Dissertationes Forestales 116

European aspen and hybrid aspen under changing environment – Leaf traits, growth and litter decomposition

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Academic dissertation

To be presented, with the permission of the Faculty of Biological and Environmental Sciences of the University of Helsinki, for public examination in Auditorium XV, Main Building, Unioninkatu 34, on May 5th 2011 at 12 o'clock noon.

Title of the dissertation:
European aspen and hybrid aspen under changing environment – Leaf traits, growth and litter decomposition

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Dissertationes Forestales 116

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ISSN 1795-7389
ISBN 978-951-651-321-1 (PDF)

(2011)

Publishers:
Finnish Society of Forest Science
Finnish Forest Research Institute
Faculty of Agriculture and Forestry of the University of Helsinki
Faculty of Science and Forestry of the University of Eastern Finland

Editorial Office:
Finnish Society of Forest Science
P.O. Box 18, FI-01301 Vantaa, Finland
http://www.metla.fi/dissertationes

Nikula, S. 2011. European aspen and hybrid aspen under changing environment – Leaf traits, growth and litter decomposition. Dissertationes Forestales 116. 41 p. Available at http://www.metla.fi/dissertationes/df116.htm

ABSTRACT

Understanding the responses of species and ecosystems to human-induced global environmental change has become a high research priority. The main aim of this thesis was to investigate how certain environmental factors that relate to global change affect European aspen (*Populus tremula*), a keystone species in boreal forests, and hybrid aspen (*P. tremula* × *P. tremuloides*), cultivated in commercial plantations. The main points under consideration were the acclimatization potential of aspen through changes in leaf morphology, as well as effects on growth, leaf litter chemistry and decomposition. The thesis is based on two experiments, in which young aspen (< 1 year) were exposed either to an atmospheric pollutant [elevated ozone (O_3)] or variable resource availability [water, nitrogen (N)]; and two field studies, in which mature trees (> 8 years) were growing in environments exposed to multiple environmental stress factors (roadside and urban environments). The field studies included litter decomposition experiments.

The results show that young aspen, especially the native European aspen, was sensitive to O_3 in terms of visible leaf injuries. Elevated O_3 resulted in reduced biomass allocation to roots and accelerated leaf senescence, suggesting negative effects on growth in the long term. Water and N availability modified the frost hardening of young aspen: High N supply, especially when combined with drought, postponed the development of frost hardiness, which in turn may predispose trees to early autumn frosts. This effect was more pronounced in European aspen. The field studies showed that mature aspen acclimatized to roadside and urban environments by producing more xeromorphic leaves. Leaf morphology was also observed to vary in response to interannual climatic variation, which further indicates the ability of aspen for phenotypic plasticity. Intraspecific variation was found in several of the traits measured, although intraspecific differences in response to the abiotic factors examined were generally small throughout the studies. However, some differences between clones were found in sensitivity to O_3 and the roadside environment.

Aspen leaf litter decomposition was retarded in the roadside environment, but only initially. By contrast, decomposition was found to be faster in the urban than the rural environment throughout the study. The higher quality of urban litter (higher in N, lower in lignin and phenolics), as well as higher temperature, N deposition and humus pH at the urban site were factors likely to promote decay.

The phenotypic plasticity combined with intraspecific variation found in the studies imply that aspen has potential for withstanding environmental changes, although some global change factors, such as rising O_3 levels, may adversely affect its performance. The results also suggest that the multiple environmental changes taking place in urban areas – which correspond closely with the main drivers of global change – can modify ecosystem functioning by promoting litter decomposition, mediated partly by alterations in leaf litter quality.

Keywords: urban environment, elevated O_3, resource availability, leaf morphology, leaf litter quality, inter- and intraspecific variation

ACKNOWLEDGEMENTS

This work was carried out at the Department of Biosciences of the University of Helsinki. Part of the studies were conducted at the experimental field of the Department of Environmental Science, University of Eastern Finland (formerly University of Kuopio), and at the Haapastensyrjä Breeding Station of the Finnish Forest Research Institute (FFRI). The financial support by the Helsinki University Environmental Research Centre, Jenny and Antti Wihuri Foundation, Hämäläisten Ylioppilassäätiö, Niemi Foundation, the FFRI and the University of Helsinki is gratefully acknowledged.

I would like to express my gratitude to my principal supervisor Docent Sirkku Manninen for her endless energy and guidance throughout the project. She has always had the time to discuss matters of all kinds related to the project and comment on the manuscripts, all this with remarkable efficiency. I warmly thank my other supervisor Docent Pertti Pulkkinen for the great opportunity to collaborate with the FFRI, continuous help, advice and encouraging attitude.

I am grateful to all my co-authors for collaboration and comments. Specifically, I wish to thank Professor Toini Holopainen and Professor Elina Oksanen for the possibility to use the free-air fumigation facility, Dr. Kevin Percy for contributing with the wax chemistry analyses, and Docent Elina Vapaavuori for providing the lignin analyses.

My sincere thanks to the personnel of the Haapastensyrjä Breeding Station and the Kuopio Campus Research Garden for helping with the experiments and maintaining the trees. I would especially like to acknowledge Raimo Jaatinen and Hiski Aro for their help with practical matters at all times. I also wish to thank Timo Oksanen for operating the ozone fumigations, and Niina Stenvall for providing tree material for the ozone experiment. Big thanks go to Ana Juanes del Valle and Jose Herrera Liceras for their help with the experiment at Haapastensyrjä, as well as for pleasant company during stays in Läyliäinen.

I am most grateful to Paavo Hellstedt, the master of branch scissors, who has been of invaluable help with the field work during all years. Virpi Karén, Markus Seppälä, Kaisa Jauhiainen and Monica Sanz, as well as several trainees from the FFRI have assisted me with the field work, and I am thankful to them all for their help and cheerful company. I also thank my former colleague Kaisa Siitonen for moral support especially during the early stages of the project, and Hannu Rita for statistical advice.

The pre-examiners Professor Reinhart Ceulemans and Dr. Elena Paoletti are gratefully acknowledged for their helpful and constructive comments on the thesis. I would also like to thank Sally Ulich, Marianna Parikka and Michael Hardman for revising the language of the thesis summary and the articles.

I wish to thank my friends for all their encouragement and peer support. The choir Hämäläis-Osakunnan Laulajat is also acknowledged for providing a community with such a great musical and social environment. Special thanks to Carlo for practical help and continuous support, and to Sirkku for always being there for me. Finally, I want to thank my family for their love and support at all times.

Helsinki, March 2011

Suvi Nikula

LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles, which are referred to in the text by their Roman numerals. The articles are reprinted with the kind permission of the publishers. Articles II and III are the author's versions of the manuscripts accepted for publication.

I Nikula, S., Percy, K., Oksanen, E., Holopainen, T. & Manninen, S. 2009. Effects of elevated ozone on growth and foliar traits of European and hybrid aspen. Boreal Environment Research 14 (suppl. A): 29–47.
http://www.borenv.net/BER/pdfs/ber14/ber14A-029.pdf

II Nikula, S., Manninen, S. & Pulkkinen, P. Growth and frost hardening of European aspen and backcross hybrid aspen as influenced by water and nitrogen. Accepted to Annals of Forest Science.
http://www.springer.com/life+sciences/forestry/journal/13595

III Nikula, S., Manninen, S., Vapaavuori, E. & Pulkkinen, P. Growth, leaf traits and litter decomposition of roadside hybrid aspen (*Populus tremula* L. × *P. tremuloides* Michx.) clones. Environmental Pollution, in press.
doi: 10.1016/j.envpol.2011.03.022

IV Nikula, S., Vapaavuori, E. & Manninen, S. 2010. Urbanization-related changes in European aspen (*Populus tremula* L.): Leaf traits and litter decomposition. Environmental Pollution 158: 2132–2142.
doi: 10.1016/j.envpol.2010.02.025

AUTHOR'S CONTRIBUTIONS

I Manninen designed the study together with Nikula. Oksanen and Holopainen provided the experimental facilities. Nikula was responsible for the data collection, morphological measurements and statistical analyses. Percy provided the wax chemistry analyses. Other leaf- and soil-related chemical analyses were carried out by others. Nikula prepared the manuscript and Manninen contributed with comments.

II Pulkkinen, Manninen and Nikula designed the study. Nikula was responsible for the data collection and statistical analyses. Leaf- and soil-related chemical analyses were carried out by others. Nikula prepared the manuscript and Pulkkinen and Manninen contributed with comments.

