TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Ökophysiologie der Pflanzen

Effects of elevated ground-level ozone on nitrogen acquisition of mature European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) trees

Rosemarie Barbara Weigt

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. J. Schnyder

Prüfer der Dissertation:

1. Univ.-Prof. Dr. R. Matyssek
2. Univ.-Prof. Dr. A. Göttlein
3. Prof. Dr. P. Millard,
   Scottish Agricultural College, UK (schriftliche Beurteilung)

Die Dissertation wurde am 07.06.2010 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 11.11.2010 angenommen.

Alles Wissen ist zuletzt Wissen vom Leben und alles Erkennen
Staunen über das Rätsel des Lebens – Ehrfurcht vor dem Leben
in seinen unendlichen, immer neuen Gestaltungen.

- Albert Schweitzer -

meinen Eltern

Acknowledgements - Danksagung

My deep gratitude I owe to all persons who continuously encouraged me during this work:

All den lieben Menschen, die mich während der Durchführung dieser Arbeit ermutigend und unterstützend begleitet haben ein herzliches Dankeschön:

Prof. Dr. Rainer Matyssek für die sehr engagierte und gute Betreuung meiner Doktorarbeit, und die lehrreiche und gute Zeit am Lehrstuhl für Ökophysiologie der Pflanzen

Dr. Karl-Heinz Häberle für die Zeit und Gelassenheit bei der Betreuung meiner Experimente, für die zahlreichen inspirierenden Diskussionen und Gespräche und das Verständnis bei all den Zweifeln

Dr. Helmut Blaschke für ein stets offenes Ohr, die gewitzten Sinnsprüche und als hilfreiches "wandelndes Nachschlagewerk"

Prof. Dr. Dr. Axel Göttlein (FG Waldernährung und Wasserhaushalt, TUM) für die unkomplizierte Hilfsbereitschaft bei Boden- und Nährstofffragen und für die Nutzung des Bodenanalyseinventars

Prof. Dr. Pete Millard für die herzliche Gastfreundschaft am Macaulay Land Use Research Institute, die hilfreichen Anregungen zu Isotopenfragen und die schöne Zeit in Aberdeen

Dr. Thomas Rötzer (LS Waldwachstumskunde, TUM), Ursula Metzger, Wilma Ritter, Dr. Christoph Schulz (LWF), Dr. Jürgen Esperschütz (GSF) und Dr. Michael Schloter (GSF) für die Zurverfügungstellung von Daten aus dem Kranzberger Forst zu Biomassen, Transpiration, Phloemsaft, Stickstoffein- und -austräge sowie mikrobieller Biomasse.

Dr. Wolf-Anno Bischoff (Gutachterbüro TerrAquat) für die Hilfestellung und das Interesse bei der Messung der N-Auswaschung

allen SFB607-Kollegen für die stets gute und motivierende Zusammenarbeit und den interessanten interdisziplinären Austausch

den Technischen Mitarbeitern des Lehrstuhls für Ökophysiologie der Pflanzen, Ilse Süß, Johanna Lebherz, Peter Kuba, Josef Heckmair und Thomas Feuerbach für den unermüdlichen Einsatz bei Wind, Wetter, Wald und Wurzeln, sowie Hans Lohner für die geduldige Betreuung des Massenspektrometers und die Isotopenmessungen der verschiedensten Proben.

den zahlreichen Studentischen Hilfskräften, insbesondere Dominik Farnady, Michael Goisser, Katharina Hermannsdorfer, Franziska Jäger, Angelika Kühn, Lukas Müller, Lena Penzkofer und Lenka Vojtova für die fleißige Unterstützung bei der Freiland- und Laborarbeit

der Deutschen Forschungsgemeinschaft für die finanzielle Ermöglichung dieses Projekts

Prof. Dr. Reinhard Agerer, Dr. Stefan Raidl und Rita Verma (Systematische Botanik und Mykologie, LMU) für die Eröffnung der interessanten Perspektive „Mykorrhiza“ als Anknüpfungspunkt an diese Arbeit

den gütigen und kritischen Korrekturlesern

allen derzeitigen und ehemaligen Angehörigen des Lehrstuhls für Ökophysiologie der Pflanzen für das fröhliche Arbeitsklima, das so gut getan hat

meinen Geschwistern und allen Freunden die mitgefiebert und so oft Mut zugesprochen haben, insbesondere meiner Mitstreiterin Wilma Ritter,

und meinen Eltern, die mir die Freude an der Natur und die Ehrfurcht vor dem Leben weitergegeben haben

und ganz besonders Michael Pfiz für die Zuversicht und das große Verständnis für alles Komplizierte

Table of contents

Abbreviations and Definitions

N	nitrogen
O_3	ozone
$1\times O_3$	free-air ambient ozone concentrations at the study site „Kranzberger Forst“
$2\times O_3$	twice-ambient ozone concentrations, experimentally released to the tree crowns at the study site via a free-air O_3 fumigation system
^{15}N	stable isotope of nitrogen (natural abundance = 0.37 atom%)
atom% ^{15}N	proportion of the isotope ^{15}N on the total N ($^{15}N + {}^{14}N$)
$^{15}NH_4{}^{15}NO_3$	ammonium-nitrate enriched in ^{15}N to 98 atom%, used for N labelling
$N_{labelled}$	fraction of labelled N on the total N content of a certain tissue or compartment showing newly acquired N
N_{total}	total N content of a certain tissue or compartment (including labelled N in labelled trees)
$\delta^{15}N$	ratio $^{15}N/^{14}N$ of a sample relative to the ratio $^{15}N/^{14}N$ of a standard (standard = atmospheric N_2)
$wood_{ozone}$	proportion of stem wood formed during years of experimentally enhanced $2\times O_3$ concentrations (2000-2006)
$wood_{pre\text{-}ozone}$	proportion of stem wood formed before the onset of the $2\times O_3$ fumigation
recovery	proportion of labelled N in a tissue or compartment relative to the amount of the applied labelled N
partitioning	distribution of biomass, N_{total} or $N_{labelled}$ (= new N) on the different tree organs or tissues as the result of resource allocation

Zusammenfassung

Die vorliegende Arbeit als Teil des interdisziplinären Sonderforschungsbereichs „SFB607 – Wachstum und Parasitenabwehr - Wettbewerb um Ressourcen in Nutzpflanzen aus Land- und Forstwirtschaft“, liefert einen Beitrag zu der Frage, inwiefern Bäume die Allokation ihrer Ressourcen hinsichtlich des Dilemmas Wachstum gegenüber Stressabwehr regulieren.

Ziel der Studie war es, den Einfluss chronisch erhöhter troposphärischer Ozon (O_3)-Konzentrationen auf adulte Waldbäume im Bestand zu bewerten. Der Schwerpunkt lag dabei auf der Versorgung mit Stickstoff (N) als wichtige Nährstoffressource. Die N-Aufnahme und -Allokation wurde an 62jährigen Rotbuchen (*Fagus sylvatica* L.) als wechselgrüne Baumart und an 52jährigen Fichten (*Picea abies* [L.] Karst.) als immergrüne Baumart untersucht. Beide Arten sind von großer ökologischer und ökonomischer Bedeutung innerhalb Mitteleuropas.

Folgende Hypothesen wurden zugrunde gelegt:

(i) Chronisch erhöhte O_3-Konzentrationen vermindern die Nährstoff-aufnahme(kapazität) der Bäume durch reduzierte Kohlenstoff (C)-Allokation in unterirdische Organe infolge eingeschränkter C-Assimilation und stomatärer Leitfähigkeit.

(ii) Buche reagiert v.a. im Mischbestand empfindlicher auf erhöhtes O_3 als Fichte, aufgrund ihres höheren Ressourcenumsatzes.

Über eine Freiluft-O_3-Begasungsinstallation im „Kranzberger Forst“ (nahe Freising, Bayern) wurden Buchen und Fichten in ihrem Kronenbereich über 7 Jahre zweifach erhöhten O_3-Konzentrationen ($2\times O_3$) ausgesetzt. Bäume unter unveränderten O_3-Konzentrationen ($1\times O_3$) dienten als Kontrolle. Der Standort zeichnet sich durch moderate bis hohe N-Verfügbarkeit und Mineralisierung sowie durch eine gute Wasserverfügbarkeit aus.

Im 6. Jahr des Ozonbegasungsexperiments wurden an jeweils 6 Buchen und Fichten (3 Wiederholungen pro Baumart und O_3-Behandlung) eine ^{15}N-Markierung in Form einer $^{15}NH_4{}^{15}NO_3$-Lösung (98 atom%) auf je 1 m² Bodenoberfläche appliziert. Über die folgenden 16 Monate wurde die Aufnahme des markierten ^{15}N in die Bäume und ihre verschiedenen Organe untersucht. Die Wurzeln, mykorrhizierte Wurzelspitzen sowie

der Boden der ^{15}N-markierten 1 m²-Flächen wurden am Ende des Experiments untersucht. Die Konzentrationen des neu aufgenommenen N ($=N_{labelled}$) und des gesamten N ($=N_{total}$) sowie das C/N-Verhältnis wurden für die verschiedenen Pflanzenorgane und Bodenhorizonte ermittelt. Die Gesamtaufnahme sowie die Baum-interne Verteilung des $N_{labelled}$ wurde auf der Basis modellierter Biomassen der Untersuchungsbäume (PRETZSCH und RÖTZER) berechnet. Die N-Bilanz des Bestandes wurde unter Einbezug von Untersuchungen zur N-Auswaschung und N-Mineralisierung sowie des mikrobiellen N-Vorrats (SCHLOTER und Mitarbeiter) erstellt.

Effekte der $2\times O_3$-Behandlung auf die N-Aufnahme zeigten sich vor allem im Laub. Da Blätter und Nadeln zum Zeitpunkt der Markierung (Juli 2005) bereits voll entwickelt waren, wurde im ersten Untersuchungsjahr nur wenig $N_{labelled}$ gefunden, während die Effekte im zweiten Jahr deutlicher ausgeprägt waren. Die Buchenblätter, besonders das Laub der Sonnenkrone, zeigten signifikant geringere $N_{labelled}$-Konzentrationen unter $2\times O_3$ im Vergleich zu $1\times O_3$, und dies korrelierte mit der kumulativen Transpiration. Ebenso zeigten die Knospen signifikant geringere $N_{labelled}$-Konzentrationen unter $2\times O_3$. Dieser Effekt war im Herbstlaub nicht zu beobachten. Dieses zeigte jedoch einerseits ein signifikant reduziertes C/N-Verhältnis im Sonnenlaub, andererseits jedoch ein erhöhtes C/N und eine geringere N_{total}-Konzentration im Schattenlaub unter $2\times O_3$. Die Fichte hingegen zeigte keine durch Ozon bedingten Unterschiede in den $N_{labelled}$-Konzentrationen der Nadeln und Knospen. In Bezug auf die signifikant geringeren N_{total}-Konzentrationen der Nadeln unter $2\times O_3$ war der Anteil des $N_{labelled}$ jedoch tendenziell erhöht. Die Knospen zeigten dagegen eine Tendenz erhöhter N_{total}-Konzentrationen unter $2\times O_3$. Die $N_{labelled}$-Konzentrationen der Zweige beider Baumarten zeigten keine Veränderungen durch $2\times O_3$, ebenso wie das Stammholz. In der Stammrinde der Buche waren die $N_{labelled}$-Konzentrationen unter $2\times O_3$ jedoch tendenziell geringer als unter $1\times O_3$. Dies deckte sich mit den Befunden in Blättern und Knospen sowie mit signifikant reduzierten $N_{labelled}$-Konzentrationen im Phloem der Buche.

In den Wurzeln wurden keine signifikanten O_3-induzierten Veränderungen der N-Konzentrationen festgestellt. Nichtsdestotrotz zeigten beide Baumarten in allen drei untersuchten Wurzel-Durchmesserklassen (< 2 mm, $\geq 2 < 5$ mm, > 5 mm) Trends zu reduzierten $N_{labelled}$- und N_{total}-Konzentrationen unter $2\times O_3$, bei teilweise erhöhtem C/N. Die mykorrhizierten Wurzelspitzen der Buche wiesen signifikant reduzierte N_{total}-Konzentrationen unter $2\times O_3$ auf, während die der Fichte keine Unterschiede zeigten.

Im Gegensatz zu den Wurzeln lagen die $N_{labelled}$- und N_{total}-Konzentrationen im

Gesamtboden bei 2×O_3 unter beiden Baumarten tendenziell höher als bei 1×O_3, signifikant allerdings nur im B-Horizont bis in 10 cm Tiefe. Die N_{total}-Aufnahmekapazität der Feinwurzeln mit zunehmendem Boden-N_{total} schien zudem unter 2×O_3 reduziert. Neben den N-Konzentrationen der Wurzeln unterschied sich auch die Wurzelverteilung über die Horizonte zwischen den O_3-Behandlungen. in der Feinwurzelbiomasse (< 2 mm) zeigten beide Baumarten eine leichte Verschiebung hin zu erhöhter Biomasse in den oberen Bodenhorizonten und reduzierter Biomasse in den unteren. Innerhalb des organischen Oberbodens war bei Fichte unter 2×O_3 die Feinwurzeldichte im oberen O_i reduziert und im darunterliegenden O_a erhöht. Die gesamte stehende Wurzelbiomasse pro Flächeneinheit (bis in 0.5 m Tiefe) und für alle Durchmesserklassen unterschied sich in beiden Arten jedoch nicht zwischen den O_3-Behandlungen.

Auf Gesamtbaumebene zeigten beide Baumarten unter 2×O_3 eine nicht signifikant geringere Aufnahme des $N_{labelled}$ und demgegenüber eine höhere Wiederfindung des $N_{labelled}$ im Boden im Vergleich zu 1×O_3. Pro Einheit Baumbiomasse war die $N_{labelled}$-Aufnahme unter 2×O_3 um etwa 15 % (Buche) bzw. 17 % (Fichte) reduziert. Bezogen auf die Wurzelmasse (< 5 mm) zeigte sich bei Buche jedoch mit etwa 46 % (nicht signifikant) eine deutlichere Reduktion der $N_{labelled}$-Aufnahme unter 2×O_3. Bei Fichte hingegen zeigte die $N_{labelled}$-Aufnahme pro Einheit Wurzelbiomasse keinen Unterschied zwischen den O_3-Behandlungen. Die verringerte $N_{labelled}$-Aufnahme pro Gesamtbaummasse war offenbar auf die geringere Gesamtwurzelmasse pro Baum unter 2×O_3 im Vergleich zu 1×O_3 zurückzuführen. Bezogen auf den N_{total}-Vorrat der Bäume war die $N_{labelled}$-Aufnahme unter 2×O_3 bei Buche ebenfalls um etwa 12 % tendenziell reduziert, während sie bei Fichte um etwa 42 % erhöht war.

Erhöhte O_3-Konzentrationen beeinflussten die Baum-interne Verteilung von neu aufgenommenem N. Im Vergleich zu 1×O_3 wurde bei Buche ein größerer Anteil an neuem N in die Wurzeln alloziert, oder verblieb dort (signifikant in mykorrhizierten Wurzelspitzen und als Trend in Feinwurzeln). Im Gegensatz dazu war der Anteil an neuem N in den Wurzeln bei Fichte unter 2×O_3 reduziert (signifikant in der Wurzelklasse ≥ 2 < 5 mm), in oberirdischen Kompartimenten, vor allem Nadeln aber erhöht.

Art-spezifische Unterschiede in der N-Allokation zeigten sich durch höhere N-Konzentrationen und einem höheren Anteil an neuem N ($N_{labelled}$) in Blättern und Knospen der Buche, und umgekehrt in geringeren N-Konzentrationen in den Wurzeln

im Vergleich zu denen der Fichte. Unter Berücksichtigung der leicht unterschiedlichen Biomasse-Allokation zwischen beiden Baumarten stellen die Knospen der Buche, die etwa 11 % des neu aufgenommenen N erwarben, eine bedeutende Stickstoff-Senke dar. Bei der Fichte dienen dagegen die Wurzeln als wichtigere Senke für neu aufgenommenen N, was vor allem für die Größenklasse $\geq 2 < 5$ mm gezeigt wurde. Die übrigen untersuchten Kompartimente wurden in beiden Baumarten zu ähnlichen Anteilen mit neuem N versorgt. Die Bedeutung des Stammes als N-Speicher der Buche wurde durch einen doppelt so großen N_{total}-Vorrat im Vergleich zu dem der Fichte deutlich.

Unter Berücksichtigung der Art-spezifischen physiologischen und morphologischen Unterschiede schien die wechselgrüne Buche empfindlicher auf erhöhtes Ozon zu reagieren als die immergrüne Fichte. Die länger andauernde O_3-Exposition der Blätter bezogen auf ihre Lebensdauer sowie die damit verbundene stärkere Einschränkung von stomatärer Leitfähigkeit und Transpiration bewirkten bei Buche stärkere Auswirkungen auf N-Aufnahme und -Transport als bei Fichte. In Verbindung mit veränderten C/N-Verhältnissen im Herbstlaub unter $2\times O_3$ könnten diese Effekte bei Buche von größerer Bedeutung für den Baum-internen N-Kreislauf sein als bei Fichte. Andererseits deutet die höhere Allokation des neuen N in die Nadeln – relativ zum N_{total}-Vorrat – auf einen erhöhten N-Bedarf der Fichte unter $2\times O_3$ hin, welcher durch das reduzierte Wurzelsystem offenbar nicht gedeckt werden konnte. Dies kann die Fichte langfristig empfindlicher gegenüber erhöhten O_3-Konzentrationen machen, insbesondere bei geringerer Nährstoffverfügbarkeit als am Untersuchungsstandort.

Hochskaliert auf Bestandesebene führte die leicht reduzierte N-Aufnahme beider Baumarten unter $2\times O_3$ zu einem tendenziell erhöhten N-Vorrat im Boden, was auch im signifikant erhöhten mikrobiellen N-Vorrat unter Fichte sichtbar war. Diese Zunahme der Boden-N-Verfügbarkeit unter $2\times O_3$, könnte langfristig zu einem erhöhten N-Verlust des Ökosystems, z.B. durch verstärkte Auswaschung, führen.

Zusammenfassend lässt sich festhalten, dass in der vorliegenden Studie in Übereinstimmung mit Hypothese (i) die N-Aufnahme von Buche und Fichte durch erhöhtes O_3 tendenziell reduziert war. Die Buche reagierte unter den gegebenen Standortbedingungen in ihrer N-Aufnahme sensibler auf erhöhtes O_3 als die Fichte, wodurch Hypothese (ii) bestätigt wird.

Summary

The present study as part of the interdisciplinary research program 'SFB607 - Growth and parasite defense - Competition of resources in economic plants from forestry and agronomy', contributed to the question about trade-offs in resource allocation between growth and stress defence.

The study assessed the impact of chronically elevated ground-level ozone (O_3) on mature trees under field conditions, with the focus on the use of nutrient resources, in particular nitrogen (N). N acquisition and allocation was studied on 62 yr old European beech (*Fagus sylvatica* L.) and 52 yr old Norway spruce (*Picea abies* L. [Karst.]) trees, representing the ecologically and economically most important tree species in Central Europe. It was hypothesized that (i) chronically elevated ground-level O_3 concentrations limit the nutrient supply upon reduced below-ground carbon allocation, and that (ii) beech responds more sensitive to elevated O_3 in a mixed stand than spruce, due to a higher resource turnover as compared to spruce.

At the free-air O_3 fumigation facility “Kranzberger Forst” (SE-Germany) canopies of beech and spruce were exposed to twice-ambient O_3 concentrations ($2\times O_3$) over 7 years. Trees exposed to unchanged ambient O_3 concentrations ($1\times O_3$) served as controls. The study site was characterized by high N availability and mineralisation, and ample water supply.

In the 6th year of the ozone fumigation experiment, a ^{15}N tracer was applied as $^{15}NH_4{}^{15}NO_3$ solution (98 atom%) to the soil surface of a 1 m² plot per each of the twelve study trees (three replicated per species and treatment). Subsequently, N acquisition and incorporation in trees and their different organs were studied over 16 months. Roots, mycorrhizal root tips and soil of the ^{15}N labelled plots were analysed at the end of the experiment. Concentrations of newly acquired N (as detected by the labelling = $N_{labelled}$), total N (N_{total}), as well as C/N ratio of the different plant and soil compartments were assessed. Uptake and partitioning of the newly acquired N at tree level was based on modelled whole-plant biomass (Pretzsch and Rötzer). Nitrogen balance and cycling of the stand was determined also covering N leaching and mineralisation at the study site, as well as the microbial biomass and related N pool (Schloter and co-workers).

Impact of $2\times O_3$ on N uptake was detectable in the tree foliage. As leaves and needles had already completed growth by the time of ^{15}N labelling in July 2005, incorporation of

newly acquired N was low in the first year of the study, but effects became more pronounced in the subsequent year of 2006. In beech leaves, particularly of the sun crown, $N_{labelled}$ concentrations were significantly lower under $2\times O_3$ as compared to $1\times O_3$, and correlated to cumulative transpiration. Consistently, buds showed significantly reduced $N_{labelled}$ concentrations under $2\times O_3$, although N_{total} concentrations were increased towards the end of the growing season. In contrast, $N_{labelled}$ concentrations in spruce needles and buds did not differ between the two ozone regimes. Relative to the significantly reduced N_{total} concentrations, however, the incorporation of $N_{labelled}$ tended to be increased in spruce needles under $2\times O_3$. Similar to beech, N_{total} concentrations in buds tended to be higher under $2\times O_3$ than under $1\times O_3$. Leaf litter of beech did not respond to $2\times O_3$ in terms of $N_{labelled}$ concentrations. However, C/N ratio was significantly reduced in litter from the sun crown under $2\times O_3$, and significantly enhanced in the shade crown. Woody axes showed no clear effect in N concentrations in response to $2\times O_3$, although N_{total} concentration was significantly reduced in the wood of sun crown twigs of spruce. Stem woody tissues were not affected in their N concentrations by elevated O_3, whereas in the stem bark and consistently in the phloem of beech, the $N_{labelled}$ concentrations were reduced.

In roots, no significant O_3-effects on N concentrations were observed, although a tendency of lowered N concentrations under $2\times O_3$ was found in all three root diameter classes (< 2 mm, $\geq 2 < 5$ mm and > 5 mm) of both species across the soil horizons. In mycorrhizal root tips, N_{total} concentration was significantly reduced under $2\times O_3$ in beech but not changed in spruce.

Conversely, in the bulk soil, $N_{labelled}$ and N_{total} concentrations tended to be higher underneath trees exposed to $2\times O_3$ than to $1\times O_3$ (significant only in the B horizon down to 10 cm soil depth) resulting in a lower C/N. The N uptake capacity of fine roots with increasing soil N tended to be lower under $2\times O_3$ compared to $1\times O_3$ in both species

Apart from N concentrations, root distribution across the soil horizons differed between the ozone treatments. A slight shift towards an increased fine root (< 2 mm) biomass in the upper and reduced biomass in deeper soil horizons was found in both species. Within the organic layers, the fine root density of spruce under $2\times O_3$ was decreased in the upper O_i layer but increased in the lower O_a layer. Total standing root biomass per unit ground area (down to 0.5 m of soil depth), however, did not differ between the two ozone treatments within each root diameter class of both species.

At the whole-tree level, recovery of $N_{labelled}$ (=new N) tended to be lower in beech and

spruce trees and higher in the soil under $2\times O_3$ compared to $1\times O_3$. A trend of reduced uptake of new N per unit of biomass was indicated under $2\times O_3$, by about 15 % in beech and 17 % in spruce. Related to root mass (< 5 mm), this response to $2\times O_3$ tended towards a reduction of 46 % in beech, whereas no difference was observed in spruce, as root mass decreased in parallel. In relation to the whole-tree N_{total} pool, the recovery of $N_{labelled}$ under $2\times O_3$ also tended to be decreased in beech by about 12 %, but increased in spruce by about 42 %.

Elevated O_3 affected the tree internal partitioning of new N, as in beech a higher proportion of new N was allocated to or remained in roots, being significant for mycorrhizal root tips and, as a trend, in fine roots. Conversely, in spruce allocation of new N to roots was decreased under $2\times O_3$, being significant in medium-sized roots.

Species-specific differences in N allocation were displayed by higher N concentrations and incorporation of new N in beech leaves and buds as compared to spruce, and conversely, lower N concentrations and incorporation of new N in beech roots in relation to spruce. Taking into account the differences of biomass allocation, buds served as an important sink for new N in deciduous beech at the end of the growing season, gathering about 11 % of newly acquired N, whereas roots, particularly medium-sized ones, were favoured in evergreen spruce. Other compartments received similar proportions of new N in both species. Cycling of old N storage could not be assessed, although the importance of e.g. stem N storage in beech was apparent by a two-fold greater pool size compared to spruce.

With regard to species-specific physiological and morphological differences, O_3 sensitivity seemed to be more pronounced in deciduous beech compared to coniferous spruce. The stronger effects on N uptake and transport probably resulted from longer exposure time of foliage to elevated O_3 relative to the leaf lifetime, and stronger reductions of stomatal conductance and transpiration. Together with alterations of C/N in leaf litter under $2\times O_3$ these effects may have greater importance for tree internal N-cycling in beech than in spruce. However, increased allocation of new N to needles - relative to the foliage N_{total} pool - indicated a high N demand of spruce under $2\times O_3$ which could not be covered by the reduced root system. This may in the long term render spruce susceptible to elevated O_3, in particular under lower nutrient availability than at the present study site.

