YAODAN ZHANG

Future climate change and soil contamination may affect plant performance. Plants of different origins and different sexes may respond differently to the combined stress. This thesis provides knowledge about the interaction between climate change and soil contamination and its effects on growth and defensive phenolics in two boreal forest tree species, Picea abies L. Karsten and Populus tremula L. This knowledge may be useful for predicting and evaluating the environmental risk of soil contamination to boreal ecosystems under future climate conditions.
GROWTH AND PHENOLICS OF TWO BOREAL FOREST TREE SPECIES

EFFECTS OF CLIMATE CHANGE AND SOIL CONTAMINATION ON *PICEA ABIES* L. KARSTEN AND *POPULUS TREMULA* L.
Yaodan Zhang

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EFFECTS OF CLIMATE CHANGE AND SOIL CONTAMINATION ON PICEA ABIES L. KARSTEN AND POPULUS TREMULA L.

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**ABSTRACT**

High northern latitudes are climatic sensitive areas. Chemical contaminants are easy to transport and accumulate in these areas, causing potential risk to plants. However, the combined effects of climate change and chemical contaminants on plant performance are still not well understood, especially for boreal tree species. In this thesis, I studied the effects of soil pyrene contamination (50 mg kg\(^{-1}\)) on growth and phenolics in Norway spruce (*Picea abies*) from five different origins and European aspen (*Populus tremula*) of both sexes under elevated temperature and CO\(_2\) concentration. In addition, I investigated the effects of TiO\(_2\) nanoparticles (nTiO\(_2\), 50 and 300 mg kg\(^{-1}\)) on growth and phenolics in both sexes of European aspen under elevated temperature and CO\(_2\). The studies were conducted in climate-controlled greenhouses, where the elevated temperature was set to achieve a mean increase of +2 °C and the raised CO\(_2\) concentration was set at 720 ppm, with ambient temperature and CO\(_2\) concentration of 360 ppm as the references.

In the evergreen *P. abies*, pyrene significantly decreased height growth, needle biomass, stem biomass, and the concentration of total phenolics in needles and stems compared to control plants. Elevated temperature alone did not affect plant growth but led to lower concentration of total phenolics in needles and stems in both control and pyrene-spiked soil treatments. Elevated CO\(_2\) led to higher concentration of stem phenolics compared to ambient treatments. The decrease in height growth and phenolic concentration caused by pyrene was greater under elevated temperature, while elevated CO\(_2\) only marginally modified the response. Seedlings from different origins showed different responses to the combined environmental stressors. The negative effects of combined stress were greater in seedlings from southern origins compared to those from northern origins.

For the sexually dimorphic deciduous *P. tremula*, males grew taller than females under ambient conditions. Elevated temperature increased the growth of both sexes, but females had greater growth increment than males. Elevated CO\(_2\) showed little effect on plant growth. Leaf phenolics decreased under elevated temperature, but
increased under elevated CO\textsubscript{2} in both sexes. Pyrene contamination decreased stem biomass and leaf area of both sexes under ambient climatic conditions, and the reduction in leaf area was more severe under elevated temperature (T), elevated CO\textsubscript{2} (CO\textsubscript{2}), and combined T + CO\textsubscript{2}. The negative effect of pyrene on leaf area was also greater in males than in females. Pyrene significantly increased the concentration of leaf total phenolics under elevated temperature and T + CO\textsubscript{2}. The residual pyrene in pyrene-spiked soils was higher under elevated CO\textsubscript{2} than under ambient, elevated temperature, and T + CO\textsubscript{2}. In nTiO\textsubscript{2} contaminated soils, nTiO\textsubscript{2} at 50 and 300 mg kg\textsuperscript{-1} did not affect growth of either sex of *P. tremula*. However, Ti accumulated in roots exposed to nTiO\textsubscript{2} and elevated temperature increased Ti uptake in the 300 mg kg\textsuperscript{-1} treatment. In all climate treatments, both concentrations of nTiO\textsubscript{2} increased the concentration of leaf phenolics in females, but did not affect and even tended to decrease it in males.

The two different tree species responded differently to soil contamination in combination with climate factors. Seedlings from different origins or of different sexes also showed different responses to the combined stress. In the longer run, the combined stress of climate change and soil contamination may cause changes in the competitive abilities, and thus lead to further species evolution of both boreal tree species.

*Universal Decimal Classification:* 504.7, 543.272.62, 547.681, 582.475, 582.681.82

*CAB Thesaurus:* carbon dioxide; climate change; growth; biomass; phenolic compounds; boreal forests; *Picea abies*; *Populus tremula*; polycyclic hydrocarbons; aromatic hydrocarbons; stress; contaminants; pollutants; sex differences; soil pollution; temperature; titanium dioxide; nanoparticles

*yleinen suomalainen asiasanasto:* hiilidioksidi; ilmastonmuutokset; kasvu; biomass; fenoliset yhdisteet; boreaalinen vyöhyke; haapa; PAH-yhdisteet; stressi; saasteet; maaperän saastuminen; sukupuolierot; lämpötila; titaanidioksidi; nanohiukkaset
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Joensuu, 21st December 2018
Yaodan Zhang
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>control, ambient conditions</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>carbon dioxide, elevated carbon dioxide</td>
</tr>
<tr>
<td>GVA</td>
<td>graphic vector analysis</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>ICP-OES</td>
<td>inductively coupled plasma-optical emission spectrometry</td>
</tr>
<tr>
<td>LA</td>
<td>leaf area</td>
</tr>
<tr>
<td>$m/z$</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>nTiO$_2$</td>
<td>titanium dioxide nanoparticles</td>
</tr>
<tr>
<td>PAHs</td>
<td>polycyclic aromatic hydrocarbons</td>
</tr>
<tr>
<td>PAL</td>
<td>phenylalanine ammonia-lyase</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>QTOF/MS</td>
<td>quadrupole time-of-flight mass spectrometer</td>
</tr>
<tr>
<td>RNS</td>
<td>reactive nitrogen species</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
<tr>
<td>SLA</td>
<td>specific leaf area</td>
</tr>
<tr>
<td>T</td>
<td>elevated temperature</td>
</tr>
<tr>
<td>T + CO$_2$</td>
<td>elevated both temperature and carbon dioxide</td>
</tr>
<tr>
<td>UHPLC-DAD</td>
<td>ultra-high-performance liquid chromatography with a diode array detector</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to by the Roman numerals I-III.


The above publications have been included at the end of this thesis with kind permission from their copyright holders: Springer (I) and Elsevier (II, III).
AUTHOR’S CONTRIBUTION

1) The author planned the experiments together with her main supervisors, prepared the contaminated soils, and participated in growth and biomass measurements and sampling in papers I, II, and III.

2) The author was responsible for the phenolic analyses in paper I, for the pyrene analyses in paper II, for the nTiO$_2$ analyses in paper III, and for the processing of secondary chemistry data in papers I, II, and III.

