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Soil charcoal addition affected biochemistry but not growth in European beech and Norway spruce seedlings

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Preface

This thesis is part of the larger project “From spruce to beech forests - fundamental ecosystem transformation driven by climate change” were multiple aspects of the northern migration of beech are investigated. By becoming part of this research group, I have benefitted from the attention and genuine interest of several dedicated researchers to whom I owe my great thanks. A special thanks to my supervisors Line Nybakken, Johan Asplund and Mikael Ohlson. Also, I give thanks to Marit Helene Lie, Sebastian Knutsen, and Annie Aasen for additional help with lab work, especially on the elemental analysis, and to Jorunn E. Olsen and Ola M. Heide for being very helpful with practical tips regarding keeping the plants happy and growing inside the glasshouse. Thanks also to Matthias Göhl for proofreading on several occasions, and to Christian Bianchi Strømme for many interesting discussions on ecology and statistics. And finally, a great thanks to Marte Fandrem for her general support and assistance throughout the project.

Abstract

Climate change is projected to result in European beech (*Fagus sylvatica*) expanding northwards into Norway spruce (*Picea abies*) forests where, in addition to climate, numerous local factors will determine the relative success of these two species. Among these are soil related factors, including charcoal from previous forest fires, which is present in soils in considerable amounts and exerts largely unknown and species-specific effects on plant growth and metabolism. Here I show that glasshouse grown beech and spruce seedlings responded differently to laboratory-produced charcoal addition and that the effect was dependent on plant organ, charcoal origin (beech- or spruce wood), and soil type (beech- or spruce forest). Charcoal addition had no effect on plant biomass, but caused several compound specific changes to the concentrations of low molecular weight phenolics assumed important in plant defences. Shoot:root ratios, specific leaf area, condensed tannin concentrations, and C:N ratios were also affected, but in such a way that the overall positive versus negative effect of charcoal addition could not easily be determined. This was largely due to the organ specific responses that complicates interpretations of the whole-plant response. Overall, the effects of charcoal addition fades in comparison to the effect imposed by soil origin. Results further indicate an uncoupling between growth and phenolic synthesis, contrary to predictions from the protein competition model. The common consensus that soil charcoal has unequivocally beneficial effects on plant growth is challenged.

Sammendrag

Klimaendringene er sagt å føre til at bøk (*Fagus sylvatica*) sprer seg nordover inn i områder med granskog, hvor i tillegg til klima, flere lokale faktorer vil påvirke dominansforholdet mellom gran (*Picea abies*) og bøk. Blant disse er flere jord-aspekter, bl.a. effekten av trekull fra tidligere skogbranner. Trekull finnes i jorda, ofte i betydelige mengder, hvor det har en stort sett ukjent og artsspesifikk effekt på plantevekst og -metabolisme. Her viser jeg hvordan drivhusdyrkede småplanter av gran og bøk reagerte ulikt på tilsetning av laboratorieprodusert trekull til vekstjorda og at effekten av kullbehandlingen var avhengig av opphavet til jorda (bøk- eller granskog) og trekullet (bøk- eller granved), i tillegg til at den ofte var ulik for forskjellige plantedeler. Trekull hadde ingen påvirkning på vekst (biomasse), men induserte flere endringer i konsentrasjonene av ulike lav-molekylvekt-fenoler som man antar er viktige i det kjemiske forsvaret hos planter. Tilsetning av trekull påvirket også skudd:rot forholdet, SLA (bladareal/tørrvekt), konsentrasjonen av kondenserte tanniner, samt karbon:nitrogen forholdet, men pga. kontrasterende resultater i de ulike plantedelene er det vanskelig å fastslå om den totale effekten var overveiende positiv eller negativ. Effekten av trekulltilsetning var veldig liten sammenlignet med påvirkningen av jordtype. Resultatene indikerer videre at det ikke eksisterer en enten-eller dynamikk mellom vekst og fenolsyntese, i strid med prediksjonene fra proteinkonkurransemodellen (en: protein competition model). Den rådende tanken om at trekull har en utelukkende positiv effekt på planter blir herved utfordret.

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Introduction

European beech (*Fagus sylvatica*; hereafter just beech) is a common, late successional temperate tree species, reaching its northernmost limit in Norway (Bolte *et al.* 2007) where it commonly competes with Norway spruce (*Picea abies*; hereafter just spruce) (Bjune *et al.* 2013). Biogeographic distribution models have predicted a strong north and eastward range expansion of beech as a result of climate change, especially due to increased precipitation and milder winter conditions (Sykes *et al.* 1996, Hickler *et al.* 2012, Saltré *et al.* 2014), that will alleviate the physiological restrictions which dictate the current range limit (Bolte *et al.* 2007). This would cause beech to expand further into the present boreal forest zone were spruce is the dominant species. Beech and spruce seedlings are both very shade-tolerant, and newly germinated beech seedlings under spruce canopies are more responsive to belowground resources than to light levels (Ammer *et al.* 2008). Wildfire-produced charcoal from previous fires is present in most northern forest soils (Zackrisson *et al.* 1996, Ohlson *et al.* 2009, Ohlson *et al.* 2013), often in considerable quantities, and represent a functional aspect of fire that is little understood. Soil charcoal has been shown to induce many changes in plant productivity (Pluchon *et al.* 2014), ecosystem processes (Zackrisson *et al.* 1996) and carbon stocks (Wardle *et al.* 2008), though soil- and species specific responses makes generalisations difficult.

Pluchon et al. (2014) found spruce to respond positively to 4 out of 6 charcoal types derived from angiosperm trees, but not to ericaceous- or gymnosperm-derived charcoal, including spruce-derived charcoal. Furthermore, the positive effects were only found in one out of two contrasting soils. To my knowledge, no studies have been done on the performance of beech in relation to soil charcoal levels. It is unknown whether beech would respond to charcoal addition in ways similar to the ecologically very analogous species spruce, or the phylogenetically more closely related angiosperm trees (see Pluchon *et al.* 2014).

Fire is a spectacular and ubiquitous force in most forest regions, including Canada (Bond-Lamberty *et al.* 2007), Russia (Shvidenko and Nilsson 2003), and Fennoscandia (Zackrisson 1977), and is considered a major driver of vegetation dynamics (Clear *et al.* 2013). During pyrolysis, typically 1-3% of the biomass is turned into charcoal (sometimes referred to as char, black carbon, or biochar) (Preston and Schmidt 2006). Landscape variation in soil charcoal is considerable, even over very short distances (Zackrisson *et al.* 1996, Ohlson *et al.* 2009, Kasin *et al.* 2013, Ohlson *et al.* 2013). Ohlson *et al.* (2009) found site averages ranging from zero to 400 g m^{-2} , with a maximum value of 5137 g m^{-2}. This can be a result from the clumped production of charcoal from logs and stumps (Ohlson *et al.* 2013), unequal transport

(Ohlson and Tryterud 2000), or of later soil disturbances such as tree uprooting (Gavin 2003), bioturbation or freeze-thaw events (Carcaillet 2001).

The influence of fire on Fennoscandian forest have declined over the last centuries, mainly due to active anthropogenic suppression and the presence of non-fire prone spruce forests (Zackrisson 1977, Niklasson and Granstrom 2000, Niklasson and Drakenberg 2001, Tryterud 2003, Ohlson *et al.* 2011, Clear *et al.* 2013), but climate change is expected to increase fire risk in the future (Flannigan *et al.* 2009), a trend that has already been observed in North America (Gillett *et al.* 2004, Westerling *et al.* 2006). As the amount of soil charcoal is related to fire history and the surrounding area that has been burned (Ohlson *et al.* 2009, Kasin *et al.* 2013), this can lead to a higher impact from charcoal on forest ecosystems in the future.

Both beech and spruce are regarded as fire sensitive due to their thin bark, although beech can potentially regenerate quickly after fire (Packham *et al.* 2012). Many studies have documented a negative correlation between beech pollen and charcoal fragments, indicating that beech abundance decreases under a more severe fire regime (Tinner *et al.* 1999, Hannon *et al.* 2000, Bradshaw and Lindbladh 2005, Bjune *et al.* 2013, Bradley *et al.* 2013). However, Bradshaw and Lindbladh (2005) also demonstrated the importance of stand replacing fires as precursors to beech establishment in southern Sweden.

Soil charcoal affects the growth of plants. Meta-analysis have showed variable, but overall positive effects of charcoal addition on aboveground productivity, but mainly for annual plants in not too acidic soils (Jeffery *et al.* 2011, Biederman and Harpole 2013). Possible effects of soil charcoal include fertilisation and input of nutrients (Pluchon et al. 2014), increased microbial activity and decomposition (Zackrisson *et al.* 1996, Wardle *et al.* 2008), increased nitrification (DeLuca *et al.* 2006), adsorption of allelopathic compounds (Nilsson 1994, Zackrisson *et al.* 1996, Wardle *et al.* 1998, Keech *et al.* 2005, but see Lau *et al.* 2008); improved water storage capacity, and increased cation exchange capacity, pH and bulk density (Laird *et al.* 2010).

Besides growth, plant responses to the soil environment also include changes in plant biochemistry and resource allocation (Chapin 1980). Stress and environmental disturbance, as defined by Grime (1977), can both act to limit production (i.e. growth and reproduction). Resource allocation is often seen as a trade-off between production and defence, where one comes at the expense of the other in a world of limited resources (Bryant *et al.* 1983, Coley *et al.* 1985, Fine *et al.* 2006). Fast growth is generally predicted when there is strong competition between plant species for resources and light, whereas high defence investments are expected when disturbance levels are high.

Phenolics are carbon based plant secondary metabolites (PSM) which are ubiquitous in plants and probably serve defensive roles as diverse as herbivore deterrents (Bryant *et al.* 1983, Coley *et al.* 1985, Dübeler *et al.* 1997), antioxidants (Iason and Hester 1993, Hagerman *et al.* 1998, Close and McArthur 2002), pathogen protection (Tomova *et al.* 2005), and UV-filtration (Lois 1994). High levels of phenolics in plant tissues are generally interpreted as an investment in chemical defence (Tomova *et al.* 2005), although they may serve several other functions outside the plants, for example as allelopathic elements or by affecting decomposition rates (Kraus *et al.* 2003). Older theories devised to predict growth-defence allocation patterns have focused on constitutive defences in an evolutionary framework (Bryant *et al.* 1983, Coley *et al.* 1985, Herms and Mattson 1992). Decades of hypothesis testing have failed to confirm any of these models as they consistently fail to predict actual concentrations of PSM (Hamilton *et al.* 2001). The protein competition model is a mechanistic model that assumes a process-based competition through scavenging between protein and phenolic synthesis for their common precursor compound, phenylalanine (Jones and Hartley 1999). Environmental conditions may cause one pathway to become favoured over the other (i.e. an induced response), thus growth is predicted to be negatively correlated to concentrations of phenolics, regardless of their function in the plant.

The concentrations of condensed tannins (high molecular weight polyphenolics) in leaves of beech (Påhlsson 1992) and *Betula pendula* (Keinänen et al. 1999) have been found to decrease in response to fertilisation, and similarly decreased along a natural gradient with increasing leaf mineral nutrient content (Påhlsson 1989). As plant phenolics are so diverse, compound specific analysis can yield idiosyncratic results. Tomova *et al.* (2005) found that individual low molecular weight phenolic compounds in roots of beech and spruce responded very differently to nitrogen fertilisation, with some increasing, and others decreasing in concentration. Biochemical changes in northern forest trees as a response to charcoal addition has to my knowledge not been investigated.

Charcoal origin affects plant responses to charcoal addition. Pluchon *et al.* (2014) found angiosperm-derived charcoal to be more beneficial to plant growth, than gymnosperm-derived charcoal, possibly due to higher levels of phosphorous (fertilising effect). Angiosperm-derived charcoal also possess greater quantities of macro-pores which is more prevailing in angiosperm trees (Keech *et al.* 2005), and that enhance its ability to adsorb chemical compounds, notably allelopathic phenols, in the soil (Zackrisson *et al.* 1996). In addition, angiosperms trees are assumed to be more responsive to edaphic factors, consistent with their dominance on resource-

rich soils (see Bond 1989), and this is supported by studies with charcoal additions (e.g. Wardle *et al.* 1998, Pluchon *et al.* 2014).

By using an integrated species distribution and migration model, Saltré *et al.* (2014) predicts only a very small colonisation ability for beech in the future, seen as a large discrepancy between potential and realised niche space, due to limiting migration velocity, larger climatic variability and extinction risk, land-use changes, and a low life-history. Detailed information on several aspects of beech autecology can help improve this model and make it more locally explicit. The notorious difficulty of predicting the contribution of long distance dispersal events to migration rates is still a large challenge and weakness for all species distribution models (Vittoz and Engler 2007), and this is also very true and highly relevant for beech specifically (Kunstler *et al.* 2007). In addition, edaphic factors is usually absent in bioclimatic models that focus on larger, often continental scales, and therefor lack this level of detail that can vary at site or stand-level. The presence of soil charcoal has the potential to affect, and perhaps shift, the relative competitive advantage between species and could thus influence the local establishment success of beech, which is expanding into forest regions dominated largely by spruce. Zhu *et al.* (2012) found from examining the demographic structure of 92 North-American tree species that climate-mediated migration is essentially not happening. This further highlights the need to understand the limitations for both migration and establishment in order to predict the colonisation ability of trees in the face of environmental change.

Here I present an experiment that tested the effect of soil charcoal addition on the growth and biochemistry of beech and spruce seedlings. To accomplish this, a full factorial experiment was conducted, with two different charcoal types (beech- and spruce-derived) and two soil types (from beech and spruce forests) as explanatory variables. By growing the seedlings in two contrasting soil types, both the level of detail and the generality of the response to charcoal treatment is enhanced. In addition, correlations of the response variables are explored further in order to investigate potential trade-offs in resource allocation between growth and defence. The questions to be answered are: (1) Do the seedlings respond to charcoal treatment? (2) Does charcoal origin make a difference? (3) Is the effect of charcoal treatment soil specific? (4) Are there signs of a trade-off between growth and defence? The results are discussed in light of the potential northward expansion of beech.

Methods

2.1 Sampling

Soils were sampled on 4 April 2014 from a beech (59°13'N; 10°21'E; 41 m.a.s.l.) and spruce forest (59°13'N; 10°19'E; 74 m.a.s.l.) 1.9 km apart, in an area around Melsomvik, Vestfold, S Norway. Both soils had supported their respective forest types in excess of two forestry cycles (>140 years). Sampling included the entire depth of the humus layer. The beech forest supported a low herb vegetation and showed an abundance of *Anemone nemorosa* and earthworms at the time of sampling. The soil was a brown soil with no signs of podsolisation, and the humus was about 15 cm thick. The spruce forest was of the *Vaccinium* type and the forest floor was sparsely vegetated. The soil had clear podsolisation and the humus layer was variable, from 2 cm deep in shallow areas, to very deep in areas of peat formation. The wetter areas were not sampled. The soil was stored in plastic bags in a cool room (~10°C) for three days before being sieved through a 1 cm mesh and homogenised by hand, and then stored in plastic containers until they were added to pots. Pots with spruce soil had a dry weight of 116,5 g (N=1) compared to 206,1 g (N=1) for beech soil.

Beech seeds, marketed for use in Götaland, S-Sweden, were purchased from Svenska Skogsplantor AB (Hallsberg, Sweden), while spruce seeds of provenance CØ1 were provided by The Norwegian Forest Seed Center (Hamar, Norway).

2.2 Charcoal production

Fresh spruce and beech branches were sampled from a small number of individuals growing in Ås, S-Norway (59°39'N; 10°47'E; 94-126 m.a.s.l) The bark was stripped off and the wood dried for 48 hours at 70°C. Maximum diameter of the wood was 2 cm, and larger branches were split lengthwise. Charcoal was prepared in a muffle furnace (B170; Nabertherm GmbH, Lilienthal/Bremen, Germany). Sticks of wood were covered in sand and placed in a steel box with a lid and the box inserted into a cold oven that gradually heated up to 450°C within 40 min, and was left there for 30 min after which the oven was turned off and the wood was allowed to cool inside the oven. Although time intervals differ slightly, the maximum temperature is the same as used by Pluchon *et al.* (2014) and Keech *et al.* (2005), and is within the temperature range commonly encountered in forest fires (Schimmel and Granström 1996). The charcoal was crushed and sieved to retain fragments between 0.6 and 2.0 mm, which is representative of the charcoal dimensions commonly found in soils (Zackrisson *et al.* 1996, Ohlson *et al.* 2009).

2.3 Experimental design

A full factorial glasshouse experiment was conducted with ten replicated blocks, each containing all treatment combinations (Figures 1, 2). Factors were soil type (beech and spruce soils), charcoal type (beech, spruce and control), and seedling species (beech and spruce). Pots were assigned random identity numbers. Plants were grown in *near*-natural light conditions (58° north) for 86 days between 28 May (latest 9 June) and 3 September 2014. Favourable light conditions and day length was ensured by accentuating the far red light spectrum using 12 normal 60W incandescent light bulbs placed approx. 1.5 m above the plants day-round from 10 June. Relative humidity was constant at 65%. Temperature was stable at 20 °C during the day and 16 °C between midnight and 6am. Blocks, and individual pots within blocks, were systematically shifted around every 2-3 weeks to remove any non-random room- or neighbour effects, respectively. Approx. 2 dl water was supplied every two days.

Pots (9 x 9 x 11 cm high) were filled with soil (23 April) to 9 cm with charcoal added as a single layer two thirds from the bottom of the pot, which is an arrangement shown to give the best utilisation of charcoal benefits by plants (Makoto *et al.* 2010). Then the pots were left for a minimum of 35 (maximum 45) days to allow time for secondary compounds to become absorbed (Makoto *et al.* 2010). The soil was kept moist during this time to ensure continued microbial activity. Seedlings were germinated in a sand medium were they were kept until the unfolding of the cotyledons, then four at a time were continuously transplanted into each pot in order of their assigned block number. The smallest of the seedlings were gradually removed until only the largest remained after approx. 14 days.

Charcoal was added to equate 300 g m^{-2}, which corresponds to 2.43 g per pot, as calculated from the top surface of the pots (81 cm^2). This is in the upper range of what is found in natural boreal soils when averaged across landscapes, but still much lower than what is reported from individual soil samples (Ohlson *et al.* 2009). Del Olmo (2014) reported similar charcoal levels from beech forests in Larvik, Vestfold, Norway.

After transplanting the beech seedlings from a sand to soil medium, all plants initially underwent apical bud formation, most likely due to root stress (Figure 3). Plants continued apical growth within 8 to 28 days (median 18), the timing of which was not explained by any treatment factor (Kruskal-Wallis rank sum test; χ^2= 0.7817, df=5, p=0.8538).