III Nikula and Manninen designed the study. Nikula was responsible for the data collection, morphological measurements and statistical analyses. Vapaavuori provided the lignin analyses. Other leaf- and soil-related chemical analyses were carried out by others. Nikula prepared the manuscript and Manninen and Pulkkinen contributed with comments.

IV Manninen designed the study together with Nikula. Nikula was responsible for the data collection, morphological measurements and statistical analyses. Vapaavuori provided the lignin analyses. Other leaf- and soil-related chemical analyses were carried out by others. Nikula and Manninen prepared the manuscript.

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LIST OF ABBREVIATIONS

ANCOVA	analysis of covariance
ANOVA	analysis of variance
AOT40	accumulated O_3 exposure over a threshold of 40 ppb
C	carbon
CO_2	carbon dioxide
CV	coefficient of variation
DBH	diameter at breast height
N	nitrogen
NO_x	nitrogen oxides
NRE	nitrogen resorption efficiency
O_3	ozone
ppb	parts per billion
SLA	specific leaf area
SO_2	sulfur dioxide

1. INTRODUCTION

1.1. Global environmental change

During this century ecosystems all over the world will be exposed to a variety of parallel environmental changes due to human activities. Increases in the atmospheric concentrations of greenhouse gases, for example carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3), mainly due to combustion of fossil fuels and land-use changes, are estimated to result in a rise in the global annual mean temperature of 1.1–6.4 °C by the end of the century (IPCC 2007). Global precipitation patterns are also expected to change during the next 100 years, with increases in mean precipitation in some regions and decreases in others (IPCC 2007). At the same time as the Earth’s climate is undergoing change, the concentrations of many pollutants in our environment are increasing. Some of the most important ones include various forms of inorganic nitrogen (N) and the secondary pollutant and greenhouse gas O_3. Although N is an essential resource for plants on one hand, high levels of wet and dry N deposition can adversely affect vegetation. The concentrations of air pollutants and greenhouse gases are often at their highest in urban environments (Shen et al. 2008), where road traffic is one of the major pollutant sources (Honour et al. 2009).

These changes in the environment have profound impacts on plant growth, although assessing their combined effect is challenging (Bytnerowicz et al. 2007). Plants may be able to acclimatize to changes in the environment in different ways, including alterations in leaf morphology, such as changes in specific leaf area (SLA; leaf area per leaf dry mass) and the amount of epicuticular waxes protecting the leaf (Gratani et al. 2000, Bussotti et al. 2005, Shepherd and Griffiths 2006, Bussotti 2008). The chemical composition of leaves, such as the concentration of nutrients and secondary metabolites, can also be altered by these environmental factors (Fenn et al. 1996, Kopper and Lindroth 2003, Bussotti et al. 2005, Valkama et al. 2007). As a result of modifications in green leaves, leaf litter quality may also change, potentially affecting litter decay rate (Fenn 1991, Grime et al. 1996, Magill and Aber 1998, Cotrufo et al. 1998, Carreiro et al. 1999, Liu et al. 2005). Litter decomposition, in turn, is a key process in the functioning of ecosystems, enabling the recycling of carbon (C) and nutrients (Swift et al. 1979).

1.1.1. Changes in water and nitrogen inputs

Water and N are fundamental resources for plants and among the most important environmental factors regulating tree growth. However, ecosystems are expected to face alterations in their supply. Global precipitation patterns are projected to change during this century: mean precipitation is estimated to increase in tropical and high latitude areas, whereas most sub-tropic and mid-latitude regions are predicted to experience decreases in precipitation (IPCC 2007). Also, extreme weather events, such as heavy rains and droughts, will occur more frequently and severely (Tebaldi et al. 2006, IPCC 2007). In Finland, the mean temperature is projected to increase by 3 to 6 °C and the annual precipitation by 12 to 22% by the year 2100 (Jylhä et al. 2009). The increase in precipitation is largely attributed to an increase in winter rains, whereas summer drought periods may become more frequent (Jylhä et al. 2009).

The anthropogenic inputs of N to ecosystems have been increasing substantially since pre-industrial times, with a 9-fold increase from 1890 to 1990 (Galloway and Cowling 2002). The escalating use of fossil fuels is a major source for increases in nitrogen oxide (NO) emissions, whereas the intensification of agriculture mainly accounts for the increases in emissions of ammonia (NH_3) (Reay et al. 2008, Bobbink et al. 2010). Although existing legislation is likely to reduce emissions – at least in Europe – on a global scale the emissions of NO and NH_3 are predicted to increase during the 21st century, resulting in an approximate doubling of the annual average N deposition over forests (Lamarque et al. 2005, Reay et al. 2008). In the atmosphere, NO and NH_3 are transformed during transport and deposited onto vegetation and soil both as wet and dry deposition (Harrison et al. 2000). Nitrogen oxide is readily oxidized to nitrogen dioxide (NO_2) by O_3. Ammonia is either subjected to dry deposition close to its emission source, or rapidly converted to ammonium (NH_4^+), which is transported for longer distances and may in turn react with other compounds (Krupa 2003 and references therein).

Trees take up N mainly from the soil via roots as NH_4^+ and nitrate (NO_3^-), and to a lesser extent as organic N compounds (Rennenberg et al. 2010), but uptake of N can also take place in the canopy mainly through stomata (Harrison et al. 2000). N additions usually result in a rise in N concentration of foliage and promote the growth of trees, especially of the portion above ground, for example by stimulating photosynthesis (Coleman et al. 1998, Cooke et al. 2005). Drought stress, in turn, often limits photosynthetic gas exchange and consequently growth (Ibrahim et al. 1997). Improvement in tree growth caused by N additions can especially be observed in environments that are limited by N availability (Nohrstedt 2001), such as temperate and boreal forests (Vitousek et al. 1997). However, elevated N deposition may result in decreased tree growth in the long term, mediated by soil acidification, leaching of nitrate and nutrient cations from the rooting zone, and mobilization of toxic aluminium ions (Gundersen et al. 2006). Furthermore, high levels of gaseous NO_x and NH_3 often have adverse effects on plants (reviews by Wellburn 1990, Krupa 2003).

The supply of water and N can also modify frost hardiness, which is a vital trait for the adaptation of trees in cold climates, and influences their growth potential during vegetative periods. However, the findings of research on their effects have not always agreed. Both increases (DeHayes et al. 1989, Rikala and Repo 1997) and decreases (Aronsson 1980, Hellergren 1981) in frost hardiness have been reported following N inputs. Mild water stress can promote frost hardening, whereas the process may be hindered by prolonged periods of drought (Colombo et al. 2001). These studies have mainly been conducted on evergreen conifers, whereas information on broad-leaved species is limited, especially in the case of the interactive effects of water and N.

1.1.2. Increasing levels of tropospheric O_3

Tropospheric O_3 is currently considered to be one of the most harmful air pollutants for vegetation, causing the most damage to plants (Paoletti et al. 2007a, Wittig et al. 2009). In the troposphere, O_3 is a secondary pollutant, formed through photochemical reactions from several precursor molecules including nitrogen oxides (NO_x), carbon monoxide (CO), and volatile organic compounds (VOCs) (Fowler et al. 1999). Both O_3 precursors and O_3 itself can be transported over long distances in the atmosphere. The background O_3 concentration has more than doubled since pre-industrial times, to the present concentration of 20–45 ppb in the Northern Hemisphere, and is currently increasing at an annual rate of 0.5–2%

(Vingarzan 2004, The Royal Society 2008). In Northern Europe, the mean annual O_3 concentration is relatively low – in Finland about 30 ppb (Laurila 1999) – compared with for example, Central and Southern Europe (Klumpp et al. 2006). However, the long summer days and rather humid environmental conditions in Northern Europe promote stomatal opening and subsequent O_3 uptake, meaning that northern forests can be negatively affected at lower O_3 concentrations than their Southern European counterparts (Karlsson et al. 2009).

Ozone enters plants mainly through open stomata, and cuticular uptake of O_3 has been considered negligible. Once inside leaves, O_3 rapidly reacts with cell wall and membrane components, forming reactive oxygen species (ROS) that induce defense mechanisms in plants (Sandermann et al. 1998). Harmful effects of O_3 can be manifested as visible leaf injuries, often resulting from exposure to acute, high O_3 peaks (Krupa and Manning 1988). Exposure to O_3 can accelerate leaf senescence, and cause reductions in stomatal conductance, photosynthetic capacity, growth, and C allocation to roots (Noormets et al. 2001, Yamaji et al. 2003, Karnosky et al. 2005, King et al. 2005, Wittig et al. 2007, 2009). O_3-related changes in leaf traits, such as increased levels of secondary metabolites and alterations in epicuticular wax chemistry (Karnosky et al. 2002, Yamaji et al. 2003, Valkama et al. 2007), may in turn alter plant susceptibility to herbivores and diseases (Percy et al. 2002), and retard the decay rate of leaf litter (Kim et al. 1998).