3) The author conducted statistical analyses and is the main author in all of the three papers (I, II, III).
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1 INTRODUCTION

1.1 CLIMATE CHANGE AND SOIL CONTAMINATION

The increased human activities caused by rapid population growth and economic development have resulted in environmental changes, including climate change and soil contamination. According to most climate scenarios, atmospheric CO$_2$ concentrations are predicted to rise to 430–1000 ppm by the year 2100 compared to pre-industrial levels of 280 ppm (IPCC, 2014). This will lead to a rise in global average air temperature of 1.5–4 °C by the end of the 21st century relative to the level in 1850–1900, and the arctic region will continue to warm more rapidly than the global mean (IPCC, 2014). Simultaneously with increases in atmospheric temperature and CO$_2$ concentrations, soil contaminated with polycyclic aromatic hydrocarbons (PAHs) and TiO$_2$ nanoparticles (nTiO$_2$) is becoming a global environmental problem. PAHs, a class of toxic persistent organic pollutants, are derived from natural activities (e.g., forest and grassland fires) and anthropogenic activities (e.g., incomplete combustion of fossil fuels and biofuels) (Haritash and Kaushik, 2009). They can be transported over long distances by atmospheric transport, enter soils by atmospheric deposition, and are likely to accumulate and remain long in soils due to their hydrophobic properties (Maliszewska-Kordybach et al., 2009; Kuśmierz et al., 2016). Forests are primarily responsible for increasing the atmospheric deposition of PAHs to the terrestrial environment, which reduces atmospheric concentrations at the expense of increased concentrations in the forest soils (Wania and Mcclachlan, 2001). Thus, forest soils are also known to be a storage reservoir of PAHs (Syed et al., 2017). PAHs have been detected in forest soils in the UK, Norway, Germany, and other European countries (Desaules et al., 2008; Nam et al., 2008; Holoubek et al., 2009; Aichner et al., 2013). In China, rapid industrialization and urbanization have also resulted in high PAH emissions with possible subsequent depositions to soils, including forest soils (Syed et al., 2017; Zhang and Chen, 2017). In addition, future climate change can lead to alterations in transport and re-volatilization of PAHs, which will affect concentrations of atmospheric PAHs (Friedman et al., 2014). Thus, PAHs concentration in soils may also be affected. TiO$_2$ nanoparticles are extensively used in industrial and commercial products (e.g., cements, asphalts, paints, sunscreens, cosmetics, and coating) because of their photocatalytic properties (Minetto et al., 2014). The widespread use of nTiO$_2$ will inevitably lead to its continuous release into the environment, especially into soil, which makes it a potential soil contaminant (Sun et al., 2017). Soil is one of the biggest sink for nTiO$_2$ (Simonin et al., 2015). TiO$_2$ nanoparticles may enter soil through biosolids originating from waste treatment or a spill during manufacturing production (Ge et al., 2011). Generally, nTiO$_2$ is insoluble in soil and its nanoparticulate form is also the main chemical form that has the potential to interact with plants (Gardea-Torresdey et al., 2014). With the constant accumulation of PAHs and nTiO$_2$ in soil and increasing temperature and CO$_2$
concentrations, plants will be exposed to combined stress. However, little is known about the combined effects of climate change and chemical contaminants on plant performance.

1.2 GROWTH AND PHENOLICS IN NORWAY SPRUCE AND EUROPEAN ASPEN

Norway spruce (*Picea abies* L. Karst.) and European aspen (*Populus tremula* L.) are two important species in boreal forests. *P. abies* is a slow-growing, abundant and economically important evergreen species in the Nordic countries, as well as in Central Europe and Russia (Jaakkola et al., 2006; Röder et al., 2010). It can be used as raw material for the pulp and paper industry and wood panel industry (Jaakkola et al., 2006). In addition, it can also act as host tree for arthropod species, such as beetles (Coleoptera), true bugs (Hemiptera), lacewings and snakeflies (Neuroptera), spiders and harvestmen (Arachnida), and bees and wasps (Hymenoptera) (Röder et al., 2010). *P. tremula* is a fast-growing, sexually dimorphic deciduous species with a wide distribution in Eurasian boreal and temperate ecosystems (Myking et al., 2011). The populations of *P. tremula* are male dominated in most of the distribution regions (Myking et al., 2011). *P. tremula* has great ecological value, as aspen trees host hundreds of herbivorous invertebrates (e.g., macrolepidoptera, beetles, and sawflies), saproxylic beetles, polypore fungi, epiphytic lichens, and also some vertebrates (e.g., woodpeckers and flying squirrels), which are at least partly dependent on the aspen (Kouki et al., 2004).

In addition to primary metabolism such as photosynthesis and respiration processes which are required for cell maintenance and proliferation, plants produce high amounts of secondary metabolites that are important for their adaptability to the complex environment (Kliebenstein, 2004; Cheynier et al., 2013). One of the most widely distributed and abundant secondary metabolite group in plants is phenolic compounds, with thousands of different structures identified (Dai and Mumper, 2010). Phenolics possess one or more aromatic rings with one or more hydroxyl groups (Figure 1), and play a vital role in plant ecology and plant physiology (Lattanzio et al., 2008; Dai and Mumper, 2010). Phenolic compounds are the major secondary metabolites in *P. abies* and *P. tremula*. *P. abies* tissues contain a wide range of phenolics including flavonoids, acetophenones, stilbenes, lignans, and phenolic acids (e.g., Virjamo et al., 2013). *P. tremula* accumulates high levels of salicylates, as well as flavonoids and phenolic acids (e.g., Randriamanana et al., 2014). Salicylates play an important role in protecting plants against generalist herbivores (e.g., Ruuhola et al., 2001; Boeckler et al., 2011). Many flavonoids, lignans, and phenolic acids have been proved to be good antioxidants that can scavenge or suppress the formation of reactive oxygen and/or nitrogen species (ROS/RNS) (Dai and Mumper, 2010). Acetophenones and stilbenes have antifungal properties and can also act as phytoalexins (Osswald and Benz, 1989; Ganthaler et al., 2017a; Ganthaler et al.,
2017b). Phenolics, as carbon-based secondary metabolites, are derived from primary metabolism: erythrose-4-phosphate produced by the pentose phosphate pathway and phosphoenolpyruvate produced by the glycolytic pathway synthesize phenylalanine through the shikimate pathway; the general phenylpropanoid metabolism that produces cinnamic acid derivatives and \( P \)-coumaroyl CoA, and different phenolic compounds (Figure 2) (Lattanzio et al., 2012; Cheynier et al., 2013). The first enzyme for the synthesis of shikimate-derived phenolic metabolites is phenylalanine ammonia-lyase (PAL), which deaminates the phenylalanine (Matsuki, 1996; McDonald et al., 1999). PAL activity is positively correlated with the supply of phenylalanine (Matsuki, 1996). Plant growth and phenolics are thought to compete for phenylalanine, as both protein synthesis and phenolic synthesis use phenylalanine as a precursor (Jones and Hartley, 1999). Thus, there may be trade-offs between growth and secondary metabolism because plants have limited resources to support the physiological processes.

Figure 1. Chemical structures of some phenolic compounds
Plants from different origins can exhibit different ecotypes and may have different growth performances, such as different height and biomass (Förster et al., 2015). Their chemical defenses may also differ as a result of different genetic adaptations (Jaakola and Hohtola, 2010). Previous studies have reported that plants of different genotypes showed great variation in their growth parameters and production of phenolic metabolites (Lavola et al., 2013; Nybakken and Julkunen-Tiitto, 2013; Randriamanana et al., 2014; Randriamanana et al., 2015a). Martz et al. (2009) also noted that the levels of phenolics were higher in plants from more northerly origins than those from southern origins in the boreal zone. For dioecious plant species, such as Salicaceae, females and males prioritize the allocation of resources differently (Nybakken et al., 2012). In general, females invest more resources in reproduction and accumulation of secondary metabolites for defense than growth, while males invest more in growth and have higher herbivore abundance and damage (Randriamanana et al., 2015b; Maja et al., 2016).