Height measurements were conducted every two weeks by moderately stretching the plants into upright position and taking the length from the soil surface to the plant apex. Soil pH was measured immediately after transplantation and after harvest (inoLab pH 720 with SenTix 81 pH electrode; WVW GmbH, Weilheim, Germany).

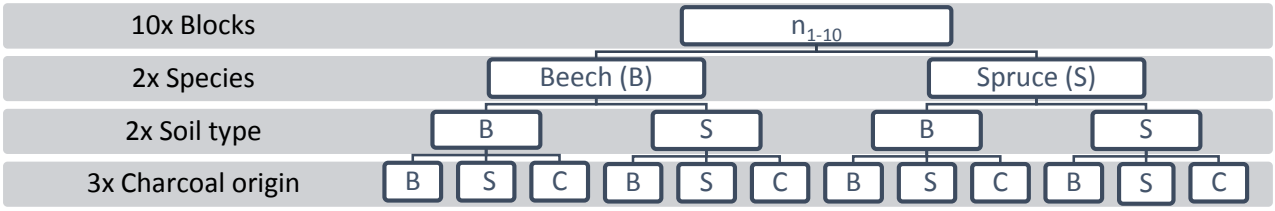

Figure 1. The layout of the experimental design. C = control.


(1)

(2)

Figure 2. Experimental design: (1) Beech and spruce seedlings were grown side by side in a randomised full factorial setup, with metal trolleys defining the blocks. (2) Each block had 12 pots and contained all the treatment combinations.

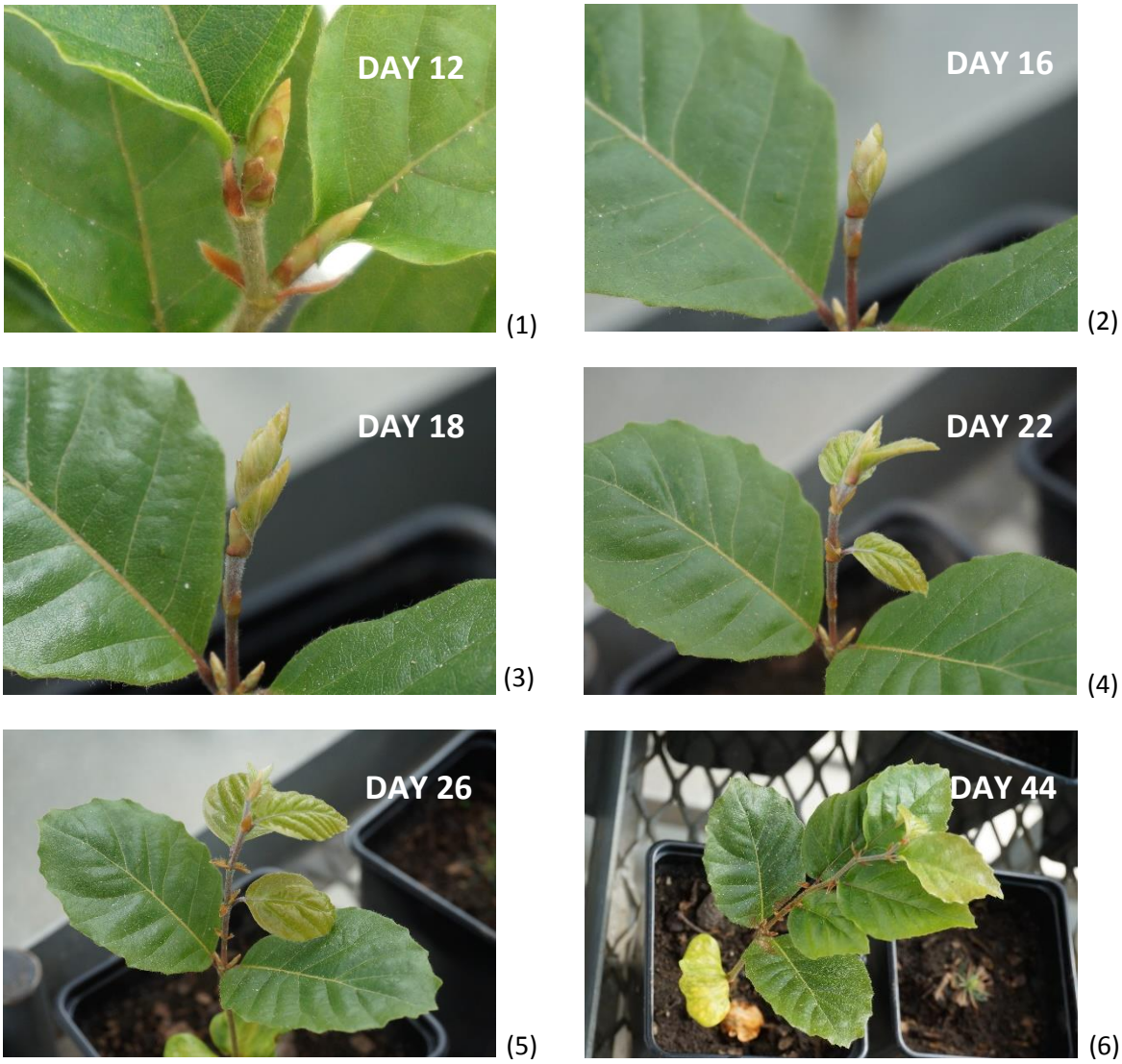

Figure 3. Sequence of photos showing a beech seedling undergoing bud break and forming new leaves: (1) Bud scales developed around the buds of all transplanted beech seedlings; (2) After a couple of weeks the apex started elongating; (3) Leaves started breaking out; (4) The buds eventually opened and new leaves emerged, often in clusters; (5) New leaves folded out quickly as they were already largely formed inside the buds. (6) A healthy and vigorous beech seedling with several darkening leaves and one remaining cotyledon.

2.4 Harvesting

Following harvesting, individual plant parts were put into separate and marked paper bags and dried at 30°C for a minimum of 48 hours. Due to time restrictions, some pots were kept in a dark fridge for a maximum of one day before they could be cut and dried. All spruce needles, or two fully developed beech leaves between 3 and 13 cm from the apex, were kept for chemical analysis. Leaf surface area was measured on the same two beech leaves used for chemical analysis (LI-3100 Area Meter; LI-COR inc., Lincoln, NE, USA). The top three cm of beech seedlings and the apical bud of spruce seedlings were excluded from chemical analysis done on

plant stems. The roots were washed to remove the soil. After drying, the plants were weighed and stored in a freezer (-20°C).

2.5 Chemical analysis

2.5.1 Extraction and elemental analysis

Plant material was removed from the freezer and grinded to fine powder using a Retsch MM400 ball mill (Retsch, Haag, Germany) at 30 revolutions per sec. for 30 – 180 sec. From this we determined total nitrogen (N) and carbon (C) with a Micro Cube (Elementar Analysen, Hanau, Germany), using 5-6 mg plant meterial. Further sub-samples of ca. 10 mg were extracted with 600 µl methanol (MeOH) and homogenised with 3-4 zirconium oxide balls at 5000 rpm for 20 sec. on a Precellys 24 homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France). Samples were then cooled on ice for 15 min before being centrifuged at 15000 rpm for 3 min (Eppendorf centrifuge 5417C, Eppendorf, Hamburg, Germany). The supernatant was transferred to a 10 ml glass tube with a Pasteur pipette. The residue was again dissolved in 600 µl MeOH, homogenised, and centrifuged in the same manner as above; the supernatant was removed, and the same extraction process was conducted two more times until both the residue and the supernatant was completely colourless. The combined supernatants were evaporated in a vacuum centrifuge (Eppendorf concentrator plus; Eppendorf, Hamburg, Germany), sealed, and stored in a freezer (-20°C) until high performance liquid chromatography (HPLC) analysis. The residues were also stored in a freezer for further analysis on MeOH-insoluble condensed tannins.

2.5.2 HPLC

The extracts were dissolved in 200 µl MeOH with the help of a VWR ultra sonic cleaner (mod. no. USC200TH; VWR International LLC, Randor, USA), and diluted with 200 µl ultra-clean water (USF ELGA Maxima HPLC; Veolia Water Technologies, Saint-Maurice, France). Samples were poured into 2 ml Eppendorf tubes and centrifuged at 15000 rpm for 3 min before being forced through a syringe filter (GHP Acrodisc 13 mm Syringe Filter with a 0.45 µm GHP membrane; PALL Corporation, Washington, USA) and sealed inside HPLC vials.

Low molecular weight phenolics were analysed using a HPLC system (Agilent Series 1200, Agilent Technologies, Waldbronn, Germany) with a G1312A binary pump, a G1329A autosampler, a G1316A thermoregulated column heater, and a G1315D diode array detector. As the stationary phase a Thermo Scientific column type was used (Thermo Fisher Scientific

Inc, Waltham, USA), with a 50 x 4.6 internal diameter and filled with ODS Hypersil (3 µm) particles. The mobile phase consisted of two solvents that eluted the samples by way of a gradient shown in Table 1 (Julkunen-Tiitto and Sorsa 2001). Injection volume varied from 20 – 60 µl depending on phenolic contents of the samples.

The absorption spectra at 270 and 320 nm, along with respective retention times, were used to identify the chemical compounds and to calculate concentrations by comparing with the commercial standards listed in Table 2:

Table 1. Gradient of solvents used in HPLC analysis. Solvent A = 1.5% tetrahydrofuran + 0.25% phosphoric acid + ultra clean water. Solvent B = 100% HPLC grade methanol.

Time (min)	%A	%B
start	100	0
5	100	0
10	85	15
20	70	30
40	50	50
45	50	50
46	0	100
58	0	100
60	100	0

Table 2. List of commercial standards used to identify and compute concentrations of phenolic compounds. Supplier 1; Sigma-Aldrich (St. Louis, USA). Supplier 2; Polyphenols (Sandnes, Norway).

Standards	Applied to the following compounds	Supplier
Myrectin-3-rhamnoside	Myricetin derivatives	1
Quercetin-3-glucoronide	Quercetin derivatives	1
Neochlorogenic acid	Chlorogenic acid and derivatives; p-OH-cinnamic acids; ellagic acid	1
(+)-catechin	(+)-catechin and derivatives	1
Kaempferol-3-glucoside	Kaempferol-3-glucoside and derivatives	1
Picein	Picein	1
Reservatrol	Resveratrol	1
Luteolin-7-glucoside	Luteolin-7-glucoside	1
Apigenin-7-glucoside	Apigenin-7-glucoside	1
Tannic acid	Condensed tannins	1
E-astringin	E-astringin and derivatives	2
Isorhaphontin	Isorhaphontin	2

2.5.3 Condensed Tannins (CT)

Concentrations of both MeOH-soluble and MeOH-insoluble condensed tannins (CTs) were identified using the acid butanol assay for proanthocyanidins described in Hagerman (2002). This is the most commonly used method for determining concentrations CTs in plant tissues

(Schofield *et al.* 2001). The HPLC-vials were removed from the auto sampler maximum 48 hours after analysis and from these 50-140 µl were used, depending on predicted CT concentration, to determine the amounts of MeOH-soluble CTs. The amount of MeOH-insoluble CTs were analysed from the residues left after the extraction process. The samples were put in 15 ml glass tubes along with enough MeOH to equal 1ml in total (1 ml MeOH regardless for MeOH-insoluble tannins), then further mixed with 6 ml butyric acid (95% butanol, 5% hydrochloric acid), and 200 µl iron reagent (2 M HCL with 2% ferric ammonium sulphate). The glass tubes were properly sealed, mixed, and placed in boiling water for 50 min. Duplicate samples was prepared when extract amounts allowed. After cooling, the light absorption at 550 nm was determined using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan). The average between duplicate samples was used as one data value. Samples were discarded when obvious evaporation had taken place from the glass tubes. Purified tannins from *Betula nana* (dwarf birch) leaves was were used as standards to calculate concentrations.

2.6 Statistical analysis

Statistical tests were performed in R-Studio (version 0.98.501; R version 3.0.2) and figures were prepared in Veusz (version 1.21) and R-Studio.

Linear mixed effects models (*lmer*-function; *lmerTest*-package) were used to look for any effects of soil type, charcoal type, and their interaction, on plant biomass (total-, aboveground-, and belowground biomass), shoot:root ratio (S:R), specific leaf area (SLA; cm^2 per gram dry weight), plant height, soil pH, and the concentrations of chemical compounds. Species were analysed separately, except when analysing changes in soil pH. Block was included as a random factor with random intercept. Interaction terms were excluded from the minimal adequate model if they did not cause a significant increase in diversion upon removal, as determined by chi-square ANOVA deletion tests. Main effects were analysed by using ANOVA's on the model terms (reported in tables). Within-factor contrasts were investigated directly from the mixed effect model output (reported in figures). All data was tested for assumptions of normality and homogeneity of variance and transformed if these requirements were not met. If problems persisted, a Kruskal-Wallis rank sum test was performed using a single explanatory variable with six levels (the six treatment combinations). When the initial test yielded significant results, a post hoc analysis was performed (*kruskalmc*-function*; pgirmess*-package).

Initial soil pH was analysed using Welch t-test for unequal variance. Due to lack of normality, Kendall's tau correlation tests were performed to look for associations between response variables (*corr.test*-function*; psych*-package).

Results

3.1 pH

Beech-derived charcoal had a mean pH of 8.21 (N=2), and spruce derived charcoal had a mean pH of 7.30 (N=2). There was a small but significant difference in initial mean soil pH between beech soil (4.20 ± 0.007 SE, N= 30) and spruce soil (4.12 ± 0.009 SE, N= 30) (Welch t-test; t=7.3, df =53.9, p<.0001). Changes in soil pH under the seedlings after 86 days (Figure 4) was explained by soil type (ANOVA on mixed model terms; $F_{1, 46}$=52.9, p<.001), seedling species ($F_{1, 46}$=18.3, p<.001), but not charcoal ($F_{1, 46}$=0.5, p=.633). The pH increased by 0.09 units in beech soils and decreased by 0.01 units in spruce soils, and similarly decreased under beech seedlings by 0.05 units and increased under spruce seedlings by 0.06 units.

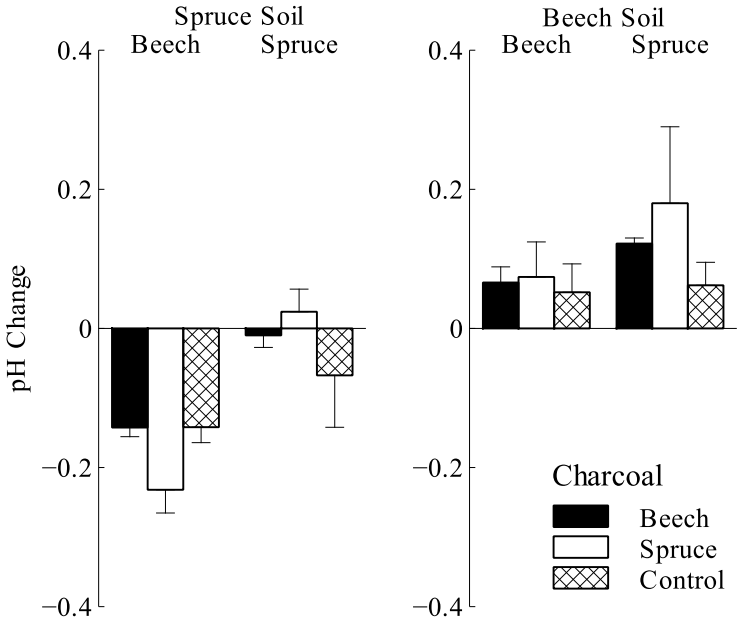

Figure 4. Mean change in soil pH under beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings grown for 86 days in two contrasting soils and charcoal types. Error bars are 1SE. No contrasts between charcoal types were significant within each soil type (mixed effects model; 0.05 sig. level).

3.2 Seedling growth

Soil, but not charcoal, had a significant effect on plant biomass (Table 3; Figure 5). For both spruce and beech seedlings, total plant biomass was greatest in spruce soil (Table 3). For beech seedlings, this was largely due to increased root growth (Figure 5). Spruce seedlings remained very small when grown in beech soil, and showed only a slight tendency for etiolation after week 6-8 (Figure 6). Contrastingly, beech seedlings grew taller in beech soil compared to spruce soil and had a higher S:R ratio (Figure 7) and a SLA (Figure 8). The soil-charcoal interaction significantly explained differences in S:R ratios of beech seedlings (Table 3) and beech derived charcoal significantly lowered the S:R ratio for beech seedlings grown in beech soil (Figure 7). For spruce seedlings, charcoal addition had contrasting, though non-significant effects on S:R in the different soils.

The addition of beech-derived charcoal, in combination with beech soil, caused a significant decrease in beech SLA compared to both spruce derived charcoal and to the control, making them similar to leaves from plants grown in spruce soil (Table 3; Figure 8).

Table 3. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on growth characteristics of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) seedlings. Numerator and denominator degrees of freedom are given in subscript. Response variables that were analysed using Kruskal-Wallis rank sum test (KW) are reported with a Chi-square statistic and degrees of freedom in subscript. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B indicate whether spruce or beech soil had the highest values of the response variable, respectively. Non-significant interaction terms that are excluded from the minimal adequate model (marked ‡) are reported as chi-square statistics from ANOVA chi-square deletion tests.