The responses of trees to O_3 are modified by other co-occurring environmental factors, such as soil N and water availability (Landolt et al. 1997, Schaub et al. 2003, Häikiö et al. 2007). Furthermore, the effects of O_3 depend on tree species as well as on genotypes within species. Deciduous species are generally considered more susceptible to O_3 impact than conifers, with fast-growing pioneer trees more susceptible than climax species (Wittig et al. 2007, Matyssek et al. 2010). In the genus *Populus*, several studies on the effects of O_3 have been carried out using trembling aspen (*Populus tremuloides* Michx.) and various poplar species (e.g. Dickson et al. 1998, King et al. 2005, Bussotti et al. 2007, Kubiske et al. 2007, Darbah et al. 2010). In contrast, few studies have addressed the O_3 sensitivity of European aspen (*Populus tremula* L.) (Matysssek et al. 1993, Häikiö et al. 2007).

1.1.3. Multiple environmental changes in urban environments

Along with the population growth, urbanization is an accelerating phenomenon in almost every part of the world (Grimm et al. 2008). Urbanization directly modifies landscapes and brings about a variety of environmental alterations, including changes in climate, atmospheric chemistry and soil properties. Urban environments are often characterized by higher mean temperatures, caused by the urban heat island effect, increased concentrations of greenhouse gases and atmospheric pollutants, such as CO_2 and NO_x, as well as higher depositions of heavy metals compared with surrounding rural areas (Pouyat and McDonnell 1991, Galloway et al. 1995, Idso et al. 1998, Pickett et al. 2001, Grimm et al. 2008). Although tropospheric O_3 levels are in general high around metropolitan areas, concentrations are typically at their highest in suburban and rural areas outside big cities due to the nature of the O_3 formation process (The Royal Society 2008).

Road traffic is a major source of air pollution in urban as well as in many rural areas, and on a global scale traffic volumes continue to increase (Colvile et al. 2001, Bignal et al. 2007). Motor vehicles emit a complex mixture of gaseous pollutants including NO_x, VOCs, sulphur dioxide (SO_2) and polycyclic aromatic hydrocarbons (PAHs) (Colvile et al. 2001, Harrison et al. 2003, Cape et al. 2004). Particulate matter containing metals originates from exhaust emissions as well as from vehicle wear and road surface abrasion (Harrison et al. 2003).

Urban areas are also characterized by severe forest fragmentation, often creating small, open forest patches (McDonnell et al. 1997, McKinney 2006). Compared with intact rural forests, forest stands in urban areas as well as those close to roads often contain an increased proportion of edge habitat with altered microclimatic conditions – trees closer to forest edges are often more exposed to solar radiation and moisture stress (Chen et al. 1993, Matlack and Litvaitis 1999, Delgado et al. 2007). Taken together, urban forests have been exposed for decades to environmental stress factors acting potentially as selective pressures (Grimm et al. 2008). This may have led to reduced genetic variation in urban tree populations, which could be manifested as smaller phenotypic variation than in the populations of surrounding rural forests (Grulke 2010).

The net effect of the multiple environmental changes taking place in cities on ecosystems can be difficult to predict based on manipulative experiments, which can only include a limited number of environmental factors in the experimental design. For instance, many studies conducted mainly in controlled environments reported adverse effects of traffic-related pollution on vegetation (e.g. Wellburn 1990, Viskari et al. 2000a, Grantz et al. 2003, Honour et al. 2009), but N-containing pollutants have also been shown to enhance plant growth near roads due to their fertilizing effect (Angold 1997, Laffray et al. 2010). Despite the importance of litter decomposition for the functioning of ecosystems, limited knowledge exists on how it is modified in urban habitats. Furthermore, previous studies reported both acceleration (McDonnell et al. 1997, Carreiro et al. 1999) and retardation (Cotrufo et al. 1995, Pavao-Zuckerman and Coleman 2005) of litter decay in urban settings. The conflicting results necessitate further studies.

1.2. European aspen and hybrid aspen as ecologically and economically important species

European aspen is one of the most widespread tree species in the world, with its distribution area covering most of Eurasia (Worrel 1995). It is the only endemic *Populus* species in Finland, where it can be found throughout the country (Hämet-Ahti et al. 1998) growing typically in mixed stands with spruce, pine and birch. Only 0.3% of the Finnish forest land area is covered with aspen-dominated stands. As a light demanding pioneer species, European aspen is able to grow in a suite of habitats ranging from dry to mesic sites, provided that it does not face intense interspecific competition (Worrell 1995).

In Fennoscandian boreal forests, European aspen is an ecologically important keystone species for biodiversity and ecosystem functioning, and various other species including animals, mosses, lichens and fungi, depend upon it (Siitonen 1999, Kouki et al. 2004). Compared with other tree species in Finland, it hosts the highest proportion of critically endangered species (Tikkanen et al. 2006). The leaf litter of aspen is particularly rich in calcium, and tends to raise the pH of otherwise typically acidic soils of boreal forests, which can consequently affect soil-related biota and processes (Valovirta 1968, Suominen et al. 2003). The economic value of aspen has previously been rather low, the species being mainly used in the match industry in the 1950-60’s. However, aspen has been systematically eliminated from the managed forests in Finland, because it serves as an intermediate host for *Melampsora pinitorqua* (Braun) Rostr., a rust disease of young pine stands (Kouki et al. 2004). In the 1990’s, economic interest in aspen started a new increase, along with advances in the paper industry (Viherä-Aarnio 1999). Nowadays, aspen is mainly cultivated for the uses of the pulp, paper and plywood industries, as well as for bioenergy production (Rytter 2006). Furthermore, *Populus* species can grow rather well on moderately polluted habitats and efficiently remove certain metals from soil, notably Cd and Zn (Laureysens et al. 2004, Hermle et al. 2006, Hassinen et al. 2009). This has made them interesting candidates for use in phytoremediation.

Hybrid aspen (*Populus tremula* L. × *Populus tremuloides* Michx.) is made by crossing European aspen with North American trembling aspen. It has proved to grow faster and to have more suitable fiber quality for papermaking than the native aspen (Yu et al. 2001b, Tigerstedt 2002), and is therefore utilized in commercial aspen plantations. However, it has also been demonstrated that hybrid aspen can cross with European aspen in nature, and that backcrosses between hybrid and European aspen may have some advantages over the pure native species concerning seed production and viability, as well as early competition (Suvanto et al. 2004, P. Pulkkinen, personal communication). Otherwise, the behavior of such backcrosses is largely unknown. In general, hybridization between introduced and native tree species may increase competition and narrow the gene pool of native species, thus threatening their overall fitness (Vanden Broeck et al. 2005). Although the performance of secondary hybrids is generally considered inferior to that of the parent species (Burke and Arnold 2001), sometimes – especially in new habitats and environmental conditions – secondary hybrids may be better adapted (Burke and Arnold 2001, Kimball et al. 2008).

In Finland, climate change has been predicted to benefit broadleaved trees such as birch (*Betula* sp.) and aspen more than conifers (Kellomäki et al. 1996). Overall, high genetic variation within natural populations and the ability for phenotypic plasticity improve the capability of a species to adapt to environmental changes (Aitken et al. 2008, Jump et al. 2009, Grulke 2010). Phenotypic plasticity – or acclimatization potential (Somero 2010) – means the ability of a genotype to change its phenotype in response to changes in the environment (Price et al. 2003). Trees generally tend to show high levels of genetic variation (Petit and Hampe 2006), and within *Populus* species, significant clonal differences have been found for a variety of attributes, including growth and leaf traits (Lindroth and Hwang 1996, Ceulemans and Deraedt 1999, Lindroth et al. 2001b, Yu 2001, Yu et al. 2001b, Marron et al. 2005). Furthermore, within *Populus* species, genotypes often vary in their response to environmental conditions, for example to soil properties (King et al. 1999, Yu and Pulkkinen 2003), as well as to stress factors such as O_3 (Dickson et al. 1998, Häikiö et al. 2007).