1.3 EFFECT OF CLIMATE CHANGE ON PLANT GROWTH AND PHENOLICS

Elevated temperature and CO$_2$ concentration have large impacts on plant
performance (Veteli et al., 2002; Dusenge et al., 2018). High northern latitudes are climate sensitive areas, where tree growth is usually temperature-limited and thus benefits from moderate elevations of temperature (Way and Oren, 2010). Previous field and greenhouse studies have reported that elevated temperature (+ 2 °C) increased height and diameter growth and shoot biomass of *P. tremula* in Finland (Randriamanana et al., 2015a; Sobuj et al., 2018). However, different plant species show variable responses to elevated temperature. For example, studies on *P. abies* have shown that elevated temperature (1.3–3.9 °C) had no effects on height growth or plant biomass (Kivimäenpää et al., 2013; Sigurdsson et al., 2013; Virjamo et al., 2014). On the other hand, elevated CO$_2$ has positive effects on the growth of C$_3$ plants in a relatively short time, as it enhances net photosynthesis and suppresses photorespiration (Cole et al., 2010; Lavola et al., 2013). However, the growth increase caused by elevated CO$_2$ is not continuous. With time, photosynthetic acclimation occurs due to the end-product inhibition, caused by an insufficient demand for carbohydrates to balance the enhanced supply under elevated CO$_2$ (Zhao et al., 2012).

The quality and quantity of phenolic compound synthesis are often affected by changing climate factors, and connected to effects on growth, as both processes require high amounts of carbon. Previous studies have shown that the concentrations of phenolic compounds in plants decreased under elevated temperature, and increased under elevated CO$_2$ concentration (Nybølken et al., 2012; Lavola et al., 2013). Moreover, plant growth and phenolics are also affected by the interactions between elevated temperature and elevated CO$_2$, as one factor can modify the effect of the other (Veteli et al., 2007; Zhao et al., 2012).

Plants of different origins respond differently to climate change. Kellomäki et al. (2008) applied model simulations to study the sensitivity of boreal forest trees in Finland to climate change. The results showed that compared to trees in northern Finland, *P. abies* trees in southern Finland may suffer from more competition with other species because of the small or even negative growth increase and decreased share in this area under climate change (Kellomäki et al., 2008). Egli et al. (1998) detected a significant CO$_2$ × provenance interaction effect on aboveground growth of *P. abies*, which means that the response of *P. abies* growth to elevated CO$_2$ differed among different provenances. In addition, plants from different areas have different photosynthetic rates, and the amount of fixed carbon available for secondary metabolites may differ (Jaakola and Hohtola, 2010). Thus, they may also have different responses in phenolic accumulation to elevated temperature and CO$_2$.

In the case of dioecious plant species, there are sex differences in plant growth and defensive phenolics in response to elevated temperature and CO$_2$ concentration. For instance, Randriamanana et al. (2015a) noted that although males of *P. tremula* were more growth-oriented under ambient conditions, the growth of females increased more under elevated temperature, and females had better chemical defense than males. Zhao et al. (2012) reported that elevated CO$_2$ enhanced plant growth in both males and females of *P. cathayana* Rehd., but males benefited more from elevated CO$_2$. Therefore, if elevated temperature and CO$_2$ increase only growth and not the
chemical defense in males, but increase both growth and chemical defense in females, males may be more susceptible to herbivore damage in the long run. However, sex differences in plant growth and phenolics response to enhanced temperature and CO$_2$ may vary with the plant species, plant tissues, growing stage, and phenolic compound in question (Xu et al., 2008; Nybakken et al., 2012; Zhao et al., 2012; Nybakken and Julkunen-Tiitto, 2013).

1.4 EFFECT OF SOIL CONTAMINATION ON PLANT-SOIL SYSTEM

1.4.1 Effect of PAHs on plant-soil system

As a group of ubiquitous environmental pollutants, PAHs can be absorbed by plants through both soil-root-shoot pathways and air-plant pathways, resulting in biochemical and physiological effects on plants (Tao et al., 2009; Song et al., 2012). At the cellular level, PAHs may stimulate the formation and accumulation of ROS in plant cells, which may cause lipid peroxidation of membranes, loss of membrane permeability and integrity, cell organelle deformities, and ultimately collapse of cellular structure (Desalme et al., 2013). This can induce morphological symptoms such as growth reduction of shoots and roots, deformed trichomes, reduced root hairs, chlorosis, necrosis, and mesophyll collapse, and also disturbances in photosynthesis, primary carbon metabolism, antioxidant enzyme activities, protein synthesis, and signal transduction (Alkio et al., 2005; Song et al., 2012; Zhang et al., 2013). In addition, the PAH phenanthrene has been reported to cause enhanced carbon allocation from primary metabolism to secondary metabolism (Desalme et al., 2011). However, the effects of PAHs on plant growth and chemical defense under climate change are not yet known.

PAHs that enter soils may be lost or degraded by physicochemical and biological processes, and the major means is biotic, i.e., through degradation or co-degradation processes mediated by bacteria and fungi (Kuppusamy et al., 2017). Plants can promote microbial biodegradation of PAHs in rhizosphere soil by plant-secreted enzymes in the root zone (Lu et al., 2014). Moreover, secondary plant metabolites may influence biodegradation of PAHs in soils (Meng and Zhu, 2011). For example, flavonoids have an important role in developing organic contaminant-degrading enzymes (Qiu et al., 2004). Salicylates has also been linked to PAH biodegradation, as they can enhance the survival of PAH-degrading microorganisms and induce the genes encoding enzymes involved in PAH degradation (Singer et al., 2003). Elevated temperature and CO$_2$ can lower the pH of rhizosphere soil, and thus affect the rhizosphere conditions by altering the composition or amount of root exudates and change soil microbial communities (Rajkumar et al., 2013; Zhao et al., 2016; Du et al., 2017a). However, whether elevated temperature and CO$_2$ will affect the degradation
of PAHs in soils is very little studied.

### 1.4.2 Effect of TiO$_2$ nanoparticles on plant-soil system

Previous soil studies have shown that nTiO$_2$ can affect the plant-soil system (Gardea-Torresdey et al., 2014; Du et al., 2017a). Metal oxide nanoparticles, including nTiO$_2$, can be absorbed by the root endodermis through apoplastic pathways and then transferred to the vascular cylinder through symplastic pathways, and further translocated to plant shoots (Du et al., 2017b). Servin et al. (2013) demonstrated the soil-root-fruit translocation of nTiO$_2$ in cucumber (Cucumis sativus L.) without biotransformation. Physiological studies of higher plants have reported that nanoparticles larger than the size of root cell wall pores accumulate in the apoplastic space, adhere to root cell walls causing mechanical damages, block the pores, reduce root hydraulic conductivity and, therefore, reduce water absorption and nutrient uptake capacity of intact plants (Asli and Neumann, 2009; García-Sánchez et al., 2015; Khan et al., 2017). However, nanoparticles smaller than the cell wall pore sizes of roots may penetrate through lateral root junctions and travel through the vascular system by symplastic pathways, hence affecting the whole plant physiology (Khan et al., 2017). A number of studies have reported on the effects of nTiO$_2$ on physiological and biochemical parameters of plants, including germination rate, elongation, biomass accumulation, antioxidant activities, photosynthetic parameters, and biochemical compositions, but the effects may vary with plant species, soil properties, nTiO$_2$ concentration, and particle size of nTiO$_2$ (Du et al., 2011; Servin et al., 2013; Zahra et al., 2017; Larue et al., 2018). In addition to the nanoparticulate form, the component metal (Ti) can also be accumulated by plant tissues from nTiO$_2$ treatments, and thus affect plants (Gardea-Torresdey et al., 2014).