Scaled up to stand level, the indicated reduced N uptake under $2\times O_3$ in both species and, conversely, higher N concentration in the bulk soil, tended to increase the soil N pool,

as also shown by the significantly increased microbial N pool under spruce. In the long term, the increased soil N availability under elevated O_3 may therefore enhance N loss from the ecosystem, e.g. via leaching.

Thus, N uptake tended to be reduced under $2\times O_3$ in both beech and spruce, although below-ground C allocation appeared to be increased, particularly in beech, supporting hypothesis (i). Under the present site conditions, beech responded more sensitive to $2\times O_3$ in N acquisition than spruce, confirming hypothesis (ii).

Introduction

Forests with their rough surface are an important filter of air pollutants, and detailed studies on the effects of long-term exposure to pollutants such as ozone (O_3) are of great importance in view of understanding changes in this filter function. Tropospheric ozone is known as an air pollutant that can damage plants by affecting photosynthesis and growth, which may lead to yield reductions (e.g. REICH & AMUNDSON 1985, REICH 1987, HECK *et al.* 1988, LEFOHN 1992, ASHMORE 2005, FELZER *et al.* 2007). In industrialized countries, its concentrations today range between 20 and 45 nl l^{-1} in comparison to a pre-industrial level of around 20 nl l^{-1}, and are prognosticated to increase by 0.5 – 2 % per year in the Northern Hemisphere (VINGARZAN 2004). Thereby, O_3 exceeds international thresholds for both human health and environment (e.g. FOWLER *et al.* 1999, PRATHER *et al.* 2003, VINGARZAN 2004, IPCC 2007), depending on the development of emissions of NOx, CH_4, CO and Volatile Organic Compounds (VOCs) as the main precursors of tropospheric O_3 formation as well as on meteorological conditions (IPCC 2007). While some studies on agricultural crops report yield reductions (EMBERSON *et al.* 2001, MORGAN *et al.* 2006) the long-term effects on forest ecosystems are unclear, and the mechanisms of tree reactions in forests are hardly understood (MATYSSEK & INNES 1999, MATYSSEK & SANDERMANN 2003, ASHMORE 2005, KARNOSKY *et al.* 2005).

Effects of elevated tropospheric O_3 on tree growth and physiology have been described by many authors (e.g. REICH 1987, MATYSSEK & INNES 1999, MATYSSEK & SANDERMANN 2003, KARNOSKY *et al.* 2003, 2005, ASHMOORE 2005, NUNN *et al.* 2006, KITAO *et al.* 2009). These effects include reductions in photosynthesis, Rubisco acitivity and stomatal conductance; reduced carbon (C) allocation from leaves into other (e.g. below-ground) organs as well as acceleration of leaf senescence.

Species differ in their susceptibility to abiotic or biotic stress and hence, any energy and resource consuming defence demands directly influence the competitiveness e.g. for nutrients. Studies found that plant defence reactions against O_3 are similar to those against pathogens, e.g. fungi that enter leaves via the stomata to cause infections (HELLER *et al.* 1990, MATYSSEK & SANDERMANN 2003). Conversely, there is evidence that O_3 stress may lead to enhanced susceptibility of plants to pathogens (HEAGLE 1973, MANNING & KEANE in: HECK *et al.* 1988, MANNING & V. TIEDEMANN 1995), although reduced infections by fungi at elevated O_3 concentrations have also been reported

(HEAGLE 1973, LUEDEMANN *et al.* 2005). Such findings support the concept that plants balance the allocation of resources between growth and defence, irrespective of ecosystems and 'growth types', such as grasses, herbaceous or woody plants (HERMS *&* MATTSON 1992, MATYSSEK *et al.* 2002, MATYSSEK *et al.* 2005). Species that differ in growth habit, such as deciduous beech and evergreen spruce, may in the long term be affected in their competitiveness in response to stress. For example, ZAK *et al.* (2007*b*) found decreased nitrogen (N) acquisition of aspen trees under elevated ozone and underlined the influence of inter- and intra-specific competition when assessing the effects of ozone on nutrient uptake. Young European beech trees showed reduced N uptake and plant biomass when growing under elevated ozone and in competition with Norway spruce, while the latter was not affected (LUEDEMANN *et al.* 2005, KOZOVITS *et al.* 2005*a, b*).

The present study aimed to clarify effects of chronically enhanced O_3 levels on N uptake and cycling in mature trees of deciduous European beech (*Fagus sylvatica* L.) and evergreen Norway spruce (*Picea abies* [L.] Karst.) at the forest site. As most physiological studies regarding O_3 effects in relation to carbon or nutrient allocation on trees have focussed on juvenile trees, the case of mature trees remained unclear. However, knowledge of stress impact on mature trees in the stand is essential in view of developing sustainable forest management practices. Few studies report decreased plant growth of mature trees under chronic ozone exposure (OLLINGER *et al.* 1997, PRETZSCH *et al.* 2009). Yet, mature trees in the field seem to be less sensitive to O_3 stress as compared to young trees or trees in chamber experiments, and show no severe visible injuries on leaves (NUNN *et al.* 2005*a*). Growth reduction in response to ozone was also reported from mature beech and spruce trees of other European sites (BRAUN *et al.* 1999, 2007, KARLSSON *et al.* 2006), and was found also for other tree species at different developmental stages (e.g. MATYSSEK *et al.* 1992, 1993, CHAPPELKA *&* SAMUELSON 1998, KARNOSKY *et al.* 2005).

The uptake and metabolism of nitrogen, as one of the most important nutrients, can be affected by above-ground O_3 stress. Since photosynthesis and N concentration in foliage are positively correlated (FIELD *&* MOONEY 1986), an O_3-induced reduction of photosynthesis may lower leaf N demand, e.g. by reduced concentration and/or activity of Rubisco (PELL *et al.* 1994, 1997). Conversely, leaf N demand may be increased due to increased enzyme production and turnover for repair and defence processes at the leaf level, e.g. production of antioxidants (ANDERSEN 2003).

Reduced stomatal conductance in response to ozone can reduce transpiration which in turn may affect N-transport into above-ground organs. Conversely, a loss of stomatal control under elevated ozone, as reported by some authors (reviewed by MATYSSEK & SANDERMANN 2003), accompanied by a reduced photosynthetic water use efficiency might increase N upward transport via the xylem and lead to a reduced photosynthetic nitrogen use efficiency.

As a consequence of premature foliage senescence under elevated ozone, retranslocation of nitrogen from leaves may increase the internal N translocation (WRIGHT *et al.* 1991, MANDERSCHEID *et al.* 1992) and change the allocation of newly acquired nitrogen (BIELENBERG *et al.* 2002). However, retranslocation may be incomplete under O_3 stress and result in increased nitrogen loss when leaves are shed (ANDERSEN 2003).

Moreover, even below-ground processes have been found to be indirectly affected by ozone. Carbon allocation to roots and soil can be reduced in response to above-ground O_3 exposure due to stomatal closure and enhanced C demand for defence mechanisms or maintenance respiration (ANDERSEN 2003). Lower below-ground supply of photosynthates may then lead to secondary effects on roots and soil organisms, as e.g. decreases in soil and root respiration (EDWARDS 1991, COLEMAN *et al.* 1996), and changes in mycorrhizal communities (GREBENC & KRAIGHER 2007). Conversely, increased soil and root respiration (ANDERSEN 2003) as well as enhanced fine root turnover (NIKOLOVA *et al.* 2010) in response to elevated ozone also represent higher costs in plants. Reduced below-ground C allocation under elevated ozone causing impairment of the root system and soil organisms, can curtail nutrient acquisition and reduce the radius of exploitation around trees. Moreover, the plant nutrient status can influence the way trees cope with O_3 stress, e.g. via altered C allocation (MAURER & MATYSSEK 1997). For example, increased leaf N in hybrid poplar was accompanied by O_3 injury (HARKOV & BRENNAN 1980 in: LEFOHN 1992).

In forest ecosystems, nitrogen availability often limits tree growth, so there is strong competition between plants, mycorrhizae and microbes for soil nutrients (SCHIMEL & BENNETT 2004). However, increased atmospheric nitrogen deposition may also lead to N saturated forests and influence the nitrogen cycle, e.g. increase fine root turnover (NADELHOFFER 2000) and reduce retranslocation of N from senescing leaves which in turn contributes to higher N mineralisation (ABER *et al.* 1989). Nitrogen acquisition by plants depend on many factors as e.g. physical and chemical soil properties,

mineralisation rates, and mycorrhization. Apparently, both micro-organisms and plants can influence the nitrogen cycle and therefore the environmental conditions for accessing nitrogen (VITOUSEK 1982, HOBBIE 1992, CHAPMAN *et al.* 2006), e.g. via leaf litter properties especially in nitrogen-limited ecosystems such as temperate forests. As trees are characterised by a long life-span, their pool of plant internal nitrogen reflects the plant's storage capacity (MILLARD 1996) and must not be neglected. The plant-internal N cycling which also serves to some extent as a buffer to changes in soil nitrogen availability, might be affected if storage pools are changed in response to stress. Effects of altered retranslocation in response to O_3, as indicated also in mature trees (SAMUELSON *et al.* 1996), may become more severe with tree ageing, as internal N cycling processes then become increasingly important (e.g. MILLER 1984, NAMBIAR *&* FIFE 1991). As a consequence of O_3 stress, a change in leaf C/N ratio can influence the litter decomposition which in turn may alter the nitrogen availability (ANDERSEN 2003).

In the present study, the dynamics of N uptake and distribution in trees under elevated O_3 were examined by the use of ^{15}N as a tracer to follow uptake and allocation processes. To study element cycling in plants or ecosystems, such as the N cycle, the use of stable isotopes has become a widely used method (NADELHOFFER *&* FRY 1994, DAWSON *et al.* 2002). Besides studies of the natural isotope ratios in system pools, the experimental enrichment of isotopes with low natural abundance (as e.g. ^{15}N) can unveil element cycles in ecosystems. The investigated system generally remains undisturbed in its processes, while small changes in element fluxes can be quantified *in situ*. Studies on mature trees using ^{15}N-enriched or depleted soil solutions have been used to improve understanding of plant internal N cycling (e.g. WEINBAUM *et al.* 1994, WEINBAUM *&* KESSEL 1998). In forests, tracer studies with ^{15}N have clarified the contribution of ecosystem components to the N cycle (e.g. NADELHOFFER *et al.* 1995, 1999, 2004, GEBAUER *et al.* 2000, JÄGGI *et al.* 2004).

Beech and spruce trees – both of high ecological and economical importance in Central Europe – differ sharply in their growth strategies. With its shade tolerance and high plasticity in growing, beech can out-compete faster but more rigidly growing trees like spruce (MATYSSEK *et al.* 2005). Together with its relatively wide ecological amplitude in terms of the edaphic and climatic conditions in the temperate zone, beech represents the predominant tree species of Central Europe under potentially natural conditions (ELLENBERG 1996). Furthermore, due to its plasticity in growing, beech can benefit from disturbances in canopy structure better than spruce (REITER *et al.* 2005). However,

resource demand and turnover per growing season seems to be higher in deciduous than in coniferous trees as they rely on the yearly replacement of their foliage (MATYSSEK 1986). This may render beech susceptible under stress-induced resource limitation.
Seasonal N cycling and storage pools are different in beech and spruce trees and the regulation of nitrogen uptake is complex in both species (GESSLER *et al.* 1998*,* 2004*b*). Under chronic stress, trees may have to change their resource allocation in favour of defence, and in the long term this may change their competitiveness. Responses to O_3 as found in mature beech and spruce trees, were inconsistent in space (e.g. within tree crown) and time (NUNN *et al.* 2005*b*, 2006, MATYSSEK *et al.* 2007*a*). Nevertheless, beech seems to be more sensitive to chronic ozone exposure than spruce in terms of accelerated leaf senescence, O_3-induced leaf injury as well as enhanced fine root turnover and reduced stem volume increment (NUNN *et al.* 2002, 2005*b*, PRETZSCH *et al.* 2009, NIKOLOVA *et al.* 2010). Furthermore, in short-term studies, beech showed lower N uptake rates under $2\times O_3$ compared to controls (HABERER *et al.* 2007, spruce was not tested here).

Within the interdisciplinary research program 'SFB607 - Growth and parasite defense - Competition of resources in economic plants from forestry and agronomy', sub-project B4, the present study was part of a long-term project in which mature trees were exposed to enhanced levels of O_3 during the growing seasons of 2000 through 2006 via a free-air O_3 fumigation system (NUNN *et al.* 2002, WERNER *&* FABIAN 2002). Experiments were performed during 2005 and 2006, the 6^{th} and 7^{th} year of $2\times O_3$ exposure, respectively. In this approach, plots of soil underneath mature beech and spruce trees were labelled each with ^{15}N in July 2005, and uptake and distribution of the labelled nitrogen was followed in trees, mycorrhizal root tips and soil over the subsequent 16 months. In a further step, the N balance and cycle of the stand were assessed and consequences of the O_3 impacts in view of species-specific responses were discussed.
It was hypothesized that with regard to the above described O_3-induced impairments, (i) chronically elevated O_3 concentrations limit the nutrient supply and/or demand upon reduced below-ground carbon allocation of the mature trees. Secondly, it was stated that (ii) beech may react more sensitive in response to elevated O_3 regarding N acquisition due to a higher resource turnover as compared to spruce, the latter being regarded as more conservative in its resource allocation.

Material and Methods

Study site

The study site is located in a mixed forest of mature European beech (*Fagus sylvatica* L.) and Norway spruce trees (*Picea abies* [L.] Karst.) in south-eastern Germany (48°25´12´´N, 11°39´42´´E) at an elevation of 485 m a.s.l. In 2005, the trees were, on average, 62 (beech) and 52 (spruce) years old (Wipfler *et al.* 2005). The stand had a stem density of 711 trees ha^{-1} (Dieler, pers. comm.) as it had not been thinned.
The annual mean temperature of the site is 7.0 to 7.5°C and the precipitation 730 to 890 mm per annum, with a mean temperature during the growing season of 14.5 to 15 °C and corresponding precipitation of 410 to 520 mm (Pretzsch *et al.* 1998). The soil is a luvisol derived from loess over tertiary sediments, with pH-values of 3 to 4 in the upper soil and >4 in the deeper soil (Pretzsch *et al.* 1998, Schuhbäck 2004). The site had been under forestry practices for centuries as no agricultural activities were documented (Galsterer 1979).
Research activities within the integrative research centre of SFB607 were carried out in an area of 0.5 ha. Scaffolding allowed for access to the tree canopies at different levels up to a height of about 28 m (*Fig. 3, A)*. Vertically suspended tubes installed within the stand canopy served as a 'free-air canopy O_3 exposure system' (Nunn *et al.* 2002, Werner *&* Fabian 2002). In addition, a crane was used for tree sampling. Throughout the growing seasons (April through September) of the years 2000 - 2007, ozone was released to the canopies of five beech and spruce trees each at twice-ambient ozone levels ($2\times O_3$) monitored continuously, but limited to 150 nl O_3 l^{-1}. Five trees of each species under unchanged ambient ozone levels ($1\times O_3$) served as controls. 110 evenly distributed ozone passive samplers documented the spread of the experimentally released ozone regime within the stand canopy at the study site (Werner *&* Fabian 2002, Heerdt 2007). Mean annual O_3 concentrations and calculated annual O_3 uptake by the trees under both ozone regimes in 2005 and 2006 are given in *Tab. 14 (Appendix)*.

Experimental setup

The experiment reported here was performed during the years 2005 and 2006, ending with the harvest of root and soil samples in March 2007. In total, twelve trees were selected for large-scale ^{15}N labelling, three per species and ozone treatment each (*Fig. 1, 2*). One year prior to the ^{15}N labelling, one plot of 1 m² soil surface was chosen at a distance of about 50 cm from the trunk of each tree.

In July 2005, the plots were labelled with 10 g $^{15}NH_4{}^{15}NO_3$ (enrichment of 98 atom % ^{15}N) dissolved in 70 l of rain water. The labelled solution was applied over seven dates with an amount of 10 l water (i.e. 1.43 g $^{15}NH_4{}^{15}NO_3$) per date, over a period of three weeks, in order to reduce rapid leaching and denitrification as well as preventing toxic conditions for roots and microbes. The total amount of applied N per plot corresponded to an input of around 36.6 kg N per ha, which is regarded as too low for inducing a fertilizing effect as compared to the current background N input at the study site of 15 kg N ha^{-1} a^{-1} (wet and dry deposition, SCHULZ, pers. comm.). Before labelling, leaf litter of the square-shaped plots was removed and kept in plastic bags during the time of labelling in order to ensure homogeneous infiltration into the rooting zone. Hence ^{15}N was directly released into the mineral soil. To avoid desiccation, the plots were covered with 1 m² polystyrene plates during labelling (*Fig. 3, C*). A wooden frame of 10 cm in height, wrapped in aluminium foil was put on the 1 m² plot during application to prevent the solution from spilling outside the plot. The label solution was poured carefully and homogeneously onto the plots with a watering can. After the last date of application, the conserved leaf litter was spread again on the respective plots.

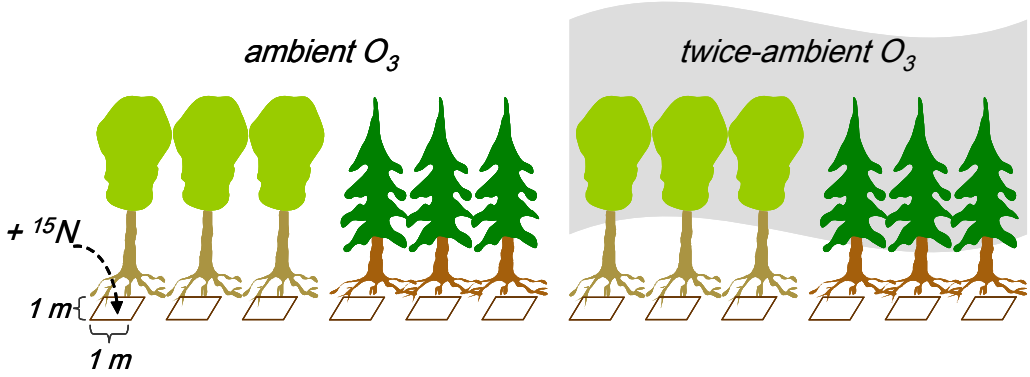

Figure 1: Scheme of the experimental design.

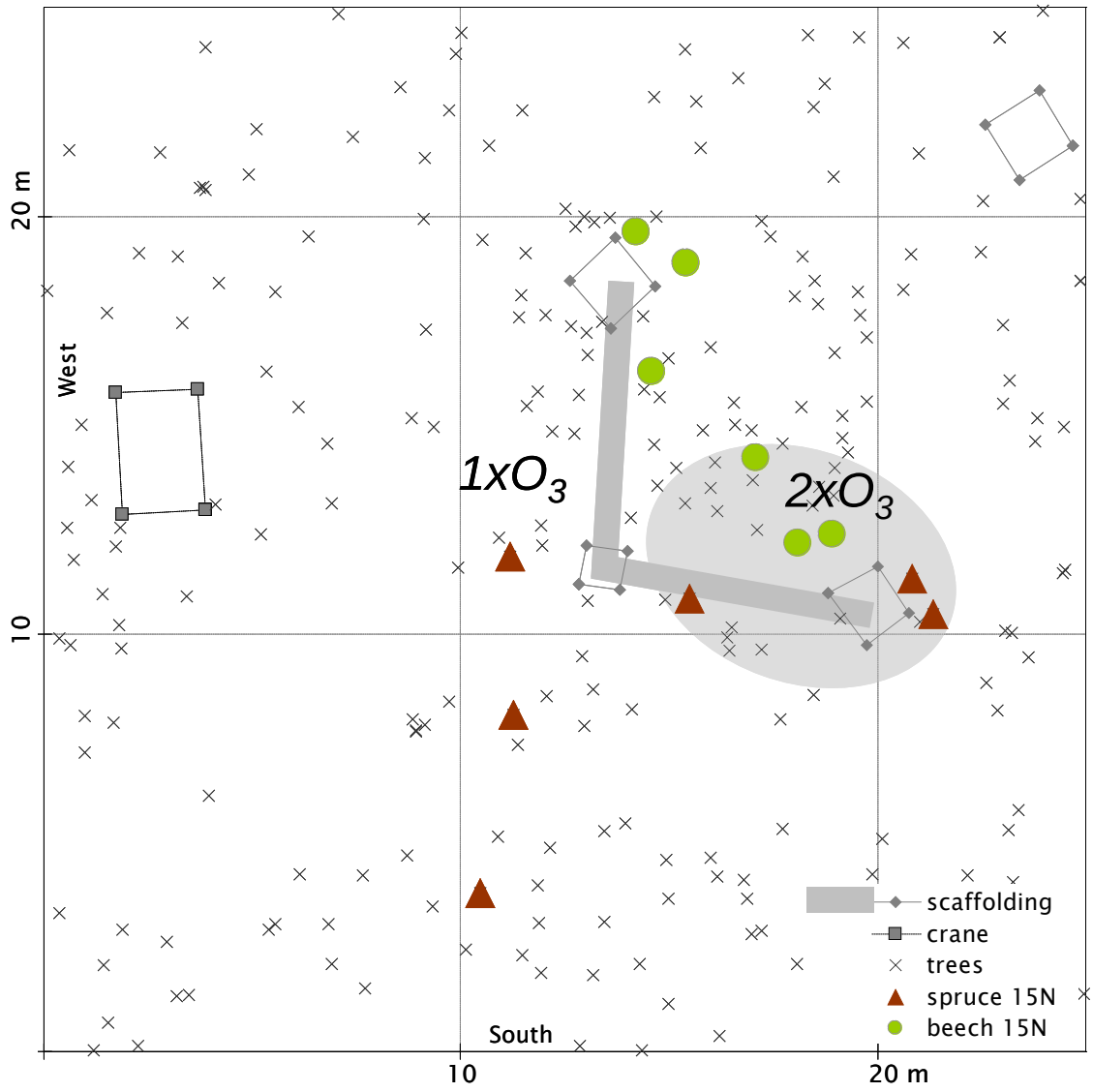

Figure 2: Study site and position of the ^{15}N-labelled beech (circles) and spruce (triangles) trees exposed to ambient ozone concentrations ($1\times O_3$) or to the twice-ambient free-air ozone fumigation ($2\times O_3$).

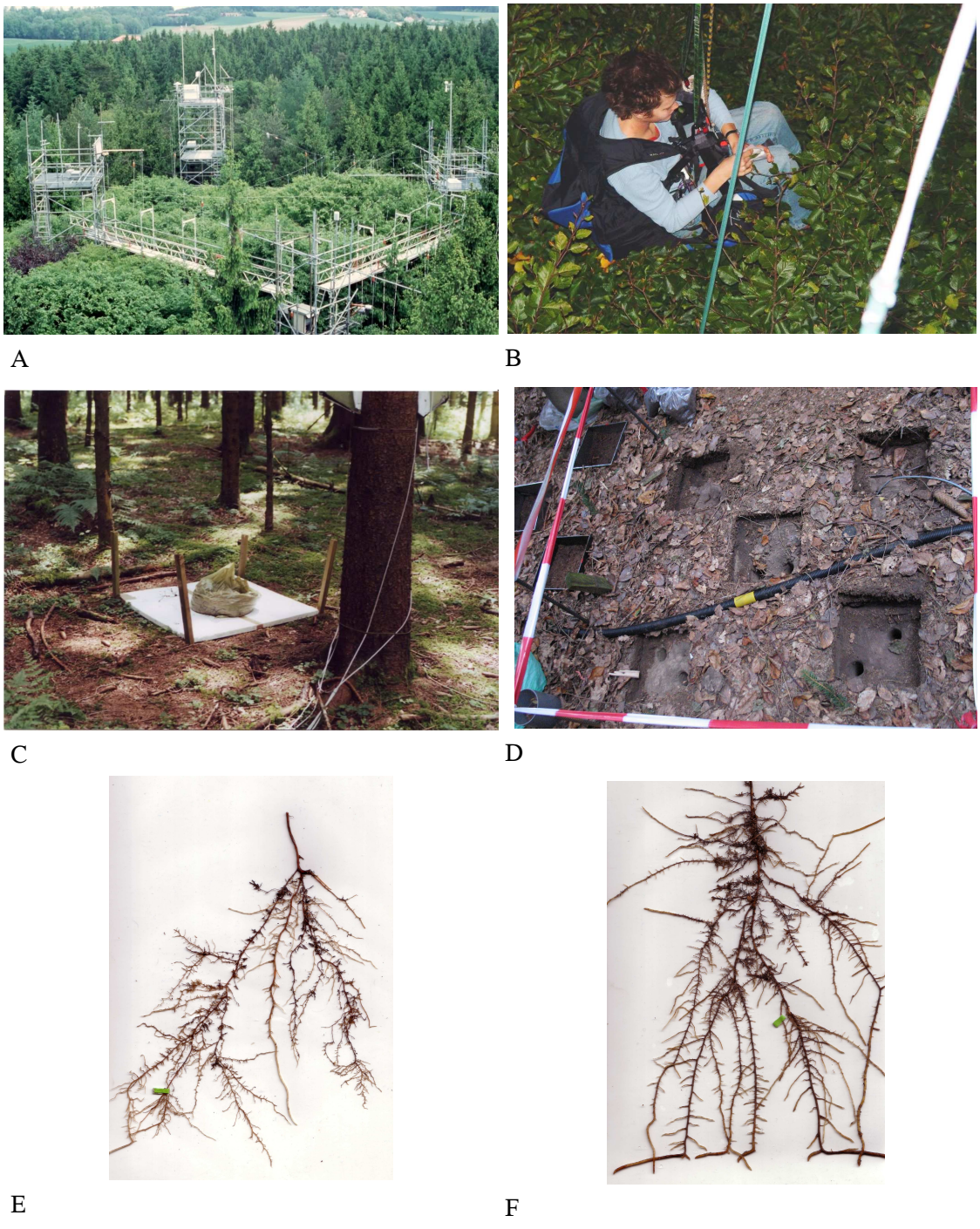

Figure 3: Study site "Kranzberger Forst" with scaffolding (A), leaf sampling in tree canopy (B), example of a ^{15}N-labelled plot during labelling, July 2005 (C), example of a ^{15}N-labelled plot at root and soil sampling, Oct. 2006 (D), example of a beech rootlet (E), example of a spruce rootlet (F).