2. AIMS OF THE THESIS

The main aim of this thesis was to investigate how certain environmental factors that relate to global environmental change affect European aspen and hybrid aspen. More specifically, the aim was to examine whether aspen acclimatizes to these environmental factors through changes in leaf morphology, and how the factors modify the growth of aspen. By also studying intraspecific variation in leaf and growth traits, and in responses to the environmental factors examined, the work aimed to contribute to the assessment of how well aspen can survive in a changing environment. Furthermore, a comparison was made of the responses of European and hybrid aspen. In addition, the work aimed to assess how potential environment-mediated changes in aspen leaf litter quality affect ecosystem functioning through litter decomposition. The environmental factors studied were elevated O_3 (I), variable water and N supplies (II), roadside environment (III) and urban environment (IV).

The specific aims of the study were:

- To observe whether leaf morphology is modified by elevated O_3 (I) and in roadside (III) and urban (IV) environments.
- To study how growth and growth-related factors are affected by elevated O_3 (I), variable water and N supplies (II) and a roadside environment (III).
- To compare the native European aspen with hybrid aspen, including both F_1 hybrid aspen (I) and backcrosses between hybrid aspen and European aspen (II), in their responses to elevated O_3 (I) and variable water and N supplies, as well as in terms of their rate of frost hardening (II).
- To assess intraspecific variation within the traits studied (I–IV) and in responses to environmental factors (I–III).
- To investigate whether leaf litter quality is altered by elevated O_3 (I), and how leaf litter quality and decomposition are modified in roadside (III) and urban (IV) environments.

3. MATERIALS AND METHODS

This thesis is based on four substudies (Table 1). Two of the substudies were conducted under controlled conditions and investigated the effects of an atmospheric pollutant (I: elevated O_3) and resource availability (II: water and N) on young aspen clones or seedlings. In the other two substudies, mature trees growing under natural field conditions in environments exposed to multiple environmental stress factors were studied (III: roadside environment; IV: urban environment). The variables examined in each substudy are indicated in Table 1 and the methods described in more detail in the corresponding chapters. Additional environmental data (temperature, precipitation, air quality) were derived from the measurements conducted by the Helsinki Metropolitan Area Council (YTV) / Department of Regional and Environmental Information (IV).

3.1. Exposure to elevated O_3 (I)

The effects of elevated O_3 on two European aspen and two hybrid aspen clones were studied using a free-air fumigation system. This investigation was conducted at the experimental field of the University of Kuopio (62°37’N, 26°11’E), central Finland, in the summer of 2006. The European aspen clones were of southern Finnish origin. One hybrid aspen clone was a cross between female *P. tremula* from southern Finland and male *P. tremuloides* from southern Canada. The other hybrid aspen clone was a cross between female *P. tremuloides*, originally from Canada but grown in southern Sweden, and male *P. tremula*. These two hybrid aspen clones were also among the clones in study III. The clones were produced from root cuttings as described in Stenvall (2006) and were 2 months old at the start of the experiment.

The experimental field consisted of four ambient air treatment plots (control) and four elevated O_3 treatment plots (elevated O_3). Each plot contained four to five trees of each clone. Elevated O_3 plots were fumigated daily from 08:00 to 22:00 h for the period from 6 July to 19 September 2006. The 14-h mean O_3 concentrations were 29 ppb (control) and 45 ppb (elevated O_3). The elevated O_3 concentration (1.5 × ambient O_3 concentration) was chosen to simulate the ambient concentration predicted for Finland in about 2050, with an annual increase of 0.2–2% (Vingarzan 2004). The AOT40 (accumulated O_3 exposure over a threshold of 40 ppb) value in elevated O_3 treatment (10.2 ppm h) was almost ten-fold compared with control treatment (1.1 ppm h) and two-fold compared with the critical level of O_3 set for forest trees (AOT40 5 ppm h) (ICP 2004). The trees were grown in 15 l pots and irrigated and fertilized (139 kg N ha^{-1} yr^{-1}) regularly. The positions of the pots were randomized each week throughout the experiment.

3.2. Exposure to variable water and N supplies (II)

The effects of water and N supply on seven European aspen and seven backcross hybrid aspen families were investigated in a greenhouse experiment, which also included freezing tests. This was carried out at the Haapastensyrjä Breeding Station of the Finnish Forest Research Institute (60°36’N, 24°36’E) in November 2006 – May 2007. All European aspen families were of southern Finnish origin. The backcross hybrid aspen families (referred to

Table 1. Description of the studies I–IV and the variables investigated.

	I	II	III	IV
Abiotic factor studied	Elevated O_3	Water, N	Roadside environment	Urban environment
Description of the study	Free-air fumigation experiment, potted young clones	Greenhouse experiment, potted young seedlings	Field study, 8–9 year old clones	Field study, mature trees
Species used[a] **(no. clones / families)**	EA (2), HA (2)	EA (7), HA × EA (7)	HA (13), EA (1)	EA
Green leaves				
Specific leaf area (SLA)	x		x	x
Epicuticular wax amount	x		x	x
Epicuticular wax chemistry	x			
N and C concentration	x	x	x	x
Concentrations of other nutrients and metals			x	
Visible O_3 injury	x			
Melampsora sp. infection	x			
Growth				
Height growth (rate)	x	x	x	
Diameter growth (rate)	x		x	
Biomass	x			
Root/shoot ratio	x			
Gas exchange				
Photosynthesis	x	x		
Stomatal conductance	x	x		
Phenology				
Leaf senescence	x			
Budset, budbreak		x		
Frost / Freezing injury	x	x		
Leaf litter				
Nitrogen resorption efficiency (NRE)	x		x	x
N and C concentration	x		x	x
Concentrations of other nutrients and metals			x	x
Concentrations of phenolics and lignin			x	x
Mass loss			x	x
Palatability			x	x
Soil analyses				
N and C concentration	x	x	x	x
Concentrations of other nutrients and metals			x	x
pH	x		x	x

[a] EA = European aspen (*Populus tremula*); HA = hybrid aspen (*P. tremula* × *P. tremuloides*); HA × EA = backcross hybrid aspen [(*P. tremula* × *P. tremuloides*) × *P. tremula*]

as hybrid aspen × aspen in the text) were produced by crossing F_1 hybrid aspen females with European aspen males. The F_1 hybrid aspens were crosses between southern Finnish *P. tremula* and southern Canadian *P. tremuloides*. All seedlings were 1–1.5 months old at the start of the experiment.

The potted seedlings were grown under three water and two N treatments, using a full factorial design – i.e. six treatments – for seven weeks. Each treatment consisted of eight replicates, each with five seedlings per family. The three water levels, low (LW), medium (MW), and high (HW), were chosen to simulate the amount of precipitation during the growing season in southern Finland at present (320 mm; MW), a 50% reduction (160 mm i.e. drought; LW) and a 50% increase (480 mm; HW). The two N levels were chosen to provide the seedlings with 20 kg N ha^{-1} (low N; LN) and 120 kg N ha^{-1} (high N; HN) during the treatment period. The LN dose corresponds to the average annual N deposition in central Europe and is about twice the N deposition in southern Finland (Lorenz et al. 2008), whereas the HN dose is similar to a normal nursery N fertilization level (Juntunen and Rikala 2001). The mean photosynthetic photon flux density in the greenhouse was 150 µmol m^{-2} s^{-1}, provided by artificial lighting. The position of the seedlings was changed three times during the treatment period.

After five weeks of water and N applications, the environmental conditions in the greenhouse were changed to simulate a frost hardening period by gradually decreasing day length and temperature. During the frost hardening period, one replicate from each water and N treatment was freeze-tested each week over a period of seven weeks. The freeze-tests were carried out in a dark, air-cooled chamber by gradually decreasing the temperature to −10 °C and back during 24 h. The first seedling group was freeze-tested after a hardening period of 15 days and the last one after 64 days. At the beginning of April 2007, after the freeze-tests and storage in a dark room (mean temperature 2 °C), the seedlings were allowed to deharden in a greenhouse without controlled lighting or heating.

3.3. Aspen in a roadside environment (III)

Two hybrid aspen plantations near the city of Hyvinkää, southern Finland, were used to compare 13 hybrid aspen clones and one European aspen clone growing in a roadside environment and a rural field site. Site “M” was located adjacent to the four-lane E12 motorway (60°35’N, 24°48’E), which has a daily traffic volume of 26 500 vehicles and a speed limit of 120 km h^{-1}. The average annual NO_x concentration on this section of motorway was 42 µg m^{-3} at 50 m from the road edge (K. Lovén, personal communication). This measure includes a background concentration of 6 µg m^{-3}. A rural background site “R” was located in a rural area 11 km west of the motorway (60°36’N, 24°36’E), under no influence of major roads or other sources of pollution. The soil type on site R was silt/loam and site M was gravelly sandy clay.