Response variable	Soil	Charcoal	Soil x Charcoal
Beech seedlings			
Height	$31.095_{1, 54.0}$ ***B	$1.222_{2, 54.0}$	0.884_{2}‡
Total biomass†	$21.709_{1, 47.0}$ ***S	$0.358_{2, 47.0}$	0.616_{2}‡
Aboveground biomass	$0.356_{1, 56.0}$	$0.271_{2, 56.0}$	1.216_{2}‡
Belowground biomass	$99.909_{1, 47.0}$ ***S	$0.439_{2, 47.0}$	1.161_{2}‡
Shoot:root††	$77.624_{1, 54.0}$ ***B	$2.673_{2, 54.0}$	$4.690_{2, 54.0}$ *
SLA†	$32.184_{1, 54.0}$ ***B	$2.404_{2, 54.0}$	$2.937_{2, 54.0}$
Spruce seedlings			
Height†	$104.368_{1, 47.0}$ ***S	$1.808_{2, 47.0}$	0.798_{2}‡
Total biomass $_{(KW; 43, 5)}$	Sig. ***S	Non-Sig.	-
Aboveground biomass†	$148.694_{1, 56.0}$ ***S	$0.978_{2, 56.0}$	2.244_{2}‡
Belowground biomass†	$176.867_{1, 56.0}$ ***S	$2.286_{2, 56.0}$	0.235_{2}‡
Shoot:root	$18.634_{1, 47.0}$ ***S	$0.094_{2, 47.0}$	4.572_{2}‡

† = log-transformed
†† = sqrt-transformed
‡ = Chi-square statistic from deletion test

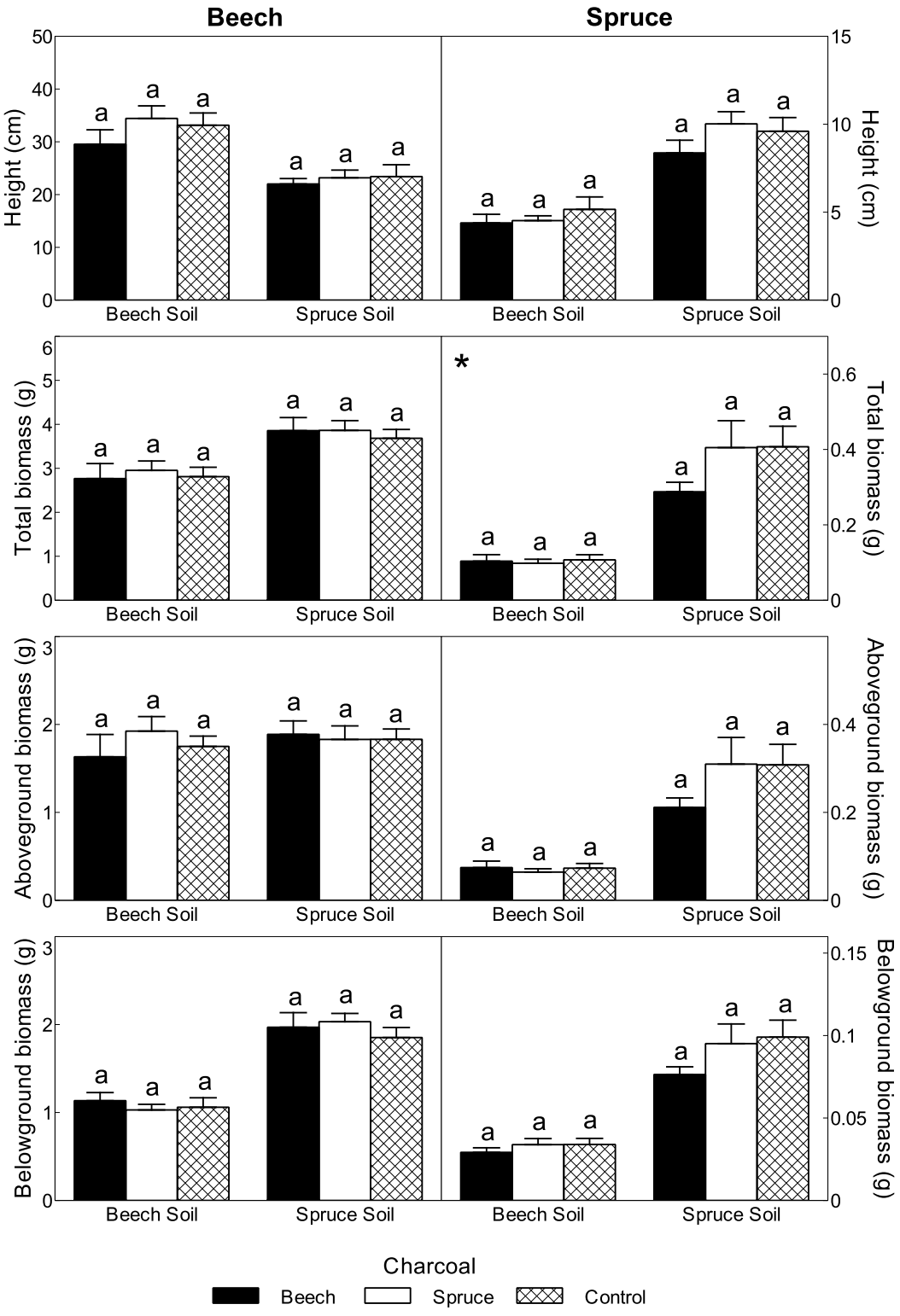

Figure 5. Mean plant height, total plant biomass, aboveground biomass, and belowground biomass for beech *Fagus sylvatica* (left column) and spruce *Picea abies* seedlings (right column). Error bars are 1SE. Different lower-case letters indicate significant differences for each soil type (mixed effects model or Kruskal Wallis rank sum test (latter marked with star (*)); 0.05 sig. level).

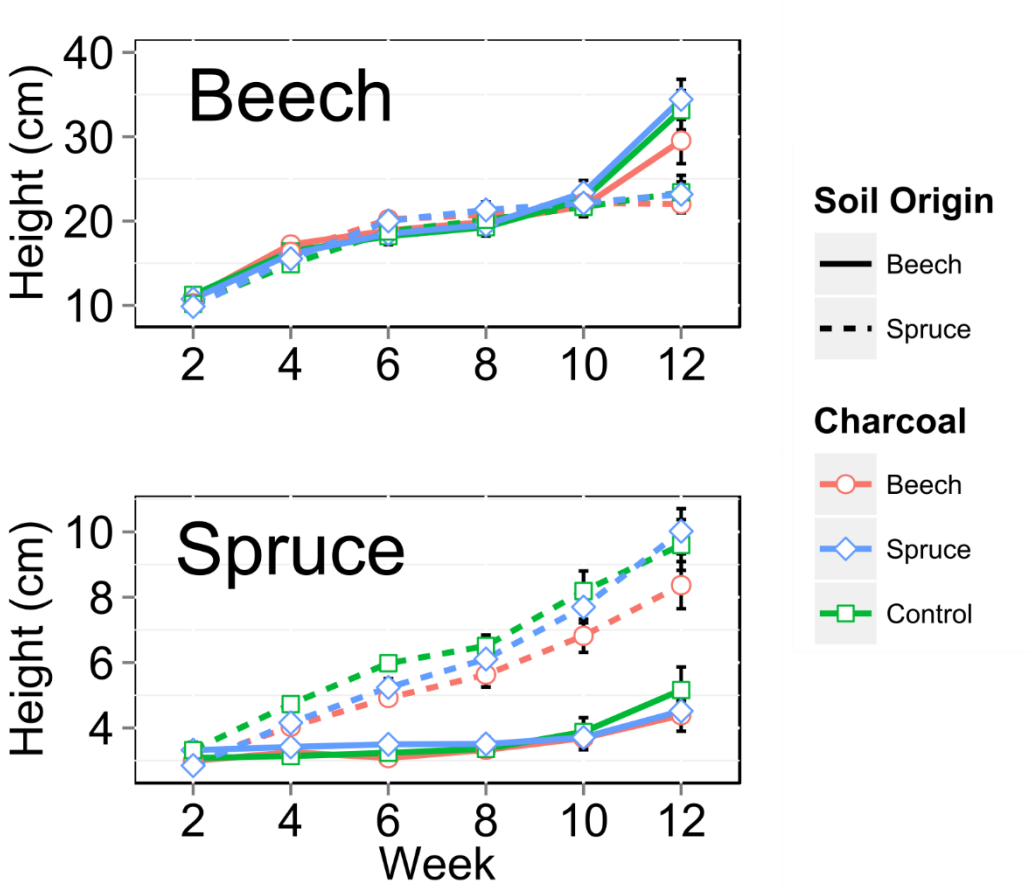

Figure 6. The cumulative height growth of beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings grown for 86 days in different soil and charcoal types. Error bars are ± SE.

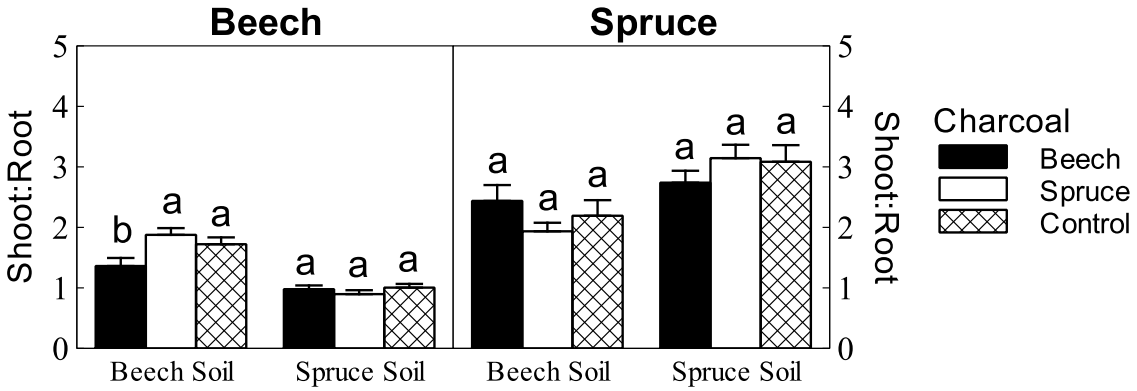

Figure 7. Mean shoot:root ratios for beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings grown in two different soils and with two different charcoal types. Error bars are 1SE. Different letters indicate significant differences within each soil type (mixed effects model; 0.05 sig. level).

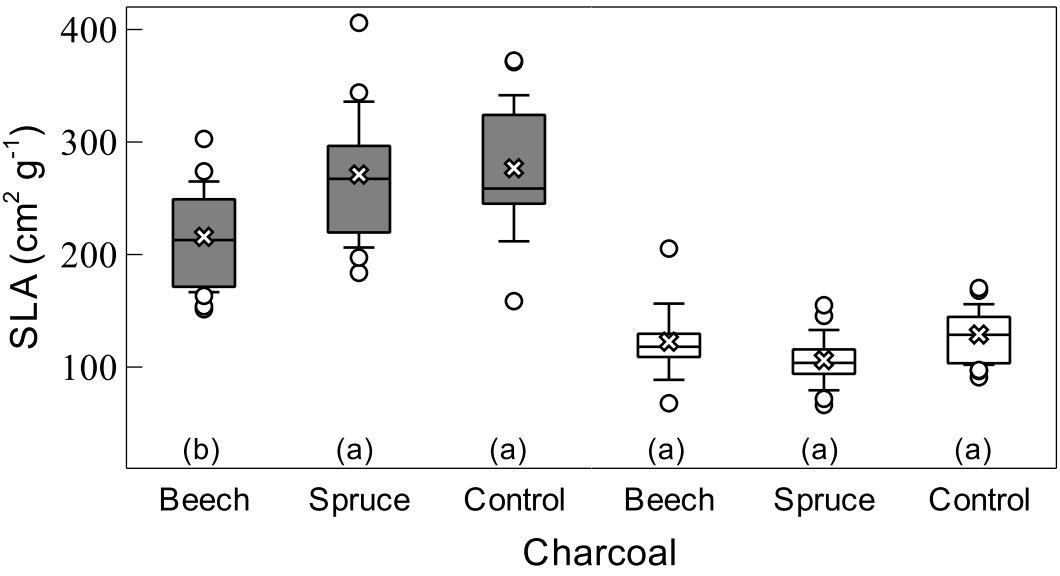

Figure 8. Specific leaf area (SLA) of beech (*Fagus sylvatica*) seedlings grown in either beech soil (gray boxes) or spruce soil (white boxes) and with different charcoal types. For each soil type, different lower-case letters (in parentheses) indicate significant differences in mean (mixed effects model; 0.05 sig. level). Center line is the median, crosses represent the mean, boxes represent the interquartile range, and whiskers are 1 SD.

3.3 Condensed tannins

Soil type significantly effected CT concentrations, but often in opposite directions for different plant parts and for the two classes of CTs (Table 4). Charcoal type explained variations in CTs only for beech stems (Table 4) where charcoal addition generally lead to reduced concentrations compared to controls (Figure 11). In addition, the ratio of MeOH-soluble vs. insoluble CTs (the sol:ins ratio) was lowered in spruce leaves by the addition of beech-derived charcoal. CT concentrations were approximately equal between beech and spruce seedlings in roots (Figure 9), but was notably higher for beech seedlings in both leaves (Figure 10) and stems (Figure 11). Insufficient plant material was available to test for soil effects on CT concentrations in spruce stems.

Table 4. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on condensed tannin concentrations in beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings. Numerator and denominator degrees of freedom are given in subscript. Response variables that were analysed using Kruskal-Wallis rank sum test (KW) are reported with a Chi-square statistic and degrees of freedom in subscript. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B indicate whether plants grown in spruce or beech soil had the highest concentrations, respectively. No interaction terms were significant and are therefore omitted from the table.

Response variable	Soil	Charcoal
Beech seedlings		
Total condensed tannins		
Roots	$13.127_{1, 53.0}$ ***S	$0.285_{2, 53.0}$
Leaves$_{(KW; 4.68, 5)}$	Non-Sig.	Non-Sig.
Stems	$9.556_{1, 52.0}$ **B	$3.176_{2, 52.0}$ *
MeOH-insoluble tannins		
Roots†	$0.012_{1, 43.3}$	$1.319_{2, 43.4}$
Leaves†	$29.142_{1, 53.7}$ ***B	$1.348_{2, 53.7}$
Stems	$111.398_{1, 52.0}$ ***B	$4.124_{2, 52.0}$ *
MeOH-soluble tannins		
Roots	$19.511_{1, 56.0}$ ***S	$0.363_{2, 56.0}$
Leaves $_{(KW; 17.2, 5)}$	Sig. ***S	Non-Sig.
Stems	$4.191_{1, 44.4}$ *B	$2.953_{2, 44.3}$
Ratio sol:ins		
Roots	$10.036_{1, 44.5}$ **S	$2.444_{2, 44.5}$
Leaves$_{(KW; 28.3, 5)}$	Sig. ***S	Non-Sig.
Stems	$59.993_{1, 52.0}$ ***S	$0.871_{2, 52.0}$
Spruce seedlings		
Total condensed tannins		
Roots	$10.758_{1, 53.0}$ **S	$0.011_{1, 53.0}$
Leaves	$12.395_{1, 45.7}$ ***B	$0.104_{2, 45.8}$
Stems	*excl.*	$0.959_{2, 6.3}$
MeOH-insoluble tannins		
Roots	$15.819_{1, 53.0}$ ***S	$0.255_{1, 53.0}$
Leaves	$3.116_{1, 45.7}$	$0.888_{2, 45.7}$
Stems	*excl.*	$0.795_{2, 8.7}$
MeOH-soluble tannins		
Roots	$9.317_{1, 55.0}$ **S	$0.049_{2, 55.0}$
Leaves	$49.663_{1, 55.0}$ ***B	$0.906_{2, 55.0}$
Stems	*excl.*	$0.526_{2, 7.2}$
Ratio sol:ins		
Roots	$0.610_{1, 53.0}$	$0.185_{2, 53.0}$
Leaves	$32.812_{1, 43.3}$ ***B	$2.002_{2, 43.4}$
Stems	*excl.*	$3.974_{2, 8.7}$

† = 1/y -transformed

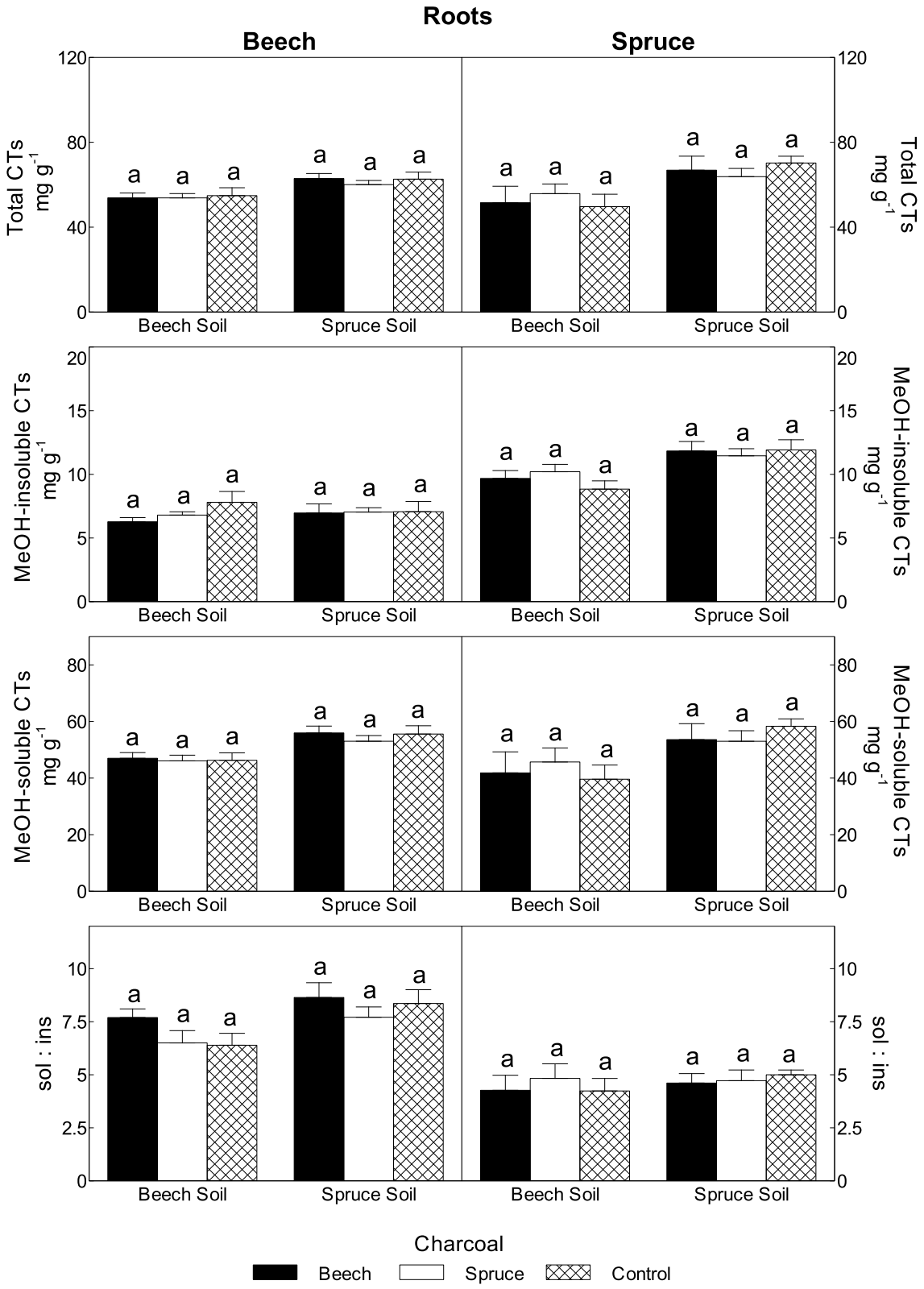

Figure 9. Mean concentrations of condensed tannins (CTs) in roots of beech (*Fagus sylvatica*; left column) and spruce (*Picea abies*; right column) seedlings. Error bars are 1SE. Different letters indicate significant differences within each soil type (mixed effects model; 0.05 sig. level).