Sampling and measurements

Plant and soil compartments were sampled at different dates before and after ^{15}N labelling as described below, with the main focus on leaves and needles. An overview of all sampled compartments is given in *Tab. 1* (see page 32).

Leaves and needles

Prior to the ^{15}N labelling, reference samples of leaves and needles were taken from each of the study trees to determine the natural ^{15}N abundance. Upon application of the ^{15}N-labelled solution, leaves, current-year- (0 yr) and one-year-old (1 yr) needles in sun and shade crowns were taken at six dates throughout 2005 and 2006 (*Tab. 1*) from the twelve labelled trees. Sun and shade leaves or needles were distinguished due to their different physiology and anatomy (*Fig. 3, B)*. Additionally, surrounding non-labelled trees were sampled in order to assess ^{15}N label distribution and to enhance replicate number for N_{total} and C/N analysis. Pooled samples of four leaves (beech) or 15 to 20 needles (spruce) per sun or shade branch were used for analysis of incorporated labelled N ($=N_{labelled}$), total N and C/N ratio. In 2006, sampling was extended because of uneven $N_{labelled}$ distribution within crowns (*Fig. 4*). Therefore, four branches were chosen from different compass directions per tree in the sun crown, and one to two in the shade crown. For comparison between species and ozone treatments in terms of incorporation of the $N_{labelled}$, branches were used with highest concentration of $N_{labelled}$ at each sampling date, as such branches were regarded to be best supplied by the roots of the labelled plots. At the last sampling of beech leaves, in November 2006, the shed leaves were collected from nets wrapped around one branch of the sun and one of the shade crown per tree.

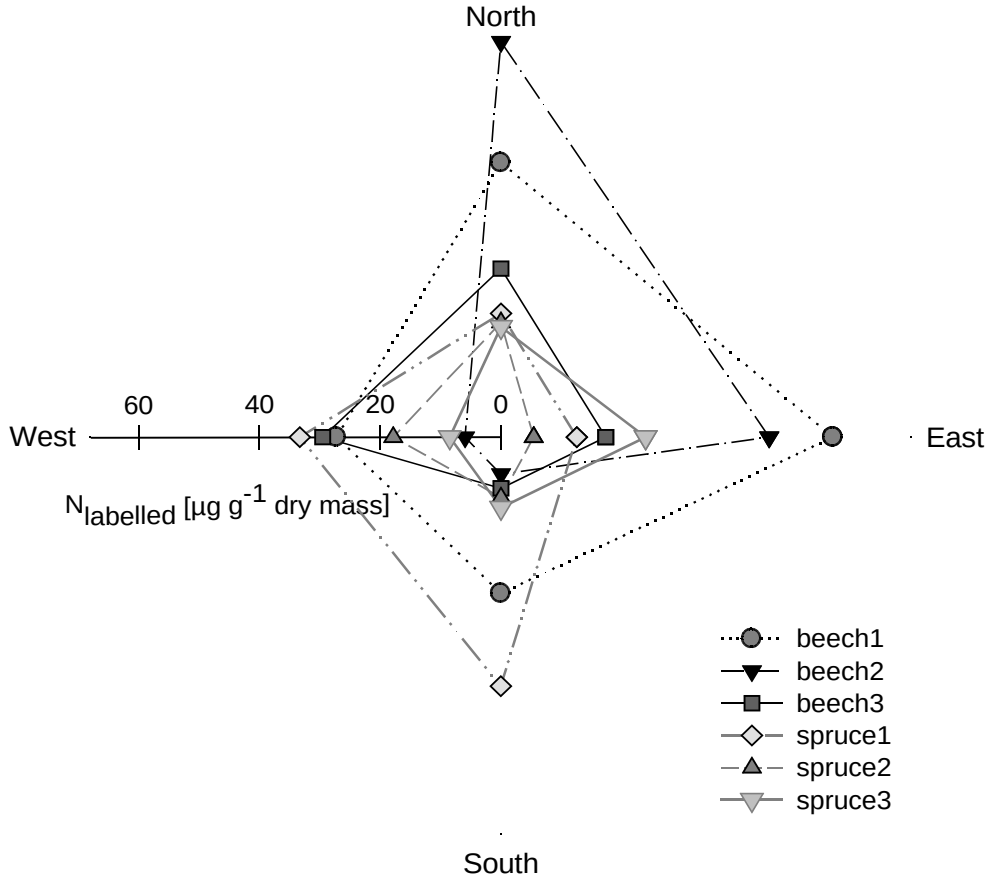

Figure 4: Distribution of $N_{labelled}$ in leaves and needles within tree sun crowns of three exemplified beech and spruce trees, each, as means with n=3 sampling dates per tree and compass direction.

Buds

Buds were sampled together with leaves and needles from same branches, but less frequently at three sampling dates. Four to five buds per sample were pooled for N and C measurements.

Fruits and cones

Fruits were found only in two beech trees, whereas no cones were available from spruce during the two sampling years.

Twigs

Young twigs up to about 5 mm in diameter were sampled once in December 2005 from the sun and shade crown of the trees. In spruce, current-year and one-year-old twigs were sampled separately as done with needle samples. Twigs from the sun crown were further separated into bark (i.e. secondary phloem, primary tissues and periderm) and wood (including parenchyma cells) for detailed labelling assessment, whereas shade twigs were analysed as a whole.

Stem cores

Stem cores were taken in December 2006 at breast height using a wood borer of 5.15 mm in diameter and 30 cm in length. The cores were dried at 65 °C to constant weight and separated into bark and wood as described above. In spruce, the dead outer bark (i.e. non-living peridermal layers or rhytidome), of about 2-3 mm in width, was removed macroscopically from the other bark tissues and excluded from analysis. Such multi-layered dead outer bark is not produced by beech trees. The wood was further separated into the total incremental part of the years 2000 through 2006, which were the years of the experimental $2\times O_3$ treatment ($wood_{ozone}$), and the wood formed before 2000 ($wood_{pre\text{-}ozone}$). These three parts of the stem cores were analysed separately for N and C.

Xylem sap and sap flow measurements

Xylem sap was collected in July 2006 from twigs of the sun crown of ^{15}N-labelled trees. A Scholander pressure bomb was used for extracting the sap. To this end, the twigs were enclosed into the pressure cylinder in a way that the cut end protruded from the cylinder by about 2 cm of their length. The bark of the upper end (about 1 cm of length) was removed to prevent contamination with phloem sap. A piece of silicon tube was slid over the base twig surface to take up the xylem solution. When pressure was raised in the cylinder, the sap was pressed through the vessels and appeared at the cut surface. It was then carefully collected from the silicon tube with a pipette and transferred into an Eppendorf tube. The pressure was increased slowly up to a maximum of 1 MPa above the level compensating the xylem water potential of the twigs. The sap was freeze-dried for N and C analysis (see below).

To correlate transpiration and the labelled N taken up by the trees, xylem sap flow of sun crown branches of same trees or, in case of spruce $1\times O_3$, of other trees from the same study site, were provided by U. METZGER (Ecophysiology of Plants, Department of

Ecology- and Ecosystem Management, TU München). Sap flow was measured every 10 minutes over two growing seasons (2005 and 2006) by means of Granier sensors (METZGER, pers. comm.). Transpiration during winter time (i.e. when beech had no foliage) was not measured.

Phloem sap

Phloem sap measurements and data were provided by the SFB partner project B5 (RITTER, GRAMS, Ecophysiology of Plants, Department of Ecology- and Ecosystem Management, TU München). Phloem sap of the studied trees was sampled by extraction of stem bark cores that were incubated in a buffer solution containing 15 mM polyphosphate (RITTER, pers. comm.). For N and C analysis, phloem exudates were freeze-dried as in the case of xylem sap.

Roots and soil

Reference samples on the natural ^{15}N abundance in fine roots and soil were taken once prior to ^{15}N labelling. In October 2006, about 1.5 years upon application of the ^{15}N labelling solution, roots and soil were sampled from the plots and separated by soil horizons or layers (*Fig. 3, 5*) according to the FAO soil taxonomy (FAO 2006). The upper organic layer of undecomposed or partially decomposed organic matter (O_i)[1] was separated from the lower organic layer of decomposed organic matter (O_a)[1], which was distinctly developed only under spruce. The mineral B horizon was further separated into layers of 10 to 20 cm width up to a depth of 90 cm. B down to ~ 30 cm is equivalent to E (FAO 2006)[2], a horizon lessived in clay as typical for luvisols. Five replicates per plot and horizon or layer were taken using metal frames (20 x 20 cm, 15 cm in height) for the upper horizons (*Fig. 5*). Soil cores from the B horizon were taken down to 30 cm depth with five replicates, whereas in the depth of 30 to 90 cm samples were taken only in the centre of each plot (*Fig. 5*). Two cores per layer and replicate were pooled to attain sufficient root biomass from the B horizon. In addition to the twelve labelled plots, those of four non-labelled trees, two per species and O_3 treatment each, were sampled accordingly.

1 Equivalent to $L+O_f$ and O_h, respectively, according to the German nomenclature, AG Bodenkunde (1982)

2 Equivalent to A_l/B_v according to the German nomenclature, AG Bodenkunde (1982)

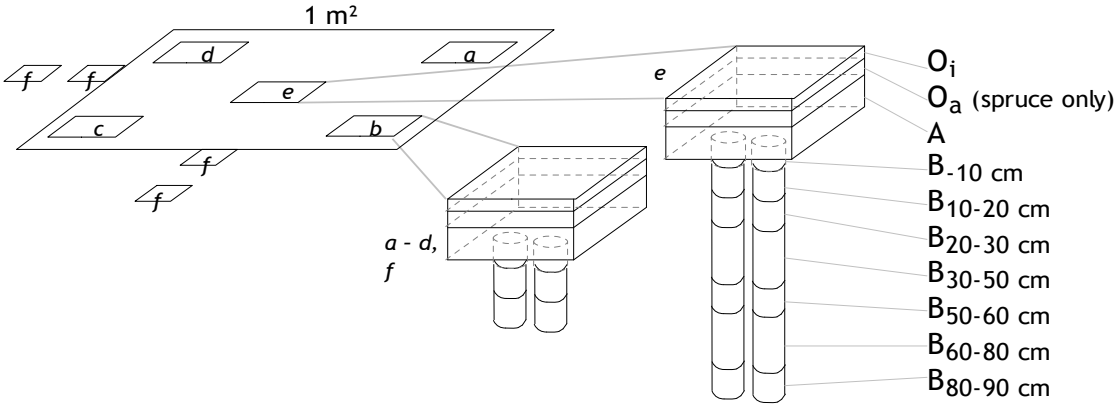

Figure 5: Protocol of root and soil sampling in 1 m²-plots. O_i and O_a represent organic soil layers, A and B represent mineral soil horizons. B was further separated into layers of 10 to 20 cm width. Five replicates per horizon or layer were taken down to 30 cm depth (a-d), and one sample per layer in 30 to 90 cm depth (e). Samples adjacent to the plots (f) were taken at a distance of 0 – 10 cm and 20 – 30 cm from plot border and down to 60 cm depth.

For testing ^{15}N-tracer distribution outside of the labelled plots, additional soil samples were taken at two locations adjacent to one beech and one spruce plot each, at a distance of 0 – 10 cm and 20 – 30 cm (*Fig. 5: f)* down to 60 cm depth.

In March/April 2007, roots and soil of the labelled 1 m²-plots were completely harvested down to 50 cm depth, without distinction of different soil horizons, in order to determine the total biomass of roots.

Soil cores including roots were stored at 7 °C prior to preparation to prevent microbial degrading processes or respiration of roots, and, in addition, to be able to distinguish between alive and dead roots in the samples. Roots were separated from the soil, washed carefully and sorted by species (*Fig. 3, E, F)*, root diameter classes ≤ 2 mm, ≥ 2 < 5 mm and ≥ 5 mm, and soil horizon or layer (sampling Oct. 2006). For analysis of coarse roots, those of diameter size of ≥ 5 < 20 mm were taken. During the washing procedure, roots that swam on water surface and/or were decayed in their structure were defined as dead and were removed. Roots were dried then at 65 °C to constant weight and the dry mass was determined. In the A horizon, mycorrhizal root tips as well as bark and wood of coarse roots were analysed to account for tissue-specific N concentration. Therefore, mycorrhizal root tips were separated from fine roots by carefully crushing

the dried roots and collecting the readily crumbling material. From this fraction, root tissue that had been peeled off was removed to the extent possible. Coarse roots were separated into bark and wood as described for twigs.

All soil samples were weighed in order to estimate the total soil mass per plot, and dried at 65 °C to constant weight for further analysis. For determination of soil water content, fresh and dry weight (standardized at 105 °C to constant weight) of soil aliquots from each horizon or layer per plot were measured.

For N and C assessment in roots and soil, one (B horizon below 30 cm) to three (upper horizons and B above 30 cm depth) replicates per horizon or layer and plot were used.

Table 1: Sampled compartments and sampling dates.

Sampled compartment	**Sampling date**	**Subsamples and Replication**
Sampling per tree		
Leaves (beech), sun & shade	End of June '05 (before ^{15}N labelling); beginning of Aug. '05; end of Aug. '05; June '06; Aug. '06; Sept. '06; Nov. '06	Two sampled branches per tree (sun and shade): pooled sample of four leaves per branch; in 2006, extended sampling of four branches with different orientations within sun crown
Needles (spruce), sun & shade, current-year-old (0yr) & one-year-old (1yr)	End of June '05 (before ^{15}N labelling); beginning of Aug. '05; end of Aug. '05; beginning of Dec. '05; June '06; Aug. '06; Sept. '06	Two sampled branches per tree (sun and shade): pooled sample of 15-20 needles per branch and needle age; in 2006, extended sampling of four branches with different orientations within sun crown
Buds (beech & spruce), sun & shade	Dec. '05; Aug. '06 (beech only); Sept. '06	Two sampled branches per tree (sun and shade): pooled sample of 2-3 buds per branch; in 2006, extended sampling of four branches with different orientations within sun crown

Table 1: continued.

Sampled compartment	**Sampling date**	**Subsamples and Replication**
Fruits (beech only)	Aug. '06	Fruits of only one tree available (beech), one exemplary fruit separated into capsule, seed coat and seed
Twigs sun & shade current-year (beech & spruce), one-year old (spruce)	Dec. '05	One per sun or shade crown and tree; twigs of sun crown separated into bark and wood
Stem wood & bark	Dec. '06	One core per tree; separated into bark, $wood_{ozone}$ and $wood_{pre\text{-}ozone}$
Xylem sap	July '06	One twig per tree within sun crown
Phloem	July '06; Sept. '06	Three cores of stem bark per tree and sampling date (morning, midday, evening), provided by partner project B5 (RITTER, pers. comm.)
Sampling per 1 m² plot		
Roots	June '05 (before ^{15}N labelling); Oct. '06; March '07	Five replicates per plot and soil horizon or layer down to 30 cm depth, and one sample (two cores pooled) per plot of layers 30 – 90 cm depth; samples separated into three different root classes (< 2 mm, ≥ 2 < 5 mm, ≥ 5mm)
Mycorrhizal root tips	Oct. '06	2 to 3 replicates per plot; only A horizon
Soil	June '05 (before ^{15}N labelling); Oct. '06	Five replicates per plot and soil horizon or layer down to 30 cm depth, and one sample (two cores pooled) per plot of layers 30 to 90 cm depth; samples adjacent to plots: two replicates per plot (of one beech and one spruce plot each) and horizon or layer at a distance of 0 to 10 cm from plot border down to 60 cm depth and at a distance of 20 to 30 cm down to 30 cm depth

N and C analysis

Solid plant material was oven-dried at 65 °C to constant weight and powdered in a ball mill (model MM2, Retsch GmbH, Haan, Germany) using polypropylene Eppendorf tubes (2 ml) and steel balls of 4 mm in diameter. For analysis of beech leaves, an aliquot was taken from the four leaves per sample in a way so that same regions of the leaf blade were cut from each leaf to ensure representativeness (*Fig. 6*).

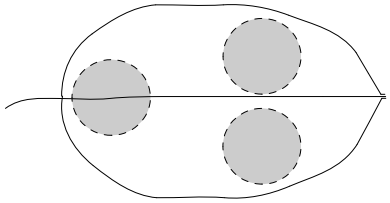

Figure 6: Leaf material of beech for ^{15}N-analysis. Regions of the leaf blade used for analysis are displayed in grey.

For analysis of spruce needles, about 10 to 15 needles per sample were ground, as described above. Other plant material of beech and spruce was ground as a whole or as representative aliquots. Roots were cut with scissors before being ground. Soil material was milled using 10 mm steel balls (hardened steel). Abrasion of the balls was assumed to be negligible compared to the amount of soil material and no elemental analysis was performed with the sample material other than that of C and N. The xylem sap was freeze-dried in a lyophiliser (model Beta 1-8, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and concentrated to fit into the tin cups prior to analysis in the mass-spectrometer.

For N and C analysis, all samples were weighed into tin cups and measured in a stable isotope ratio mass spectrometer IRMS (Isoprime Ltd., former GV Instruments Ltd., Manchester, UK) connected to an elemental analyser (EA 3000, Euro Vector Instruments and Software, Milan, Italy). Depending on the average concentration of nitrogen for each organ, a minimum dry weight of 2 mg for leaves, needles, buds and twigs, 3 mg for roots and stem samples and 5-10 mg for soil (according to the average N content of each horizon) was necessary to warrant precise measurement. For δ^{15}N, the precision of measurement was ± 0.2 ‰ based on the laboratory standard. Rye flower was used as the laboratory standard after measurement of every 12th sample each. To

prevent contamination with labelled material, the labelled and non-labelled samples were prepared with different equipment where appropriate (steel balls, instruments to weigh in ground material) or cleaned with alcohol and ultrasound (tweezers, scissors).

Soil texture and soil density

Analysis of soil texture was performed from two plots, one under beech and spruce each, from the mineral soil horizons. The proportion of the fine soil fraction was determined by hydrometer analysis (ISRIC 1992) on the basis of the settling velocity of different particle size classes in a liquid, while the coarse fraction was measured by wet sieving. Humus-rich soil samples (> 3 % C) were pre-treated with H_2O_2 to destroy the organic matter.

For soil density measurement, one soil profile was excavated down to 70 cm depth, as undisturbed soil was sampled with cutting cylinders (100 cm^3) along the profile.

Nitrate leaching and denitrification

Possible loss of labelled N by leaching was assessed through nitrate leaching measurements at different trees at the same study site under $1\times O_3$ using non-labelled NH_4NO_3. The area-based method by Self Integrating Accumulators (SIA, TerrAquat, Stuttgart, Germany) was used which consist of cylinders of 10 cm height and 10 cm diameter with a fine net at the bottom to allow water passage (*Fig. 7*). Cylinders were filled with a mixture of quartz sand, quartz silt and an anion-exchange resin as NO_3 adsorber (Bischoff, 2007). Plots of 1 m^2 were chosen with ten beech and spruce trees each, with sampling at a distance of around 50 cm from the trunk (as in labelled plots). Adjacent to each plot in opposite to the corresponding tree, soil was excavated on an area of 40 x 60 cm to a depth of 60 cm. Two horizontal tunnels underneath each plot were excavated at 40 to 60 cm depth leaving the above soil undisturbed. A SIA cylinder was placed in each horizontal tunnel with the top edge of the cylinder at a depth of 40 cm (below the main rooting zone). By this placement, vertical soil water flux could pass through the cylinders at a defined area of 78.5 cm^2 thereby adsorbing the dissolved NO_3. The cylinders were surrounded by sand to create a barrier for water flow (for details see Bischoff & Kaupenjohann 1998, Bischoff 2007). The pits were refilled with soil and the SIAs remained in the soil for 9 months. After installation of the adsorber cylinders, in August 2005, non-labelled NH_4NO_3 (10 g dissolved in 70 l of rain water) was applied to the plots over seven dates comparable to the ^{15}N-labelled plots with a

time delay of four weeks compared to the ^{15}N labelling. In May 2006 (snow cover remained until April), the SIAs were removed from the soil and analysed for NO_3 content (see BISCHOFF 2007).

Denitrification was assessed via N_2O emission although N_2 release from the soil can not be resolved due to the high background concentration of N_2 in the atmosphere (78 %). According to SCHLESINGER (1997), about 5 % of N resulting from denitrification is released via N_2O. N_2O release from the soil was measured on the plots used for NO_3 leaching measurements and was carried out over 1 year (2005 to 2006) by the SFB partner project B12 (SCHLOTER, MUNCH, Institute of soil ecology, Helmholtz Zentrum München).

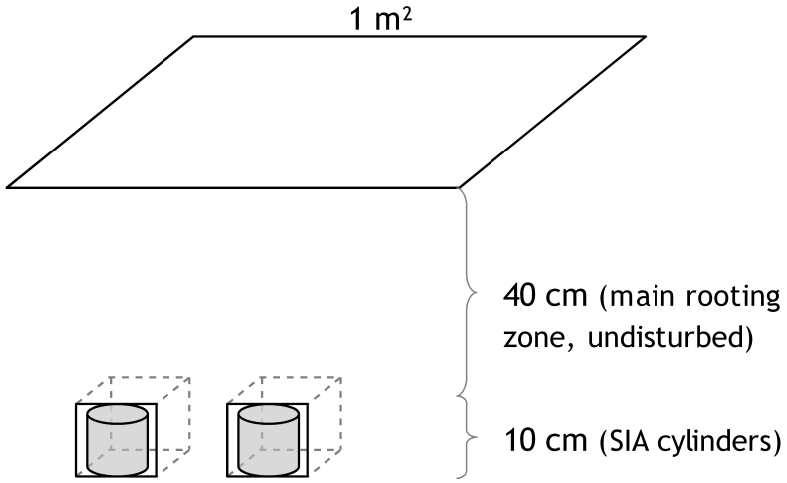

Figure 7: Scheme of the area-based nitrate leaching measurements underneath 1 m²-plots of beech and spruce. SIA cylinders were installed below the main rooting zone at 40 cm soil depth, with two replicates per plot.

Nitrogen mineralisation

Net mineralisation of nitrogen of the study site was measured over one year between June 2006 and June 2007, by exposing soil samples of the horizons O, A and B (down to 12 cm depth) in polyethylene bags to the temperature regime of the stand at the corresponding soil depth (according to RUNGE 1970). The monthly collected samples (except for January to March 2007 where no samples were taken) were incubated in the field for four and eight weeks in order to evaluate the nitrogen mineralisation over time. Concentrations of total nitrogen, ammonia and nitrate were determined in $CaCl_2$ extracts

of the soil samples before and after incubation. The difference in concentrations of ammonia and nitrate between sampling date and end of incubation represent the net mineralisation during the incubation time.

*Calculation*s

$N_{labelled}$, total N and C/N ratio

The incorporation of labelled N into plant organs or soil was calculated using the following formula, according to MILLARD & NEILSEN (1989):

$$N_{labelled\ (compartment)} = \frac{{}^{15}N\ atom\%\ excess_{(compartment)} * N_{total\ (compartment)}}{{}^{15}N\ atom\%\ excess_{label\ solution}}$$

where $N_{labelled\ (compartment)}$ is the concentration of labelled N in a certain tissue or compartment per unit of dry mass; ${}^{15}N\ atom\%\ excess_{(compartment)}$ is the ^{15}N enrichment of the labelled sample minus the natural ^{15}N abundance of the appropriate tissue or compartment; $N_{total\ (compartment)}$ is the total nitrogen concentration of the specific tissue or compartment per unit of dry mass; ${}^{15}N\ atom\%\ excess_{label\ solution}$ is the ^{15}N enrichment of the applied $^{15}NH_4{}^{15}NO_3$-solution minus the natural mean background of ^{15}N, taken as 0.37 atom% (according to MARIOTTI 1983, IUPAC 2002). For natural ^{15}N abundance of the each tissue or compartment, non-labelled material was sampled before ^{15}N labelling (see above). Discrimination against ^{15}N during N uptake and cycling was regarded as negligible here since the labelled nitrogen was applied as readily available N and was highly enriched in ^{15}N. C/N ratio was calculated using the measured concentrations of C and N_{total} per dry mass.

Transpiration per unit of time period

Daily transpiration rates were summed up from the beginning of the ^{15}N labelling in July 2005 until September 2006, the last date of leaf and needle sampling before autumnal leaf fall (*Tab. 1*). The cumulative transpiration was correlated with the concentrations of $N_{labelled}$ in sun leaves of beech and current-year needles of spruce in September 2006, 16 months after the ^{15}N labelling.

Root density and root distribution along the soil profile

Root density was calculated by summing up the dry mass of all root samples per plot (October 2006 and March/April 2007) down to 0.5 m depth, expressed as g m^{-2}. Root distribution was scaled along the soil profile as based on the root biomass sampled in October 2006 from the different soil horizons or layers.