The hybrid aspen clones were crosses between *P. tremula* from southern Finland and *P. tremuloides*, originally from southern Canada. The European aspen clone was of southern Finnish origin. The trees were produced by micropropagation as described in Yu et al. (2001a) and clone plantations were established with one-year-old plants. At site M, three trees per clone were sampled at 15 m and 30 m from the motorway edge, where elevated levels of gaseous and particulate pollutants would still be expected (Glasius et al. 1999, Bignal et al. 2007, Gadsdon et al. 2009). Six trees per clone were sampled at site R. The trees were eight (site M) or nine (site R) years old when leaf sampling and growth measurements were conducted in July–October 2007. Since some of the trees at each site were flowering in the spring of 2007, the tree populations as a whole were considered to be mature, where mature is defined as the capacity to reproduce.

3.4. Aspen in an urban environment (IV)

In order to compare mature European aspen growing in urban forests to those in rural forests, a field study was conducted over three years (2006–2008) in the Helsinki Metropolitan Area on the southern coast of Finland. Six forest sites – three in each category – were selected to represent either an urban or a rural land-use type based on their distance from the urban core. Urban sites were located 3–7 km and rural sites 24–30 km away from Helsinki city center (60°10’N, 24°57’E). The urban environment had on average 0.9 °C higher air temperature, four times greater NO_x concentration, 2.5 times greater SO_2 concentration, and double the AOT40 value, compared to the rural environment. No difference was detected in total rainfall between the land-use types. All field sites represented mesic *Myrtillus* type forests (Cajander 1949) with Norway spruce (*Picea abies* (L.) Karst.) and Scots pine (*Pinus sylvestris* L.) as dominant tree species. European aspen was distributed as scattered individuals or as small clusters at all sites.

At each field site, 30 trees were selected for leaf sampling (with the exception of one urban site, where only 23 trees were sampled), avoiding individuals growing close to paths. Leaf sampling took place in late August and early September in 2006–2008. The diameter at breast height (DBH) of the trees ranged from 6 to 27 cm, thus excluding very young or old individuals, since DBH can be used as an indirect indicator of tree age in managed forests (Latva-Karjanmaa et al. 2007). Most of the trees were observed to flower in the spring of 2007. As the flowering trees were represented across the range of DBH measured, the populations as a whole were considered to be mature.

The trees were genotyped using a microsatellite method according to Koivuranta et al. (2008). The genotyping showed that most aspen clones (77%) were represented by one tree individual (ramet). Consequently, the clone sizes, indicating the number of ramets per clone, were small (on average 1.7). Therefore, it was not possible to statistically test how clones differed in the variables measured, or in their response to temporal variation in environmental conditions. Instead, to quantify the range of intraspecific variation present in the populations studied, the coefficient of variation (CV: standard deviation/mean) was determined for each leaf trait per study site. The average values of each clone were used in calculating the CVs.

3.5. Litter decomposition and feeding experiments (III, IV)

Abscised hybrid aspen (III) and European aspen (IV) leaf litter was collected in the first week of October 2007 from each study site and used in litter decomposition experiments using the litterbag technique. In study III, litter collected from both field sites was incubated at site M and site R. In study IV, litter collected from each of the six field sites was incubated at one urban and one rural field site. Litterbags were placed under the litter layer (III) or into the humus layer (IV) in early October 2007. The litterbags were sampled twice, in early May (after 7 months) and early November 2008 (after 13 months). At all decomposition sites, 10 litterbags per collection site were collected at both sampling dates.

Aspen leaf litter was also used in laboratory experiments, where litter was fed to the land snail *Arianta arbustorum* (L.). Land snails form a part of the macrofaunal decomposer community in the upper soil compartment, and in boreal forests, European aspen leaf litter provides an important food and calcium source for them (Valovirta 1968). In the feeding experiments, litter from each study site was placed on a petri dish and three snails per dish were left to feed for 90 h. In study III, each petri dish contained one litter leaf from each of the two sites (unpublished results, for a more detailed method description, see IV). In study IV, the palatability of leaf litter was determined relative to green aspen leaves collected from a reference forest, and one litter leaf and one green leaf were placed on each dish. Based on the consumption by snails, a palatability index was calculated for each litter type (III, IV).

In addition, leaf litter was used to determine the nitrogen resorption efficiency (NRE) of trees in studies I, III and IV. NRE was calculated using the N concentrations of green leaves (N_g) and senescent leaves (N_s) as follows:
$\%NRE = (N_g - N_s)/ N_g \times 100$

3.6. Statistical analyses

With regard to most of the tree-related variables, factorial ANOVA or ANCOVA was used to test the main effects and interactions of abiotic factors (I: O_3; II: water, N; III: distance from motorway; IV: land-use type), species (I-II), clone/family (I-III), time since the start of frost hardening (II: only for freezing injury), and study year (IV). The factors were used as fixed factors, except for family (II) and clone (III), which were used as random factors. To eliminate the possible effects of tree age on the variables measured, growth rate was used as a covariate in the analyses of gas exchange, bud phenology and freezing injury in study II, and DBH as a covariate in the analyses of leaf traits in studies III and IV. Repeated measured ANOVA was employed in the case of time-repeated measurements (I).

The effects of O_3 (I) and distance from motorway (III) were also tested separately for individual clones with Student's t-test (I) and one-way ANOVA/ANCOVA (III). Student's t-test was used to test for differences in soil properties (III-IV); climate and air quality (IV); green leaf elements other than N and C (III); CV, NRE, and chemical composition of initial litter (IV); and leaf litter palatability (III-IV); between the study sites (III) and land-use types (IV). Factorial ANOVA was used to test the main effects and interactions of decomposition site, litter origin and incubation time on litter mass loss and N content (III-IV). Pearson or Spearman's correlation tests were employed to examine the relationships between different variables (I-IV). The data were log- or arcsin-square-root-transformed when necessary to obtain a normal distribution. The results were considered significant at $P < 0.05$. All statistical analyses were performed with SPSS 15.0 for Windows.

4. RESULTS AND DISCUSSION

4.1. Xeromorphic leaves in roadside and urban environments

Elevated O_3 alone did not affect the morphological leaf traits studied of young aspen trees in experimental study I. However, under field conditions mature trees of both European aspen and hybrid aspen acclimatized to roadside and urban environments by producing leaves with higher amounts of epicuticular waxes (III, IV). In addition, hybrid aspen formed leaves with lower SLA in the roadside habitat (III). These morphological changes are indications of xeromorphism, which is considered to be an adaptation to stress conditions, particularly to drought (Bussotti 2008). The increased leaf xeromorphism may have been induced by changes in microclimatic conditions and soil properties, typical of habitats close to road edges (Forman et al. 2003, Delgado et al. 2007) and in fragmented urban forests (Murcia 1995). These environmental alterations include higher solar radiation, temperature and wind action, as well as lowered soil moisture, which can promote water stress (Chen et al. 1993, Matlack and Litvaitis 1999, Forman et al. 2003, Delgado et al. 2007). The xeromorphic adaptations could also be related to the findings of Viskari et al. (2000a), who observed that exposure to exhaust gases caused a loss of stomatal control in *P. abies*, which can increase tree susceptibility to drought.

In the 3-year field study (IV), interannual variation in climatic factors was manifested as variation in these leaf traits between study years, which further indicates the ability of aspen for phenotypic plasticity. For example, the wax quantity was highest in the warmest and driest growing season with the highest O_3 indices, and lowest in the wettest one. This supports the view that epicuticular waxes may serve as an adaptation against natural and O_3-related water stress (Shepherd and Griffiths 2006, Manninen et al. 2009).

Trees in study I were grown with optimal water and N supplies, which may counteract potential O_3-related (moisture) stress (Manninen et al. 2009), rendering xeromorphic adaptations unnecessary. Differences in growth conditions between different studies may also partly explain why our results (I) contrast with some previous reported increases in xeromorphic leaf traits under O_3 exposure (Karnosky et al. 2002, Ribas et al. 2005, Manninen et al. 2009), being consistent with others that observed no effect of O_3 (Paoletti et al. 2007b).

Epicuticular waxes, particularly their structure and composition, have been employed as indicators of air pollution mainly in conifers (Cape and Percy 1998, Viskari 2000), but less often in deciduous species. SLA have often been used as a bioindicator of environment quality, especially of urban air quality (Carreras et al. 1996, Gratani et al. 2000, Balasooriya et al. 2009, Kardel et al. 2010), but in contrast to the results in study III, these studies reported increases in SLA at more polluted sites, and sites exposed to high traffic volume. The results of studies III and IV suggest that the amount of epicuticular waxes could also be considered as a non-specific indicator of habitat quality, and that for aspen it may be a more suitable indicator than SLA.