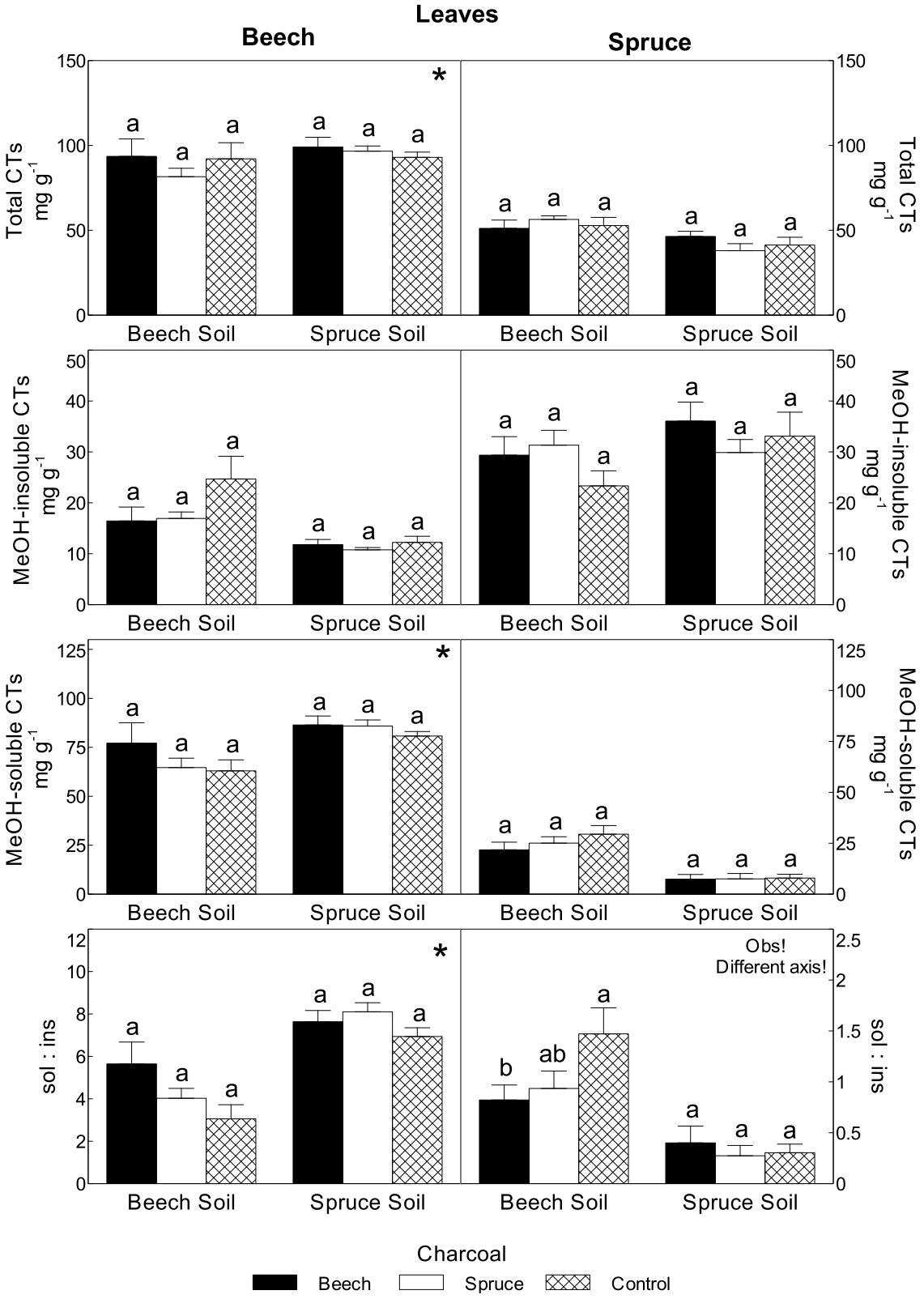

Figure 10. Mean concentrations of condensed tannins (CTs) in leaves of beech (*Fagus sylvatica*; left column) and spruce (*Picea abies*; right column) seedlings. Error bars are 1SE. Different letters indicate significant difference within each soil type (mixed effects model or Kruskal Wallis rank sum test (latter marked with star (*)); 0.05 sig. level).

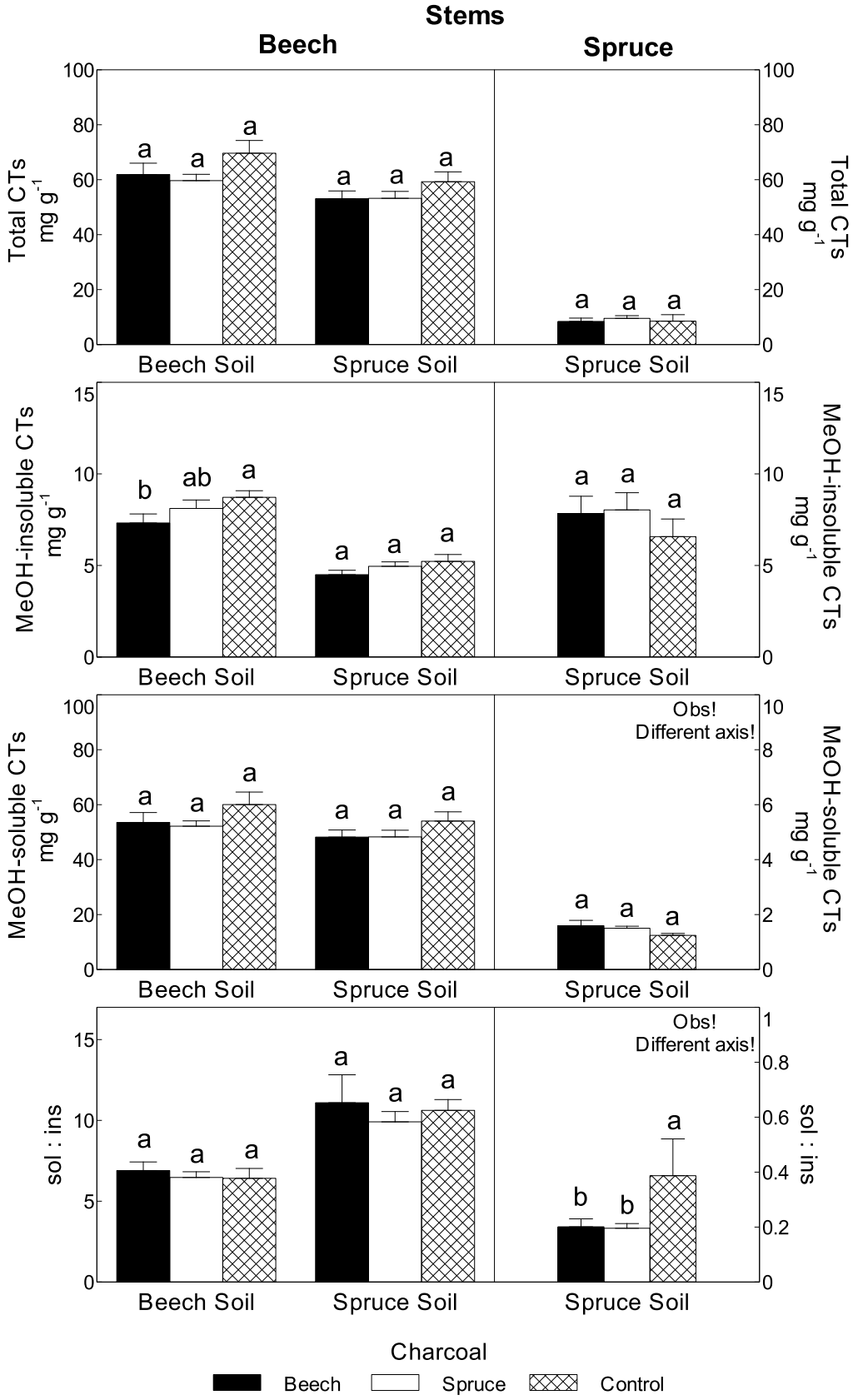

Figure 11. Mean concentrations of condensed tannins (CTs) in stems of beech (*Fagus sylvatica*; left column) and spruce (*Picea abies*; right column) seedlings. Error bars are 1SE. Different letters indicate significant difference (mixed effects model; 0.05 sig. level), within each soil type.

3.4 Low molecular weight phenolics

In total, forty-four individual low molecular weight (l.m.w.) phenolic compounds were identified from roots, leaves and stems of beech and spruce seedlings (Tables S1, S2). Compounds were analysed individually and/or was grouped with related compounds when thought appropriate. Names of compounds refers back to tables S1 and S2 where the concentrations of each single compound is listed.

3.4.1 Roots

Two l.m.w. phenolic compounds were identified from beech roots: a catechin derivative (der. 1) and ellagic acid (Table S1). Catechin concentrations were higher in spruce soil compared to beech soil (Table 5). Charcoal had no influence on the concentrations of either compound (Table 5; Figure 12).

Eight compounds were identified from spruce roots: Three chlorogenic acid derivatives (ders. 5-7; phenolic acids); E-astringin, one astringin derivative (der. 1), resveratrol, and isorhaphontin (stilbenes); and (+)-catechin (Table S2). Charcoal addition often had opposite effects in the two soil types, causing main effects to show as non-significant (Table 5). Beech-derived charcoal, in combination with beech soil, significantly reduced the concentration of several compounds found in spruce roots, compared to either controls or spruce-derived charcoal (Figure 13). Spruce-derived charcoal addition did not result in any differences on concentrations compared to controls. Spruce soil caused significantly higher concentrations of phenolic acids and (+)-catechin in spruce roots compared to beech soil (Table 5).

Table 5. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on the concentrations of low molecular weight (l.m.w.) phenolics in roots of beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B (B not relevant here) indicate whether plants grown in spruce or beech soil had the highest concentrations, respectively. Non-significant interaction terms that are excluded from the minimal adequate model (marked ‡) are reported as chi-square statistics from ANOVA chi-square deletion tests. Numerator and denominator degrees of freedom are given in subscript. *Notes: 1,"Eight 'zero-values' (out of 60 total) were regarded artefacts and treated as missing values"; 2, "All eight identified compounds"; 3, "Chlorogenic acid derivatives 5-7"; 4, "Resveratrol, isorhaphontin, E-astringin and an astringin derivative (der. 1)"; 5, "E-Astringin + one astringin derivative (der. 1)".*

Response	Soil	Charcoal	Soil x Charcoal	Notes
Beech seedlings				
Total l.m.w. phenolics	$6.833_{1, 40.7}$ *S	$0.790_{2, 42.3}$	2.737_{2}‡	
Catechin der. 1	$5.019_{1, 46.4}$ *S	$0.092_{2, 46.4}$	4.103_{2}‡	
Ellagic acid	$0.567_{1, 47.7}$	$1.143_{2, 47.7}$	1.999_{2}‡	1
Spruce seedlings				
Total l.m.w. phenolics	$1.948_{1, 37.6}$	$1.096_{2, 37.6}$	$2.078_{2, 37.8}$	2
Total phenolic acids†	$41.232_{1, 47.0}$ ***S	$0.860_{2, 47.0}$	3.294_{2}‡	3
Total stilbenes	$0.017_{1, 54.0}$	$0.387_{, 54.0}$	$4.505_{2, 54.0}$ *	4
Total astringins†	$1.066_{1, 45.0}$	$0.723_{2, 45.0}$	$5.616_{2, 45.0}$ **	5
Resveratrol††	$3.931_{1, 56.0}$	$0.941_{2, 56.0}$	4.193_{2}‡	
Isorhaphontin	$2.630_{1, 54.0}$	$0.292_{2, 54.0}$	$3.128_{2, 54.0}$	
(+)-catechin	$55.219_{1, 56.0}$ ***S	$0.917_{2, 56.0}$	3.094_{2}‡	

† = log-transformed

†† = sqrt-transformed

‡ = Chi-square statistic from deletion test

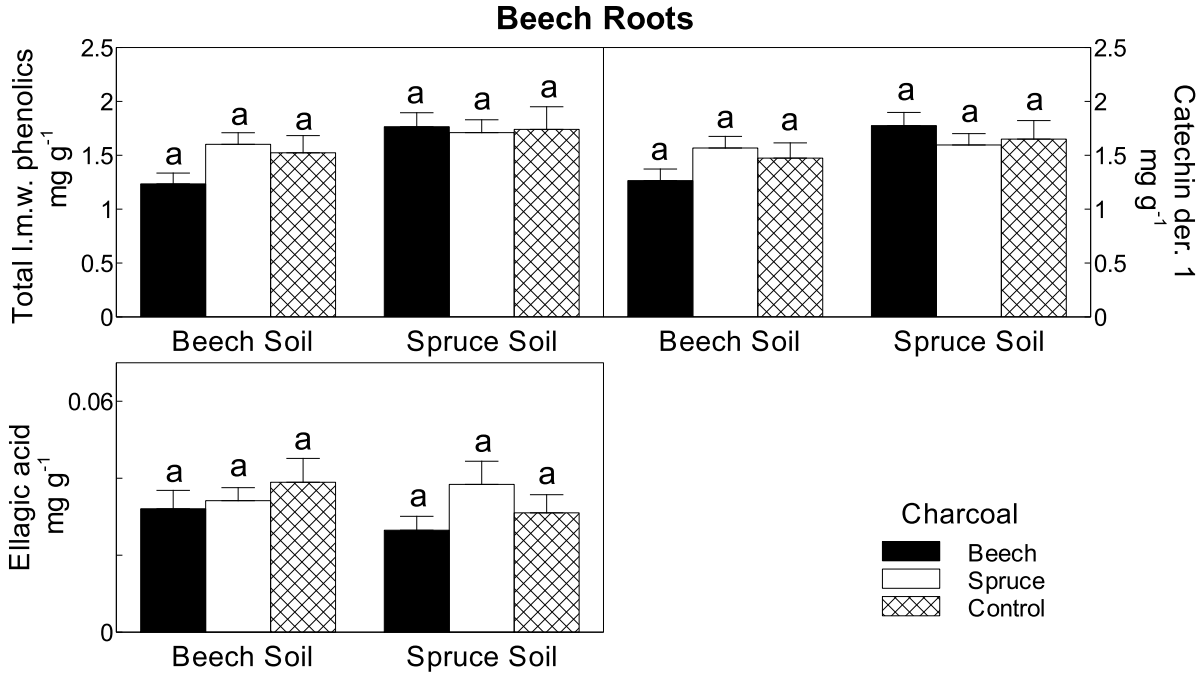

Figure 12. Mean concentrations of a catechin derivative and ellagic acid in roots of beech (*Fagus sylvatica*) seedlings. Error bars are 1SE. Different letters indicate significant difference, within each soil type (mixed effects model; 0.05 sig. level).

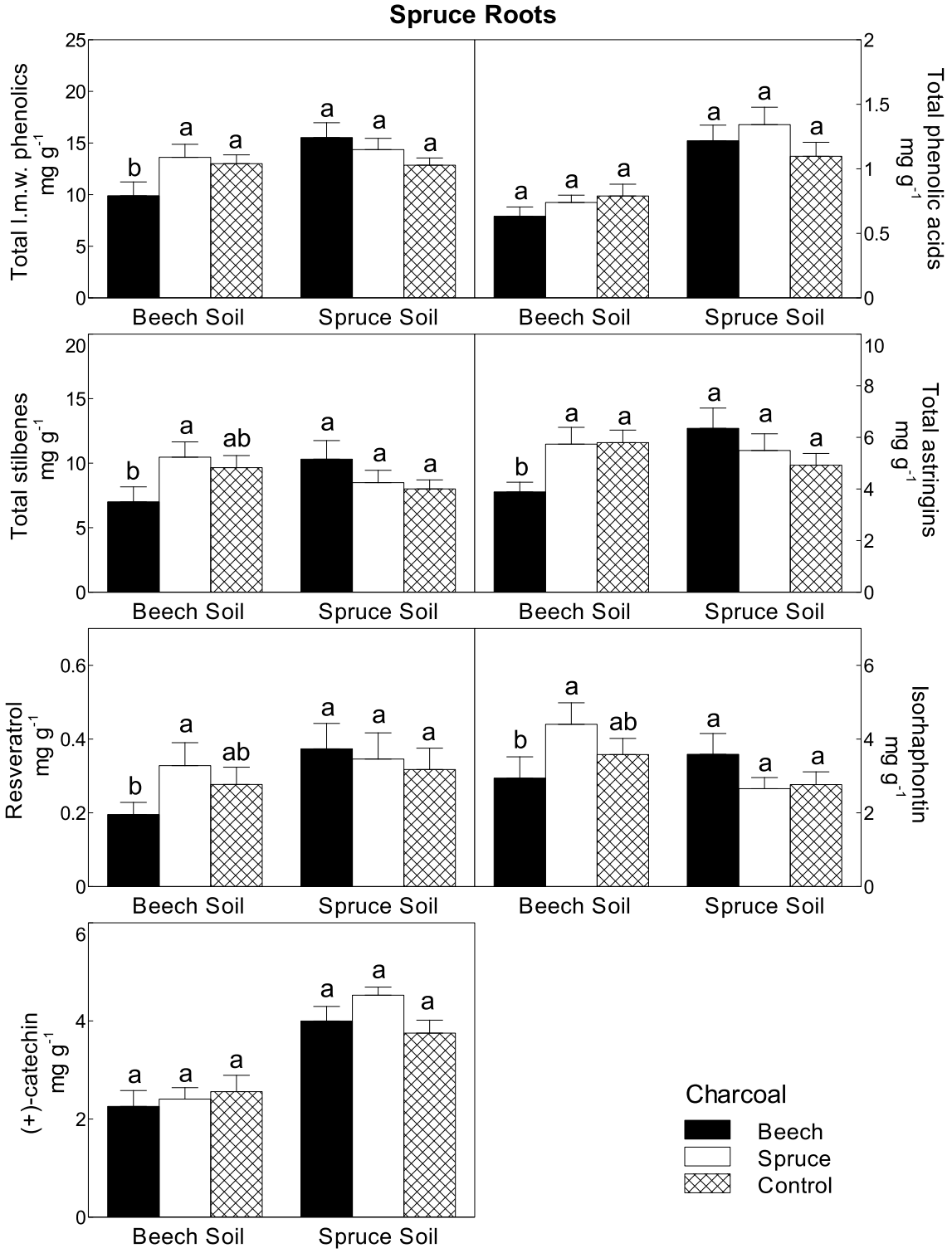

Figure 13. Mean concentrations of low molecular weight (l.m.w.) phenolics in roots of spruce (*Picea abies*) seedlings. Error bars are 1SE. Different letters indicate significant difference, within each soil type (mixed effects model; 0.05 sig. level).