TiO$_2$ nanoparticles also affect the soil environment. Du et al. (2011) found that nTiO$_2$ at about 90 mg kg$^{-1}$ significantly inhibited soil protease, catalase, and peroxidase activities, i.e., changed the soil quality and health. In addition, nTiO$_2$ may result in direct and indirect adverse effects on soil bacteria (Ge et al., 2013). It may directly disrupt cell membrane, induce ROS production and oxidative stress, leading to genotoxicity and DNA damage thereby causing cell death (Gou et al., 2010). The indirect effects of nTiO$_2$ on soil bacteria include altering the physical environment of the soil, which is vital for bacteria survival and growth; or food web impacts, such as toxicity to protozoa that consume bacteria (Ge et al., 2013). The impacts of nTiO$_2$ on the soil environment may also influence the composition and quality of plant root exudates, which can further affect plant absorption, utilization, and production of nutrient (Du et al., 2017a), and thus affect plant growth and phenolic production.

One could expect that nTiO$_2$ could possibly interact with climate change factors, but this has been scarcely studied. So far, only Du et al. (2017a) investigated the effects of nTiO$_2$ on rice (Oryza sativa L.) under elevated CO$_2$ concentration using a full-size free-air CO$_2$ enrichment system. They found that nTiO$_2$ at 50 and 200 mg kg$^{-1}$...
did not induce visible signs of toxicity in rice plants under ambient CO$_2$ level, but it significantly reduced rice biomass and grain yield, and also changed the composition of soil microbial communities under elevated CO$_2$ concentration (Du et al., 2017a).

Apart from this, little is known about the effect of nTiO$_2$ on plant performance under elevated temperature and CO$_2$ concentration, and, to our knowledge, nothing about the effect on forest tree species.

1.5 AIMS OF THE THESIS

Boreal forest ecosystems are simultaneously affected by multiple environmental stressors, including ongoing climate change and soil contamination. Levels of atmospheric temperature and CO$_2$ concentrations both affect plant photosynthesis and carbon uptake, and hence affect the amount of carbon available for plant growth and carbon-based defensive compounds. Soil contamination may also affect plant carbon allocation, but little is known about how this may vary under different climatic conditions. The main aim of this thesis was to study the effects of climate change (elevated temperature and CO$_2$ concentration) and soil contamination (pyrene and nTiO$_2$) on two boreal forest tree species: the evergreen _P. abies_ and the deciduous _P. tremula_. In addition, I also wanted to study the responses of _P. abies_ from different origins and _P. tremula_ of both sexes to these multiple stressors, which could further affect the competitive abilities and adaptive evolution of the species.

The following questions were addressed:

1. Will elevated temperature and CO$_2$ concentration affect growth and defensive phenolics in _P. abies_ (I) and _P. tremula_ (II, III)?

2. Will soil pyrene contamination affect growth and defense of _P. abies_ (I) and _P. tremula_ (II), and how will elevated temperature and CO$_2$ concentration affect the responses of _P. abies_ (I) and _P. tremula_ (II) to pyrene?

3. Will elevated temperature and CO$_2$ concentration affect the residue of pyrene in soil (II)?

4. Will soil nTiO$_2$ contamination affect growth and defense of _P. tremula_, and will there be interactions between nTiO$_2$ and climate factors (III)?

5. Will plant tissues accumulate Ti from nTiO$_2$ treatments, and how will elevated temperature and CO$_2$ concentration affect the Ti uptake in _P. tremula_ (III)?

6. Will there be origin-related differences in the response of _P. abies_ (I) and/or sex-related differences in the response of _P. tremula_ to the multiple stressors (II, III)?
2 MATERIALS AND METHODS

2.1 EXPERIMENTS

Three experiments were conducted over the summer growing seasons in 2014 (I) and 2015 (II, III) in climate-controlled greenhouses at Mekrijärvi Research Station, Ilomantsi, University of Eastern Finland (62° 47′ N, 30° 58′ E, 145 m a.s.l.) (Figure 3, Figure 4a). Sixteen greenhouses (16 m² each) were randomly assigned to four climate treatments: ambient temperature + ambient CO₂ concentration (C), elevated temperature + ambient CO₂ concentration (T), ambient temperature + elevated CO₂ concentration (CO₂), and elevated temperature + elevated CO₂ concentration (T + CO₂). Based on IPCC (2014), temperature in the elevated treatment greenhouses was set to 2 °C above the ambient level, and the ambient temperature was achieved by following the outside air temperature through a modulated system. Ambient and elevated CO₂ concentrations were set at 360 and 720 ppm, respectively. The relative humidity was maintained at 60% in all greenhouses. The photoperiod in the experimental greenhouses followed the outside natural day length. More technical details are described in Zhou et al. (2012).

Figure 3. Sixteen greenhouses at Mekrijärvi Research Station

2.2 SOIL PREPARATION AND PLANT MATERIALS

The peat soil (Kekkilä Oy, Vantaa, Finland) used in experiments I and II was treated
with pyrene (purity > 98%) to have the concentration of 50 mg kg\(^{-1}\). In experiment III, nTiO\(_2\) (purity > 97%, particle size < 100 nm) was added into the peat soil to have concentrations of 50 and 300 mg kg\(^{-1}\). Pyrene in n-hexane or nTiO\(_2\) was added to 10% (by volume) of the required quantity of soil. The pyrene-containing (after n-hexane evaporated) and nTiO\(_2\)-containing soils were progressively diluted with the remaining 90% (by volume) of untreated soils and mixed thoroughly. The contaminated soils were incubated for two weeks before planting. Soil without pyrene or nTiO\(_2\) was used as the control soil.

The *P. abies* seedlings (one-year old) used in experiment I originated from five different locations in Finland (A: Sodankylä, 67° 43’ N, 26° 11’ E; B: Tuusniemi, 62° 48’ N, 28° 29’ E; C: Virrat, 62° 12’ N, 24° 07’ E; D: Joutsa, 61° 39’ N, 26° 16’ E; and E: Mikkeli, 61° 41’ N, 27° 16’ E) (Figure 4a, b). Seedlings from A, C, and D were grown in a nursery in Rovaniemi (northern Finland, 66° 29’ N, 25° 33’ E) (Figure 4a) and those from B and E were grown in a nursery in Tuusniemi (southern Finland, 62° 52’ N, 28° 21’ E) (Figure 4a) for the first growing season. The *P. tremula* seedlings (Figure 5) used in experiments II and III were originally micropropagated from the dormant axillary buds of adult European aspen trees from different locations in eastern and southern Finland (Table 1). The mother trees were selected from distant locations to make sure that they did belong to different genotypes. A summary of experiments conducted in this thesis is presented in Table 2.