4.2. Changes in leaf N concentration

In experimental study II, seedlings responded to the increase in soil N supply with enhanced leaf N concentration, a response typical of *Populus* (Cooke et al. 2005, Häikiö et al. 2007). In addition to soil-derived N via roots, trees can carry out direct foliar uptake of N from the atmosphere (Schmutz et al. 1995). In the field study on urban and rural forests (IV), urban aspens had a higher leaf N concentration and a lower C:N ratio than the rural ones. This partly relates to the higher N concentration of humus layer at urban sites, implying a higher soil N supply (see II). The higher N levels in urban trees and humus can mainly be attributed to the higher NO_x concentrations, which were four times that of the air in the rural area. This suggests a higher N deposition in the urban environment (e.g. Lovett et al. 2000). Tissue N concentrations have been shown to increase with atmospheric inputs of N, and therefore high foliar as well as soil N concentrations and low C:N ratios have often been used as indicators of elevated N deposition (Fenn et al. 1996, Manninen and Huttunen 2000, Pitcairn et al. 2001, Thimonier et al. 2010).

In study III, the leaf N concentration of trees at the motorway site was similar to that in urban trees in study IV. However, although leaf N concentration appeared to reflect N deposition on a larger scale (IV), in study III it did not increase with increasing proximity to the motorway, a major source of N-containing pollutants. Indeed, at the motorway site, leaf N concentration did not differ between trees grown at 15 m and 30 m from the motorway. Viskari et al. (2000b) related the lack of changes in N metabolism observed in *P. abies* grown next to a motorway with a similar traffic density to that used here, to a relatively low average NO_x concentration. Some authors suggest that other N-related variables, such as $\delta^{15}N$ in plants, are better indicators of traffic-related N deposition than total tissue N concentration (Pearson et al. 2000, Marsh et al. 2004). This could also be the case here, but needs further analyses. However, the higher leaf N:P ratio at the motorway site could be a sign of increased N input, as high tissue N:P ratio is often used as an indicator of nutrient imbalance caused by elevated N deposition (Franzaring et al. 2010). In study III, higher leaf and soil N concentrations were observed at the rural site than at the motorway site. The source for this, however, is unlikely to be atmospheric N, because of the absence of nearby pollutant sources. It could be accounted for by differences between the two sites in previous land use practices, such as fertilization. This idea is supported by the higher Cd levels in leaves at the rural site (III), Cd being a trace element found in phosphate fertilizers (Grant and Sheppard, 2008).

In the case of fertilized young aspen, exposure to elevated O_3 decreased leaf N concentration and increased C:N ratio (I), which has also been reported by other authors (Lindroth et al. 2001a, Yamaji et al. 2003, Kopper and Lindroth 2003). This has been linked to O_3-induced activation of senescence-related processes, such as degradation of chlorophyll, Rubisco and other proteins (Ribas et al. 2005).

4.3. O_3 induced leaf injuries but improved tolerance against rust disease

In recent meta-analyses, *Populus* species were shown to be one of the most sensitive tree genera to current and elevated O_3 concentrations in terms of photosynthetic gas exchange, growth and biomass production (Wittig et al. 2007, 2009). In study I, the sensitivity of aspen to O_3 was manifested as O_3-specific leaf injury, seen as bifacial necrotic lesions, on all clones by the end of the experiment. The emergence of visible injuries may have been promoted by the regular irrigation and N fertilization that the trees received. These practices were likely to favor stomatal opening, as shown in study II for N, and thus increase O_3 flux (Schaub et al. 2003). Two of the clones even showed visible O_3 injuries under ambient air (I), which is rare in the O_3 climate prevailing in Fennoscandia (Timonen et al. 2004). This suggests that young aspen may also be adversely affected by current O_3 concentrations under conditions that promote O_3 flux into the leaves, such as in nurseries, where irrigation and N fertilization are common practices.

By the end of experiment I, all aspen clones were naturally infected by *Melampsora* sp. rust fungus. These fungi are among the most widespread and damaging diseases of *Populus*, capable of causing heavy defoliation (Christersson et al. 1992). O_3-exposed trees were found to be less sensitive to the infection than the control ones – the rust was twice as abundant under ambient air. This is in contrast with some previous observations on O_3-exposed *Populus* (Beare et al. 1999, Karnosky et al. 2002). The increased rust tolerance did not seem to be explained by the amount or chemistry of epicuticular waxes (I). It may be linked to O_3-induced general defense reactions against oxidative stress (Sandermann et al. 1998).

4.4. Growth of young aspen under variable water and N supplies and elevated O_3

The effects of water and N on tree growth are well studied in commercially important species – which in the genus *Populus* include trembling aspen and various *Populus* hybrids – whereas limited research exists on European aspen. The results of study II on aspen growth conform to previous observations: Increased N supply stimulated photosynthetic gas exchange and growth of both European aspen and hybrid aspen × aspen, responses that are typical of pioneer trees, and have frequently been reported for *Populus* species (Ibrahim et al. 1997, Coleman et al. 1998, Häikiö et al. 2007). Cooke et al. (2005) showed with young hybrid poplar that these responses can be seen after even relatively short N treatment periods (14–28 d). Although pioneer species are often adapted to variable soil water conditions, drought and wet conditions limited aspen growth (II), in accordance with results reported by others (e.g. Ibrahim et al. 1997, Guidi and Labrecque 2010).

Despite visible O_3 injuries in leaves, no negative effects of elevated O_3 on growth and biomass production were observed in study I, which may partly be related to the relatively short exposure time of only one growing season (Oksanen 2003, Karnosky et al. 2005). Instead, the height growth of one European aspen clone was slightly stimulated by O_3 exposure, a response previously observed in *Populus* and *Betula* under relatively low concentrations of O_3 (Karnosky et al. 1996, Oksanen et al. 2001, Yamaji et al. 2003). However, previous studies have shown that under prolonged O_3 exposure, the initial growth enhancement may disappear (Pääkkönen et al. 1993), and be followed by cumulatively impaired growth (Oksanen 2003). One factor related to the lack of negative growth responses to elevated O_3 may be the growth-stimulating effect of the regular N fertilization (see II) that the trees received. Soil N amendment has been shown to compensate for adverse growth effects of O_3 in European aspen and hybrid aspen in a study by Häikiö et al. (2007), in which the N fertilization level was similar to that in study I. In any case, exposure to O_3 tended to cause a shift in biomass allocation towards the above-ground part of the plant. Similar responses are common in tree species with an indeterminate growth pattern (Dickson et al. 1998, Oksanen et al. 2001, Yamaji et al. 2003), such as aspen. The impaired root/shoot balance may negatively affect growth in the long term, if the trees were exposed to elevated O_3 for several growing seasons, for example by weakening below-ground competition and predisposing trees to drought stress, winter damage and parasites (Oksanen et al. 2001).

Plant phenological events, such as budbreak, leaf senescence, and budset, are important determinants of growth as they are involved in determining the length of the vegetative period. The timing of budset is also in close connection to the development of frost hardiness, which starts around the time of growth cessation (Hurme et al. 1997). In study II, enhanced N supply lengthened the vegetative period by promoting budburst and delaying budset, which can increase the annual growth increment. However, high N supply – especially when combined with drought during the growing season – also postponed the development of frost hardiness. The delayed formation of terminal buds suggests this to be at least partly mediated by an N-related prolongation of the vegetative period. These results suggest that high N inputs, such as doses used in nursery fertilization, may predispose aspen to damage by both autumn and spring frosts, which in turn can adversely affect subsequent growth (Silfver et al. 2008). However, the negative effect of high N supply on frost hardening was evident mainly at the beginning of the frost hardening period, implying that if the hardening period is long enough, N supply does not notably affect frost hardiness later in the autumn / winter. This is in accordance with some previous studies on conifers (Jalkanen et al. 1998, Puértolas et al. 2005). Exposure to elevated O_3, in turn, shortened the vegetative period by accelerating leaf senescence (I), as previously observed in *P. tremuloides* (Karnosky et al. 2005). However, elevated O_3 did not affect the amount of frost injury (I), which is compatible with the inconsistent results reported on O_3 effects on frost sensitivity of trees (Skärby et al. 1998).

While these results have significance for example to seedlings growing in nurseries receiving regular N fertilization, further studies would be necessary to validate the results in mature trees in nature. Previously, the results from studies on frost hardening of conifer seedlings using a similar freeze-test technique to study II, have correlated fairly well with survival test results from field experiments on mature trees (Andersson 1992). In the case of exposure to O_3, the response of mature trees to O_3 stress may differ from that of seedlings (Samuelson and Kelly 2001).