3.4.2 Leaves

Fourteen compounds were identified from beech leaves: chlorogenic acid and four derivatives (ders. 1-4; phenolic acids); three quercetin derivatives (ders. 1-3); five kaempferol derivatives (ders. 1-5); and a myricetin derivatives (der. 1) (Table S1). All but the quercetins excisted in significantly higher concentrations in plants grown in beech soil (Table 6). Several significant contrasts were found in beech soil only: beech-derived charcoal reduced the concentrations total flavonoids and total phenolic acids, and spruce-derived charcoal reduced the concentrations of quercetins (Figure 14). Contrastingly, charcoal had no effect on concentrations of phenolic compounds in leaves of beech seedlings grown in spruce soil.

Nine compounds were identified from spruce leaves: E-astringin, a p-OH-cinnamic acid derivative (der. 1), luteolin-3-glucoside, a quercetin derivative (der. 6), apigenin-7-glucoside, kaempferol-3-glucoside and another kaempferol derivative (der. 10), picein, and a myricetin derivative (der. 2) (Table S2). Spruce-derived charcoal addition caused increased concentrations of three different compounds (a kaempferol derivative, luteolin-3-glucoside, and a p-OH-cinnamic acid derivative), whereas beech-derived charcoal caused both reduced (E-astringin) and increased (a p-OH-cinnamic acid derivative) concentrations (Figures 15, 16).

Table 6. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on the concentrations of low molecular weight (l.m.w.) phenolics in leaves of beech (*Fagus sylvatica*) and spruce (*Picea abies*) seedlings. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B (S not relevant here) indicate whether plants grown in spruce or beech soil had the highest concentrations, respectively. Numerator and denominator degrees of freedom are given in subscript. No interaction terms were significant and are therefore omitted from the table. Notes: 1, "Chlorogenic acid + four derivatives (ders. 1-4)"; 2, "Myricetin der. 1+ quercetin ders. 1-3 + kaempferol ders. 1-5"; 3, "Quercetin ders. 1-3"; 4, "kaempferol ders. 1-5".

Response	Soil	Charcoal	Notes
Beech seedlings			
Total l.m.w phenolics†	$43.528_{1, 56.0}$ ***B	$2.255_{2, 56.0}$	
Total chlorogenic acids†	$40.969_{1, 56.0}$ ***B	$2.024_{2, 56.0}$	1
Total flavenoids	$17.826_{1, 56.0}$ ***B	$2.494_{2, 56.0}$	2
Total quercetins†	$1.944_{1, 56.0}$	$0.875_{2, 56.0}$	3
Total kaempherols†	$33.814_{1, 56.0}$ ***B	$2.256_{2, 56.0}$	4
Myricetin der. 1 †	$8.343_{1, 47.0}$ **B	$1.684_{2, 47.0}$	
Spruce seedlings			
Total l.m.w phenolics†	$1.295_{1, 54.0}$	$0.090_{2, 54.0}$	
E-astringin	$16.083_{1, 52.0}$ ***B	$0.569_{2, 52.0}$	
p-OH-cinnamic acid der. 1†††	$0.029_{1, 56.0}$	$1.828_{2, 56.0}$	
Luteolin-7-glucoside	$0.351_{1, 56.0}$	$1.313_{2, 56.0}$	
Quercetin der. 1 †	$4.256_{1, 47.0}$ ***B	$0.467_{2, 47.0}$	
Apigenin-7-glycoside††	$1.597_{1, 47.0}$	$2.852_{2, 47.0}$	
Kaemferol-3-glucoside†	$0.657_{1, 56.0}$	$0.175_{2, 56.0}$	
Kaemferol der. 1	$2.032_{1, 46.9}$	$10.338_{2, 46.9}$ ***	
Picein	$3.070_{1, 44.2}$	$0.192_{2, 44.3}$	
Myricetin der. 2†	$10.372_{1, 47.0}$ ***B	$0.219_{2, 47.0}$	

† = log-transformed

†† = sqrt-transformed

††† = 1/y -transformed

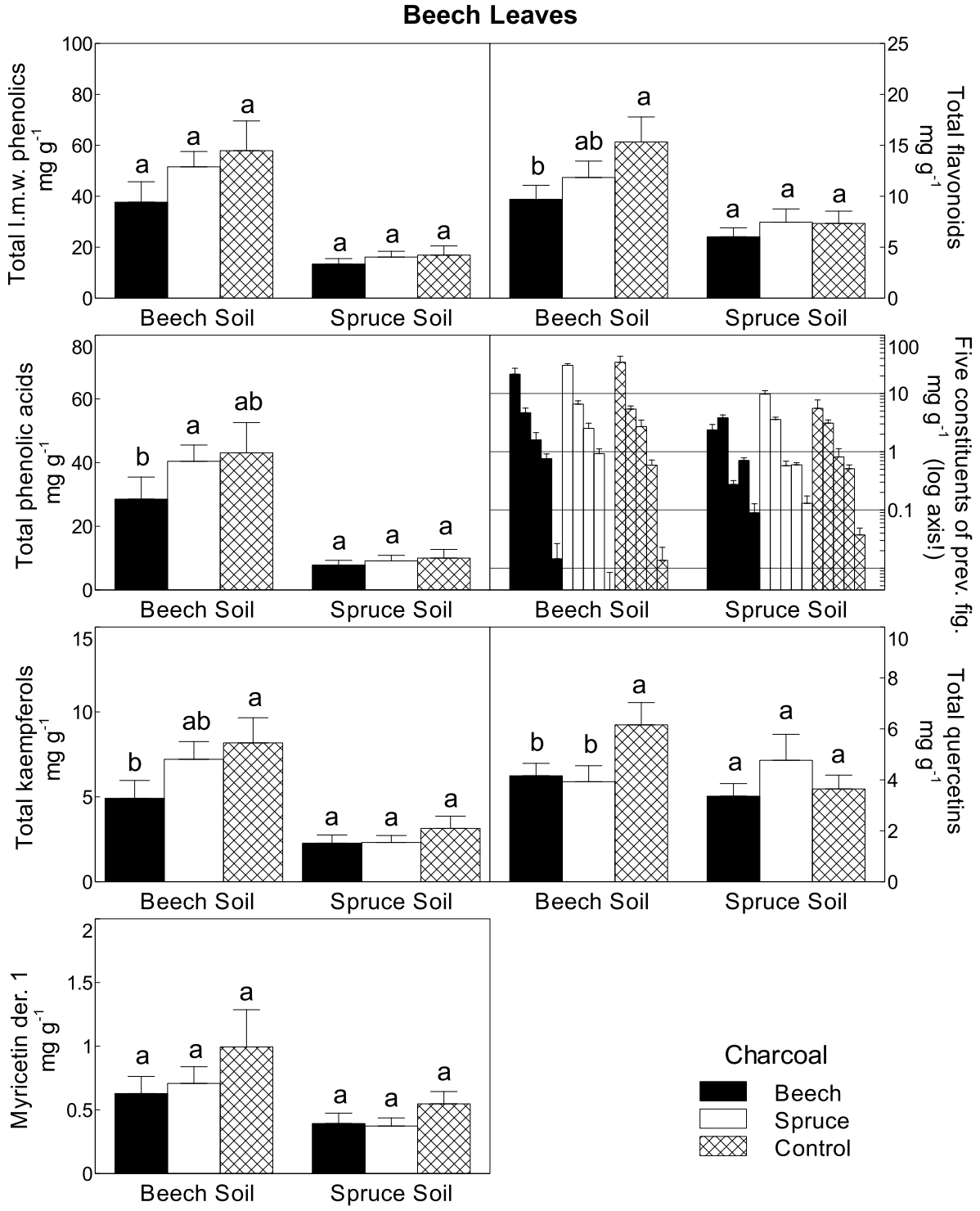

Figure 14. Mean concentrations of low molecular weight (l.m.w.) phenolics in leaves of beech (*Fagus sylvatica*) seedlings. Error bars are 1SE. Different letters indicate significant difference (mixed effects model; 0.05 sig. level), within each soil type.

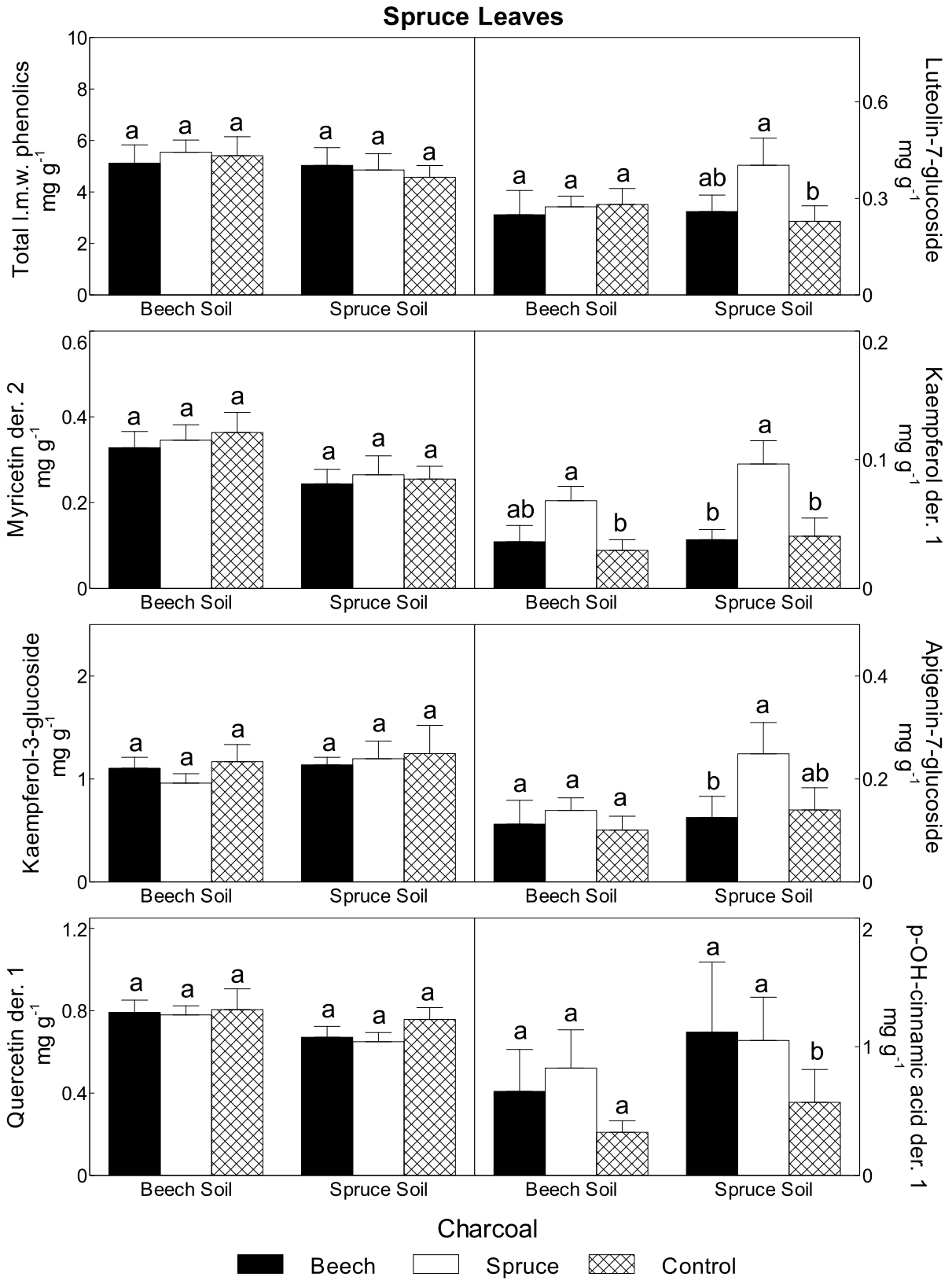

Figure 15. Mean concentrations of low molecular weight (l.m.w.) phenolics in leaves of spruce (*Picea abies*) seedlings. Error bars are 1SE. Different letters indicate significant difference (mixed effects model; 0.05 sig. level), within each soil type.

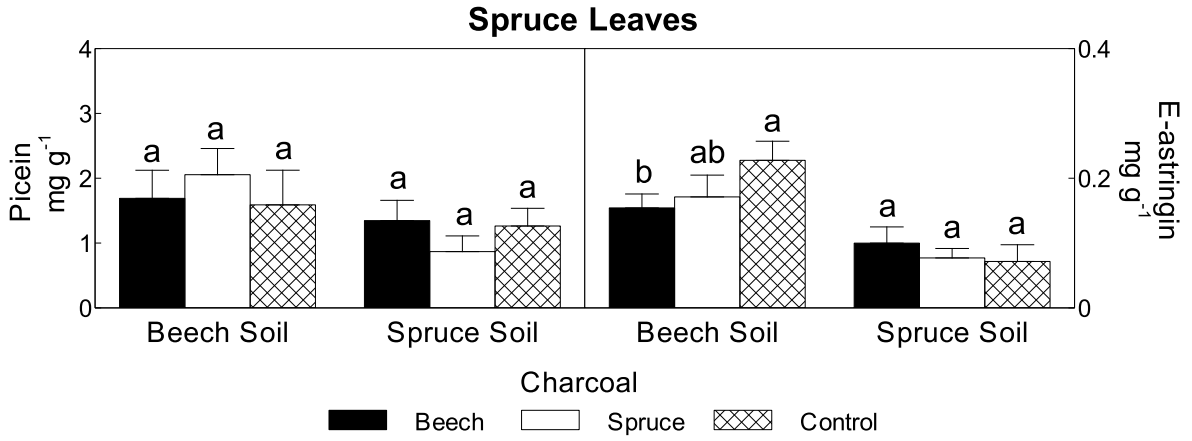

Figure 16. Mean concentrations of picein and E-astringin in leaves of spruce (*Picea abies*) seedlings. Error bars are 1SE. Different letters indicate significant difference within each soil type (mixed effects model; 0.05 sig. level).

3.4.3 Stems

Seven compounds were identified from beech stems: a catechin derivative (der. 2), two quercetin derivatives (ders. 4-5), and four kaempferol derivatives (ders. 6-9) (Table S1). Four compounds were identified from stems of spruce: E-astringin, isorhaphontin, a chlorogenic acid derivative (der. 8), and kaempferol-3-glucoside (Table S2). Total quercetin and kaempferol concentrations were higher in stems of beech seedlings grown in beech soil (Table 7). Spruce-derived charcoal, but not beech-derived, reduced the concentration of a catechin derivative in beech seedlings (Figure 17), and increased the concentration of isorhaphontin in spruce seedlings (Figure 18).

Table 7. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on the concentrations of low molecular weight (l.m.w.) phenolics in stems of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) seedlings. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B (S not relevant here) indicate whether plants grown in spruce or beech soil had the highest concentration, respectively. Numerator and denominator degrees of freedom are given in subscript. No interaction terms were significant and are therefore omitted from the table. *Notes: 1, "Quercetin ders. 4-5"; 2, "Kaempferol ders. 6-9; 3, "Soil is omitted as explanatory variable due to insufficient plant material".*

Response	Soil	Charcoal	Notes
Beech seedlings			
Total l.m.w phenolics	$3.741_{1, 55.0}$	$2.362_{2, 55.0}$	
Catechin der.2	$0.004_{1, 46.4}$	$3.685_{2, 46.4}$ *	
Total quercetins ††	$13.3593_{1, 47.0}$ ***B	$1.182_{2, 47.0}$	1
Total kaempferols ††	$20.310_{1, 47.0}$ ***B	$1.178_{2, 47.0}$	2
Spruce seedlings			3
Total l.m.w phenolics		$0.974_{2, 29.0}$	
E-Astringin	-	$0.153_{2, 27.0}$	
Isorhaphontin††	-	$2.964_{2, 27.0}$	
Chlorogenic acid der. 8	-	$0.399_{2, 18.0}$	
Kaemferol-3-glucoside††	-	$0.983_{2, 18.0}$	

†† = sqrt-transformed

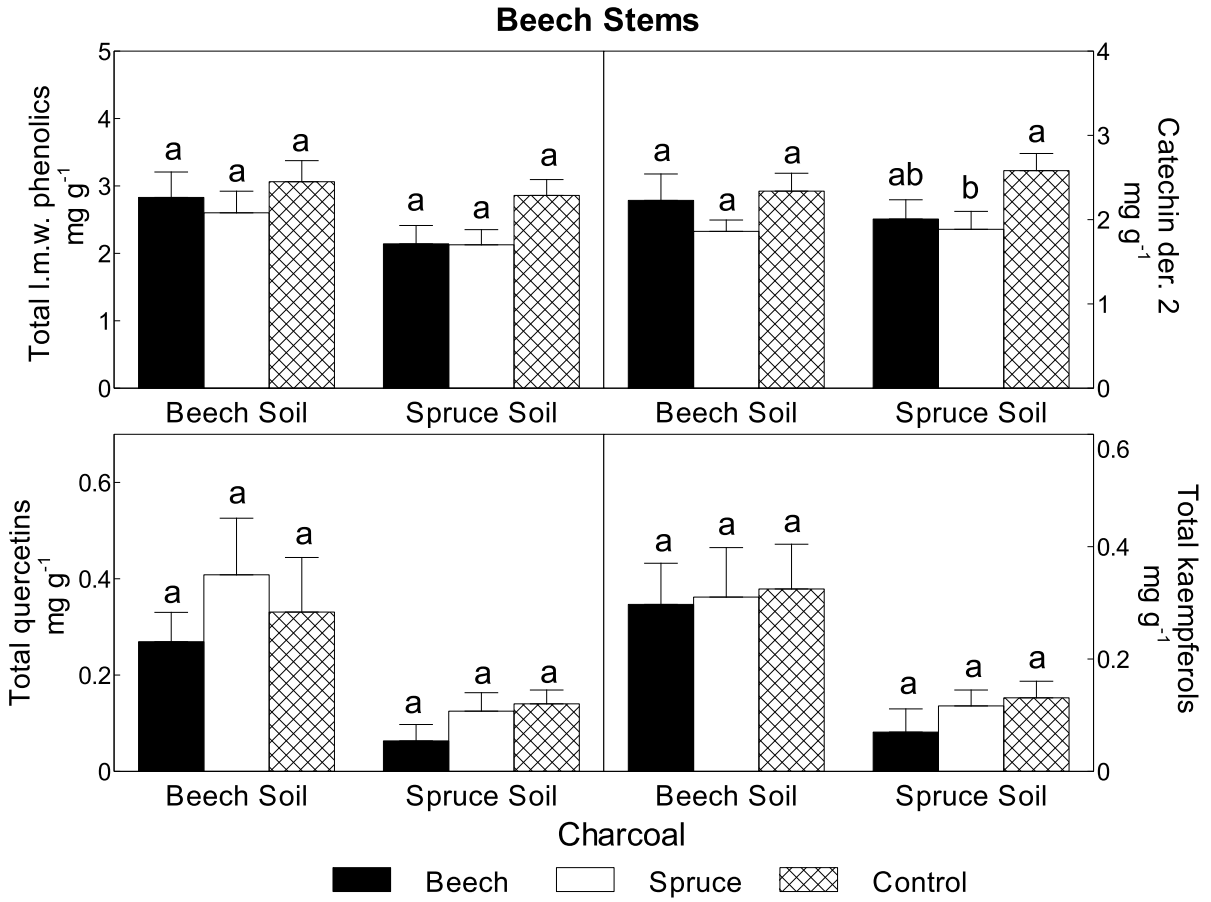

Figure 17. Mean concentrations of low molecular weight (l.m.w.) phenolics in stems of beech (*Fagus sylvatica*) seedlings. Error bars are 1SE. Different letters indicate significant difference within each soil type (mixed effects model; 0.05 sig. level).