N budget of trees

The N budget of the trees was calculated on the basis of the measured N_{total} concentrations of each plant compartment and the biomass per whole tree for each of the studied trees. Biomass data were provided by the SFB partner project C1 (RÖTZER, PRETZSCH, Forest yield science, Department of Ecology- and Ecosystem Management, TU München) from a modelling approach ("BALANCE", see RÖTZER *et al.* 2009) as based on biogeochemical and physiological processes that drive tree growth (PRETZSCH *et al.* 2008, RÖTZER *et al.* 2009). An overview on N concentrations and corresponding biomass data is given in *Tab. 2*. Where the model did not provide biomasses for compartments covered in N assessments, the following approaches were employed instead:

Since the proportion of sun and shade crown biomass varies among trees depending on stem diameter, stand density and light regime (e.g. LARCHER 1994, CERMAK 1998), means of measured N concentrations of sun and shade leaves were used here to calculate the N content in the tree crown. REITER *et al.* (2005) reported for sun branches of beech at the same study site a higher carbon investment of foliage mass per canopy volume compared to shade branches by a factor of 1.4, but found no difference in respective investments of sun and shade foliage in spruce (factor 1.1). Investment in woody axes per volume was also higher in the sun than in the shade crown in beech but did not differ in spruce. Similarly, FARNADY (2008) found a higher proportion of sun than shade foliage mass per branch in beech and to a lower extend also in spruce. However, the

proportions of sun and shade of the total crown volume was not clear. On the other hand, projected foliage area and specific leaf area (m^2 kg^{-1}) of sun foliage is about half that of shade foliage each in beech and similar in sun and shade of spruce (PATZNER 2004). Upscaling can lead to a similar amount of foliage biomass in the sun and shade crown but depends on the above mentioned factors, as well as on classification of sun and shade characteristics, particularly in the intermediate zone within the canopy. Because of these uncertainties in quantification of sun and shade crown biomass, a proportion of 50 : 50 of sun and shade crown biomass (buds, foliage, twigs, see *Tab. 1* and *13*) was employed in this study as an approximation for the total tree crown.

N concentration in needles and twigs of spruce was measured in current-year and one-year-old needles or twigs (*Tab. 1* and *13*). At the present study site, 91 % of total needle mass in the spruce trees comprises needles younger than five years old (NUNN *et al.* 2006) and these age classes are generally characterised by similar N concentrations per dry mass (SCHULZE *et al.* 1989). Therefore, the mean of N concentrations of both measured age classes, current-year and one-year-old (see *Tab. 1* and *13*), was used in the present study to calculate the N content per tree foliage and woody axes as an approximation.

The modelled total stem biomass was divided into the compartments bark, $wood_{ozone}$ and $wood_{pre\text{-}ozone}$ (definition see above, *Fig. 8*). For bark biomass a proportion of 7.2 % (beech) and 9.6 % (spruce) of total stem dry mass was used according to DIETZ (1975). Biomass proportion of $wood_{ozone}$ and $wood_{pre\text{-}ozone}$ was calculated on a stem volume basis, with the stem being regarded as cone-shaped in both species (including the crown height as vertical prolongation of the stem). Measured tree height in 2006 was used as cone height for $wood_{ozone}$ and tree height in 1999 was used for $wood_{pre\text{-}ozone}$. The diameters for these calculations were derived from the measured breast height diameter of each tree (WIPFLER, pers. comm.), taking into account the mean thickness of living bark (2.6 and 3 mm for beech and spruce[3], respectively) and extrapolating linearly to the stem base. Thickness of wood formed during the years of the experimental O_3 treatment (2000 through 2006) was measured on stem cores per each tree (see above), being on average 30 and 25 mm for beech and spruce, respectively. Wood density was regarded as similar between the two distinguished stem wood compartments. The dead outer bark of spruce which had been excluded from the N and C analysis (see above) was included in the modelled total stem biomass, thus leading to some possible overestimation of N

3 Bark of spruce measured without dead outer bark.

in the bark of spruce.

Total root biomass as provided by the model, was further distinguished into coarse roots (≥ 5 mm), medium-sized roots (≥ 2< 5 mm) and fine roots (< 2 mm), according to the biomass proportion of these three root classes assessed through the harvest of the ^{15}N-labelled plots (*Tab. 1*). Additionally, biomass of mycorrhizal root tips, which were not included in the modelled data, were added to fine root biomass according to the biomass proportion of fine roots and mycorrhizal root tips measured in the A horizon.

Total N budget and partitioning per tree was calculated for the winter season, after leaf fall of beech, since N concentration of most compartments was measured only once, in autumn or winter when trees had reached dormancy (*Tab. 1*).

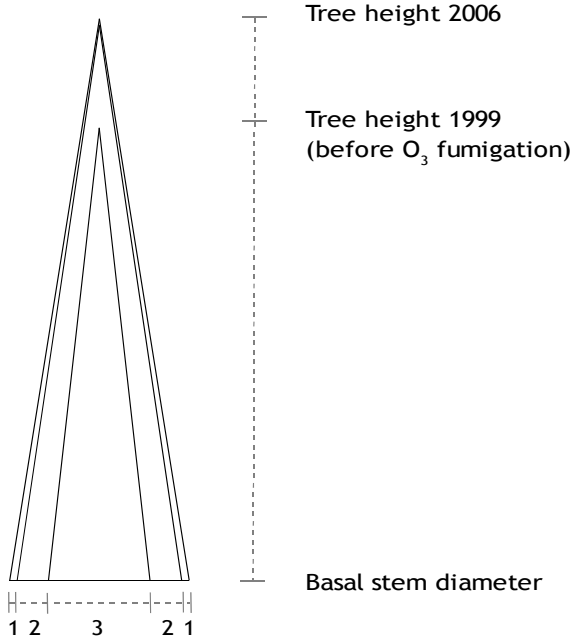

Figure 8: Scheme of the estimated volume proportion of bark (1), $wood_{ozone}$ (2), and $wood_{pre\text{-}ozone}$ (3), used for calculation of biomass proportions and N pools in beech and spruce trees.

Recovery of $N_{labelled}$ at the whole-tree level

The recovery of the labelled nitrogen 16 months after application was calculated for each measured tissue or compartment as:

$$\text{recovery}_{(\text{compartment})} = \frac{N_{\text{labelled (compartment)}} \times \text{biomass}_{(\text{compartment})}}{N_{\text{label solution}}}$$

where

$\text{recovery}_{(\text{compartment})}$ is the proportion of labelled N in a whole tissue or compartment per tree or soil relative to the amount of labelled N applied per each 1 m² plot; $N_{\text{labelled (compartment)}}$ is the concentration of labelled N in a certain tissue or compartment per unit of dry mass (see above)[4]; $\text{biomass}_{(\text{compartment})}$ is the total mass of a certain tissue or compartment per tree, or the soil mass; $N_{\text{label solution}}$ is the amount of labelled N applied per each 1 m² plot (i.e. 3.66 g N plot^{-1}). In the case of roots the biomass refers to the root mass assessed per 1 m² plot (see above), in the case of soil it refers to the soil mass down to 90 cm depth per 1 m² plot and down to 60 cm depth for the area surrounding the plots up to 30 cm distance from plot border according to *Fig. 5* (masses were extrapolated from soil cores of each plot, see above).

Recovery of labelled N in non-labelled neighbourhood trees was calculated from an average recovery of five measured non-labelled trees which were situated at distances of 1-2 m from labelled plots, multiplied by all surrounding trees per each plot up to a distance of about 2 m.

Understorey vegetation which was present only on some spruce plots (mosses) and adjacent to one of the spruce plots (*Dryopteris filix-mas* L., *Rubus fruticosus* L. agg., juvenile *Acer pseudoplatanus* and *Abies alba*), was not quantified here as their biomass was small.

For assessing loss of labelled N via leaching, a proportion of 2 % of nitrogen in bulk soil was assumed as maximum plant available N dissolved in the soil solution (according to SCHROEDER 1972), since the N concentration of soil solution was not measured. The dilution of labelled N was calculated by assuming that principally all labelled N was still dissolved in the soil solution at 16 months after application.

4 For recovery of labelled N in foliage and buds per individual tree, the unequal distribution of the label within the tree crowns were taken into account by applying the mean of four orientations measured per tree in 2006 (see above).

Table 2: Overview on the biomass and element data of beech and spruce used for N budget and recovery of $N_{labelled}$. Tree biomass data were derived from the model "BALANCE" or calculated, where modelling was not available (see text).

Compartment	*Biomass*	*N_{total} concentrations*	*$N_{labelled}$ concentrations*
Foliage	per tree and date	per tree and date; mean of sun&shade, mean of orientations (in 2006), mean of 0yr and 1yr old needles	per tree and date; mean of sun&shade, mean of orientations (in 2006), mean of 0yr and 1yr old needles
Buds	per tree species and date -> proportion calculated for each tree	per tree and date; mean of sun&shade, mean of orientations (in 2006), mean of 0yr and 1yr old needles	per tree and date; mean of sun&shade, mean of orientations (in 2006), mean of 0yr and 1yr old needles
Branches	per tree and date	per tree and date; mean of sun&shade, in spruce: mean of 0yr and 1yr old twigs	per tree and date; mean of sun&shade, in spruce: mean of 0yr and 1yr old twigs
Total stem	per tree and date		
Stem bark	calculated as proportion of total stem biomass (7.2 % for beech and 9.6 % for spruce acc. to DIETZ, 1975)	per tree, measured in Dec. 2006[5]	per tree, measured in Dec. 2006[5]
Stem $wood_{ozone}$	calculated as: total stem – (stem bark + stem $wood_{pre\text{-}ozone}$)	per tree, measured in Dec. 2006	per tree, measured in Dec. 2006
Stem $wood_{pre\text{-}ozone}$	calculated as proportion of total stem biomass via volume of stem wood without bark and $wood_{ozone}$	per tree, measured in Dec. 2006[5]	per tree, measured in Dec. 2006[5]

5 Missing values of stem tissues from single trees were extrapolated via an average ratio in N concentrations of bark : $wood_{ozone}$ or $wood_{ozone}$: $wood_{pre\text{-}ozone}$ from same species.

Table 14: continued.

Compartment	*Biomass*	*N_{total} concentrations*	*$N_{labelled}$ concentrations*
Total roots	per tree and date		
Coarse roots (bark, wood) ≥ 5 mm	calculated as proportion of total roots per tree acc. to measurements from 1 m²-plots	per 1 m² plot, measured in Oct. 2006	per 1 m² plot, measured in Oct. 2006
Roots ≥ 2< 5 mm	calculated as proportion of total roots per tree acc. to measurements from 1 m²-plots	per 1 m² plot, measured in Oct. 2006	per 1 m² plot, measured in Oct. 2006
Roots < 2 mm	calculated as proportion of total roots per tree acc. to measurements from 1 m²-plots	per 1 m² plot, measured in Oct. 2006	per 1 m² plot, measured in Oct. 2006
Mycorrhizal root tips	calculated as proportion of fine roots < 2 mm per tree acc. to measurements from 1 m²-plots (A horizon)	per 1 m² plot, measured in Oct. 2006	per 1 m² plot, measured in Oct. 2006
Soil	per 1 m² plot down to 90 cm depth, extrapolated from soil core measurements	per 1 m² plot, measured in Oct. 2006	per 1 m² plot, measured in Oct. 2006
Neighbouring trees	per tree and date of five exemplified trees	organs measured as for ^{15}N labelled trees	organs measured as for ^{15}N labelled trees

Partitioning of new N (=$N_{labelled}$) on whole-tree level

Partitioning of $N_{labelled}$ per tree, as the result of the allocation of newly acquired nitrogen within the tree, was determined on the basis of the recovery in late autumn/winter since most compartments were measured after leaf fall of beech. The partitioning of $N_{labelled}$ in all compartments was calculated as:

$$q_{N\ labelled\ (compartment)} = \frac{recovery_{(compartment)}}{recovery_{(total\ tree)}}$$

where

$q_{N\ labelled\ (compartment)}$ is the proportion of $N_{labelled}$, i.e. new N recovered, in each studied tissue or compartment relative to the total of $N_{labelled}$ recovered per tree and date; $recovery_{(total\ tree)}$ is the sum of the recovery of $N_{labelled}$ of all studied tissues or compartments per tree. Since not all tissues or compartments were analysed for $N_{labelled}$ before and after beech leaf fall, some approximations were used as shown in *Tab. 3*. In order to take into account beech foliage in the partitioning of $N_{labelled}$ in late summer, the proportions of $N_{labelled}$ in branches and stem bark (both measured in winter) were corrected for N retranslocated from leaves before leaf fall (see *Tab. 3*).

Table 3: Sampling dates of measured concentrations of $N_{labelled}$ [µg g^{-1}] used for partitioning of new N.

Sampling dates applied for partitioning of new N in late summer	
Foliage	Sept. 2006
Buds	Sept. 2006
Branches	Dec. 2005
Stem bark	Dec. 2006, corrected for retranslocated $N_{labelled}$ of senescent leaves in beech by subtraction of a proportion of $N_{labelled}$ retranslocated into stem bark according to: $N_{labelled}$ retranslocated = $N_{labelled}$ foliage (Sept. 2006) – $N_{labelled}$ shed leaves (November 2006); apportionment of $N_{labelled}$ retranslocated between stem bark and branches was estimated according to the proportion of $N_{labelled}$ recovered in stem bark vs. branches
Stem $wood_{ozone}$	Dec. 2006
Stem $wood_{pre\text{-}ozone}$	Dec. 2006
Coarse roots (bark, wood) ≥ 5 mm	Oct. 2006
Roots ≥ 2< 5 mm	Oct. 2006
Roots < 2 mm	Oct. 2006
Mycorrhizal root tips	Oct. 2006
Sampling dates applied for partitioning of new N in winter	
Foliage	(spruce only) Sept. 2006
Buds	Sept. 2006
Branches	Dec. 2005, corrected for additional uptake of $N_{labelled}$ in 2006 in beech: as approximation, an estimated proportion of $N_{labelled}$ retranslocated from senescent leaves into branches according to the proportion of $N_{labelled}$ recovery in stem bark vs. branches added as minimum increase in $N_{labelled}$ in 2006
Stem bark	Dec. 2006
Stem $wood_{ozone}$	Dec. 2006
Stem $wood_{pre\text{-}ozone}$	Dec. 2006
Coarse roots (bark, wood) ≥ 5 mm	Oct. 2006
Roots ≥ 2< 5 mm	Oct. 2006
Roots < 2 mm	Oct. 2006
Mycorrhizal root tips	Oct. 2006

N cycle of the stand

The N cycle at the stand level was estimated on the basis of the basal stem area at the study site of 50.77 m^2 ha^{-1} in 2005, with beech accounting for 13.14 and spruce for 35.95 m^2 ha^{-1} (DIELER, pers. comm.). N uptake was calculated from growth increment of 2005 to 2006 (based on modelled biomasses in both years). The nitrogen pool of the bulk soil was calculated as the sum of total nitrogen per soil horizon or layer down to 90 cm depth, as derived from N concentration measurements and soil mass extrapolated from soil core assessments (see above).

Nitrogen deposition and interception at the stand level were derived from continuous recordings at the local climate station, approximately 1 km away from the study site (SCHULZ, LWF 2006, pers. comm.).

Nitrogen in microbial biomass of the study site was determined by chloroform-fumigation-extraction of soil samples (ESPERSCHÜTZ *et al.* 2007), the measurements being provided by the SFB partner project B9 (PRITSCH, ESPERSCHÜTZ, Institute of soil ecology, Helmholtz Zentrum München). The nitrogen pool in microbial biomass was calculated as the difference of total bound nitrogen per dry mass between chloroform-fumigated and non-fumigated soil extractions, divided by 0.54 (factor for the extractable part of nitrogen in microbial biomass, JOERGENSEN *&* MUELLER 1996).

For assessing N loss via leaching, measured nitrate leaching under beech was corrected for the stem flow as leaching was measured close to the stem base. Stem flow in beech is significantly higher than in spruce due to the funnel-shaped architecture and the smooth surface of stem bark (e.g. ÇEPEL 1967, REYNOLDS *&* HENDERSON 1967, NIHLGARD 1970). The higher water infiltration thus increases input and downward movement of nutrients such as N into the soil in the proximity of the stem base (KOCH *&* MATZNER 1993) within a radius of about 1.5 m around the stem (FALKENGREN-GRERUP 1989). Additional N input at the stem base was calculated from the yearly stem flow to be 15.5 % of the total precipitation above-stand (ÇEPEL 1967), assuming a minimum N concentration of the stem flow similar to that of the precipitation (total N input via wet deposition was 8.8 kg ha^{-1} yr^{-1} at the study site in 2005, SCHULZ, LWF, pers. comm.). N leaching due to increased water input at the stem base (142 mm a^{-1}) was estimated from the difference in leaching between plots treated with NH_4NO_3 addition in 70 l rain water and respective non-treated controls. It was assumed that the NH_4NO_3 -N was negligible and the additional water was mainly responsible for enhanced N leaching under the treated plots (see chapter *Results 3 Nitrogen balance and cycling*).

Leaching of NH_4-N was not measured, since NO_3 as highly mobile ion is the main form of inorganic N loss in soils. However, at a neighbouring site, the concentration of NH_4-N below the main rooting zone was still about 26 % of total inorganic N. Therefore, an equivalent proportion was added to the measured loss of NO_3-N in order to estimate leaching of total inorganic N. Loss of dissolved organic N by leaching was not measured, but may be important in forest soils although highly variable and depending on soil type and nitrogen input (NEFF *et al.* 2003).

Statistical analysis

Statistical tests were employed according to *Tab. 13*. Data were tested with linear mixed models or univariate two-way and three-way analysis of variance (ANOVA) with the factors “ozone”, “position” and “sampling date” for leaf, needle and bud data; “ozone”, “sampling date” and “needle age” for needle data; and “ozone” and “species” for soil, root and the other data. The individual trees represented the minimum replication unit with n=3 (for $N_{labelled}$) and n=5 (for N_{total} or C/N ratio) in each treatment ($1\times O_3$, $2\times O_3$) and species (beech, spruce), while for most of the tested compartments, subsamples with several replicates per tree were taken. Therefore, 'tree individual' was used as a random factor when enough data were available, taking into account the limited number of trees per treatment or species as well as the repeated sampling within each tree (e.g. tree crown position, root classes). Least Square Means Student’s t-test or Least Square Means Contrasts were applied in the case of multiple or individual comparisons. Tests were performed at a significance level of 5 %, using JMP INTRO, Version 5.0.1a (SAS Institute Inc., Cary, NC, USA) and R, Version 2.7.1 (Free Software Foundation, Inc., Boston, MA, USA).

Results

The uptake and distribution of the labelled nitrogen ($N_{labelled}$) as a tracer is described in the following in comparison to the total nitrogen (N_{total}) of the investigated system components: above-ground tree organs, particularly the foliage, as well as below-ground organs and soil. Nitrogen concentrations, N pools, and the partitioning of new N are compared in terms of species differences as well as elevated and ambient O_3 levels. Finally, the N cycle of the stand is described with respect to consequences of O_3 effects on trees.

1 Nitrogen in above-ground organs

Nitrogen in leaves and needles

Sun leaves of beech exhibited significantly higher concentrations of $N_{labelled}$ than shade leaves ($p = 0.013$), although the N_{total} concentration was lower in the sun leaves ($p = 0.0005$).

In 2005, first assessment of the $N_{labelled}$ concentration in beech leaves was done four weeks after the beginning of the ^{15}N application. Although the leaves were fully developed by the time of labelling, the $N_{labelled}$ as new nitrogen ranged on average between 8 µg g^{-1} (sun) and 1 µg g^{-1} (shade), respectively, in August 2005 (*Fig. 9*, left). This corresponds to 0.392 atom% ^{15}N (sun) and 0.368 atom% ^{15}N (shade) versus the natural ^{15}N abundance in leaves of 0.364 atom%. However, during the following growing season (2006), the labelled nitrogen became apparent in the leaves with about 10fold higher concentrations than in the preceding year, although a high variation was observed, both within (*Fig. 4*) and between trees (*Fig. 9*, left). Leaf N_{total} concentrations decreased during the course of the growing season in 2006 ($p < 0.0001$) with lowest concentrations found in leaf litter in autumn upon nitrogen retranslocation into storage organs before leaf abscission (*Fig. 9*, right).

In July and August 2006, the concentrations of $N_{labelled}$ were significantly reduced under $2\times O_3$, and to a lower extent also in September 2006, whereas no difference was observed in 2005 and in leaf litter in November 2006. In contrast, leaf N_{total} concentrations were rather stable except for lower concentrations under $2\times O_3$ in the litter of shade leaves. In leaf litter, the C/N ratio varied between the ozone treatments, being lower in the sun ($p < 0.0001$) and higher in the shade crown ($p < 0.0001$) under $2\times O_3$ compared to $1\times O_3$.

Similar to beech leaves, $N_{labelled}$ concentrations in spruce needles were higher in the sun than in the shade foliage (significant at three sampling dates for current-year needles), whereas N_{total} was significantly lower in the sun than in the shade in both age classes (0yr: $p = 0.0003$; 1yr: $p = 0.0073$).

Four weeks after label application, $N_{labelled}$ amounted in needles on average to 5.7 µg g^{-1} in the sun crown and 2.6 µg g^{-1} in the shade, or 0.405 atom% ^{15}N and 0.382 atom% ^{15}N,

respectively. The natural abundance was 0.364 atom% both, in sun and shade needles. Similar to beech, allocation of $N_{labelled}$ into spruce needles increased during the course of the two consecutive growing seasons, although the increase was more pronounced in current than in one-year-old needles (*Fig. 10* and *11*). Higher $N_{labelled}$ concentrations were observed in the sun than in the shade foliage, being significant only in current-year needles in July and August 2006 ($p < 0.05$ and $p < 0.01$, respectively). Compared to beech, the $N_{labelled}$ of needles was less than 50 % of the level in beech sun leaves and about 50 % in the shade leaves.

The N_{total} concentrations in needles remained stable throughout the two investigated growing seasons. Compared to beech, N_{total} was about 50 % of the level in beech leaves.

Although a tendency towards higher levels of $N_{labelled}$ was observed in needles of the sun foliage under $2\times O_3$, the two ozone treatments showed no significant difference in $N_{labelled}$. In contrast, N_{total} concentrations were significantly lower under $2\times O_3$ compared to $1\times O_3$ at several sampling dates in the sun and shade foliage of current-year needles, and at most sampling dates of one-year-old needles ($p = 0.0185$ for one-year-old needles over all sampling dates). However, in June 2006, the current-year shade needles showed higher N_{total} concentrations under $2\times O_3$ compared to $1\times O_3$ (*Fig. 10* and *11*, right).

The C/N ratio was significantly increased under $2\times O_3$ in current-year needles in the sun crown ($p = 0.056$) as well as in one-year-old needles in both, in sun and shade crown ($p = 0.0002$).

The two investigated needle age classes of spruce differed in $N_{labelled}$ in both years in that newly formed needles incorporated significantly more new (shown as labelled) nitrogen than one-year-old needles. However, needles formed in 2005, the year of labelling, still increased their $N_{labelled}$ uptake in the subsequent year by almost doubling the concentrations of $N_{labelled}$ (first three sampling dates in *Fig. 10* and last three dates in *Fig. 11*).

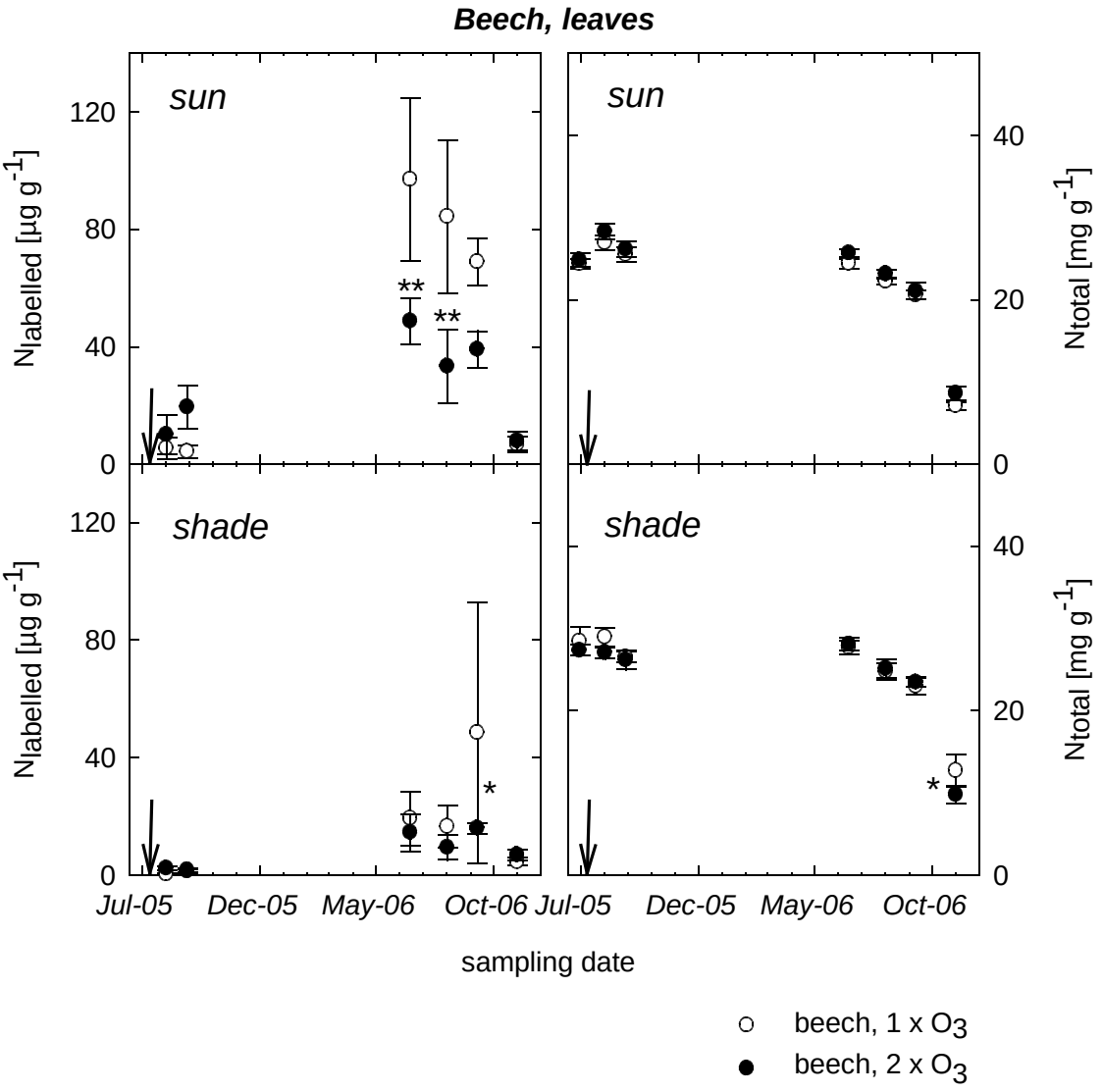

Figure 9: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of beech leaves of the sun and shade foliage throughout 1 ½ growing seasons, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. November data represent leaf litter. Arrows indicate the time of labelling, asterisks indicate significant differences between the ozone treatments at $p < 0.05$ (*), 0.01 (**) or 0.001 (***).