4.5. Growth of mature aspen in the roadside environment

In study III, mature hybrid aspen grew more slowly in the roadside environment, but growth reductions were only observed in some clones at a distance of 15 m from the motorway. Although the element analyses of green leaves suggested higher metal deposition at the motorway than at the rural site (III), the metal concentrations were generally well below those reported as toxic for plants (Kabata-Pendias 2001), as well as at the lower end of the range observed in other roadside environments (Cuny et al. 2001, Bakirdere and Yaman 2008, Tomašević et al. 2008, Peachey et al. 2009). Therefore it seems unlikely that metal deposition alone is responsible for the reductions in growth. Microclimatic and soil alterations caused by road edge effect, as discussed in section 4.1., could have contributed to these growth responses. However, the growth reductions at 15 m can be considered as minor, as the height growth rate at all three test locations fell within the range reported for hybrid aspen growing on land previously used for agriculture (Hynynen 1999, Tullus et al. 2009). Taken together, the results thus support previous studies suggesting *Populus* species as suitable for plantations in moderately polluted areas

(Laureysens et al. 2004, Hermle et al. 2006, Hassinen et al. 2009), such as alongside roads carrying heavy traffic.

4.6. European aspen is more sensitive to stress than hybrid aspen

Hybrid aspen was superior to native European aspen in terms of growth and biomass production, even though no difference between species was found in the photosynthesis of young trees (I). Faster growth of hybrid aspen was also observed in older, field-grown trees (III), although it must be noted that only one European aspen clone was studied here. However, hybrid aspen was observed to have a lower root/shoot ratio in the pot experiment (I), which may make it more vulnerable, for example to drought, if expressed in mature trees in nature, under competition for water and nutrients.

With regard to the abiotic stress factors studied, European aspen was more sensitive to elevated O_3 than hybrid aspen (I), in terms of both visible foliar O_3 injuries and O_3-induced decreases in leaf N concentration and root/shoot ratio. Furthermore, with respect to biotic stress factors, European aspen was about twice as sensitive to the *Melampsora* rust infection than hybrid aspen (I). The *P. tremuloides* material may confer better resistance to O_3 upon hybrid aspen, as well as higher resistance to rust fungus, relative to the native European aspen. It has been suggested that in North America, where O_3 concentrations are higher than in Finland, the prevailing O_3 climate has already resulted in natural selection for O_3 tolerance in *P. tremuloides* populations (Berrang et al. 1989). Previous studies such as by Gallo et al. (1985) have also shown *P. tremuloides* to be less sensitive to *Melampsora magnusiana* Wagner than *P. tremula*.

In study II comparing European aspen and backcross hybrid aspen, European aspen grew faster during their first growing season under greenhouse conditions. However, hybrid aspen × aspen tended to experience earlier budburst, which may allow more growth than in European aspen during subsequent growing seasons. This would agree well with observations on mature F_1 hybrid aspen: the faster growth of F_1 hybrid aspen compared with European aspen has been attributed particularly to its longer vegetative period (Yu et al. 2001b). With respect to abiotic stress, hybrid aspen × aspen was more tolerant of frost and developed frost hardiness faster than the native European aspen (II). This response was rather unexpected, as the parent material of *P. tremuloides* used in the experiment originated from more southern locations (45–55° N) than that of *P. tremula* (60° N), and southern ecotypes generally develop frost hardiness later than northern ones when grown in the same environment (Hurme et al. 1997). On the other hand, the climate in the area of *P. tremuloides* origin – southern continental Canada – consists of lower daily mean and average minimum winter temperatures than in the more maritime southern Finland. This difference may have contributed to tolerance to frost in hybrid aspen × aspen. The combined effect of high N supply and drought in delaying the development of frost hardiness (see section 4.4.) was more pronounced in European aspen. It thus appears that in environments with high N supply and periods of drought, a situation likely in forthcoming decades (Lamarque et al. 2005, Jylhä et al. 2009), the difference in tolerance to autumn frosts between the aspen species studied may become more pronounced. Further studies would be needed in order to see if these results also apply to mature trees.

4.7. Intraspecific variation in response to environmental changes

Populus species are known for their considerable genetic variation in growth and leaf traits (Lindroth and Hwang 1996, Ceulemans and Deraedt 1999, Lindroth et al. 2001b, Yu 2001, Yu et al. 2001b, Marron et al. 2005). The results of studies I and III with clonal plant material support these findings. In contrast, in study II with aspen families grown from seeds, significant variation among families was only recorded in the frost hardiness of backcross hybrid aspen. When compared with clones, the higher heterogeneity within seed-originating families – which consist of slightly genotypically different individuals – may mask part of the among-family variation. Additionally, intraspecific differences possibly become more evident as trees age (Yu et al. 2001b).

In contrast to some previous *Populus* studies (e.g. Lindroth et al. 2001b, Yu and Pulkkinen 2003, Häikiö et al. 2007, Marron et al. 2007), the aspen clones and families studied here responded rather similarly to the different abiotic factors, as generally no significant clone/family × environment interactions were recorded (I-III). Yet, the clone-based analyses (I, III) suggest some differences between clones in their response to O_3 (I) and motorway proximity (III). More sensitive clones to O_3 and roadside environment could be separated according to visible O_3-injuries (I) and growth response (III). The more O_3-sensitive clones within both aspen species manifested visible O_3-injuries even under ambient air treatment (I). The clone that was found least responsive to O_3 (clone 34) (I), was also one of the hybrid aspen clones that was not affected by the proximity to the motorway for any of the variables studied (III). Previously, this clone has been classified as sensitive or of intermediate sensitivity to O_3 based on growth response, whereas a clone that was one of the two most sensitive clones to the proximity to the motorway (clone 17) (III) has been classified as O_3-tolerant (Oksanen et al. 2001, Häikiö et al. 2007). Comparing the results from different studies indicates that the sensitivity of a certain clone to abiotic stress depends on the stress factor, making it difficult to predict how a particular genotype will perform under multiple stresses prevailing in nature.

With respect to the field study of mature European aspen growing in natural forest stands (IV), the range of variation (CV) in all leaf traits was found to be similar in both the urban and rural aspen populations. This implies that urbanization-related environmental change has not been sufficient to have functioned as a selective force on the European aspen studied here, or has not been operating for long enough. Alternatively, it can also imply that natural aspen populations can acclimatize and/or adapt to environmental changes, and consequently maintain the genetic variation underlying the phenotypic variation in a changing environment.

4.8. Ecosystem-level effects

4.8.1. Changes in the quality of leaf litter

In our studies nitrogen resorption efficiency (NRE) was unaffected by elevated O_3 (I), roadside (III) or urban (IV) environment, suggesting that aspen is able to maintain normal internal N cycling under various environmental conditions. It also implies that the N levels in leaf litter reflect that of green foliage. In study I, this occurred only partially: European aspen, but not hybrid aspen, tended to produce leaf litter with a lower N concentration and higher C:N ratio under elevated O_3. It therefore appears that O_3 effect on leaf chemical composition is greater in green leaves than in litter, as also found by Kasurinen et al. (2006). Neither did Lindroth et al. (2001a) find any influence of O_3 on the N resorption of *P. tremuloides*. However, Uddling et al. (2006) observed impaired N resorption in O_3-exposed *Betula pendula* Roth. Although changes in litter C:N ratio can possibly affect litter mass loss, so far the majority of field studies have found minor or no effects of elevated O_3, alone or in combination with elevated CO_2, on leaf litter decomposition rates (reviewed by Kasurinen et al. 2007).

Although traffic-related pollutants are major components of air pollution in urban areas, in our studies the effects of roadside (III) and urban (IV) environment on aspen leaf litter quality differed to some extent. In study IV, the higher atmospheric N deposition in urban areas appeared to be reflected not only in the green leaves of European aspen (see section 4.2.), but also in leaf litter. Urban litter had a higher N concentration (+27%) and lower C:N ratio, as well as lower concentrations of lignin and total phenolics than rural litter, although the difference was not statistically significant (IV). The higher soil nutrient supply in urban compared to rural forests (IV) may have decreased C partitioning to leaf phenolics (Osier and Lindroth 2001, Häikiö et al. 2009). However, in study III, the hybrid aspen leaf litter contained less N, P and K and had a higher C:N ratio and concentration of total phenolics at the motorway site than the corresponding rural litter. As discussed in section 4.2, this is more likely to be related to such factors as the fertilization histories of the study sites than to the proximity of the motorway. The concentrations of metals Al, Fe, Ni and Zn were 60–200% higher in both motorway and urban litter compared with the rural litter types, reflecting the higher metal deposition close to roads, also demonstrated in green leaves (III), and the greater amount of other pollutant sources present in urban compared with rural areas.