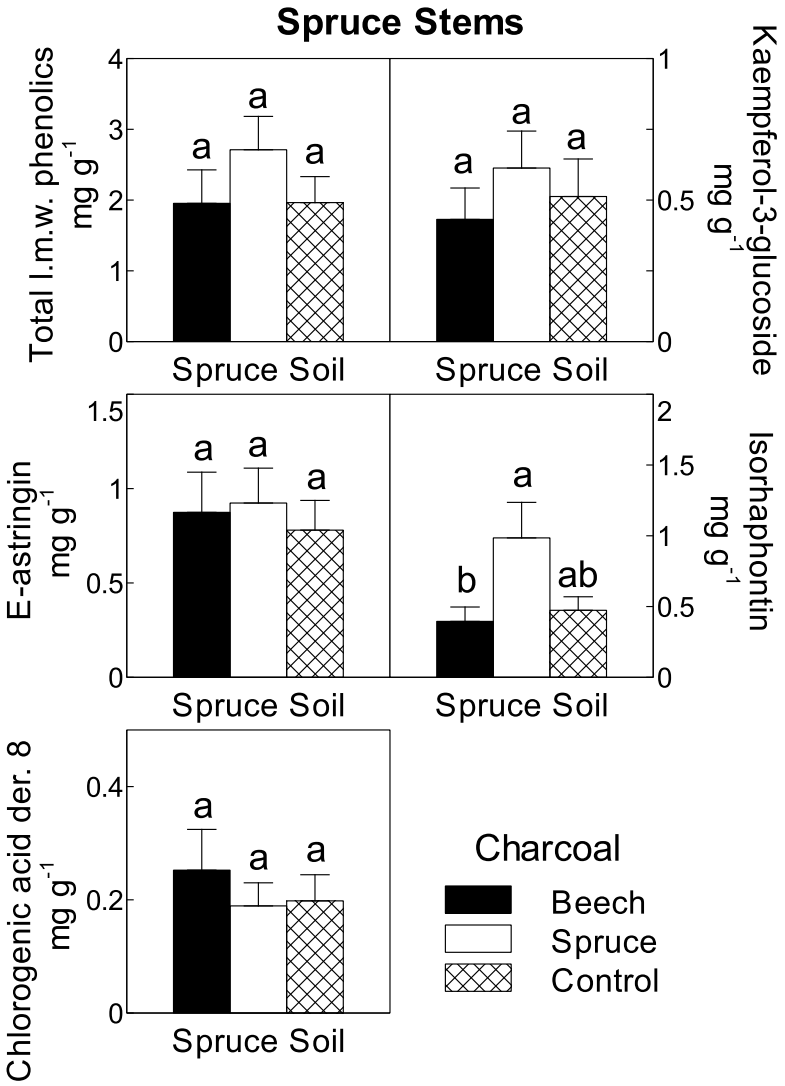

Figure 18. Mean concentrations of low molecular weight (l.m.w.) phenolics in stems of spruce (*Picea abies*) seedlings. Error bars are 1SE. Different letters indicate significant difference (mixed effects model; 0.05 sig. level).

3.5 C : N ratios

Soil type and both charcoal types affected C:N ratios in beech and spruce seedlings. Beech seedlings had much higher C:N ratios in spruce soil, for all plant parts (Table 8). Beech-derived charcoal addition significantly increased the C:N ratio beech roots in spruce soil (Figure 19). For spruce, much the opposite pattern was found, with greater C:N ratios in beech soil for both roots and leaves (Table 8). In addition, spruce-derived charcoal increased the C:N ratio in spruce stems, and decreased it in leaves (Figure 19).

Table 8. F-values obtained from ANOVA's on the model terms of linear mixed effects analysis on the C:N ratio of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) seedlings. Asterix refer to p-values and denotes the significance level (*P<0.05; **P<0.01 and ***P<0.001). Letters S and B indicate whether plants grown in spruce or beech soil had the highest values, respectively. Numerator and denominator degrees of freedom are given in subscript. No interaction terms were significant and are therefore omitted from the table.

Response	Soil	Charcoal
Beech seedlings		
Roots	$113.621_{1,\ 56.0}$ ***S	$3.711_{2,\ 56.0}$ *
Leaves	$8.227_{1,\ 56.0}$ **S	$0.325_{2,\ 56.0}$
Stems	$100.241_{1,\ 56.0}$ ***S	$0.782_{2,\ 56.0}$
Spruce seedlings		
Roots	$27.078_{1,\ 47.0}$ ***B	$0.624_{2,\ 47.0}$
Leaves	$6.908_{1,\ 44.0}$ *B	$2.028_{2,\ 44.1}$
Stems	$2.702_{1,\ 51.0}$	$0.605_{2,\ 51.0}$

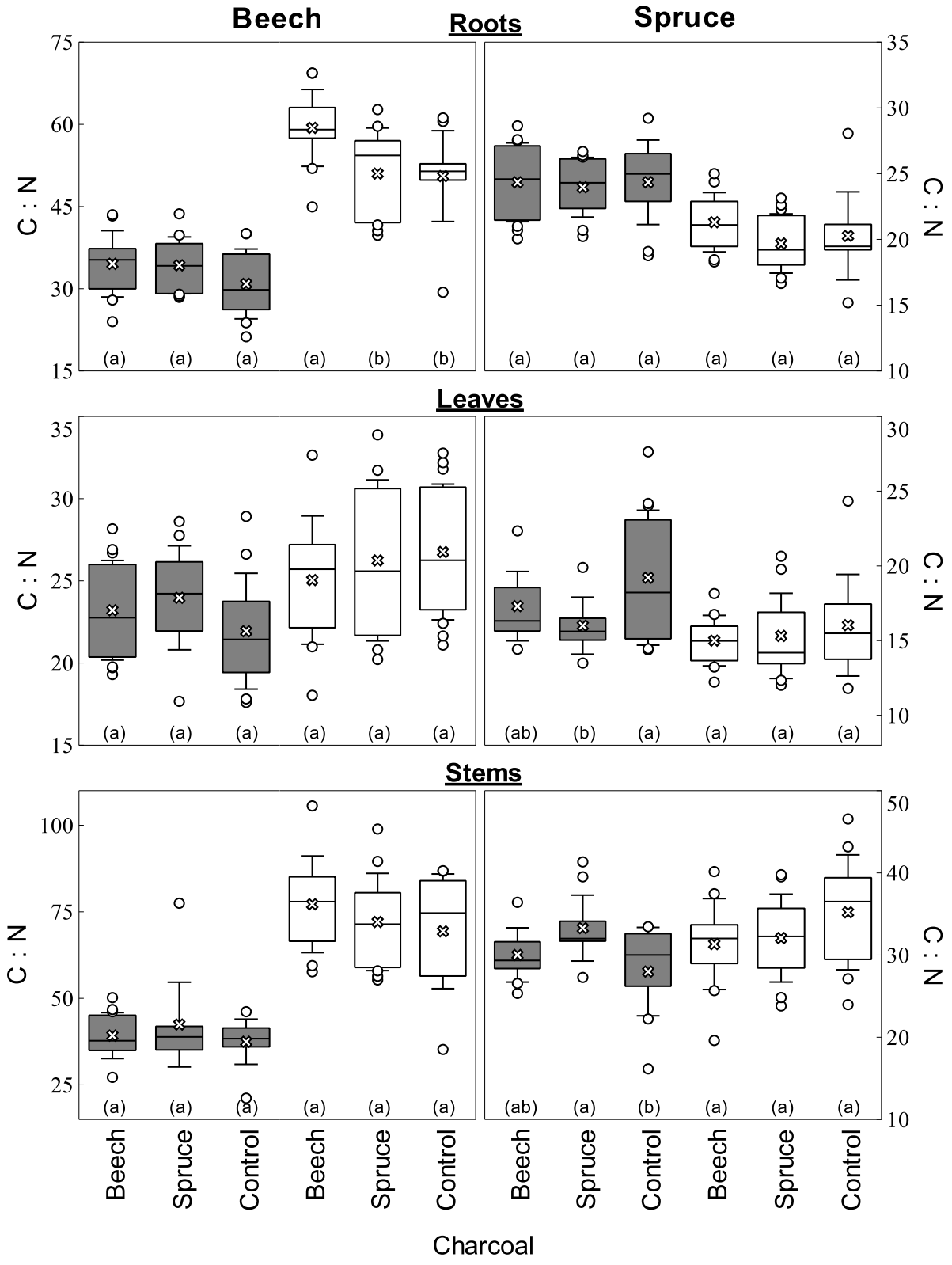

Figure 19. C:N ratios in roots, leaves, and stems of beech (*Fagus sylvatica*, left column) and spruce (*Picea abies*, right column) seedlings. Seedlings were grown in either beech soil (gray boxes) or spruce soil (white boxes) and with different charcoal types. For each soil type, different lower-case letters (in parentheses) indicate significant differences in mean (mixed effects model; 0.05 sig. level). Center line is the median, crosses represent the mean, boxes represent the interquartile range, and whiskers are 1SD.

3.6 Correlations

In beech seedlings, plant height, SLA and S:R generally had similar associations to other variables, whereas coefficients for biomass, and especially belowground biomass, very often had opposite signs (Figure 20). Where the correlations were significant, beech height, S:R and SLA were associated with reduced C:N and sol:ins ratios, increased levels of l.m.w. phenolics, and organ specific responses to total CTs. For spruce seedlings the pattern was more simple: Plant height, biomass, and S:R were all positively correlated to each other, and had similar associations to other response variables, which included reduced CTs and sol:ins ratios in leaves, as well as reduced C:N ratios in stems (Figure 21).

In addition, when looking for signs of internal metabolic trade-offs between different classes of phenolics, negative correlations were found between the concentrations of MeOH-soluble and insoluble CTs within beech leaves (tau=-0.23, p=.008), and spruce leaves (tau=-0.23, p=.010), and positive correlations within beech stems (tau=0.26, p=.004) and spruce roots (tau=0.27, p=.004).

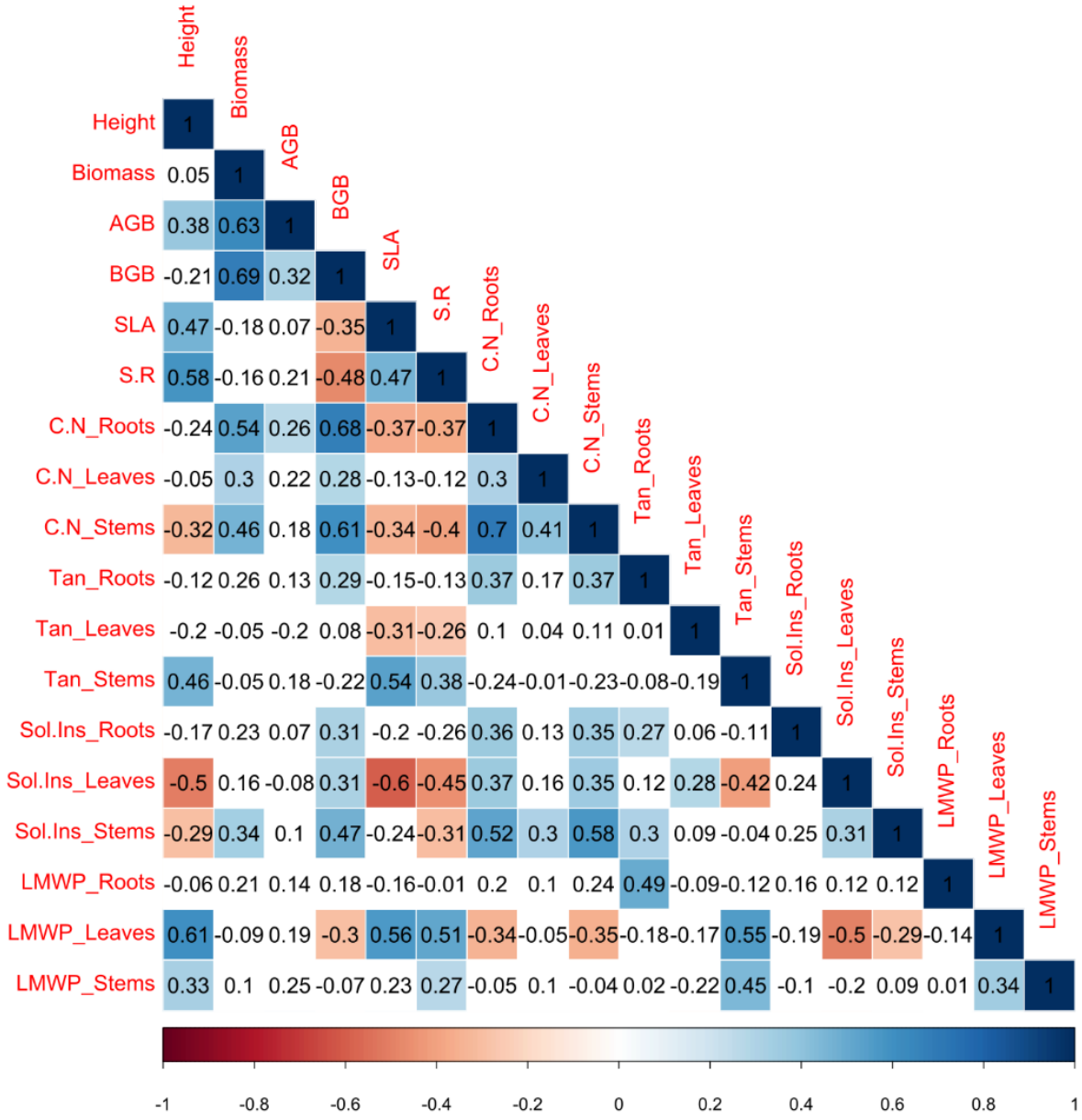

Figure 20. Kendall's tau rank correlation coefficients for associations between the measured response variables on beech (*Fagus sylvatica*) seedlings. Colours refer to the size and direction of coefficients. Non-significant correlations are in white. Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; SLA, specific leaf area; S.R, shoot:root ratio; Tan, total condensed tannins; Sol.Ins, ratio of MeOH-soluble and insoluble condensed tannins; LMWP, low molecular weight phenolics.

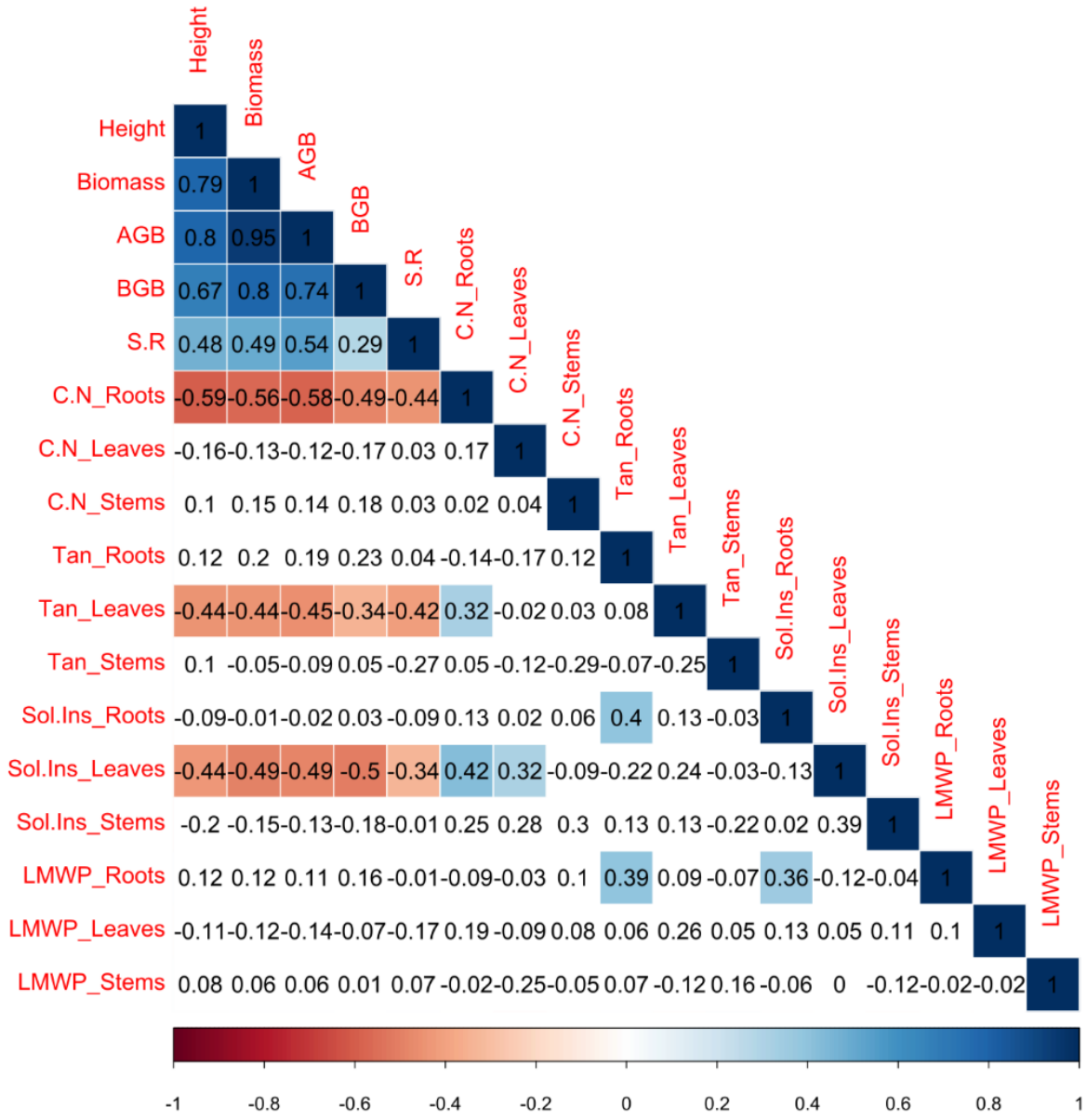

Figure 21. Kendall's tau rank correlation coefficients for associations between measured response variables on spruce (*Picea abies*) seedlings. Colours refer to the size and direction of coefficients. Non-significant correlations are in white. Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; S.R, shoot:root ratio; Tan, total condensed tannins; Sol.Ins, ratio of MeOH-soluble and insoluble condensed tannins; LMWP, low molecular weight phenolics.