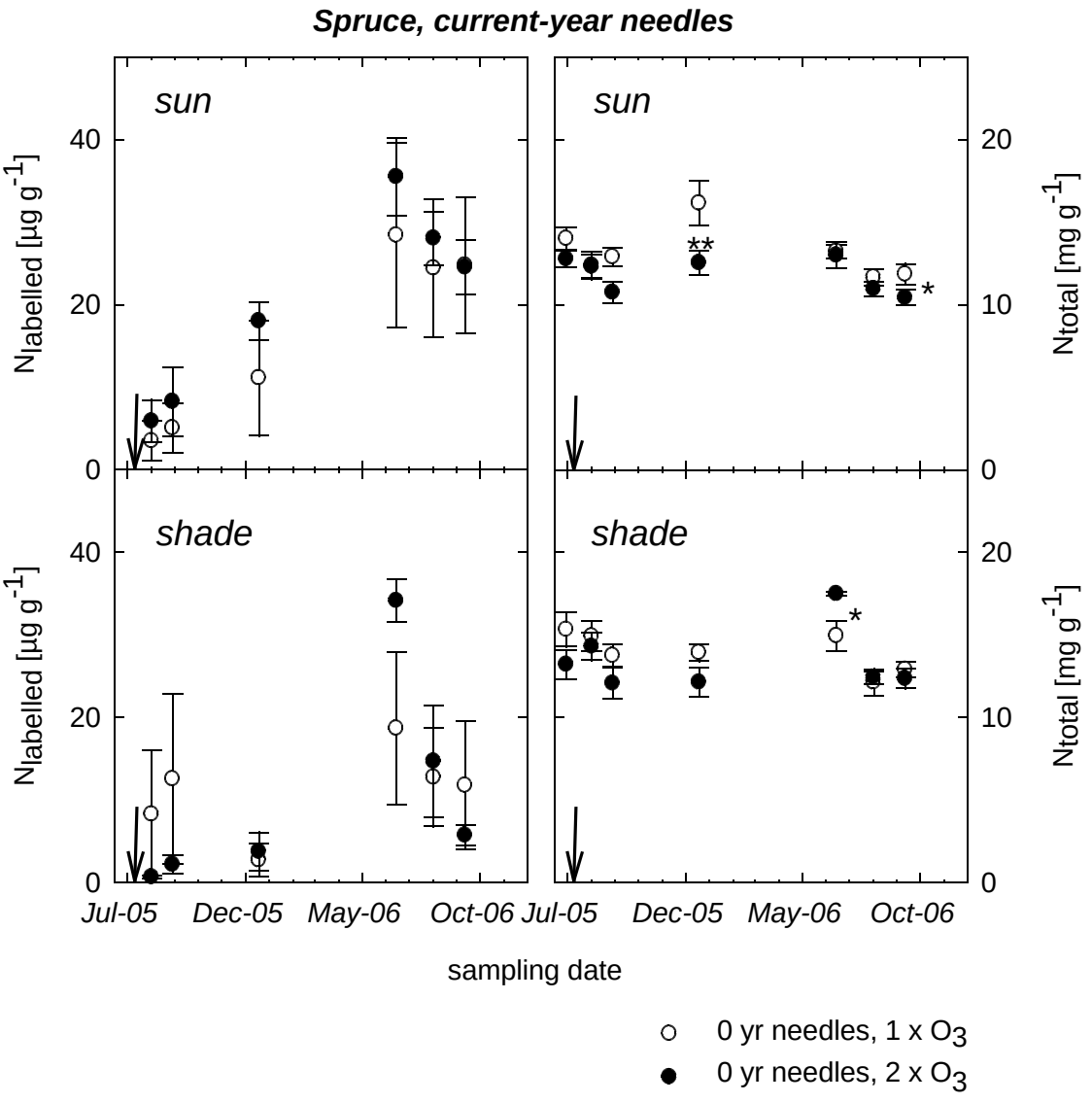

Figure 10: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of current-year needles (= 0 yr) of sun and shade foliage in spruce throughout 1 ½ growing seasons, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. Arrows indicate the time of labelling, asterisks indicate significant differences between the ozone treatments at p < 0.05 (*), 0.01 (**) or 0.001 (***).

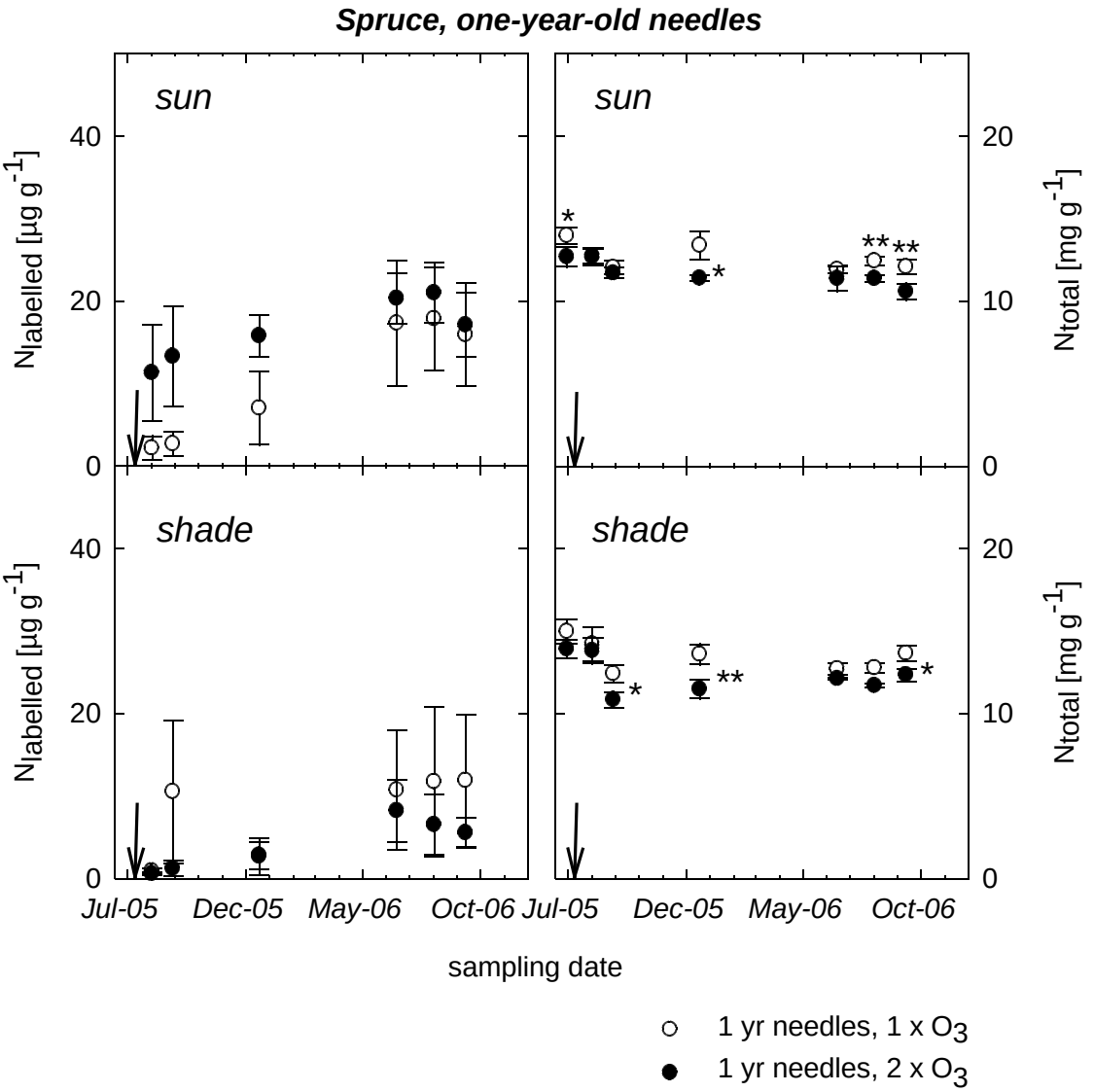

Figure 11: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of one-year-old needles (= 1 yr) of sun and shade foliage in spruce throughout 1 ½ growing seasons, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. Arrows indicate the time of labelling, asterisks indicate significant differences between the ozone treatments at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen in buds

In buds of beech, similar to the leaves, $N_{labelled}$ concentrations were significantly higher in the sun crown compared to the shade ($p = 0.0408$), while N_{total} was significantly lower in the sun compared to the shade ($p = 0.0007$). Accordingly, the C/N ratio was significantly higher in the sun than in shade ($p = 0.0079$).
As in leaves, $N_{labelled}$ increased towards the second year upon labelling whereas N_{total} did not differ between the sampling dates (*Fig. 12*). In contrast, the C/N ratio seemed to depend on the seasonal course and was highest in June 2006 averaging 46.
Buds of $2\times O_3$ first showed similar (sun crown) or higher (shade) $N_{labelled}$ compared to levels under $1\times O_3$ in December 2005. In the second year, however, the concentrations under $2\times O_3$ were about half the level of $1\times O_3$ and significantly reduced in the sun crown in June 2006. In contrast, N_{total} became higher under $2\times O_3$ than $1\times O_3$ in September 2006. Additionally, the C/N ratio was lower under $2\times O_3$ compared to $1\times O_3$ in September 2006 ($p = 0.039$).

Similar to beech buds, $N_{labelled}$ concentrations in buds of spruce were higher in the sun crown than in the shade (significant in August and September 2006), while N_{total} concentrations in the sun crown were almost half the levels of the shade crown ($p < 0.0001$). Also, the C/N ratio was significantly higher in the sun compared to the shade crown ($p < 0.0001$). $N_{labelled}$ in buds increased towards the second year as was the case in needles, although the concentrations slightly decreased from August to September in 2006 (*Fig. 13*). N_{total} also was lower in December 2005 compared to the August and September sampling dates in 2006 (*Fig. 13*), which may be due to seasonal variations as the small amount of tracer N is unlikely to elevate nitrogen concentrations in the plant tissue.
Although $N_{labelled}$ was slightly higher in buds under $2\times O_3$ compared to $1\times O_3$, no significant effect of ozone was found. This was consistent with the findings in needles (see above). Similarly, N_{total} tended to be higher under $2\times O_3$ at all sampling dates ($p = 0.0531$) being significant in the shade in December 2005, which contrasts with the N_{total} in needles. Accordingly, the C/N ratio in buds was lower under $2\times O_3$ compared to $1\times O_3$ in some cases (sun: $p = 0.0185$ in September 2006; shade: $p = 0.0083$ in December 2005).

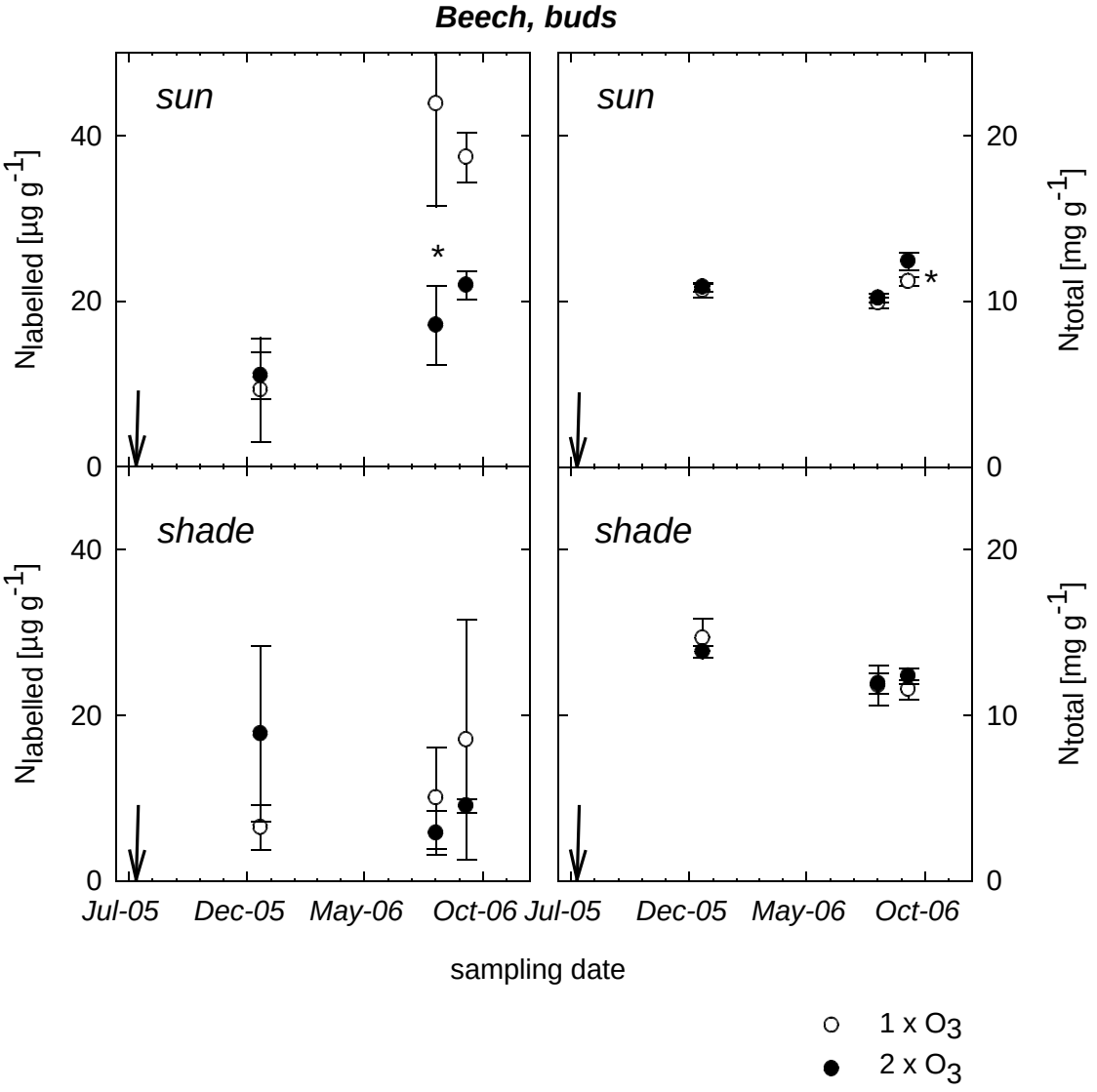

Figure 12: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of beech buds of sun and shade crown throughout 1 ½ growing seasons, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. Arrows indicate the time of labelling, asterisks indicate significant differences between the ozone treatments at p < 0.05 (*), 0.01 (**) or 0.001 (***).

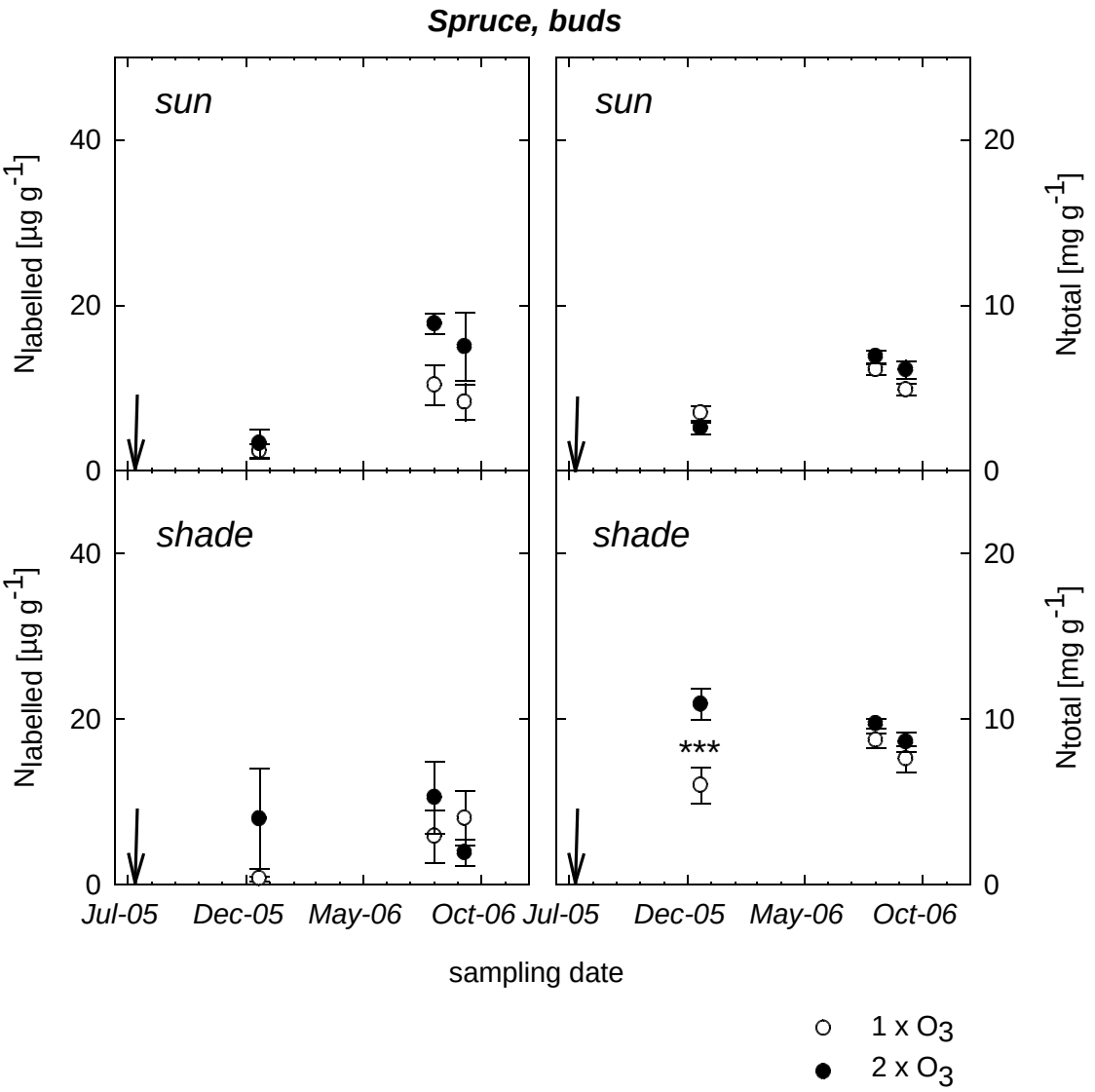

Figure 13: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of spruce buds of sun and shade crown throughout 1 ½ growing seasons, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. Arrows indicate the time of labelling, asterisks indicate significant differences between the ozone treatments at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen in twigs

In December 2005, a considerable amount of $N_{labelled}$ was incorporated into twig tissue, particularly in the sun crown (*Fig. 14*). As twigs serve as storage organs in trees during winter, the newly incorporated nitrogen most likely represents stored N retranslocated from leaves or needles. Besides, the twigs might have covered part of their own metabolic N demand from the soil prior to autumnal retranslocation, since the beginning of labelling. In contrast, N_{total} concentrations of twigs were higher in the shade than in the sun crown, especially in beech, whereas spruce showed highest N_{total} in the wood of sun twigs.

On average, $N_{labelled}$ concentrations of twigs were not different between beech and spruce, however, in the sun crown, wood of beech tended to have lower concentrations than of spruce ($p = 0.0741$). In contrast, N_{total} was significantly higher in shade twigs of beech compared to spruce. And, similarly to $N_{labelled}$, wood of sun twigs had significantly higher concentrations of N_{total} in spruce than in beech. The two species also differed in their C/N ratio, with lower levels in beech shade twigs ($p = 0.0016$) but by about 50 % higher C/N in wood of beech sun twigs compared to spruce ($p = 0.0459$).

Under $2\times O_3$, both species showed slightly lower concentrations of $N_{labelled}$ in sun twigs compared to $1\times O_3$ although this was not significant. Similarly, N_{total} of twigs was reduced under $2\times O_3$, although being significantly lower only in the wood of spruce sun twigs. Consistently, the C/N ratio was higher under $2\times O_3$ compared to $1\times O_3$ in the wood of sun twigs of both species, although this effect was not significant ($p = 0.0802$).

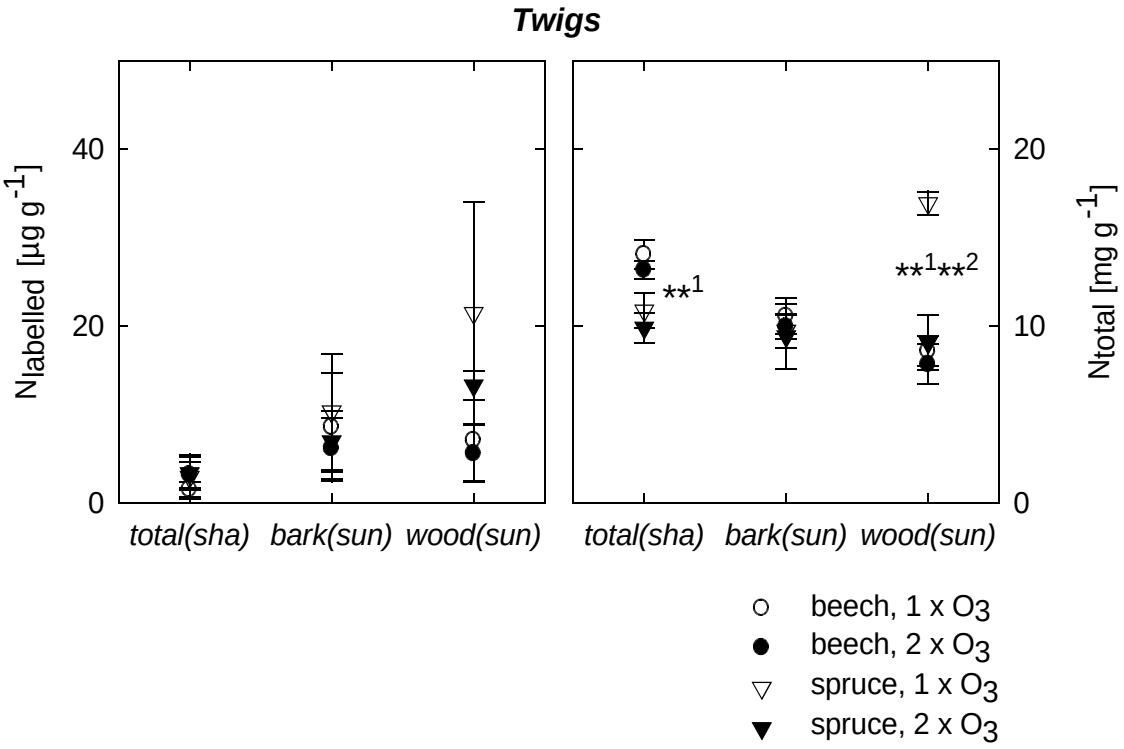

Figure 14: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of current-year twigs of December 2005 in sun and shade crown, as means ± SE, with n=3 trees for $N_{labelled}$ and n=5 for N_{total}. Asterisks indicate significant differences either between species ([1]) or ozone treatment ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen in stem, xylem and phloem sap, and beech fruits

Further tissues exemplified were stem, beech fruits, xylem and phloem sap (*Fig. 15*). Such tissues showed enrichment of $N_{labelled}$ with the beech seeds exhibiting the highest concentrations. In spruce, there was no seed production during the two study years.
In stem tissues, the two species did not differ in the concentrations of $N_{labelled}$, whereas in terms of N_{total}, the stem showed higher concentrations in beech compared to spruce, being significant in the bark and in the wood of the years of ozone treatment ($wood_{ozone}$), but not before ($wood_{pre\text{-}ozone}$). Under $2\times O_3$, $N_{labelled}$ in the bark of beech was slightly lower compared to $1\times O_3$, however, this is based on only one sample available for $1\times O_3$. In contrast, N_{total} concentration was significantly higher in $wood_{ozone}$ in beech ($p = 0.0311$), with a trend of lower C/N. Wood tissues of spruce showed no O_3 effects in N concentrations or C/N.