![Figure 4. Locations of Mekrijärvi Research Station, Rovaniemi nursery, Tuusniemi nursery, and five origins (A, B, C, D, E) of *P. abies* (a), and *P. abies* seedlings used in experiment I (b)](image)
Figure 5. *P. tremula* seedlings used in experiments II and III

Table 1. Clone number, sex, and origin of the clones of *P. tremula* seedlings used in experiments II and III

<table>
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<th>Clone number</th>
<th>Sex</th>
<th>Location of parent tree</th>
<th>Paper</th>
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<td>II, III</td>
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<tr>
<td>2</td>
<td>Female</td>
<td>Loppi (Uotila), southern Finland</td>
<td>II, III</td>
</tr>
<tr>
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<tr>
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<tr>
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</tr>
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### Table 2. Summary of the experiments conducted in this thesis

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<th>II</th>
<th>III</th>
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<td><em>Populus tremula</em></td>
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<td>C, T, CO₂, T + CO₂</td>
<td>C, T, CO₂, T + CO₂</td>
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<tr>
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<td>Pyrene (0, 50 mg kg⁻¹)</td>
<td>nTiO₂ (0, 50, 300 mg kg⁻¹)</td>
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<td>Stem biomass</td>
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<td>Residual pyrene in soil</td>
<td>Ti uptake</td>
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### 2.3 GROWTH MEASUREMENTS AND SAMPLING

The height growth and basal diameter growth of all seedlings were measured every second week during the experiments I, II, and III. At the end of the experiment I, the top shoots (about 10 cm) of all *P. abies* seedlings were cut and separated into needles and stems for phenolic analyses. Stem samples were cut into two longitudinal halves. At the end of the experiments II and III, two mature *P. tremula* leaves from each individual were collected for leaf area measurements and leaf phenolic analyses. All samples were dry-air dried at room temperature in a drying room (with 10% relative humidity), and then stored at −20 °C until chemical analyses. The remaining aboveground parts of all seedlings in experiments I, II, and III were harvested for biomass measurements. They were air-dried at room temperature, and then separated into needles/leaves and stems, and weighed. Following removal of aboveground parts, the soil samples in each pot in experiment II and root samples in experiment III were collected, air-dried at room temperature for pyrene and Ti
analyses, respectively.

### 2.4 LABORATORY ANALYSES

#### 2.4.1 Phenolic analyses

Phenolic compounds in dried needles and stems of *P. abies* seedlings (I) and leaves of *P. tremula* seedlings (II, III) were extracted with methanol and analyzed by high-performance liquid chromatography (HPLC, 1100 series, Agilent, Santa Clara, CA, USA) following the procedures described in Nybakken et al. (2012). Identification of phenolics was performed by mass spectrometry using a quadrupole time-of-flight mass spectrometer (QTOF/MS, 6540 series, Agilent, Santa Clara, CA, USA) with an ultra-HPLC with a diode array detector (UHPLC-DAD, 1200 series, Agilent, Santa Clara, CA, USA) according to Randriamanana et al. (2014). Compounds of which mass could not be successfully determined were identified according to the retention times and UV spectra of corresponding commercial standards, which were also used for quantification of phenolic compounds.

#### 2.4.2 Pyrene analyses

Soil samples in experiment II were crushed and sieved. Pyrene in soils was extracted with 1:1 (v/v) hexane/acetone and analyzed by HPLC according to Gao and Zhu (2004). Pyrene was identified according to the retention time and UV spectrum of the standard (Sigma-Aldrich Co., St. Louis, MO, USA), and quantified according to its absorbance at 234 nm.

#### 2.4.3 Ti analyses

Dried leaves, stems, and roots of *P. tremula* in experiment III were collected, weighed (about 0.5 g), and then digested with HNO₃ and HClO₄ (4:1, v/v). Concentrations of Ti in plant samples were analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES, Optima 5300, PerkinElmer, Waltham, Mass., USA) according to Du et al. (2017a). Three replications were performed for each sample. The same solution without plant samples was used as control. A calibration check standard was prepared using a titanium stock solution GSBG62014-90 (Central Iron & Steel Research Institute, Beijing, China). This was analyzed as a sample to verify analyte concentration and instrument calibration.
2.5 STATISTICAL ANALYSES

All statistical analyses were conducted with SPSS (IBM® SPSS® Statistics 22.0, Armonk, NY, USA). Biomass and phenolic data in experiment I, and all data in experiments II and III were analyzed using a linear mixed model with temperature (I, II, III), CO₂ (I, II, III), pyrene (I, II)/nTiO₂ (III), origin (I)/sex (II, III) as fixed factors, and genotype (II, III) and greenhouse (I, III) as random factors. For height and diameter data in experiment I, a repeated linear mixed model was used, where time was specified as a repeated variable. In experiments II and III, the increments of height and diameter (end values – start values) were used, and start height and start diameter were used as covariates. All data were tested for normality, and if needed, the non-normal data were sqrt(x)-, ln(x)-, or ln(x + 1)-transformed. Nonparametric tests were used when data did not meet the requirements of parametric tests.

In addition, according to published methods (Haase and Rose, 1995; Koricheva, 1999), graphic vector analyses (GVA) were carried out in order to further elucidate the effects of elevated temperature, elevated CO₂, and pyrene on phenolic production and biomass accumulation in needles and stems of P. abies (I) and leaves of P. tremula (II); and elevated temperature, elevated CO₂, and nTiO₂ on phenolic production and biomass accumulation in leaves of P. tremula (III). The GVA plots were built using SigmaPlot 12.5 (© 2011 Systat Software, Inc., Chicago, IL, USA).
3 RESULTS AND DISCUSSION

3.1 PLANT GROWTH AND PHENOLICS

The P. abies seedlings from five different origins exhibited different growth performances (I). Seedlings from southern origins B and E had the highest height, diameter, and shoot biomass, whereas seedlings from the northernmost origin A had the lowest (Figure 6, Figure 7) (I). Seedlings from origins C and D were from more southerly-located parental trees but were nursery grown in northern Finland (Rovaniemi). They were not able to reach the expected higher growth of their natural origins obviously due to their low initial growth in Rovaniemi (I). Plants of different provenances exhibit significant differences in growth performance (Lee et al., 2015). Previous field and greenhouse experiments conducted in Canada showed that black spruce (P. mariana (Mill.) B.S.P.) trees of southern provenances were considerably taller and had larger shoot weight than trees of northern provenances (Johnsen and Seiler, 1996a; Johnsen et al., 1996b). Manninen et al. (1998) compared the growth of Scots pine (Pinus sylvestris L.) seedlings from four provenances in Finland and found that the length and dry weight of shoots decreased towards the north.

