect that the increased input of litter phenolics into soils have on soil pH deserves attention in future studies, since soil pH is an important factor for earthworms (Edwards & Bohlen 1996).

6.4 A century is a long time to predict the effects of atmospheric change

To date, factorial experiments studying the effects of atmospheric change on forest trees have used only simple gradients, i.e. one elevated gas concentration level (e.g. +200 ppm or +360 ppm CO$_2$) that have been compared to control. However, environmental change is gradual by nature, and thus the current information gives only a glimpse of possible effects over time. Moreover, the modelled scenarios depend on emission abatement actions (IPCC 2001), but these agreements have not yet been undersigned by all countries.

The effects of atmospheric change on silver birch are also influenced by other abiotic factors that need to be taken into account in predictions. There are factors that may either mitigate the effects of elevated CO$_2$, such as increasing temperature (Zvereva & Kozlov 2006), or may exacerbate the effects of elevated O$_3$, such as an increase in various wavelengths of light (Ormrod et al. 1995,
of two potential defoliator insect species was not affected, indicating the minor importance of phenolics as defensive compounds in silver birch. The growth responses of silver birch and early season insect larvae and detritivorous soil macrofauna feeding on birch leaves underline the threat represented by tropospheric O$_3$ to living organisms, although the exact reason for this effect in the studied animals remains to be clarified.

6.5 Conclusions

Graphical vector analysis and the analysis of total contents are significant improvements in studies of carbon allocation in plants. Without this method, inferences on the responses of phenolic compounds to atmospheric change in silver birch could have been flawed, due to the interference of biomass change. My studies revealed how the accumulation of epicuticular phenolic compounds is different from that of other compounds. The relatively long duration of the fumigation experiment in comparison to most of the previous experiments on the subject showed that the effects of elevated CO$_2$ on phenolics are significant and stable over time, whereas the effects of elevated O$_3$ are more tissue- and compound-dependent and could accumulate over time. However, despite significant changes in phenolics under elevated CO$_2$, the growth of two potential defoliator insect species was not affected, indicating the minor importance of phenolics as defensive compounds in silver birch. The growth responses of silver birch and early season insect larvae and detritivorous soil macrofauna feeding on birch leaves underline the threat represented by tropospheric O$_3$ to living organisms, although the exact reason for this effect in the studied animals remains to be clarified.

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