In the phloem exudate, $N_{labelled}$ was more than ten times higher in beech than in spruce under $1\times O_3$ ($p < 0.05$), although with a high variation amongst beech trees. N_{total} was significantly higher in beech compared to spruce in both ozone treatments. Under $2\times O_3$, phloem of beech had significantly lower $N_{labelled}$ concentrations than the ones under $1\times O_3$ ($p = 0.0213$), whereas no difference was found in spruce between the ozone treatments. Similar outcome was reflected by N_{total}, however, significantly lower concentrations in beech occurred under $2\times O_3$ only in September ($p = 0.0197$). In the xylem sap, $N_{labelled}$ and N_{total} concentrations did not differ between species or ozone treatments.

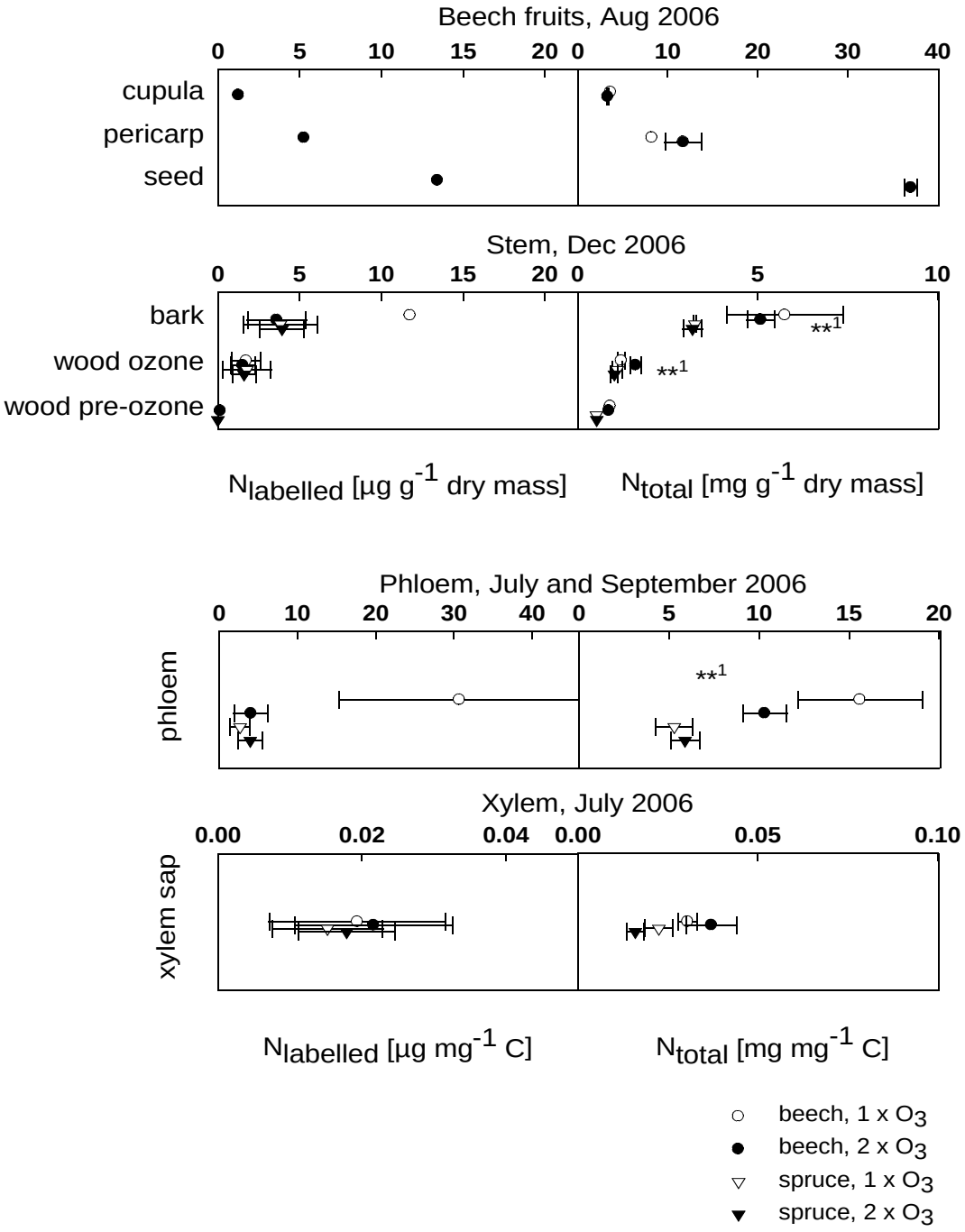

Figure 15: $N_{labelled}$ (left) and N_{total} (right) concentrations in stem, fruits of beech, phloem and xylem sap. Phloem data were taken from two sampling dates (July and September 2006). Values as means ± SE, with n=3 trees per species or treatment for $N_{labelled}$ and n=3-5 for N_{total} (xylem sap, phloem and stem) or exemplary measurements (fruits: n=1-2 trees per treatment). Symbols per category are plotted with an offset for graphical reasons. Asterisks indicate significant differences either between species ([1]) or ozone treatment ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Correlation between transpiration and uptake of labelled N

Based on the observed differences in N concentrations between species and ozone treatments, the uptake of labelled N was related to transpiration of the same trees.
The relationship was examined by means of $N_{labelled}$ concentrations in leaves and needles within the sun crown (see *Fig. 9* and 10) and xylem flux of same trees (METZGER, pers. comm.). Concentrations of $N_{labelled}$ in leaves and needles in September 2006, were linearly related to the cumulative transpiration of tree crowns since beginning of the ^{15}N labelling in July 2005 through September 2006 (*Fig. 16*).
Throughout the two study years, the foliage area-related, cumulative transpiration during growing season was about three times higher in beech compared to spruce ($p < 0.0001$). In parallel, $N_{labelled}$ in sun leaves was, on average, twice as high as in spruce needles ($p = 0.0124$, see also section *Nitrogen in leaves and needles*). Under $2\times O_3$, beech transpired by around 42 % less ($p < 0.0026$, n=3-4) and had by 49 % lower $N_{labelled}$ concentrations in sun leaves ($p = 0.0243$, n=2-3) compared to $1\times O_3$ (see *Fig. 9*). In spruce, no effect of ozone was found during the study period.

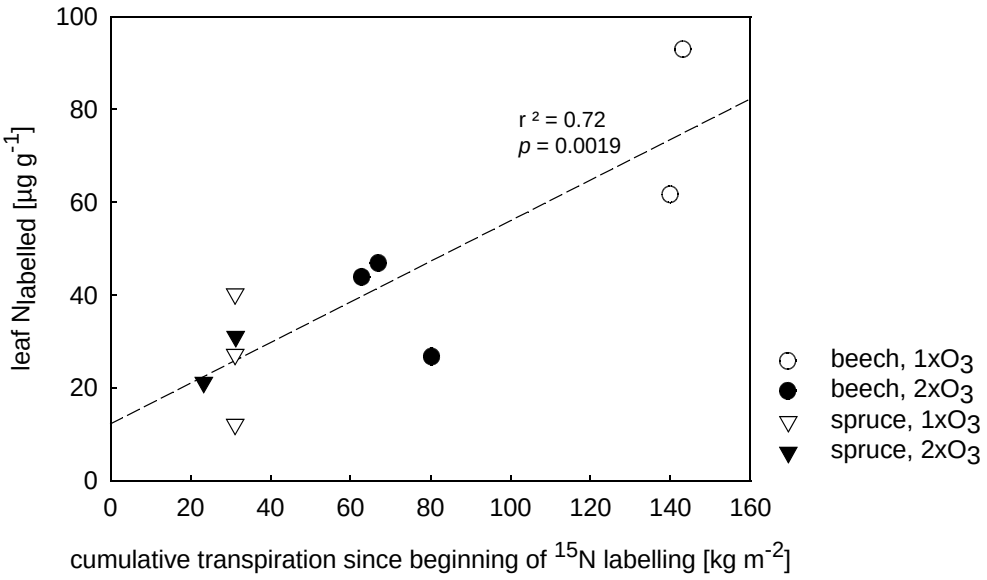

Figure 16: Correlation between cumulative transpiration per unit of leaf area since beginning of the ^{15}N labelling in July 2005 through September 2006 and $N_{labelled}$ in dry mass of sun crown leaves or current-year needles in September 2006. Respective data set re-evaluated from figures 9 and 10. Each data point represents an individual tree. In spruce, $1\times O_3$, data for transpiration and $N_{labelled}$ are derived from separate trees.

2 Nitrogen in below-ground organs and soil

Nitrogen in the soil

The soil of the study site was characterized by silty clay loam with relatively high proportions of clay (*Tab. 4*).

After application of the labelled $^{15}NH_4{}^{15}NO_3$ solution on top of the mineral soil in July 2005, the labelled N was found throughout the soil profile at the end of 2006. $N_{labelled}$ was highest in the (humus-rich) upper horizons with on average 41 µg g^{-1} and decreased with soil depth to as low as about 0.2 µg g^{-1} (*Fig. 17*). These levels correspond to 0.652 atom% ^{15}N in organic and humus-rich layer, and 0.410 atom% in B horizon, respectively, versus the natural ^{15}N abundance of 0.364 (litter and humus layer), 0.365 (A horizon) and 0.367 atom% (B horizon). Generally, $N_{labelled}$ showed high variation both within plots (data not shown) and between trees.

Species differences were reflected by the litter where $N_{labelled}$ was lower under beech compared to spruce. The C/N ratio also was generally lower under beech compared to spruce being significant in B_{-10} ($p = 0.0256$). Soil underneath $2\times O_3$ treated trees tended to have higher $N_{labelled}$ concentrations than under $1\times O_3$ for both species, which was significant in the upper layer of the B horizon (B_{-10}). N_{total} showed a similar tendency. The C/N ratio of soil tended to be lower under $2\times O_3$ compared to $1\times O_3$ trees, although significance was found only within the O_a horizon under spruce, being 21.4 ± 0.8 *(SE)* mg mg^{-1} for $2\times O_3$ versus 24.9 ± 0.3 mg mg^{-1} for $1\times O_3$ ($p = 0.002$).

The applied $^{15}NH_4{}^{15}NO_3$ leaked to a low extent out of the labelled 1 m²-plots as reflected by the horizontal distribution of $N_{labelled}$, being exemplified around two plots (*Fig. 18*). Within a range of 10 cm along plot border, $N_{labelled}$ in each horizon was about 23 % (beech) to 32 % (spruce) of the concentrations within the plots (compare *Fig. 17*). At a distance of 20-30 cm from plot border, the proportion was about 5 % irrespective of the species.

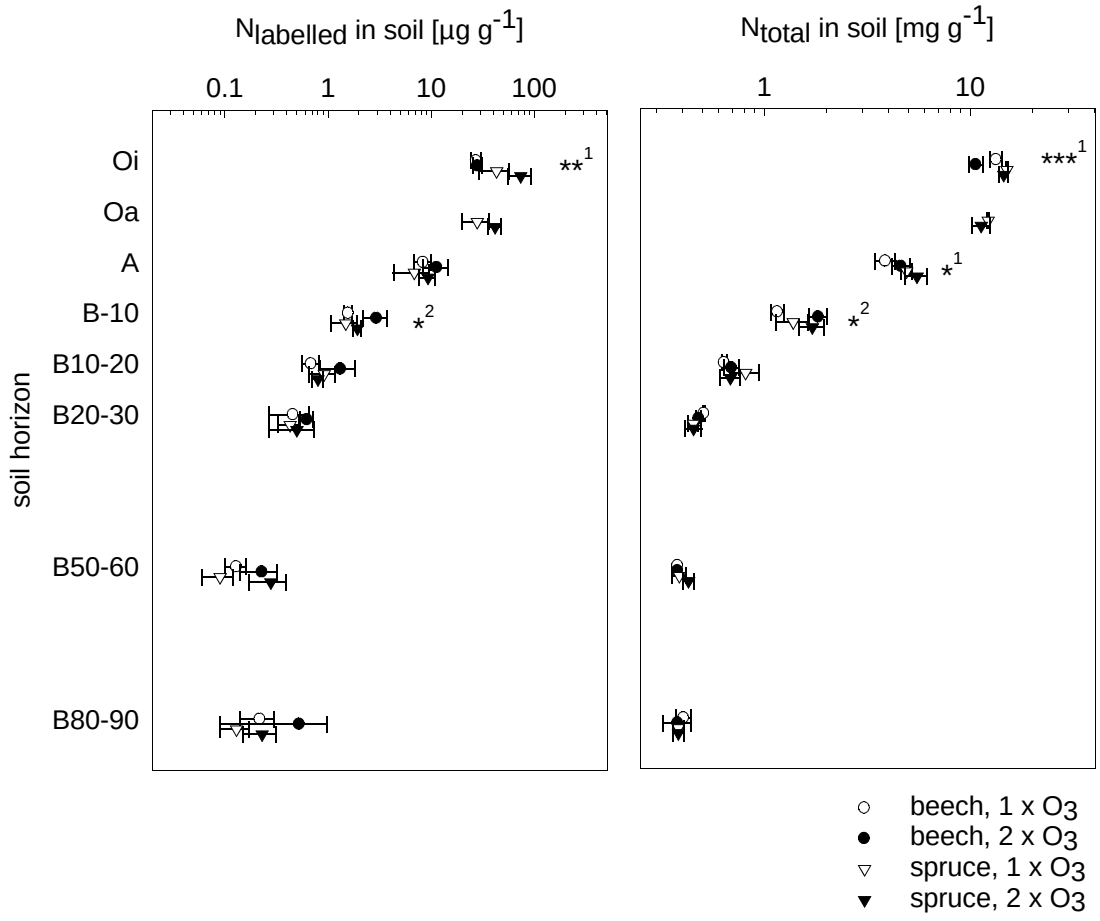

Figure 17: Concentrations of $N_{labelled}$ (left) and N_{total} (right) in soil dry mass of different soil horizons or layers down to 90 cm depth. Symbols along the y-axes are plotted with an offset in each horizon or layer for graphical reasons. Means ± SE are log-scaled, with n=3 plots for $N_{labelled}$ and n=5 for N_{total}. Asterisks indicate significant differences either between species ([1]) or ozone treatments ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

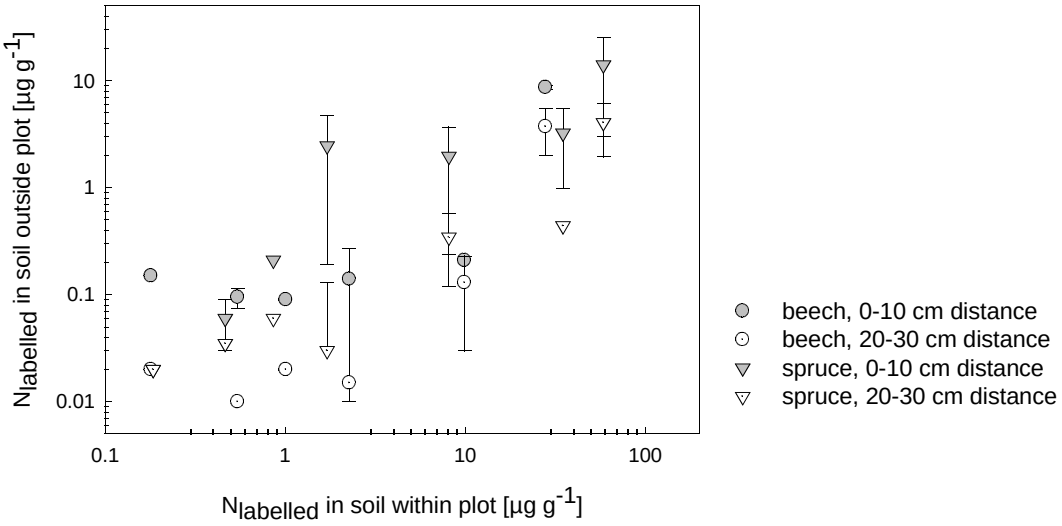

Figure 18: Horizontal distribution of $N_{labelled}$ outside the plots (n=2, means ± SE, log-scaled) at the distances 0-10 cm and 20-30 cm from the plot border, compared to $N_{labelled}$ within the plots. Data of $N_{labelled}$ within the plots were taken from Fig. 17, with $1\times O_3$ and $2\times O_3$ pooled. Data points represent different soil horizons or layers down to 60 cm depth .

Table 4: Soil properties at the study site

	Soil horizon / layer	*Clay [%]*	*Silt [%]*	*Sand [%]*	*Soil density [g cm^{-3}]*	*Soil moisture [%]*	*C [%]*	*N [%]*	*C/N*
Beech									
	A	28	62	10	0.6	28.1	8.33	0.43	19.4
	B_{-10}	36	51	13	1.0	15.4	2.65	0.15	17.5
	B_{10-20}	27	64	9	1.2	12.0	1.05	0.07	15.6
	B_{20-30}	25	66	9	1.2	11.6	0.53	0.05	10.6
	B_{30-50}	30	62	7	1.4	14.9	-	-	-
	B_{50-60}	38	56	6	1.5	15.7	0.31	0.04	8.3
	B_{60-80}	32	59	9	1.5	16.4	-	-	-
	B_{80-90}	34	54	12	-	16.8	0.34	0.04	9.0
Spruce									
	A	28	63	8	0.6	26.7	10.69	0.52	20.5
	B_{-10}	21	69	10	0.9	13.4	3.03	0.15	19.1
	B_{10-20}	21	70	9	1.2	10.4	1.17	0.08	15.0
	B_{20-30}	28	64	8	1.2	11.2	0.45	0.04	10.0
	B_{30-50}	35	57	8	1.4	14.7	-	-	-
	B_{50-60}	36	54	10	1.5	17.1	0.32	0.04	7.5
	B_{60-80}	36	53	12	1.5	17.0	-	-	-
	B_{80-90}	40	49	11	-	19.6	0.36	0.04	9.5

Root distribution

Root density, as measured by total root harvest per 1-m² plot down to 0.5 m depth, was similar in all plots independent of species or ozone treatment (fine roots < 2 mm: 193 ± 25 (*SE)* g m^{-2}, medium-sized roots ≥ 2 < 5 mm: 294 ± 36 g m^{-2}, coarse roots > 5 mm: 2307 ± 488 g m^{-2}).

Across the soil profile, roots of both species were distributed down to 90 cm depth, although main distribution varied between beech and spruce: Spruce roots of all diameter classes occupied mainly upper horizons (O and A), whereas beech roots displayed distribution mainly in the A and B horizon down to about 60 cm depth (*Fig. 19, Tab. 5*). Under 2×O_3, fine root biomass was significantly increased within the A horizon, but decreased within the B horizon (B_{20-30}). Medium-sized roots of spruce tended to be decreased under 2×O_3, being significant in the O_i layer. Similarly, coarse root biomass was decreased in beech in the B horizon (B_{20-30} and B_{30-60}) under 2×O_3.

When taking into account the variations in thickness of the upper horizons (O and A), the differences in root distribution between ozone treatments, expressed as root density per soil volume, became more pronounced in fine and medium-sized roots (*Fig. 20*): Within the O_i layer, density of fine and medium-sized roots of spruce were significantly reduced under 2×O_3, whereas in the O_a layer, fine root density of spruce was increased under 2×O_3. Fine root density in beech was also significantly increased under 2×O_3 in the A horizon, with a similar trend for medium-sized roots.

Table 5: Significant differences in the root distribution per m² across the soil profile between species (^{1}) and ozone treatments (^{2}) as main effects, indicated by asterisks at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Soil horizon/ layer	root diameter class <2 mm [g m^{-2}]	root diameter class ≥2<5 mm [g m^{-2}]	root diameter class ≥5mm [g m^{-2}]
O_i	*^{1}	*^{1}, (^{2}: spruce 0.0566)	
O_a			
A	*^{1}, *^{2}	**^{1}	**^{1}
B_{-10}			
B_{10-20}	***^{1}	*^{1}	*^{1}
B_{20-30}	***^{1}, **^{2}		*^{1}, beech: *^{2}
B_{30-60}	***^{1}	**^{1}	
B_{60-90}			

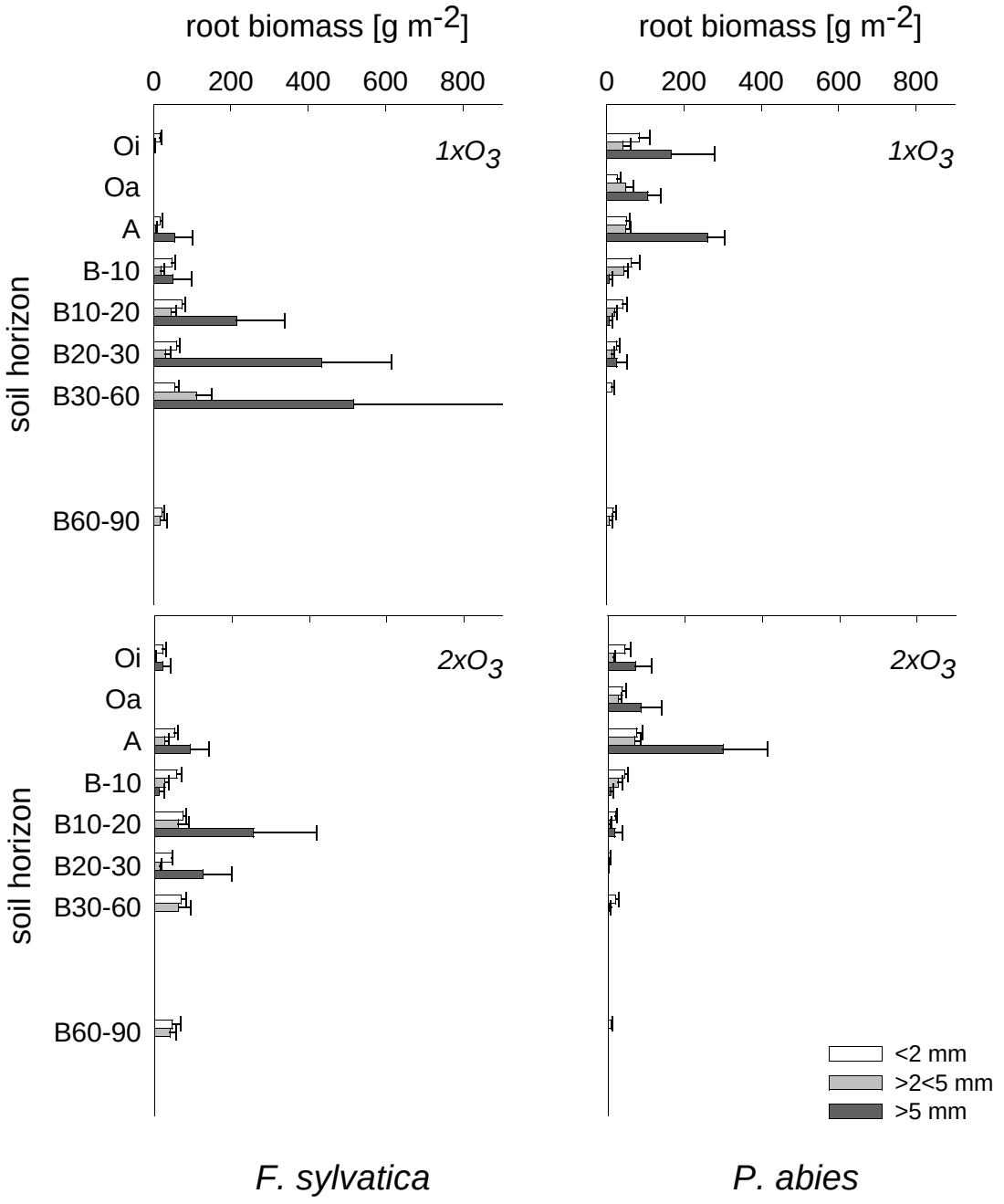

Figure 19: Root distribution of beech (left) and spruce (right) per m² and soil horizon or layer across the soil profile, separated by ozone treatments (1×O_3, 2×O_3) and the root diameter classes < 2 mm, ≥ 2 < 5 mm and ≥5 mm, given as means ± SE, with n=5 plots.