4.8.2. Urbanization accelerates leaf litter decomposition

Two separate litterbag experiments were used to investigate the early decomposition of aspen leaf litter in roadside (III) and urban (IV) environments. Litter origin (motorway, urban or rural), which relates to litter quality (see section 4.8.1.), and environmental conditions at the decomposition site were both found to influence litter mass loss in these studies. Of these factors, litter quality had a more consistent (III) or more significant (IV) effect on litter decay.

The urban environment promoted litter decay throughout the 13-month incubation period both through the effect of litter quality and site conditions: Urban litter decomposed faster than rural litter, and decomposition was faster at the urban site (IV). In contrast, litter decay was retarded in the roadside environment, but only initially (III). Motorway litter decomposed slightly more slowly than rural litter during the whole incubation period, but the effect of decomposition site on litter decay was evident only during the first seven months, when decomposition proceeded slower at the motorway site.

A low litter C:N ratio and high concentrations of N and P are often associated with faster litter decomposition, especially in the early stages of decay, whereas lignin and other phenolics are known to retard decomposition (Melillo et al. 1982, Horner et al. 1988, Berg 2000, Hättenschwiler and Vitousek 2000). Differences in these chemical attributes between urban, motorway and rural litter (see section 4.8.1.) can account for the faster decay of urban litter and the slower decay of motorway litter. Our results contrast with some previous studies comparing the decomposability of urban and rural leaf litter (Cotrufo et al.

1995, Carreiro et al. 1999, Pouyat and Carreiro 2003), which were, however, conducted on more xeromorphic *Quercus* species.

The higher humus N concentration in urban compared to rural forests (IV) could be among factors that promoted litter decay at the urban site. Conversely, the lower concentrations of N, C and organic matter in soil at the motorway than rural site (III) could have contributed to the slower decay at the motorway site. Findings on the effects of elevated N deposition or soil N on decay rate have been controversial, ranging from stimulating to no or retarding effects (Koopmans et al. 1998, Magill and Aber 1998, Kuperman 1999, Prescott et al. 1999, Berg 2000). However, the review by Fog (1988) concludes that in the case of easily degradable material, such as deciduous leaf litter, N additions often promote early decomposition. Cotrufo et al. (1995), in turn, report positive associations between soil C and N concentration, organic matter and microbial biomass.

One decay-promoting factor could be the higher temperature (0.7–1.2 °C) at the urban decomposition site (IV), probably caused by the urban heat island effect (McDonnell et al. 1997). Previously, Pouyat et al. (1997) identified soil temperature as one of the major drivers for faster leaf litter decomposition in an urban compared with a rural environment. In the urban area, dust deposition (Lovett et al. 2000) may have contributed to the higher concentrations of base cations observed in the urban litter and humus layer, as well as the slightly higher pH of humus (IV). This is also likely to have promoted litter decay in the urban environment (Swift et al. 1979).

In contrast to many studies (Berg et al. 1991, Cotrufo et al. 1995, Post and Beeby 1996, Johnson and Hale 2004), the higher total concentrations of metals observed in the urban humus layer and in urban litter (IV) appeared not to inhibit decomposition; or at least they did not override the effects of factors that promoted litter decay. The metal concentrations in motorway litter (III) were similar to or lower than those in urban litter (IV), suggesting that they may not have been high enough to inhibit litter decay. Scheid et al. (2009) observed that metal concentrations of *P. tremula* leaf litter even higher than those recorded in studies III and IV, did not influence litter decomposition. These authors explained the lack of an observable effect on decay by the low bioavailability of metals in litter. Furthermore, metal concentrations in humus and soil at all decomposition sites (III, IV) did not exceed concentrations previously reported to negatively affect soil microflora (Smith 1990).

Berg and Staaf (1981) identified three phases in the evolution of the total N content of decomposing litter: an initial leaching phase of N, a net accumulation period – mainly due to microbial immobilization of N – and a final net release of N. In our studies, all litter types first accumulated N. In study IV, N accumulation continued during the whole observation period, but in study III, some net N release occurred after 13 months of decomposition. In both studies, litter quality affected the rate of N accumulation or release. As decomposers have to meet their N requirements, the period of net N immobilization is typically longer and N release slower for litter material with lower initial N concentration (Parton et al. 2007). This probably explains the slower N release from the motorway than rural litter (III), and relatively greater N accumulation in rural than urban litter (IV), as these litter types were initially lower in N. In study III, however, N dynamics of litter in the roadside environment differed from those in the rural environment only at the beginning of the decomposition process, following the pattern of litter mass loss. Thus, the retardation of decomposition and N release in the roadside environment appeared to be only initial and temporary. However, considering the possible differences between the two sites in former land-use, it is not obvious if the results reflect only the effect of proximity to the motorway.

In any case, the results, together with minor effects on tree growth (see section 4.5), imply that roadside ecosystems under the conditions in this study may not considerably differ from surrounding rural areas.

With regard to consumption by a macrofaunal decomposer, the litter originating from the motorway and rural sites (III) were similar in terms of their palatability to the land-snail *A. arbustorum* (unpublished results), further implying similarity between the study sites. However, although no statistically significant difference was recorded, the litter produced in urban forests was twice as palatable to *A. arbustorum* as the litter originating from rural forests (IV). Higher palatability to macrofaunal decomposers was positively correlated with faster litter mass loss (IV), in accordance with the findings of others, for example Cotrufo et al. (1998). This can also be related to the differences in litter quality between urban and rural sites, as similar chemical characteristics promote both microbial litter decomposition and consumption by herbivores and macrofaunal decomposers. Environment-mediated changes in green leaves and litter thus appeared to be transmitted to other trophic levels as well.

5. MAIN FINDINGS AND CONCLUSIONS

The results illustrate that aspen may acclimatize relatively easily to environmental changes by means of its leaves, indicating the ability for phenotypic plasticity. The field studies showed that mature aspen acclimatized to the urban environment, including the roadside habitat, by producing more xeromorphic leaves. Phenotypic plasticity was further indicated by the variation in leaf morphology in response to interannual climatic variation.

With regard to the experiments on young trees, aspen appeared to be sensitive to elevated O_3 in terms of visible leaf injuries, and even to ambient O_3 levels under conditions that promoted O_3 flux into leaves. Although minor effects on growth were observed after one season's exposure to elevated O_3, the reduced biomass allocation to roots and accelerated leaf senescence suggest that growth can be negatively affected in the long term. The responses to O_3 were likely modified by water and N availability. These factors were also shown to affect frost hardening, with high N supply – especially when combined with drought – postponing the development of frost hardiness of young aspen. Delayed frost hardening may predispose trees to early autumn frosts. In the case of mature aspen (8–9 years old), the growth of some of the hybrid aspen clones was retarded close to the motorway. The growth reductions were, however, considered to be minor when compared with growth rates reported at similar sites.

Compared with hybrid aspen, including both F_1 hybrid aspen and backcrosses between hybrid aspen and European aspen, the native aspen appeared more sensitive to stresses, including elevated O_3 and infection from *Melampsora* rust fungus – a pathogen that can cause severe defoliation on *Populus*. Because of its slower frost hardening rate, European aspen can also be more susceptible to early autumn frosts. These results further support the use of hybrid aspen in commercial plantations.

Intraspecific variation was found in several of the traits evaluated in this study, including leaf morphology and growth rate, although differences between aspen clones or families in response to abiotic factors were generally small. However, some differences between clones were found in sensitivity to O_3 and the roadside environment. The phenotypic plasticity combined with intraspecific variation, if also present in natural population, imply that aspen has potential for withstanding environmental changes. This is further supported by the similar range of phenotypic variation in leaf traits found in urban and rural European aspen populations.

The decomposition of aspen leaf litter was not consistently altered in the roadside environment, suggesting minor effects on C and nutrient cycling. By contrast, litter decomposition was found to be faster in the urban than the rural environment throughout the study. Urban litter was also more palatable to a general herbivore. The higher quality of urban litter, as well as higher temperature, N deposition and humus pH in the urban site were likely to have promoted litter decay, and seem to have overridden the potential negative effects of higher litter and humus metal concentrations. The results thus indicate that the multiple environmental changes taking place in urban areas – which correspond closely with the main drivers of global change – can affect ecosystem functioning by promoting litter decomposition, mediated partly by leaf litter quality.

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