Discussion

I found that charcoal addition had several qualitative effects on plant biochemistry, including C:N ratios, but that the total levels of phenolic compounds were largely unaffected from 86 days of exposure to soil charcoal. Charcoal addition did not affect seedling biomass, but caused a reduction in the S:R ratio and SLA in beech seedlings. The response to charcoal treatment

was largely dependent on soil type, charcoal origin, target species and plant organ. In addition, soil type had a much stronger overall effect on growth and resource allocation, as compared to charcoal. Lastly, increased growth was not associated with a reciprocal reduction in the levels of phenolic compounds, as predicted by the protein competition model. In fact, rather the opposite was found.

Of the handful earlier studies documenting an effect of soil charcoal on the growth of northern forest tress (e.g. Wardle *et al.* 1998, Keech *et al.* 2005, Makoto *et al.* 2010, Pluchon *et al.* 2014), responses have been very specific to target species, soil type, and charcoal origin. Charcoal induced changes to the concentration of phenolic compounds in trees has to my knowledge not been investigated. This study provides detailed information on the effect of charcoal on both growth and secondary metabolism specific to beech and spruce seedlings, but fails to find evidence for an unequivocally beneficial effect from charcoal addition.

Condensed tannins

Effects of charcoal additions on the absolute concentrations of CTs was limited to the small, but significant reduction in the MeOH-soluble fraction within beech stems (Figure 11). Charcoal addition also affected the relative levels of MeOH-soluble versus insoluble CTs (sol:ins ratio) in spruce leaves and stems, causing a shift towards a higher dominance of insoluble tannins (Figures 10, 11). Due to the structural complexity of CTs, a completely honest spectre of relative concentrations is difficult to obtain from a single bulk analysis, and although the acid butanol assay (an acid-catalysed colorimetric reaction) is the most commonly used, several shortcomings of this method has been pointed out by Schofield *et al.* (2001). Still, MeOH-insoluble CTs made up a smaller, but considerable fraction of the total CT pool. These are typically polyphenols of higher molecular weight and possibly bound to cell walls (Schofield *et al.* 2001). Higher sol:ins ratios were found in beech seedlings grown in spruce soil (Table 4), which was also the soil type which caused the highest C:N ratios in beech (Table 8), and in addition, the sol:ins ratio was negatively correlated to several positive growth characteristics in both beech (Figure 20) and spruce (Figure 21). This supports the conclusion that fast growth and beneficial growing conditions are associated with reduced levels of MeOH-soluble, relative to insoluble, CTs. This again indicates that the charcoal-induced reduction in the sol:ins ratio of spruce leaves was a positive reaction to charcoal addition. Although it is unclear how the physiological roles of soluble versus insoluble CTs differ from each other, a possible explanation is that they exert a herbivore specific defence (e.g. Hagerman and Robbins

1993). Regardless, the qualitative chemical alteration is interesting as it reveals a more functional aspect of the composition of different fractions of CTs.

Large quantitative and qualitative differences were found between plant parts of the same species. For example, total CT concentrations responded divergently to soil type in beech stems and roots, and between spruce roots and leaves (Table 4). These organ specific responses complicates the interpretation of the role of CTs in seedlings, and it seems likely that either CTs are important to plants for reasons other than defence, or that plants utilise a strategic compartmentalisation of these compounds. It was for example contrary to initial predictions that beech soil, which was extremely detrimental to spruce growth, resulted in lower levels of root tannins compared to spruce soil. It is worth noting that studies limited to the chemical analysis of a single plant part (usually leaves) could end up concluding differently about the overall effect of charcoal on plants.

Analysis on condensed tannins also revealed marked species specific differences in response patterns as well as in chemical profiles. The total CT pool in spruce leaves was comprised of approx. equal proportions of the MeOH-soluble and insoluble fractions, whereas beech leaves had approx. 4-8 times more soluble than insoluble tannins, and in total had close to double the tannin concentrations of spruce leaves (Figure 10). The same pattern is repeated and enhanced when comparing tannin concentrations in plant stems (Figure 11), but no large interspecific difference was found within roots (Figure 9). There are several other chemical substances used by plants with somewhat similar defensive purposes as tannins. For example, spruce is well known for having large quantities of terpenoid resins (Zeneli *et al.* 2006). Interspecies differences in phenolic concentrations are therefore to be expected. Still worth noting are the large interspecific differences found in leaves. Spruce needles are retained for several years, and can also store nutrients during winter. This should imply that they merit a greater defensive investment compared to beech leaves, which are replaced every season. Spruce needles were characterised by low total CTs, but also low sol:ins ratios, indicating perhaps that MeOH-insoluble CTs are increasingly important as constitutive defence in long lived plant organs.

Low molecular weight phenolics

Individual l.m.w. phenolic compounds showed idiosyncratic responses to charcoal additions, and the effects were dependent on target species, plant organ, soil type, and charcoal origin. However, for beech seedlings, charcoal either reduced, or had no effect, on concentrations of

l.m.w phenolics compared to controls (Figures 12, 14, 17), and most of the significant differences was found in beech soils with added beech-derived charcoal, with only one compound responding to charcoal addition in spruce soil (catechin der. 2; Figure 17). Compounds in beech seedlings that responded to charcoal addition included a catechin derivative, E-astringin, as well as the total concentrations of kaempferols, quercetins, and phenolic acids. Spruce seedlings responded in many ways differently than beech to charcoal additions. When compared to controls, spruce-derived charcoal caused an increase in the concentrations of luteolin-7-glucoside, a p-OH-cinnamic acid derivative, and a kaempferol derivative in spruce leaves (Figure 15), whereas beech-derived charcoal caused reduced concentrations of astringins in both leaves (Figure 16) and roots (Figure 13), but an increase in a p-OH-cinnamic acid derivative in leaves. In addition, spruce seedlings often had diverging responses to the two charcoal types, causing them to become significant compared to each other, but not compared to controls. In all such cases, spruce-derived charcoal addition caused relatively higher phenolic levels compared to beech-derived charcoal.

Resource allocation theory predicts that increasingly beneficial growing conditions are associated with reduced accumulation and investment in PSMs, including phenolics (Grime 1977, Bryant *et al.* 1983, Coley *et al.* 1985, Herms and Mattson 1992, Jones and Hartley 1999). The results on beech seedlings are in accordance with initial predictions that soil charcoal, and especially angiosperm-derived charcoal (see Pluchon *et al.* 2014), is beneficial to plants, in this case by lowering the requirement and thus synthesis of costly phenolics assumed important in plant defences. Tomova *et al.* (2005) found a similar reduction in l.m.w. phenolics in beech and spruce roots following nitrogen fertilisation, even if also in that case individual compounds responded idiosyncratically. The results on spruce are more complex, and effects were both positive and negative (reduced or increased concentrations of phenolic compounds, respectively), although spruce-derived charcoal only had neutral or negative effects on spruce seedlings.

When concentrations of all identified compounds for each plant part were summed together, the effects of charcoal addition generally cancelled out, except in spruce roots where beech-derived charcoal caused an overall reduction of l.m.w phenolics (Figure 13). Still, these results should be interpreted carefully as not all the phenolic compounds in the plants could be identified and thus included in the total summed concentration. It assumes less to conclude that, generally, charcoal-induced changes in phenolic-associated plant defences were qualitative, rather than quantitative. This is in accordance with Keinänen *et al.* (1999) who found that total non-tannin phenolics remained unchanged, even if total CT levels were reduced.

C : N ratios

C:N ratios were only moderately affected by charcoal additions (Table 8; Figure 19). Beech-derived charcoal, in combination with spruce soil, increased the C:N ratio in beech roots. This treatment combination caused no other significant effects on any response variable tested. For spruce seedlings, C:N increased in stems due to spruce-derived charcoal addition, and similarly decreased in leaves. As for the species specific differences, C:N ratios in young seedlings are likely biased by the amount of nitrogen present in the seeds, and should therefore not be interpreted as in differences in environmentally induced differences in nutrient status.

Overall, result on C:N ratios were counter to expectations that charcoal would increase the growth rate of seedlings. Although charcoal contains little nitrogen, it has been shown to impact nitrogen mineralisation processes, notably nitrification (DeLuca *et al.* 2006). Plant relative growth rate is strongly associated with the C:N ratio (Ågren 2004, Peng *et al.* 2011), and similarly in this study, low C:N was associated with several positive indicators for growth and plant vigour (Figures 20, 21).

Interpreting conflicting results on beech seedlings

It is somewhat paradoxical that beech-derived charcoal addition caused decreased levels of several individual phenolic compounds, as well as reducing the S:R ratio and SLA and increasing the C:N ratio in beech seedlings. Plant C:N ratios has been shown to be negatively correlated to the relative growth rate (Ågren 2004, Peng *et al.* 2011) and positively correlated to defence-related PSMs (Royer *et al.* 2013). Low S:R ratios are predicted in nutrient limiting environments where plants need to allocate more photosynthates towards root growth (Grime 1977, Chapin 1980, Titlyanova *et al.* 1999). SLA is another well-known metric used to predict a plants place on a hypothetical resource availability axis (Reich *et al.* 1998, but see alsoWilson *et al.* 1999), where the theory is that small, thick leaves have low net photosynthetic capacity, but also low dark respiration rates, low turnover rates and higher levels of structural defence. Therefore, low SLA is predicted for species with conservative growth strategies, whereas high SLA is indicative of fast growth, keeping in mind the large interspecific variation. SLA is sometimes found to be positively correlated to nutrient availability (Shipley and Almedia-Cortez 2003), and leaf nitrogen content (Pierce *et al.* 1994), but for beech specifically, increased nitrogen availability has been observed to reduce SLA (Heath and Kerstiens 1997, Bouriaud *et al.* 2003), though the benefit of this strategy remains unclear. SLA is also very dependent on

light levels, and Minotta and Pinzauti (1996) found the lowest SLA values for beech grown under high light and high nutrient conditions. Here, SLA was negatively correlated to plant C:N ratios and positively correlated to the S:R ratio, which indicates that high SLA values are associated with healthy, fast-growing plants, but the underlying factor determining the variability in beech SLA remains uncertain.

In addition, beech seedlings grew tallest in beech soil, with higher S:R ratios and SLA, and much higher C:N ratios, but still accumulated a greater total biomass in spruce soil due to higher belowground biomass. This apparent contradiction is puzzling. From the correlation matrix of response variables (Figure 20, 21) we see that for spruce seedlings, all the growth parameters (height, total biomass, aboveground biomass, belowground biomass, and S:R) were positively correlated. For beech on the other hand, aboveground biomass had no significant correlation to height, S:R ratio, nor SLA, and belowground biomass had a negative correlation to these same variables. Beech biomass was positively correlated to the C:N ratio, which is opposite to what is seen in the spruce seedlings, and indicates that different mechanism are at play. Although it would not be surprising that adverse conditions could cause increased root growth it is generally assumed that this growth should not be additive to the total net assimilation, as seems to be the case here. The difference in beech seedling height was seemingly a result of a change that occurred in the last two weeks of the experiment (Figure 6), and could be due to differences in phenology. Besides this, I can offer no biological explanation for the conflicting results on beech growth characteristics.

The finding that beech-derived charcoal reduced the S:R ratio is not different from other studies who found neutral or negative effects of charcoal addition. Pluchon *et al.* (2014) found negative as well as positive effects of charcoal additions on the S:R ratio of spruce and *B. pubescens.* A large meta-analysis similarly found no significant mean change in S:R ratios as an effect of charcoal addition (Biederman and Harpole 2013).

Lack of fertilising effect from charcoal

Fertilisation through the input of mineral nutrients is considered perhaps the most important effect of soil charcoal on plants. Påhlsson (1992) found N fertilisation to cause a marked drop in total phenolic levels in beech leaves. Similarly, (Keinänen *et al.* 1999) found for *B. pendula* that fertilisation caused a drop in the level of CTs, though not in l.m.w. phenolics. No such effect was found in this study on the total level of phenolic compounds. Taken together with the lacking evidence for increased seedling growth from charcoal addition, and the failure of

charcoal addition to consistently reduce C:N ratios, it can be assumed that charcoal did not exert a strong fertilising effect on the seedlings in this study. Several explanations for this unexpected result are plausible. Gundale and DeLuca (2007) report on fundamental differences in plant responses when treated with either laboratory- or wildfire-produced charcoal, indicating a bias due to charcoal production method. Also, plants differ in their ability to respond to nutrient flushes: gymnosperms, such as spruce, are considered less responsive than angiosperms, such as beech (Bond 1989). However, as a slow-growing climax species, beech differ from many other angiosperms and may share similar characteristics with spruce.

No evidence for a growth-defence trade-off

With the exception of CT levels in spruce leaves, no clear pattern emerged in the form of negative correlation between growth and phenolic levels to indicate a trade-off between growth and defence partitioning. In fact, beech height was positively correlated to three measures of phenolic concentration. This experiment therefore fails to find any evidence for the protein competition model (see Jones and Hartley 1999), and points to an uncoupling between growth and phenolic synthesis. Besides the hypothesises competition between protein and phenolic synthesis, an internal metabolic trade-off may also exist between different classes of phenolics (see Keinänen *et al.* 1999), but similarly, no negative correlations between CTs and non-tannin phenolics was found, indication there is no strong trade-off between these two classes of phenolics. There was, however, a weak negative correlation between concentrations of MeOH-soluble and insoluble CTs in leaves of both beech and spruce, but this was not true for the other plants organs.

Effect of soil type

For all response variables analysed in this study, soil type had a greater explanatory effect than charcoal treatment, and sometimes much greater. It is as expected that soil type have a strong effect on plants, mainly due to differences in pH, mineral nutrition, soil structure, allelochemicals, and microbial communities. However, with the exception of soil pH and soil dry weight, soil characteristics have not been investigated in this study. Assuming soils to be very heterogeneous over short distances, even within one forest type, the soil main effects should not be interpreted as representative differences between the two forest types from where they were sampled. The effects of soil type are still interesting for comparing relative effect

sizes, to elucidate general resource allocation trade-offs within the plants, and for identifying interactions between soil and charcoal.

For beech as well as spruce seedlings, charcoal effects were found on both soil types, but rarely did the same variable respond simultaneously to charcoal addition in both soils. Initial soil pH was very similar (4.20 in beech soil and 4.12 in spruce soil), and, unexpectedly, changes in soil pH was not influenced by charcoal, even though charcoal pH was quite alkaline (8.2 for beech-derived, 7.3 for spruce-derived charcoal). Soils also differed markedly in weight, indicating an unequal contribution of mineral particles, and thus correspondingly different soil structures (even though only the humus layer was deliberately sampled, it is reasonable to assume the presence of at least some mineral soil). In addition, the two soils were still biologically active with presumably somewhat different microbial floras that could have been affected unequally by changes in mineralisation rates (e.g. Zackrisson *et al.* 1996, Wardle *et al.* 2008). Previous studies have also found associations between charcoal effects and the level of phosphorous in soils (Pluchon *et al.* 2014), as well as the amount of phenolic allelochemicals (Wardle *et al.* 1998). It remains unclear which aspect of soil type dictated the dependency of the charcoal effects in this study, and further analysis on soil characteristics is needed to elucidate this interaction.

Spruce growth was severely retarded in beech soil (reduced biomass, height, and S:R) which resulted in higher C:N ratios in leaves and roots (Table 8) and higher levels of tannins in leaves (Table 4) but caused no change in total l.m.w. phenolics. An in-depth interpretation of soil effects on beech seedlings is made complicated by conflicting results (see discussion above), although large differences were found both on growth and biochemistry. From the large differences between beech and spruce seedlings in their response to the two soil types, it becomes clear that soil factors are crucial in determining the relative success and competitive ability of seedlings after germination. This is in accordance with Ammer *et al.* (2008) who found that newly germinated beech and spruce seedlings compete mostly for belowground resources, followed by increasing competition for light. The early success of beech and spruce seedlings is a good predictor of their subsequent growth (Ammer *et al.* 2008), and should therefore be of great importance for the outcome in regards to species dominance.

The future for northern beech forests

Soil factors, including charcoal, are only some of a multiple of factors that could alter the relative competitive balance between spruce and beech and thus facilitate or hinder the

northward expansion of beech into spruce dominated ecosystems. These include the infection by pathogens, especially some virulent species in the Oomycote genus *Phytophora (Jung et al. 2005)*, as well as several species of the fungi *Neonectria*, which together with the beech scale *Cryptococcus fagisuga* act as the causal agents of beech bark disease (Ehrlich 1934, Houston 1994). Both *Phytophora* (Telfer 2013) and *Neonectria* spp (Talgø *et al.* unpublished, in (Telfer 2013)) have been observed in Norwegian beech forests. Besides this, the main determinant of beech expansion in some parts of the northern range limit, is no doubt forest management practices, where the projected economical cast-off is continuously monitored in order to maintain maximal yield (e.g. Madsen *et al.* 2013). The future strategies in forest management will depend on new science casting light on plant responses to climate change. This study is a small contribution to this, and indicates that beech and spruce have largely different requirements and/or tolerances for soil type, and that of these soil factors, soil charcoal mainly induce qualitative biochemical changes with largely unknown effects on fitness. This study will benefit from further analysis on soil characteristics, which will cast further light on beech and spruce edaphic preferences and tolerances relevant for making prediction concerning the future colonisation ability of beech.

In conclusion, soil charcoal addition exerted several effects on beech and spruce biochemistry, but plant biomass remained unaffected. Overall, soil type had a much stronger influence on the plants than charcoal treatment. Plant responses to charcoal addition were dependent on target species, plant organ, soil type and charcoal origin, which highlights the need for increasingly detailed and comprehensive studies in order to determine the overall effect of charcoal addition on plants.

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Supporting Information

Table S1. Range and mean concentrations of phenolic compounds (mg g^{-1}) in seedlings of beech (*Fagus sylvatica*) grown in two contrasting soils and with either beech-derived (B) or spruce-derived (S) charcoal. Bullets indicate grouping factor. C = control, CT = condensed tannins, l.m.w.= low molecular weight.