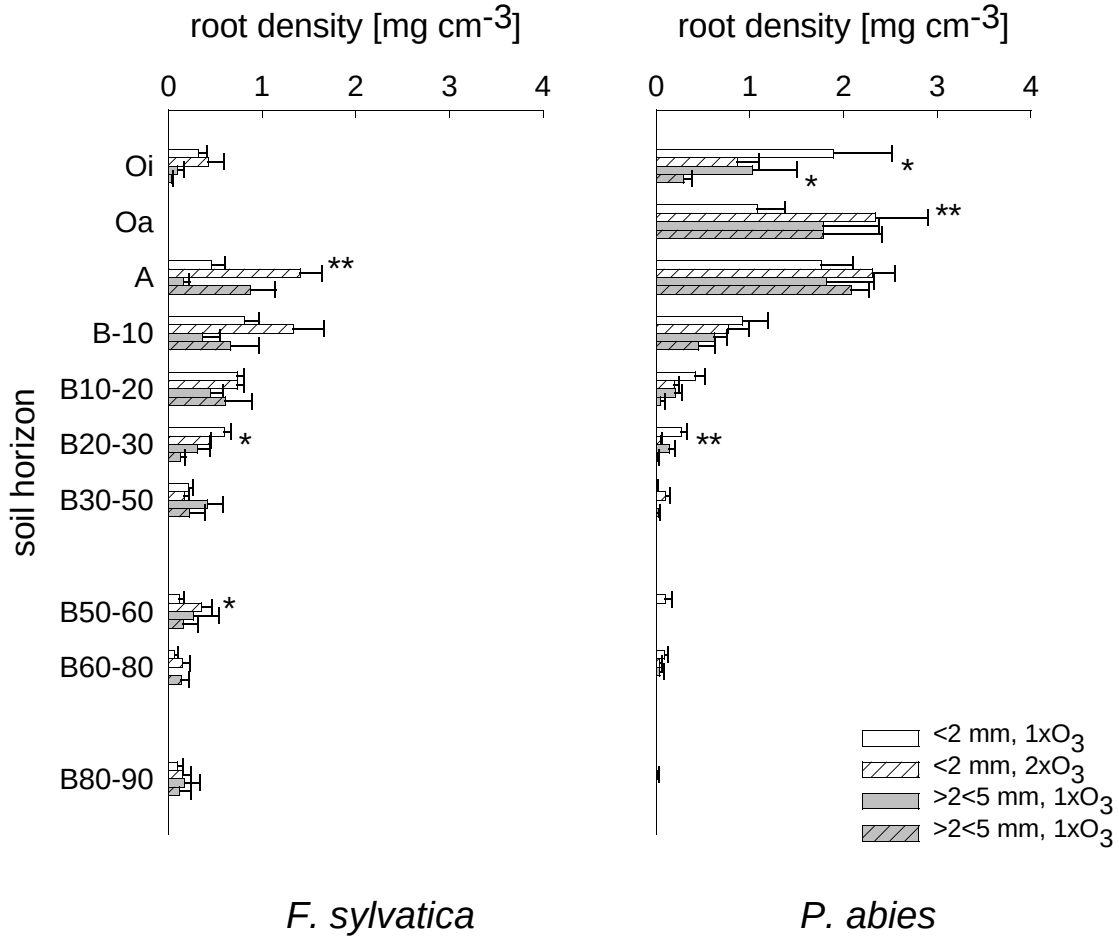

Figure 20: Root density of the root diameter classes < 2 mm and ≥ 2 < 5 mm of beech (left) and spruce (right) across the soil profile, separated by ozone treatments (1×O_3, 2×O_3), given as means ± SE, with n=5 plots. Asterisks indicate significant differences between ozone treatments at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen in the roots

Nitrogen concentrations were highest in fine roots < 2 mm and lowest in coarse roots ≥ 5 < 20 mm. The $N_{labelled}$ concentrations ranged on average between 235 µg g^{-1} (roots < 2 mm) to 42 µg g^{-1} (roots ≥ 5 < 20 mm), corresponding to 2.54 and 1.18 atom% ^{15}N, respectively, while the natural ^{15}N abundance was 0.364 atom% in the three root classes. $N_{labelled}$ and N_{total} decreased with soil depth in the fine roots < 2 mm of both species, particularly in beech, whereas no significant trend was found in the root classes ≥ 2 < 5 mm and ≥ 5 < 20 mm (*Fig. 21*). Patterns of fine roots were consistent with such of $N_{labelled}$ and N_{total} concentrations in soil along the soil profile (see also *Fig. 17*). Note that in the B horizon, samples from the different B-layers down to 90 cm depth were pooled for each root class due to low root material.

Differences in $N_{labelled}$ between species were observed in the root class ≥ 2 < 5 mm, where beech exhibited lower concentrations of $N_{labelled}$ compared to spruce, particularly in the A and B horizon. This can be explained by the different age and root morphology of both species, as roots of this diameter class can be older and thus lignified and less active in N uptake in beech as compared to spruce. Within all root classes, N_{total} was lower in beech compared to spruce, mainly in the B horizon.

Although the N concentrations appeared to be slightly lower in roots under $2\times O_3$ as compared to $1\times O_3$, the ozone treatments did not significantly affect $N_{labelled}$ concentrations in the three root diameter classes across the soil horizons (*Fig. 21*, left), which was consistent with findings on N_{total} (*Fig. 21*, right). The C/N ratio, however, was significantly higher in fine roots (< 2 mm) as well as in coarse roots (≥ 5 < 20 mm) of beech under $2\times O_3$ in the B horizon ($p = 0.0017$ and $p = 0.0078$, respectively), while in spruce, it was slightly higher the root class ≥ 2 < 5 mm ($p = 0.0881$) in the O_a horizon.

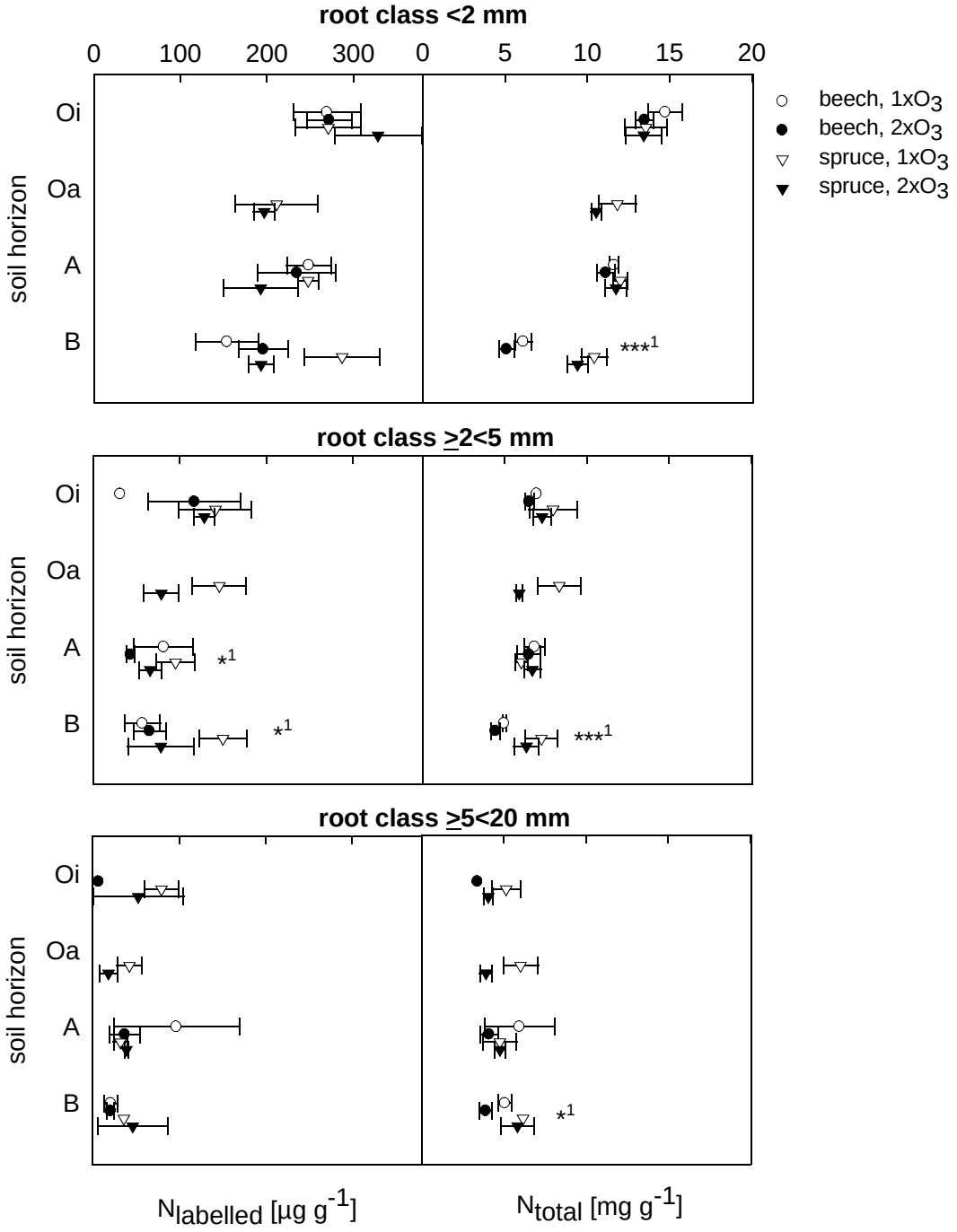

Figure 21: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of roots of different root diameter classes across soil horizons down to 90 cm depth. Symbols along the y-axes are plotted with an offset for each horizon for graphical reasons, as means ± SE, with n=3 plots for $N_{labelled}$ and n=5 for N_{total}. Asterisks indicate significant differences either between species ([1]) or ozone treatments ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen in specific root tissues: mycorrhizal root tips, bark and wood of coarse roots

Mycorrhizal root tips had the highest concentrations of $N_{labelled}$ and N_{total} of all investigated compartments. $N_{labelled}$ was, on average, 303 µg g^{-1} corresponding to 2.33 atom% ^{15}N, with a natural abundance of around 0.364 atom%. Within the A horizon, $N_{labelled}$ decreased from mycorrhizal root tips towards coarse roots by more than 90 % in beech and about 80 % in spruce (*Fig. 22*). N_{total} concentrations showed a similar pattern in beech with concentrations in the coarse roots of about 80 % less than in mycorrhizal root tips, whereas in spruce, highest N_{total} concentrations were observed in the bark of coarse roots. In both species, N_{total} concentrations were higher in mycorrhizal root tips than in the corresponding fine roots (beech: $p < 0.0001$; spruce: $p = 0.005$).

In mycorrhizal root tips, the two tree species did not significantly differ in $N_{labelled}$ although, beech tended to incorporate more new (labelled) nitrogen into mycorrhizae compared to spruce (*Fig. 22*). However, N_{total} was significantly higher in beech than in spruce, whereas the C/N ratio was lower in beech (25.7 ± 0.6 *(SE)* mg mg^{-1}) compared to spruce (34.1 ± 0.6 mg mg^{-1}) ($p < 0.0001$). Any effect of ozone was observed in $N_{labelled}$ of the mycorrhizal root tips. In contrast, N_{total} in mycorrhizal root tips of beech were significantly lower under $2\times O_3$ compared to $1\times O_3$, while C/N ratio tended to be higher ($p = 0.091$).

Bark and wood of coarse roots were significantly different between species in nitrogen status. Beech exhibited about three to five times lower $N_{labelled}$ concentrations compared to spruce and about half of the N_{total} concentrations of spruce in both tissues. Consistently, the C/N ratio was almost twice as high in beech (bark: 69.2 ± 7.3 mg mg^{-1}; wood: 139.6 ± 20.1 mg mg^{-1} compared to spruce (bark: 41 ± 1.6 mg mg^{-1}; wood: 70.6 ± 3.8 mg mg^{-1}). Ozone did not show any effect in root bark and wood.

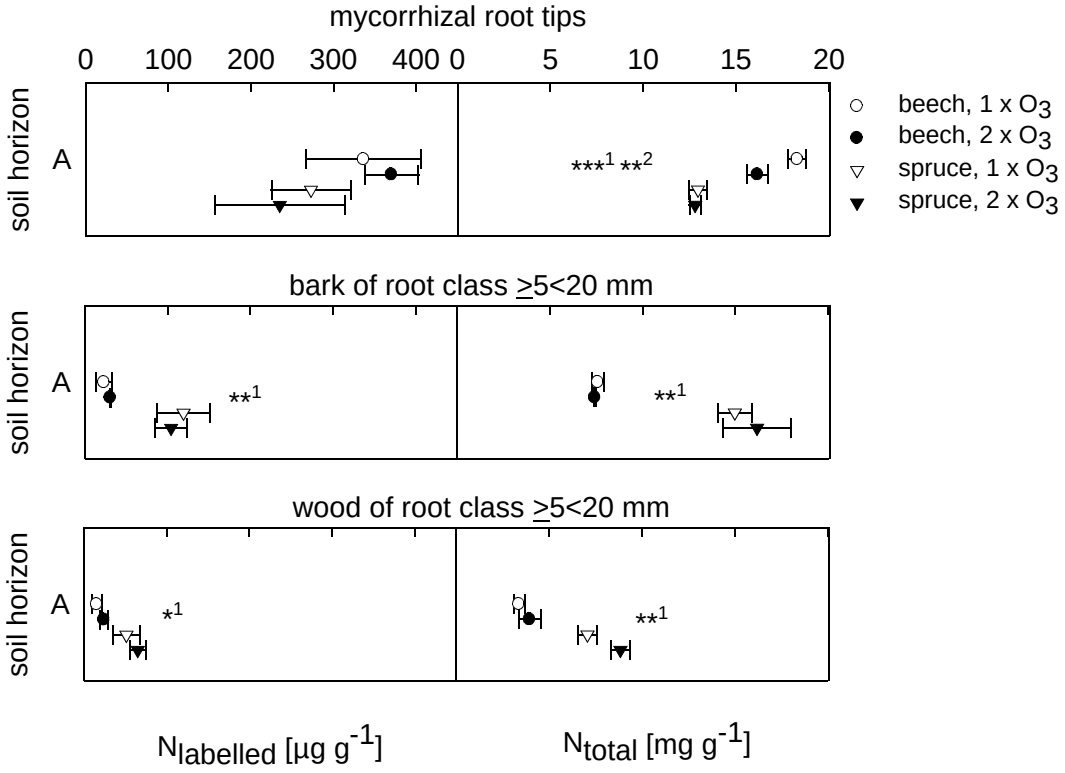

Figure 22: $N_{labelled}$ (left) and N_{total} (right) concentrations in dry mass of mycorrhizal roots tips, bark and wood of coarse roots in the mineral A horizon. Symbols along the y-axes are plotted with an offset for graphical reasons, as means ± SE, with n=3 plots for $N_{labelled}$ and n=5 for N_{total}. Asterisks indicate significant differences either between species ([1]) or ozone treatment ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Nitrogen concentrations compared between soil and roots

Correlation was found between concentrations of soil N_{total} and root N_{total} across soil horizons in the fine root classes < 2 mm of beech and spruce, and in the medium-sized roots ≥ 2 < 5 mm of beech (*Fig. 23*). N_{total} correlated positively between roots and soil in both root classes, showing a trend of saturation in roots with increasing soil N for beech and partly for spruce, particularly in the fine roots. This is assumed to reflect the capacity in N uptake. Fine roots, at the direct soil-plant-interface, showed a similar

relationship in both species, whereas medium-sized roots showed different dependency to soil N in beech and spruce, as indicated by linear regression (log-transformed, $p = 0.0111$). Under $2\times O_3$, fine roots showed a reduced N uptake capacity across both species ($p = 0.0166$) as indicated by a saturation at lower root N_{total} concentrations.
The non-linear correlations between N_{total} in roots and soil were described by a logarithmic function (*Fig. 23*). No such relationship was found for the $N_{labelled}$.

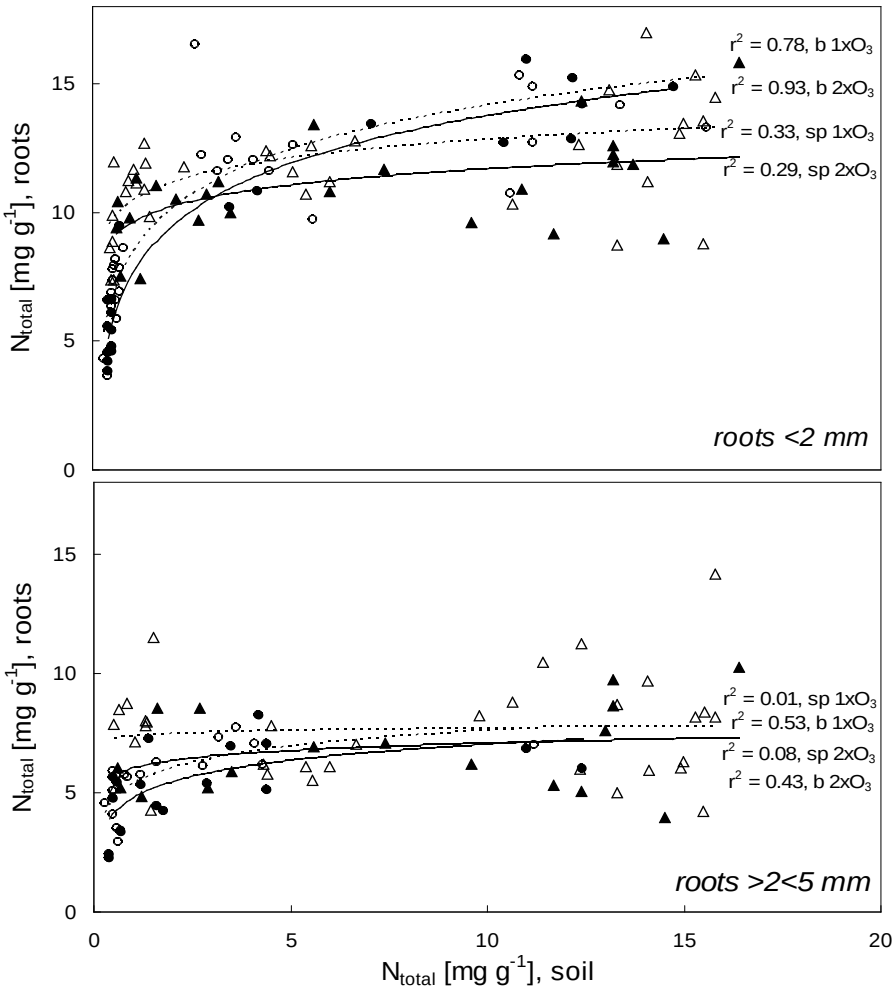

Figure 23: Correlation of N_{total} between fine roots < 2 mm (above), or medium-sized roots ≥ 2 < 5 mm (below), and soil across the soil horizons O_i, O_a (spruce only), A and B, for beech (b) and spruce (sp) under $1\times O_3$ (dashed line) and $2\times O_3$ (solid line).

3 Nitrogen balance and cycling

Following the approach of studying effects of elevated ground-level ozone on the nitrogen concentrations of single organs or tissues (chapters *1 Nitrogen in above-ground organs* and *2 Nitrogen in below-ground organs and soil*), the nitrogen budget at the whole-tree and stand level was assessed in order to determine the different nitrogen pool sizes within the soil-plant-continuum. Mostly modelled (and in some cases measured) biomass data from the study site were used (see *Material and Methods*). Partitioning of newly acquired nitrogen within trees was assessed on the basis of the labelled nitrogen recovered in the different compartments, in order to study possible changes in nitrogen allocation patterns under the impact of elevated ground-level ozone. Finally, the N balance and cycle was established for the study site for providing an overview on possible plant-mediated changes at stand level as response to chronically elevated ground-level ozone. As data were derived from up-scaling and sample sizes were low, statistical analysis is less reliable. Nevertheless, results of statistical tests were given.

Tree level N budget and partitioning

While whole-tree biomass was not significantly different between O_3 treatments and species, but showing high variation, biomass partitioning within trees differed between species and, in spruce, between O_3 regimes (*Fig. 24; Tab. 6*). The biomass proportion of buds, stem bark, coarse root bark, medium and fine roots as well as mycorrhizal root tips was significantly lower in beech compared to spruce. In contrast, total stem (wood and bark) had a higher percentage in beech biomass than in spruce. Biomass partitioning under $2\times O_3$ was not different from that under $1\times O_3$ in beech, whereas in spruce differences were observed: Above-ground, the proportion of branch biomass in spruce was significantly lower under $2\times O_3$ compared to $1\times O_3$, whereas total stem biomass, and in particular stem bark, was higher under $2\times O_3$. Below-ground, biomass proportion of each root class (except for bark of coarse roots) and mycorrhizal root tips were significantly lower in spruce under $2\times O_3$ compared to $1\times O_3$ although total root mass was only slightly reduced under $2\times O_3$ ($p = 0.06$).

Beech foliage accounted for up to 4.6 ± 0.9 *(SE)* kg tree^{-1} in summer representing only about 1 % of the total tree biomass (shown in *Fig. 24,* in addition to the biomass

partitioning in winter).

These biomasses were calculated without implementation of an "O_3-impact"-factor in the applied model, but on the basis of allometric relationships of the trees in 2002 (see *Material and Methods*). As O_3 fumigation of the trees had started in 2000, the growth development under influence of three years of elevated O_3 was taken into account.

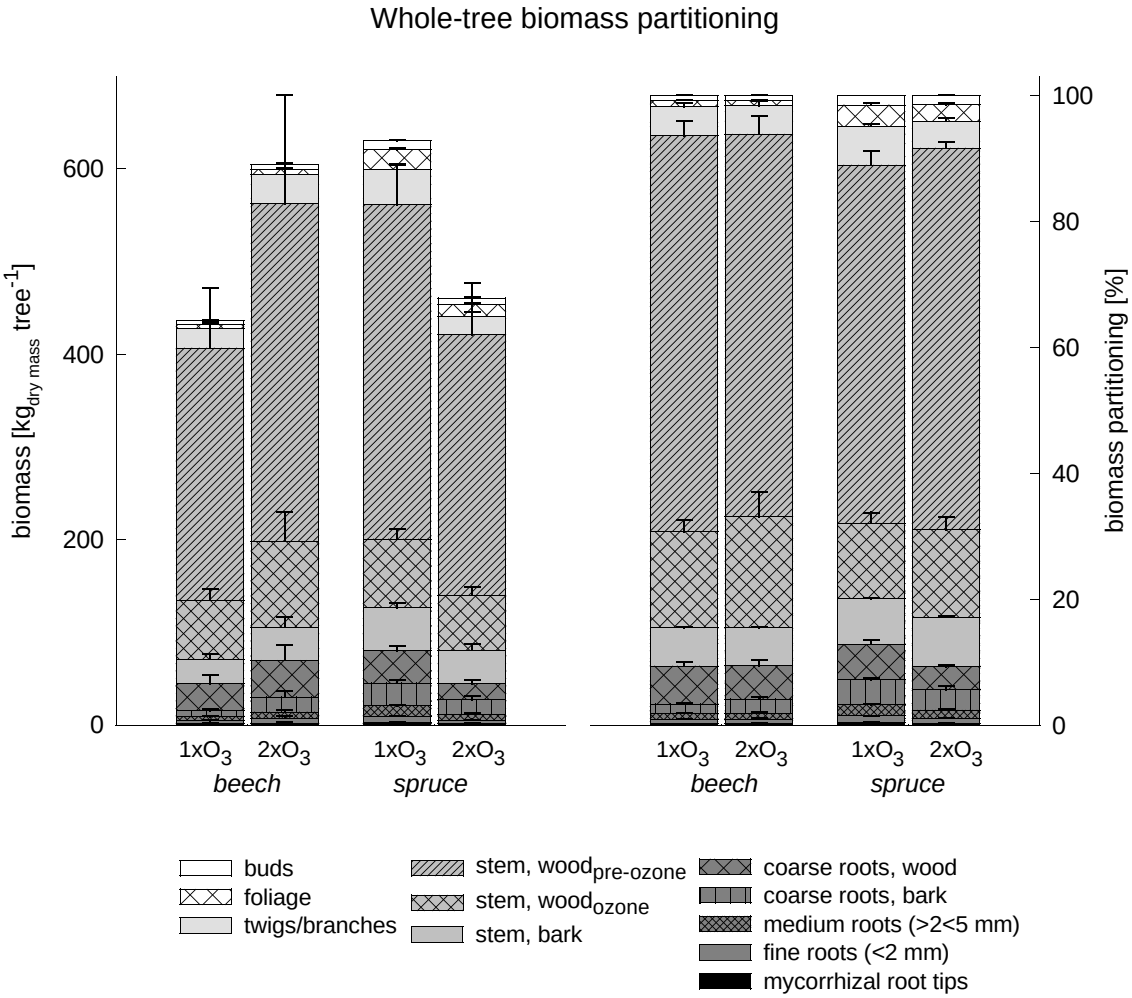

Figure 24: Total content (left) and partitioning (right) of tree biomass in winter 2006. Only biomass of beech foliage in summer was added here for ease of comparison between deciduous beech and evergreen spruce. (Means ± SE, with n=5 trees).

*Table 6: Statistical analysis of biomass partitioning within trees in winter. Effects of species and ozone treatments according to two-way ANOVA (*p-*values), n.s. = not significant, non-significant trends in parentheses. Within interacting factors, species effects under one O_3 treatment or ozone effects of only one species are detected as significant contrasts; b = beech, sp = spruce.*

	Species	**Ozone**	**Species × Ozone**	**Contrast Species**	**Contrast Ozone**
Tree total	n.s.	n.s.	n.s.	n.s.	n.s.
buds	<0.0001	n.s.	n.s.	n.s.	n.s.
foliage	-	n.s. (spruce only)	-	-	-
twigs/branches	n.s.	n.s.	(0.1219)	(0.0758, 1×O_3)	0.0309 (sp)
stem total	0.0071	n.s.	(0.1194)	0.0041 (2×O_3)	0.0331 (sp)
stem, $wood_{pre\text{-}ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, $wood_{ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, bark	<0.0001	n.s.	0.0794	<0.0001 (1×O_3) <0.0001 (2×O_3)	0.0175 (sp)
roots total	n.s.	n.s.	(0.1681)	(0.0778, 1×O_3)	(0.06, sp)
coarse roots, wood	n.s.	(0.0708)	(0.3926)	n.s.	(0.064, sp)
coarse roots, bark	0.0009	n.s.	n.s.	n.s.	n.s.
medium roots	0.0003	n.s.	(0.0994)	0.0004 (1×O_3) (0.0609, 2×O_3)	0.0254 (sp)
fine roots	(0.0568)	n.s.	(0.1490)	0.0226 (1×O_3)	0.0477 (sp)
mycorrhizal root tips	(0.0519)	n.s.	(0.1395)	0.02 (1×O_3)	0.0498 (sp)

According to the whole-tree biomass, the total N content per tree did not significantly differ between species and O_3 treatments, although being almost 2fold higher in spruce under 1×O_3 than in 2×O_3 spruce (p = 0.0724) or in 1×O_3 beech (p = 0.0449; *Fig. 25, left*). However, dry-mass related whole-tree N_{total} concentration was significantly lower in beech than in spruce, and was also lower in spruce under 2×O_3, compared to 1×O_3 (*Fig. 26*).