In the P. abies seedlings, 24 different phenolic compounds were detected in needles, and 23 in stems, and the concentration of total phenolics in needles was twice that in stems (I). Acetophenones and flavonoids were the main groups of phenolics in needles of P. abies, while flavonoids and stilbenes were the main phenolic groups in stems (Figure 8) (I). Seedlings from the northernmost origin A contained more phenolics in both needles and stems than seedlings from other origins (Figure 8) (I). Although seedlings from southern origins C and D were grown in Rovaniemi for the first growing season, phenolics in these seedlings were more similar to those from origin E than those from origin A (Figure 8) (I). According to Jaakola and Hohtola (2010), plants from different origins show different genetic adaptations, and their capabilities in chemical defenses differ. Moles et al. (2011) performed a meta-analysis and found that chemical defenses were higher in plants from higher latitudes. Studies on Juniperus communis L. and P. sylvestris in Finland also showed that plants from northern origins had higher phenolic and terpene levels than plants from southern origins (Manninen et al., 1998; Martz et al., 2009).
Figure 6. Height (a) and diameter (b) growth of *P. abies* seedlings from five origins (A–E). Bars represent mean values ± SE (n = 4).

Figure 7. Needle and stem biomass of *P. abies* seedlings from five origins (A–E). Bars represent mean values + SE (n = 4).
Figure 8. Concentrations of different phenolic groups in needles (a) and stems (b) of *P. abies* seedlings from five origins (A–E). Bars represent mean values + SE (n = 4)

For the dioecious *P. tremula* seedlings, both females and males grew fast during the growing season (II, III). Although no significant sex differences in plant growth were detected, males tended to be taller and had higher biomass than females under ambient conditions (II, III). A total of 19 phenolic compounds including salicylates, flavonoids, and phenolic acids were identified and quantified in the leaves of *P. tremula* seedlings, and salicylates were the major phenolic group (Figure 9) (II, III). Among the salicylates, salicortin and tremulacin were the most abundant compounds (II, III). Salicaceae species, such as *Salix* and *Populus*, accumulate comparatively high levels of phenolic compounds (Julkunen-Tiitto, 1986;
Randriamanana et al., 2014; Nissinen et al., 2016). Previous studies on field- and greenhouse-grown *P. tremula* and *Salix myrsinifolia* Salisb. and greenhouse-grown *S. repens* L. have also reported significant accumulation of salicylates, flavonoids, and phenolic acids in leaf samples, and especially high for salicylates (Nybakken and Julkunen-Tiitto, 2013; Randriamanana et al., 2014; Randriamanana et al., 2015a; Randriamanana et al., 2015b; Nissinen et al., 2016). Salicylate-derived phenolics are prominent foliar chemicals in Salicaceae species, and they can protect plants against generalist herbivores, pathogens, and abiotic stresses (Chen et al., 2009; Lindroth and St. Clair, 2013; Julkunen-Tiitto and Virjamo, 2017). Salicortin and tremulacin are known to be the most biologically active salicylates, because both of them contain a cyclohexenone carboxylic acid functional group (Figure 1) (e.g. Lindroth and St. Clair, 2013). They have been demonstrated to have strong negative effects on herbivore growth, development, and fecundity (e.g., Hemming and Lindroth, 1995; Chen et al., 2009; Boeckler et al., 2011). Previous studies on dioecious plants have noted that females allocate more resources to defensive phenolics than males (Nybakken and Julkunen-Tiitto, 2013; Randriamanana et al., 2015a). However, there were no significant sexual differences in leaf total phenolics in *P. tremula*, but concentrations of many individual phenolic compounds were higher in females than in males (II, III).

![Figure 9](image_url)

**Figure 9.** Concentrations of different phenolic groups in leaves of *P. tremula* seedlings in experiments II and III. Bars represent mean values + SE (n = 4 for experiment II and n = 3 for experiment III)
3.2 EFFECTS OF CLIMATE CHANGE AND SOIL CONTAMINATION ON GROWTH

Elevated temperature or elevated CO$_2$ showed little effect on height growth, diameter growth, or shoot biomass of the $P. abies$ seedlings (I), which contradicts the findings of Sallas et al. (2003), but agrees with the results of previous studies of field-grown $P. abies$ (Slaney et al., 2007; Virjamo et al., 2014). However, elevated temperature increased plant growth parameters of $P. tremula$ including height, diameter, shoot biomass, and specific leaf area, and the increments were greater in females than in males (II, III), which is in accordance with the findings of Randriamanana et al. (2015a). Elevated CO$_2$ reduced height growth and specific leaf area of $P. tremula$ (II, III), but increased (II) or did not affect (III) diameter growth. Similar results from studies on $P. tremula$ (Sobuj et al., 2018), $Larix kaempferi$ (Lamb.) Carr. (Yazaki et al., 2004), and $P. abies$ (Sallas et al., 2003) have also been reported. The results of effects of elevated temperature and CO$_2$ concentration on growth of $P. abies$ and $P. tremula$ are summarized in Table 3. Growth of trees at high northern latitudes may be temperature-limited and will benefit from moderate elevation of temperature (Way and Oren, 2010). In addition, Tjoelker et al. (1998) investigated the growth responses of five boreal tree species to elevated CO$_2$ at different temperatures and found that effects of elevated CO$_2$ on growth were minimal or even suppressed at low temperatures, but maximal towards optimal growth temperatures. The growth response to elevated CO$_2$ is expected to be small in most boreal forests where the availability of nitrogen rather than carbon is the main limiting factor for plant growth (e.g., Slaney et al., 2007). The strong growth responses of $P. tremula$ to elevated temperature indicate that $P. tremula$ is more sensitive to temperature than $P. abies$, and the optimum growth temperatures for $P. tremula$ in experiments II and III have not yet been reached. Trees from different functional groups often differ in their growth responses to climate warming (Way and Oren, 2010). Evergreen trees show little or much smaller growth responses to warming than deciduous species, because of the trade-offs between their traits that reduce nutrient losses and those that lead to high rates of dry matter production (Aerts, 1995; Way and Oren, 2010).
Plant growth can be affected by direct and indirect exposure to PAHs, but it depends on the PAH in question, exposure concentration, and plant species (Desalme et al., 2013). For example, shoot and root biomass of five plants (Brassica rapa L., C. sativus, B. campestris L., Solanum lycopersicum L., and Lactuca sativa L.) were reduced with increasing concentration of sprayed phenanthrene (Ahammed et al., 2012). However, soil pyrene contamination did not affect shoot or root biomass of Festuca arundinacea Schreb. at 50 or 100 mg kg\(^{-1}\), but it decreased the biomasses at 200, 300, and 500 mg kg\(^{-1}\) concentrations (Lu et al., 2014). For P. abies seedlings, soil pyrene contamination (50 mg kg\(^{-1}\)) significantly reduced the height growth, and the reduction was more severe under elevated temperature, especially in seedlings from southern origins C, D, and E (I). It also decreased needle and stem biomass, and this decrease was greater in seedlings from origins B and E (I). Climate change in combination with pyrene may cause a suboptimal growth environment for P. abies seedlings, especially for those from southern origins. Previous model-based studies have estimated that P. abies will suffer from competition with other species and its proportion in the southern parts of the boreal zone will seriously decrease as the climate changes, taking into account the changes in atmospheric temperature, precipitation, and CO\(_2\) concentration (Kellomäki et al., 2008; Hickler et al., 2012). For P. tremula seedlings, pyrene did not affect the height, diameter or leaf biomass of both sexes, but significantly decreased the stem biomass and leaf area under ambient conditions, and the decrease of leaf area was stronger under elevated temperature, elevated CO\(_2\), and combined elevated temperature and CO\(_2\) (II). Moreover, the decrement of leaf area caused by pyrene was greater in males than in females of P. tremula (II). Pyrene is a representative four-ring PAH with relatively high potential genotoxicity and high concentration in the soil environment (Tuhačková et al., 2001; Zhang and Chen, 2017). It has a high affinity to plant tissues because of its lipophilic character, and can be taken up into plant tissues through soil-plant pathway and thus affects plant growth (Hückelhoven et al., 1997; Tao et al., 2009).
Unlike pyrene, nTiO$_2$ at 50 and 300 mg kg$^{-1}$ did not affect any measured growth parameters of *P. tremula* seedlings under ambient conditions (III), which is in accordance with earlier studies on vegetables (*Lycopersicon esculentum* Mill.), crops (*Triticum aestivum* L. and *Phaseolus vulgaris* L.), and wetland or aquatic plants (*Rumex crispus* L. and *Elodea Canadensis* Michx.) (Jacob et al., 2013; Song et al., 2013). In addition, unlike the findings of Du et al. (2017a) that nTiO$_2$ (50 and 200 mg kg$^{-1}$) significantly reduced rice biomass and grain yield under elevated CO$_2$ concentration (570 ppm vs. 370 ppm), nTiO$_2$ showed no effects on *P. tremula* growth under elevated temperature, elevated CO$_2$, or combined elevated temperature and CO$_2$ (III). TiO$_2$ nanoparticles have been reported to be less toxic than other metal-based nanoparticles such as Ag and ZnO nanoparticles, because they are virtually insoluble in soils (Tourinho et al., 2012; Gardea-Torresdey et al., 2014). However, the effects of nTiO$_2$ on plant growth have also been positive or negative, depending on the concentrations and physicochemical parameters of nTiO$_2$, soil types, and different experimental setups and systems (Dietz and Herth, 2011; Larue et al., 2012; Larue et al., 2018). A summary of results of effects of soil pyrene and nTiO$_2$ contamination on plant growth is presented in Table 4.