	Beech Soil			Spruce Soil		
	B	S	C	B	S	C
Beech Roots						
Total CTs	45.06 - 64.96 (53.83)	45.62 - 67.55 (53.79)	41.32 - 72.72 (54.84)	56.27 - 75.97 (62.91)	51.73 - 68.56 (60.06)	42.70 - 78.76 (62.63)
MeOH-soluble CTs	39.98 - 58.39 (47.01)	37.40 - 61.11 (46.09)	36.90 - 62.62 (46.29)	44.72 - 68.43 (55.95)	43.42 - 61.62 (53.02)	35.45 - 64.97 (55.57)
MeOH-insoluble CTs	5.08 - 7.88 (6.27)	6.08 - 8.23 (6.80)	4.42 - 11.21 (7.80)	4.78- 12.47 (6.96)	5.71 - 8.51 (7.04)	5.31 - 13.79 (7.07)
• L.m.w. phenolics	0.74 - 1.54 (1.23)	1.21 - 2.10 (1.60)	0.82 - 2.44 (1.52)	1.23 - 2.50 (1.76)	1.17 - 2.16 (1.71)	1.01 - 2.83 (1.74)
Catechin der. 1	0.71 - 1.76 (1.26)	1.16 - 2.07 (1.57)	0.81 - 2.42 (1.47)	1.21 - 2.48 (1.78)	1.45 - 2.09 (1.60)	0.99 - 2.78 (1.65)
Ellagic acid	0.01 - 0.06 (0.03)	0.02 - 0.05 (0.03)	0.01 - 0.07 (0.04)	0.02 - 0.05 (0.03)	0.02 - 0.07 (0.04)	0.01 - 0.05 (0.03)
Beech Leaves						
Total CTs	45.60 - 148.25 (93.56)	51.72 - 101.17 (81.57)	48.78 - 163.23 (92.10)	79.78 - 137.25 (99.09)	82.50 - 116.64 (96.91)	77.42 - 109.75 (92.97)
MeOH-soluble CTs	28.64 - 105.96 (71.02)	36.22 - 88.20 (64.64)	37.37 - 94.00 (62.96)	69.57 - 118.62 (86.40)	68.75 - 104.79 (85.85)	67.78 - 94.60 (80.74)
MeOH-insoluble CTs	9.16 - 39.47 (16.44)	12.37 - 23.84 (16.93)	11.09 - 42.51 (24.68)	9.15 - 18.63 (11.79)	8.89 - 13.74 (10.77)	9.19 - 22.26 (12.23)
• L.m.w. phenolics	6.16 - 67.00 (37.68)	27.04 - 84.27 (51.56)	12.11 - 129-40 (57.89)	5.28 - 29.33 (13.38)	9.04 - 31.10 (16.13)	7.56 - 40.13 (16.90)
•Phenolic acids (next 5)	2.83 - 54.08 (28.53)	19.85 - 65.71 (40.41)	6.19 - 101.78 (43.06)	3.43 - 18.96 (7.83)	3.43 - 22.97 (9.12)	3.89 - 30.33 (10.01)
Chl acid	0.69 - 42.21 (21.48)	9.48 - 57.85 (30.40)	2.56 - 95.58 (34.37)	0.52 - 6.16 (2.37)	0.50 - 15.29 (4.25)	0.46 - 22.86 (5.55)
Chl. acid der. 1	0 - 4.26 (1.60)	0 - 4.87 (2.52)	0 - 7.45 (2.70)	0 - 5.94 (0.84)	0.24 - 1.45 (0.58)	0.14 - 2.75 (0.81)
Chl. acid der. 2	0.17 - 1.52 (0.76)	0.28 - 2.08 (0.93)	0.05 - 1.36 (0.59)	0.38 - 1.07 (0.71)	0.38 - 0.82 (0.60)	0 - 0.93 (0.51)
Chl. acid der. 3	1.38 - 8.98 (4.67)	2.34 - 11.04 (6.56)	2.03 - 9.23 (5.39)	1.88 - 6.17 (3.82)	2.23 - 5.41 (3.57)	1.07 - 5.66 (3.09)
Chl. acid der. 4	0 - 0.12 (0.01)	0 - 0.04 (0.00)	0 - 0.08 (0.01)	0 - 0.35 (0.09)	0 - 0.34 (0.13)	0 - 0.10 (0.04)
•Flavonoids (next 9)	3.80 - 16.60 (9.70)	3.74 - 20.15 (11.85)	3.52 - 27.63 (15.33)	2.07 - 10.76 (6.03)	2.93 - 17.85 (7.45)	3.71 - 15.05 (7.33)
•Quercetins (next 3)	1.99 - 7.51 (4.16)	1.26 - 6.35 (3.93)	1.46 - 10.51 (6.16)	0.74 - 5.83 (3.36)	1.83 - 13.08 (4.77)	2.04 - 7.18 (3.64)
Quercetin der. 1	0.71 - 6.43 (2.47)	0.63 - 4.43 (2.36)	1.14 - 7.87 (4.02)	0.33 - 3.53 (1.73)	0.51 - 6.40 (2.36)	0.91 - 3.41 (1.67)
Quercetin der. 2	1.01 - 2.43 (1.65)	0 - 3.32 (1.54)	0.32 - 4.74 (2.14)	0.41 - 2.32 (1.57)	1.13 - 6.68 (2.37)	1.03 - 3.94 (1.96)

Table cont. on next page

Table S1 continued

	Beech Soil			Spruce Soil		
	B	S	C	B	S	C
Quercetin der. 3	0 - 0.37 (0.04)	0 - 0.27 (0.03)	0	0 - 0.32 (0.06)	0 - 0.42 (0.04)	0 - 0.18 (0.02)
•Kaempferols (next 5)	1.38 - 10.90 (4.91)	2.24 - 12.74 (7.21)	1.89 - 14.39 (8.18)	0.91 - 6.29 (2.27)	0.98 - 4.80 (2.31)	1.33 - 8.39 (3.14)
Kaempferol der. 1	0 - 1.36 (0.14)	0 - 1.20 (0.33)	0 - 0.72 (0.07)	0	0 - 0.83 (0.08)	0 - 0.36 (0.04)
Kaempferol der. 2	0.93 - 6.51 (3.27)	1.22 - 9.12 (4.75)	0.94 - 11.80 (5.84)	0.43 - 4.49 (1.40)	0.58 - 3.49 (1.39)	0.70 - 6.55 (2.10)
Kaempferol der. 3	0 - 2.28 (0.78)	0 - 2.57 (1.17)	0.25 - 3.91 (1.60)	0 - 1.30 (0.13)	0 - 1.06 (0.21)	0 - 1.02 (0.36)
Kaempferol der. 4	0 - 0.46 (0.18)	0 - 1.02 (0.27)	0 - 0.33 (0.17)	0 - 0.58 (0.27)	0 - 0.53 (0.19)	0 - 0.38 (0.20)
Kaempferol der. 5	0.23 - 0.96 (0.54)	0 - 1.51 (0.69)	0 - 1.59 (0.50)	0.16 - 0.87 (0.47)	0.20 - 0.85 (0.44)	0.18 - 0.79 (0.44)
Myricetin der. 1	0 - 1.36 (0.63)	0.10 - 1.39 (0.71)	0.17 - 3.27 (0.99)	0.16 - 1.05 (0.39)	0.12 - 0.76 (0.37)	0.11 - 0.92 (0.55)
Beech Stems						
Total CTs	38.01 - 73.90 (61.88)	50.43 - 67.26 (59.66)	43.19 - 85.08 (69.64)	38.96 - 69.48 (53.09)	42.75 - 67.57 (53.22)	43.12 - 75.76 (59.26)
MeOH-soluble CTs	33.37 - 64.62 (53.55)	43.30 - 59.94 (52.16)	33.50 - 76.01 (60.04)	33.69 - 64.33 (48.21)	38.58 - 61.77 (48.27)	38.46 - 69.98 (54.04)
MeOH-insoluble CTs	4.64 - 9.40 (7.32)	5.75 - 10.46 (8.11)	6.94 - 10.46 (8.72)	3.47 - 5.61 (4.50)	3.62 - 5.93 (4.95)	3.43 - 7.02 (5.22)
• L.m.w. phenolics	1.16 - 4.63 (2.83)	1.48 - 4.80 (2.60)	1.88 - 4.43 (3.06)	0.87 - 3.59 (2.14)	1.26 - 3.28 (2.13)	1.57 - 3.79 (2.86)
•Quercetins (next 2)	0 - 0.55 (0.27)	0 - 1.07 (0.41)	0 - 1.01 (0.33)	0 - 0.35 (0.06)	0 - 0.30 (0.13)	0 - 0.26 (0.14)
Quercetin der. 4	0 - 0.38 (0.20)	0 - 0.93 (0.34)	0 - 0.86 (0.30)	0 - 0.35 (0.06)	0 - 0.29 (0.11)	0 - 0.26 (0.14)
Quercetin der. 5	0 - 0.17 (0.07)	0 - 0.16 (0.07)	0 - 0.16 (0.04)	0	0 - 0.11 (0.02)	0
•Kaempferols (next 4)	0 - 0.61 (0.30)	0 - 0.90 (0.31)	0.04 - 0.82 (0.32)	0 - 0.44 (0.07)	0.03 - 0.27 (0.12)	0 - 0.25 (0.13)
Kaempferol der. 6	0 - 0.23 (0.11)	0 - 0.23 (0.11)	0 - 0.19 (0.10)	0 - 0.12 (0.04)	0.03 - 0.11 (0.06)	0 - 0.10 (0.06)
Kaempferol der. 7	0 - 0.26 (0.11)	0 - 0.29 (0.11)	0 - 0.31 (0.11)	0 - 0.19 (0.02)	0 - 0.16 (0.05)	0 - 0.17 (0.06)
Kaempferol der. 8	0 - 0.12 (0.05)	0 - 0.26 (0.07)	0 - 0.33 (0.11)	0 - 0.08 (0.01)	0 - 0.05 (0.01)	0 - 0.03 (0.00)
Kaempferol der. 9	0 - 0.10 (0.03)	0 - 0.11 (0.02)	0 - 0.03 (0.00)	0 - 0.04 (0.00)	0	0
Catechin der. 2	1.02 - 3.62 (2.23)	1.14 - 2.74 (1.86)	1.27 - 3.41 (2.34)	0.87 - 2.99 (2.01)	1.13 - 3.19 (1.89)	1.36 - 3.37 (2.58)

Table S2. Range and mean concentrations of phenolic compounds (mg g^{-1}) in seedlings of spruce (*Picea abies*) grown in two contrasting soils and with either beech-derived (B) or spruce-derived (S) charcoal. Bullets indicate grouping factor. C = control, CT = condensed tannins, l.m.w. = low molecular weight.

	Beech Soil			Spruce Soil		
	B	S	C	B	S	C
Spruce Roots						
•Total CTs	13.57 - 97.29 (51.52)	34.34 - 75.88 (55.80)	18.16 - 86.94 (49.67)	31.50 - 87.12 (66.86)	46.05 - 76.93 (63.81)	48.74 - 79.77 (70.24)
MeOH-soluble CTs	39.98 - 58.39 (47.01)	22.41 - 65.82 (45.69)	9.69 - 65.70 (39.59)	22.82 - 73.79 (53.59)	35.38 - 65.22 (53.01)	41.46 - 68.58 (58.32)
MeOH-insoluble CTs	7.02 - 12.86 (9.68)	7.34 - 12.98 (10.21)	5.40 - 12.95 (8.84)	8.68 - 16.51 (11.83)	9.03 - 14.27 (11.46)	7.29 - 15.89 (11.92)
• L.m.w. phenolics	4.23 - 17.23 (9.89)	9.03 - 19.45 (13.61)	7.57 - 17.31 (13.00)	9.85 - 25.00 (15.53)	10.76 - 21.21 (14.36)	9.85 - 15.94 (12.85)
•Phenolic acids (next 3)	0.36 - 1.11 (0.63)	0.48 - 1.01 (0.74)	0.48 - 1.43 (0.79)	0.81 - 2.21 (1.22)	0.50 - 2.27 (1.34)	0.54 - 1.75 (1.10)
Chl. acid der. 5	0.18 - 0.80 (0.40)	0.29 - 0.52 (0.42)	0.28 - 0.84 (0.48)	0.44 - 1.41 (0.70)	0.33 - 1.78 (0.91)	0.30 - 1.18 (0.63)
Chl. acid der. 6	0 - 0.37 (0.19)	0.14 - 0.50 (0.28)	0.17 - 0.52 (0.27)	0.27 - 0.63 (0.42)	0.17 - 0.60 (0.36)	0.22 - 0.54 (0.40)
Chl.acid der. 7	0 - 0.10 (0.04)	0 - 0.07 (0.04)	0 - 0.09 (0.04)	0.05 - 0.18 (0.10)	0 - 0.13 (0.08)	0.01 - 0.12 (0.07)
•Stilbenes (next 4)	3.24 - 12.54 (7.00)	5.26 - 15.61 (10.47)	5.02 - 13.79 (9.65)	5.86 - 17.15 (10.31)	5.79 - 15.66 (8.49)	4.11 - 11.78 (8.00)
•Astringins (next 2)	1.89 - 5.94 (3.89)	3.48 - 9.25 (5.74)	3.56 - 8.20 (5.80)	3.74 - 11.88 (6.35)	3.39 - 9.97 (5.49)	2.16 - 7.78 (4.92)
E-Astringin	1.89 - 5.87 (3.87)	3.44 - 9.19 (5.70)	3.56 - 8.16 (5.77)	3.72 - 11.79 (6.29)	3.34 - 9.91 (5.45)	2.11 - 7.72 (4.87)
Astringin der. 1	0 - 0.07 (0.02)	0 - 0.08 (0.04)	0 - 0.09 (0.03)	0.02 - 0.11 (0.06)	0.02 - 0.08 (0.05)	0.01 - 0.09 (0.05)
Reservatrol	0 - 0.35 (0.18)	0.09 - 0.74 (0.33)	0.11 - 0.57 (0.28)	0.16 - 0.79 (0.37)	0.17 - 0.94 (0.35)	0.16 - 0.65 (0.32)
Isorhaphontin	1.10 - 6.26 (2.94)	1.66 - 7.82 (4.40)	1.33 - 5.72 (3.58)	1.55 - 7.30 (3.59)	1.70 - 4.75 (2.65)	0.92 - 4.24 (2.77)
(+)-catechin	0.58 - 3.80 (2.26)	1.47 - 3.73 (2.41)	1.47 - 4.72 (2.56)	2.85 - 5.65 (4.00)	3.84 - 5.56 (4.52)	2.47 - 5.15 (3.75)
Spruce Leaves						
Total CTs	24.19 - 75.25 (51.13)	44.03 - 66.84 (56.38)	34.78 - 77.96 (52.77)	32.06 - 61.89 (46.36)	19.43 - 56.77 (37.97)	25.55 - 59.72 (41.33)
MeOH-soluble CTs	3.17 - 37.97 (21.77)	6.58 - 36.75 (25.05)	14.91 - 55.83 (29.44)	2.23 - 23.25 (7.38)	2.05 - 26.86 (7.50)	2.06 - 18.03 (7.90)
MeOH-insoluble CTs	15.36 - 56.93 (29.36)	19.22 - 43.88 (31.32)	9.31 - 43.78 (23.33)	18.78 - 55.19 (36.06)	17.07 - 45.83 (29.87)	19.83 - 54.33 (33.10)

Table cont. on next page

Table S2 continued

	Beech Soil			Spruce Soil		
	B	S	C	B	S	C
• L.m.w. phenolics	2.84 - 8.37 (5.12)	3.95 - 8.57 (5.54)	3.03 - 9.22 (5.41)	2.58 - 9.30 (5.03)	2.40 - 8.00 (4.85)	2.25 - 6.60 (4.57)
E-Astringin	0 06 - 0.26 (0.15)	0.06 - 0.36 (0.17)	0.09 - 0.39 (0.23)	0 - 0.24 (0.10)	0 - 0.14 (0.08)	0 - 0.30 (0.07)
p-OH-cinnamic acid der. 1	0.12 - 2.74 (0.65)	0.07 - 2.36 (0.83)	0.10 - 0.93 (0.34)	0.12 - 5.21 (1.11)	0.08 - 2.69 (1.05)	0.06 - 2.24 (0.57)
Luteolin-3-glucoside	0.05 - 0.67 (0.25)	0.15 - 0.45 (0.27)	0 - 0.55 (0.28)	0.09 - 0.52 (0.26)	0.08 - 0.91 (0.40)	0.05 - 0.49 (0.23)
Quercetin der. 6	0.56 - 1.20 (0.79)	0.57 - 0.95 (0.78)	0.47 - 1.61 (0.81)	0.34 - 0.92 (0.67)	0.48 - 0.91 (0.65)	0.54 - 1.13 (0.76)
Apigenin der. 1	0 - 0.44 (0.11)	0.02 - 0.26 (0.14)	0.03 - 0.28 (0.10)	0 - 0.35 (0.13)	0 - 0.59 (0.25)	0.02 - 0.34 (0.14)
Kaempferol-3-glucoside	0.62 - 1.66 (1.10)	0.63 - 1.46 (1.96)	0.39 - 2.09 (1.17)	0.90 - 1.61 (1.14)	0.55 - 2.48 (1.20)	0.45 - 3.54 (1.25)
Kaempferol der. 10	0 - 0.12 (0.04)	0 - 0.13 (0.07)	0 - 0.07 (0.03)	0 - 0.07 (0.04)	0.02 - 0.22 (0.10)	0 - 0.11 (0.04)
Picein	0.42 - 4.82 (1.69)	0.56 - 3.87 (2.05)	0.25 - 4.61 (1.59)	0.44 - 3.78 (1.35)	0.25 - 2.60 (0.87)	0.44 - 2.51 (1.26)
Myricetin der. 2	0.11 - 0.57 (0.33)	0.22 - 0.54 (0.35)	0.19 - 0.60 (0.36)	0.15 - 0.62 (0.34)	0.14 - 0.52 (0.26)	0.16 - 0.46 (0.25)
Spruce Stems						
Total CTs				2.98 - 13.26 (8.42)	7.17 - 13.10 (9.54)	6.06 - 13.24 (8.52)
MeOH-soluble CTs				1.15 - 2.41 (1.59)	1.18 - 1.67 (1.50)	1.17 - 1.31 (1.24)
MeOH-insoluble CTs				5.27 - 12.11 (7.84)	6.00 - 11.48 (8.03)	4.74 - 8.45 (6.57)
• L.m.w. phenolics				0 - 3.90 (1.95)	0.54 - 5.11 (2.71)	0 - 3.21 (1.96)
E-Astringin				0 - 1.80 (0.87)	0.05 - 1.92 (0.92)	0 - 1.39 (0.78)
Isorhaphontin				0 - 0.76 (0.40)	0.05 - 2.48 (0.99)	0 - 0.87 (0.47)
Chl. acid der. 8				0 - 0.67 (0.25)	0 - 0.38 (0.19)	0 - 0.43 (0.20)
Kaempferol-3-glucoside				0 - 1.01 (0.43)	0.32 - 1.59 (0.61)	0 - 1.43 (0.51)

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