### Table 4. Effects of pyrene on growth of *P. abies* and *P. tremula*, and effects of nTiO$_2$ on growth of *P. tremula* under ambient (C), elevated temperature (T), elevated CO$_2$ concentration (CO$_2$), and combined T + CO$_2$

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</tbody>
</table>

Arrows show direction of change
- - no effect on growth parameter

### 3.3 Effects of Climate Change and Soil Contamination on Phenolics

In uncontaminated soil treatments, elevated temperature decreased the concentration of total phenolics in both needles and stems of *P. abies* seedlings from all origins (I) and in leaves of females and males of *P. tremula* seedlings (II, III).
Elevated CO₂, on the other hand, increased phenolics in stems of *P. abies* (I) and in leaves of *P. tremula* (II, III) (Table 5). Similar decreases in phenolics caused by elevated temperature and increases caused by elevated CO₂ have been noted for boreal forest tree species, for example, in the needles of field-grown *P. abies* seedlings (Virjamo et al., 2014), in the stems of greenhouse-grown *P. tremula* seedlings (Sobuj et al., 2018), in the leaves of field-grown *S. myrsinifolia* seedlings (Nybakken et al., 2012), in the leaves of greenhouse-grown *Betula pendula* Roth. seedlings (Lavola et al., 2013), and in the needles of field-grown *P. sylvestris* seedlings (Sallas et al., 2001). In addition, there were significant T × CO₂ interactions on total phenolics, which means that elevated CO₂ eliminated the negative effects of elevated temperature on total phenolics in *P. abies* (I) and *P. tremula* (II), as reported earlier by Veteli et al. (2007).

**Table 5. Effects of elevated temperature (T) and elevated CO₂ concentration (CO₂) on phenolics in *P. abies* and *P. tremula***

<table>
<thead>
<tr>
<th>Species</th>
<th>Picea abies</th>
<th>Populus tremula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climate treatments</td>
<td>T</td>
<td>CO₂</td>
</tr>
<tr>
<td>Phenolics</td>
<td>↓</td>
<td>↑</td>
</tr>
</tbody>
</table>

Arrows show direction of change
- : no effect on phenolics

Soil pyrene contamination lowered the concentration of total phenolics in needles and stems of *P. abies* seedlings from all origins under ambient conditions, and the decrease caused by pyrene was not relieved by elevated CO₂ and was even greater under elevated temperature, maybe due to a cumulative effect (I). In females and males of *P. tremula* seedlings, however, pyrene did not affect leaf total phenolics under ambient conditions, but it significantly increased them under elevated temperature and combined elevated temperature and CO₂ (II). nTiO₂, on the other hand, stimulated the accumulation of total phenolics in leaves of *P. tremula*, but only in females, and no interactions were found between nTiO₂ and the climatic factors (III). The results of effects of soil pyrene and nTiO₂ contamination on plant defensive phenolics are summarized in Table 6.
Table 6. Effects of pyrene on phenolics in *P. abies* and *P. tremula*, and effects of nTiO$_2$ on phenolics in *P. tremula* under ambient (C), elevated temperature (T), elevated CO$_2$ concentration (CO$_2$), and combined T + CO$_2$

<table>
<thead>
<tr>
<th>Effects of soil contaminants</th>
<th>Pyrene</th>
<th>nTiO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td><em>Picea abies</em></td>
<td><em>Populus tremula</em></td>
</tr>
<tr>
<td>Climate treatments</td>
<td>C T CO$_2$ T+CO$_2$</td>
<td>C T CO$_2$ T+CO$_2$</td>
</tr>
<tr>
<td>Phenolics</td>
<td>↓ ↓ ↓ ↓</td>
<td>↑ ↑ ↑ ↑</td>
</tr>
</tbody>
</table>

Arrows show direction of change

$\rightarrow$: no effect on phenolics

F: the effects were found only in females

Environmental stressors can affect the concentration of phenolics in plants by diverting substrates from primary processes of growth into secondary metabolism (Lattanzio et al., 2012). This may result in competition and trade-offs between growth and defense because plants have limited resources to support their physiological processes (Herms and Mattson, 1992; Lattanzio et al., 2012). My results can be explained by the carbon-nutrient balance (CNB) hypothesis (Bryant et al., 1983) and the growth-differentiation balance (GDB) hypothesis (Herms and Mattson, 1992). Plant growth and carbon-based secondary metabolism can compete for photosynthates. In uncontaminated soils, plants prioritized resource allocation to growth over that of carbon-based phenolics under elevated temperature, while the carbon surpluses were high enough to satisfy or to prioritize the production of phenolics over growth under elevated CO$_2$. Soil pyrene contamination might have irreversible damaging effects on *P. abies* seedlings, as indicated by the inability to utilize the resources to satisfy either growth or production of phenolics. However, *P. tremula* seedlings allocated more resources to the synthesis of phenolics than growth, and is obviously better than *P. abies* to adapt to the combined stress of pyrene and elevated temperature and CO$_2$. This indicates that different tree species respond differently to pyrene in combination with climate factors. Due to lower defense levels, *P. abies* may be more subject to pest and pathogen damages under the combination of pyrene contamination and future climate conditions. *P. tremula*, on the other hand, seems to be able to regulate growth and metabolism to adjust to the stress of the tested pyrene contamination under elevated temperature and CO$_2$. TiO$_2$ nanoparticles have been reported to alter the soil bacterial community composition under either ambient or elevated CO$_2$ conditions, which may influence the composition and quality of plant root exudates (Ge et al., 2013; Du et al., 2017a). This can further affect plant absorption and utilization of nutrient under climate change, and thus change the production of phenolics. The presence of nTiO$_2$ did not impact the growth of females or males of *P. tremula* seedlings, but induced an increase in leaf total phenolics in females. This may indicate that females of *P. tremula* have better
chemical defense against nTiO$_2$ than males under current and future climate conditions, and males may get more herbivore damages in nTiO$_2$ contaminated soils. In the end, all this may have impact on the sexual reproduction and evolution of _P. tremula_ species.

### 3.4 ENVIRONMENTAL BEHAVIOR OF SOIL CONTAMINANTS

The concentration of pyrene in pyrene-spiked soils significantly decreased during the 11 weeks after _P. tremula_ planting (II). The residual pyrene in soils was 28% and 45% higher for females and males of _P. tremula_, respectively, under elevated CO$_2$ compared with ambient conditions (II). However, there was no differences in residual pyrene concentration between ambient and elevated temperature or under elevation of both temperature and CO$_2$ (II). In the plant-soil system, plants can enhance the rate and extent of degradation of pyrene in soil due to increased microbial activity and degradation mediated by plant-secreted enzymes in the rhizosphere (Liste and Alexander, 2000; Lu et al., 2014). In addition, soil microorganisms play a vital role in the decomposition of organic matter (Drissner et al., 2007). The effect of elevated atmospheric CO$_2$ on the degradation of PAHs in soils is very little studied. Elevated atmospheric CO$_2$ may not directly influence soil microorganisms, as the concentration of CO$_2$ in soil is much higher (10–50 times) than in the atmosphere. However, it can indirectly affect root growth and rhizodeposition, which are closely associated with the soil microbial community (Kandeler et al., 1998; Tscherko et al., 2001). Ai et al. (2018) conducted a study in open top chambers and found that elevated atmospheric CO$_2$ significantly increased total PAH residuals in soil, i.e., decelerated the degradation of PAHs. This was mainly because elevated CO$_2$ had a negative impact on microbial processes linked to PAH degradation; it altered the soil microbial community composition and led to a decrease in the biomass of Gram-positive bacteria, which was the most important group of soil microbes in degrading PAHs (Ai et al., 2018). Temperature changes can directly affect soil microbial diversity, growth, mineralization rate, and enzyme activities (Tscherko et al., 2001). Iqbal et al. (2007) reported that elevated temperature (42 ± 2 °C vs. 21 ± 1 °C) accelerated the bioremediation of PAHs in soils, which may be because elevated temperature increased the ability of indigenous microorganisms to degrade PAHs and decreased the adsorption so that more organic materials were available for microorganisms to degrade. However, the temperature enhancement (about 2 °C) in my study was too small to change the degradation of pyrene in soil.

Plant tissues can accumulate Ti from nTiO$_2$ treatments, especially in roots (Jacob et al., 2013). Ti was not detected in leaves or stems in _P. tremula_ seedlings in any of the soil treatments, neither in roots in uncontaminated soils (III). However, it was significantly accumulated in roots in soils contaminated with 50 and 300 mg kg$^{-1}$ nTiO$_2$, and the concentration of Ti in roots correlated positively to the added amount of nTiO$_2$ to the soil (III). Plant roots are more sensitive to the contaminants as they...
are directly exposed to the contaminants in soils (Song et al., 2017). In addition, elevated CO$_2$ had no effect on root Ti uptake (III), which is in agreement with the results of Du et al. (2017a). The elevated temperature increased Ti concentration in roots of the 300 mg kg$^{-1}$ treatment seedlings (III). Elevated temperature may increase the number of lateral roots or production of fine roots within a certain temperature range (Pregitzer et al., 2000). Fine roots are thought to be the main site of element absorption due to their high surface area to mass ratio (Johnson et al., 2006). We also observed that _P. tremula_ seedlings had much more fine roots under elevated temperature (Figure 10) (III), which might have resulted in more absorption of Ti from nTiO$_2$ contaminated soils. However, the concentration of Ti in roots was not high enough to affect taproot and shoot growth of _P. tremula_ seedlings. No significant differences in root Ti concentration were detected between females and males of _P. tremula_ (III).

**Figure 10.** Fine roots of _P. tremula_ seedlings under control (C), elevated temperature (T), elevated CO$_2$ (CO$_2$), and elevated both temperature and CO$_2$ (T+CO$_2$)
4 CONCLUSIONS

With the constant accumulation of PAHs and nTiO$_2$ in soil, at the same time as temperatures and CO$_2$ concentration increase, plants will inevitably be exposed to combined stress. This study presents new information on the interaction between climate change and soil contamination and its effects on growth and defensive phenolics in two boreal forest tree species. Based on my results, different tree species respond differently to soil pyrene contamination in combination with climate factors. Pyrene decreased growth and defensive phenolics in *P. abies* seedlings, and the reduction was aggravated under elevated temperature and CO$_2$ concentration. Moreover, the negative effects of combined stress on *P. abies* seedlings from southern origins were greater than those from northern origins. However, although pyrene moderately decreased growth of both sexes, *P. tremula* seedlings had a stronger chemical defense against pyrene than *P. abies* seedlings under elevated temperature and CO$_2$ concentration. This indicates that *P. abies* may be more subject to pest and pathogen damages than *P. tremula* due to lower growth and defense levels under a combination of soil pyrene contamination and future climate conditions. Both sexes of *P. tremula* seem to have a high capacity to regulate growth and metabolism to acclimate or adapt to multiple environmental stressors. In addition, plants of the two tree species studied also responded differently to different soil contaminants. Although nTiO$_2$ did not affect growth of either sex of *P. tremula* seedlings, it increased leaf phenolics in females, but not in males. This suggests that females have a better chemical defense against nTiO$_2$ than males, and males may get more herbivore damages in nTiO$_2$ contaminated soils under future climate conditions. In the long run, the combined stress of climate change and soil contamination may cause changes in the competitive abilities and the further evolution of both boreal tree species.

Based on the evidence presented in this study, further studies on the effects of climate change and soil contamination on the plant-soil-microbe system are needed to explore the mechanisms behind the effects on growth and defensive systems in boreal forest tree species. In addition, studies on the responses of trees to the multiple stressors in different climatic zones are also needed in order to predict and evaluate the environmental risk of soil contamination to trees in different regions under future climate conditions.
5 BIBLIOGRAPHY


Iqbal J, Metosh-Dickey C, Portier RJ (2007) Temperature effects on bioremediation of PAHs and PCP


Sigurdsson BD, Medhurst JL, Wallin G, Eggertsson O, Linder S (2013) Growth of mature boreal Norway spruce was not affected by elevated [CO$_2$] and/or air temperature unless nutrient availability was improved. Tree Physiology 33: 1192–1205.


YAODAN ZHANG

Future climate change and soil contamination may affect plant performance. Plants of different origins and different sexes may respond differently to the combined stress. This thesis provides knowledge about the interaction between climate change and soil contamination and its effects on growth and defensive phenolics in two boreal forest tree species, Picea abies L. Karsten and Populus tremula L. This knowledge may be useful for predicting and evaluating the environmental risk of soil contamination to boreal ecosystems under future climate conditions.