Partitioning of total N within trees during winter was different between species (*Fig. 25; Tab. 7*). Above-ground, N allocated to buds was about 1.4 times higher in beech (4.9 % of total tree N) compared to spruce (3.4 %), while N in the tree stem of beech, accounting for 51 % of total tree N, was almost twice as high as in the spruce stem (26 %). In contrast, below-ground the proportion of total root N in beech (21 % of total

tree N) was about half the proportion of that in spruce (37 %), which was most pronounced in coarse and medium roots, while fine roots contained almost the same proportion of total tree N in both species. However, N in mycorrhizal root tips was significantly higher in beech (2 % of total tree N) than in spruce (1.6 %).

N_{total} partitioning in the non-green tissues was not different between the O_3 treatments in beech, whereas in spruce, significantly more N had been allocated into buds under $2\times O_3$ compared to $1\times O_3$ trees. Moreover, a tendency of more N allocated to the stem, but less to branches was found for spruce under $2\times O_3$ compared to $1\times O_3$.

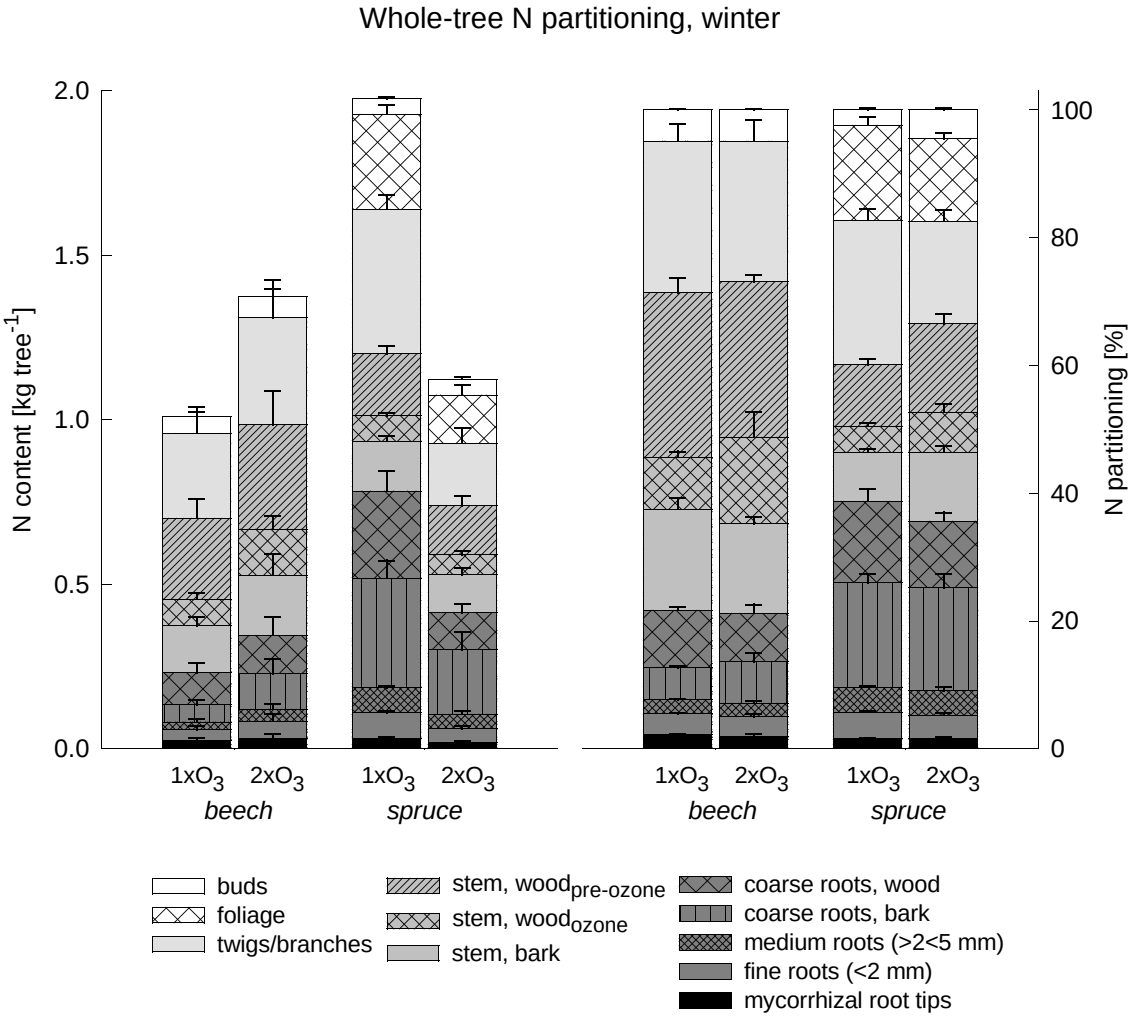

Figure 25: Total content (left) and partitioning (right) of N_{total} per tree in winter 2006, as means ± SE, with n=5 trees.

Table 7: Statistical analysis of N partitioning within trees in winter. Effects of species and ozone treatments according to two-way ANOVA (p-values), n.s. = not significant,non-significant trends in parentheses. Within interacting factors, species effects under one O_3 treatment or ozone effects of only one species are detected as significant contrasts; b = beech, sp = spruce.

	Species	**Ozone**	**Species × Ozone**	**Contrast Species**	**Contrast Ozone**
buds	<0.0001	0.0017	0.0020	<0.0001 (1×O_3) <0.0001 (2×O_3)	<0.0001 (sp)
foliage	-	n.s. (spruce only)	-	-	-
twigs/branches	n.s.	n.s.	n.s.	n.s.	n.s.
stem total	<0.0001	n.s.	n.s.	n.s.	n.s.
stem, $wood_{pre\text{-}ozone}$	<0.0001	n.s.	(0.0837)	<0.0001 (1×O_3) 0.0002 (2×O_3)	(0.0663, sp)
stem, $wood_{ozone}$	0.0174	n.s.	n.s.	n.s.	n.s.
stem, bark	0.0002	n.s.	(0.0516)	0.0002 (1×O_3)	(0.0765, sp)
roots total	<0.0001	n.s.	n.s.	n.s.	n.s.
coarse roots, wood	0.0301	n.s.	n.s.	n.s.	n.s.
coarse roots, bark	<0.0001	n.s.	n.s.	n.s.	n.s.
medium roots	0.0001	n.s.	n.s.	n.s.	n.s.
fine roots	(0.0907)	n.s.	n.s.	n.s.	n.s.
mycorrhizal root tips	0.0449	n.s.	n.s.	n.s.	n.s.

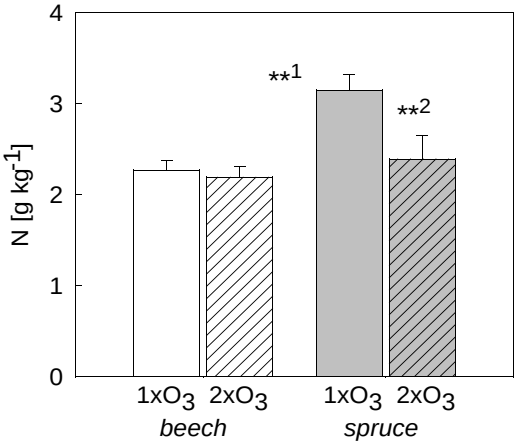

Figure 26: Proportion of N_{total} per total tree biomass (dry mass), as means ± SE, with n=5 trees. Asterisks indicate significant differences either between species ([1]) or ozone treatment ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Changes of the N status in plants or single plant organs over time or in response to a treatment can be visualized according to TIMMER & STONE (1978) and TIMMER & MORROW (1984) depicting the relationship between N_{total} concentration, N_{total} content and biomass within one diagram. Thereby, a shift along the ordinate (*Fig.* 27, *left*, A) represents a change in the nutritional status of the plant or a certain tissue as nutrient content remains similar, independent of biomass. Here, increased biomass does not improve nutrient gain, whereas decreased biomass may result in nutrient accumulation. A shift along the abscissa (*Fig.* 27, *left*, B) displays unchanged nutritional status with nutrient content being dependent on biomass. A shift in diagonal direction (*Fig.* 27, *left*, C) indicates a change in nutritional status without affecting biomass, e.g. increased nutrient acquisition does not promote growth. In the studied trees, the N status was similar in beech and spruce on the whole-tree level, except for spruce under $1\times O_3$ (*Fig. 27, right*). In beech, whole-tree N_{total} concentrations were similar under both O_3 regimes, and a shift towards higher N_{total} content corresponded to a higher biomass under $2\times O_3$. This pattern was also found for most of the single organs or tissues (*Fig. 28, 29, 30* and *31*). However, stem $wood_{ozone}$ had significantly higher N_{total} concentrations under $2\times O_3$ (*Fig. 29,* compare chapter *1 Nitrogen in above-ground organs*). Conversely, mycorrhizal root tips had significantly lower N_{total} concentrations under $2\times O_3$ (*Fig. 31;* compare *Fig. 22,* chapter *2 Nitrogen in below-ground organs and soil*) at similar or even higher biomass and total N content. Together with a similar trend observed in the bark of stem (*Fig. 29)* and coarse roots (*Fig. 30)* as well as in fine roots (*Fig. 31)*, this indicates an effect of O_3 on N acquisition and/or tree internal N allocation.

In contrast, spruce trees under $2\times O_3$ not only had lower whole-tree N content which was consistent with a lower tree biomass, but did also show a lower whole-tree N concentration. This difference between O_3 treatments, in particular in terms of N concentration, mainly reflects the N status of foliage and twigs/branches which also exhibited reduced N concentrations and lower N content under $2\times O_3$ compared to $1\times O_3$ (*Fig. 28;* compare *Fig. 10, 11* and *14*, chapter *1 Nitrogen in above-ground organs*). However, N concentration in buds of spruce was significantly higher under $2\times O_3$ than $1\times O_3$ (*Fig. 28*, but compare *Fig. 13,* chapter *1*). Stem tissues and below-ground organs of spruce were not affected in their N status but showed a lower N content according to the lower biomasses under $2\times O_3$ (*Fig. 29, 30* and *31*).

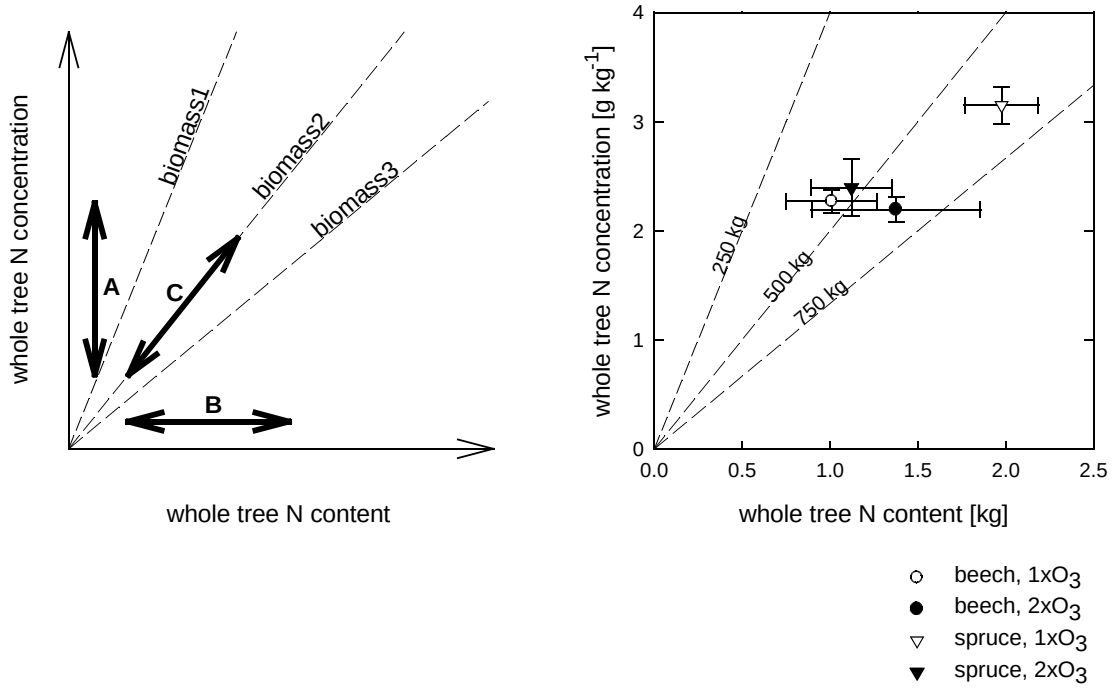

Figure 27: N_{total} concentration versus N_{total} content in relation to biomass (scheme, left) of whole trees in winter. Values as means ± SE, with n=5 trees. Whole tree biomass is indicated by dashed lines.

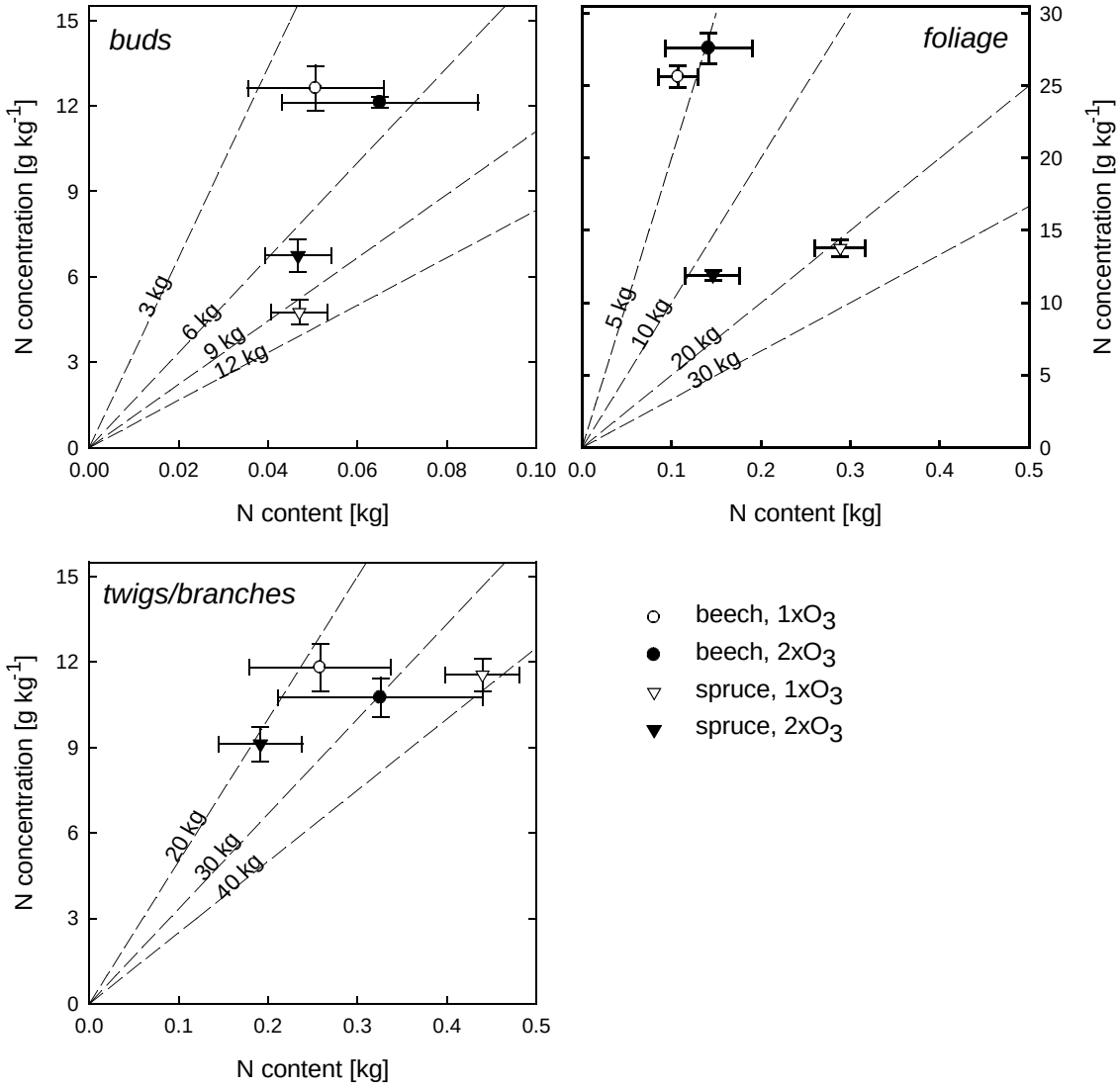

Figure 28: N_{total} concentration versus N_{total} content in buds, foliage of spruce and twigs/branches per tree in winter; only biomass of beech foliage in summer was added here for ease of comparison between deciduous beech and evergreen spruce. Values as means ± SE, with n=5 trees. Biomass of organ or tissue per tree is indicated by dashed lines.

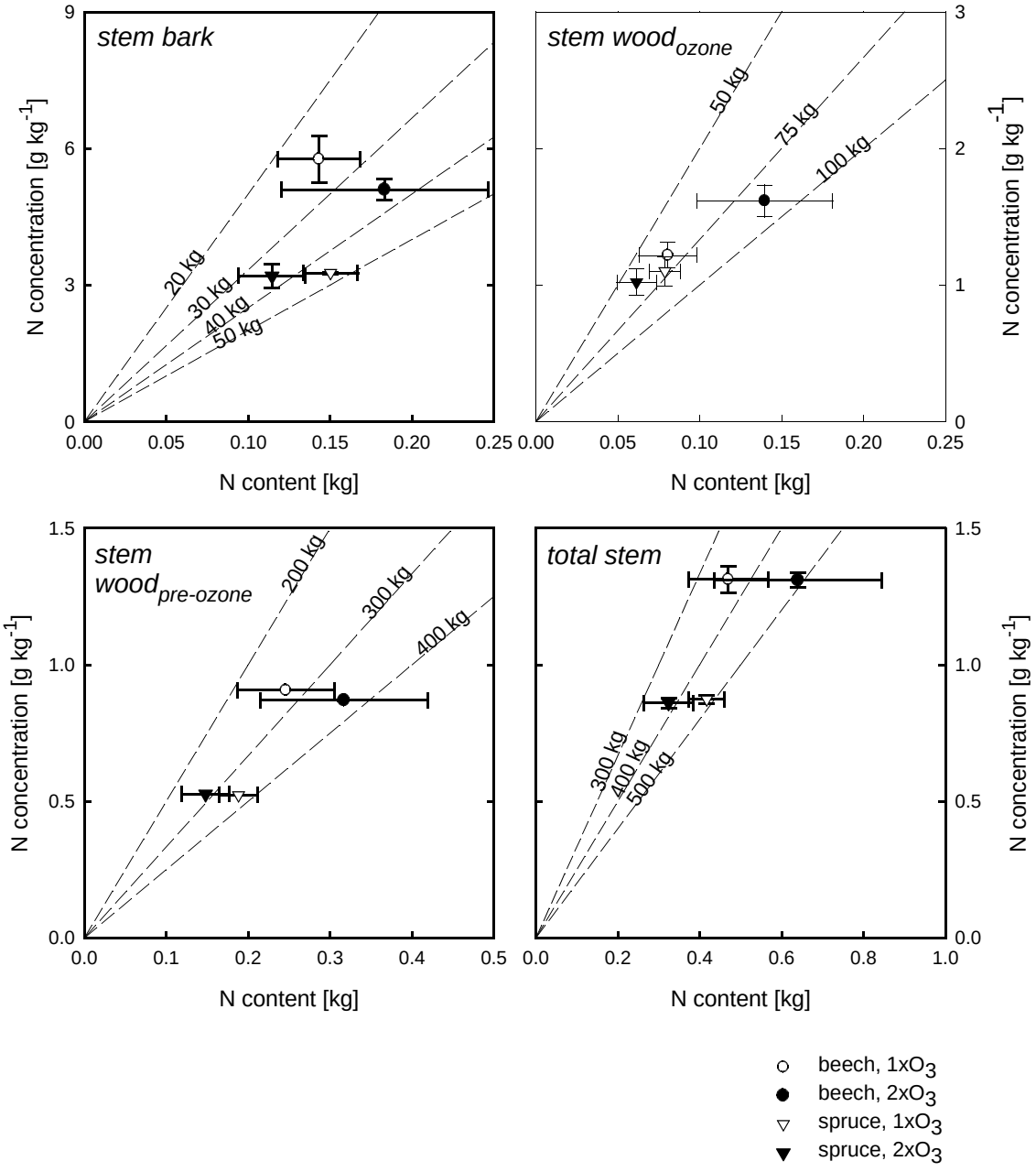

Figure 29: N_{total} concentration versus N_{total} content in the separated stem tissues bark, wood$_{ozone}$ (formed within years of O_3 fumigation), wood$_{pre-ozone}$ (formed before O_3 fumigation) and in total stem per tree in winter, as means ± SE, with n=5 trees. Biomass of tissue per tree is indicated by dashed lines.

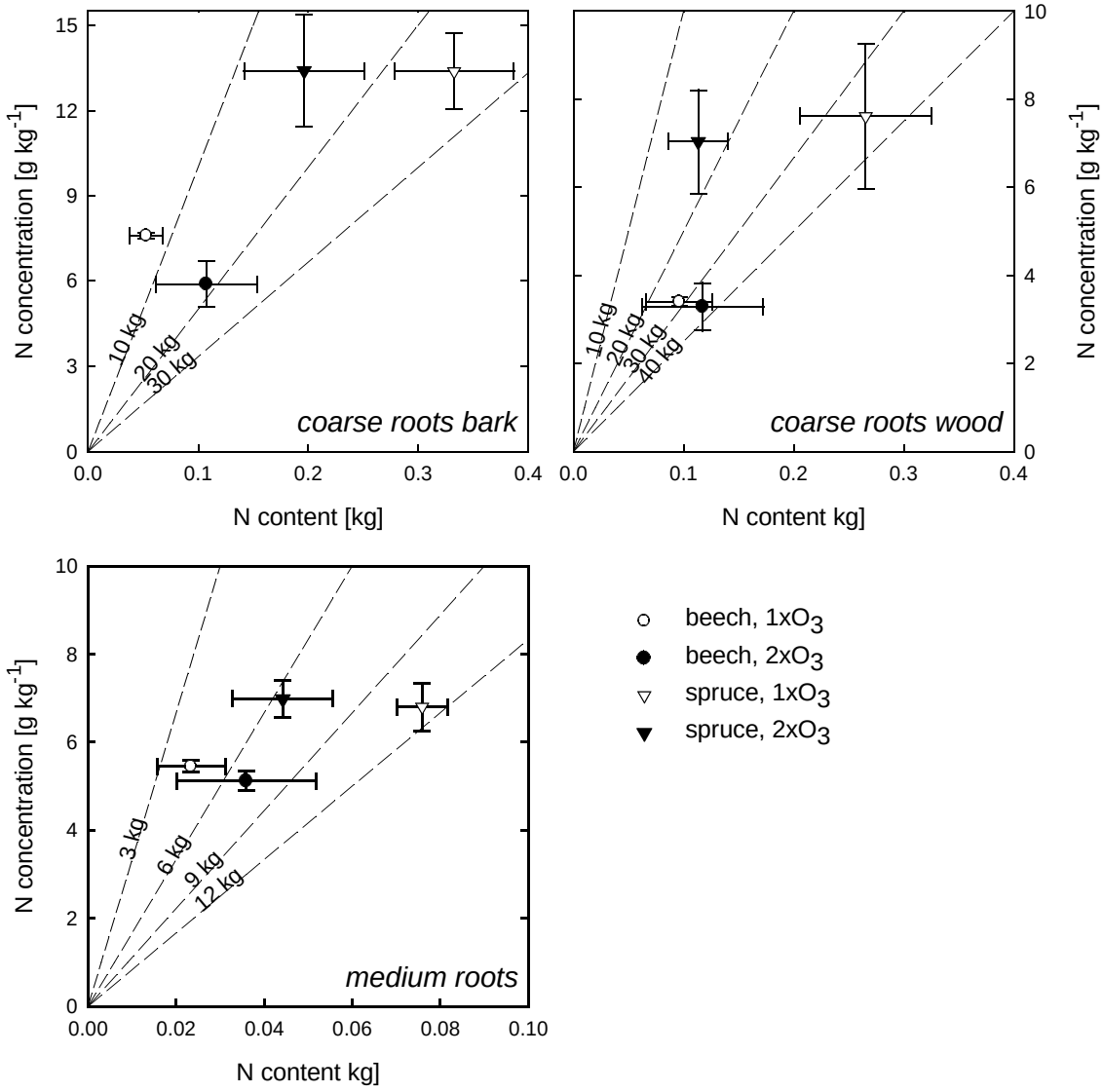

Figure 30: N_{total} concentration versus N_{total} content in bark and wood of coarse roots and in medium roots per tree in winter, as means ± SE, with n=5 trees. Biomass of tissue or root class per tree is indicated by dashed lines.

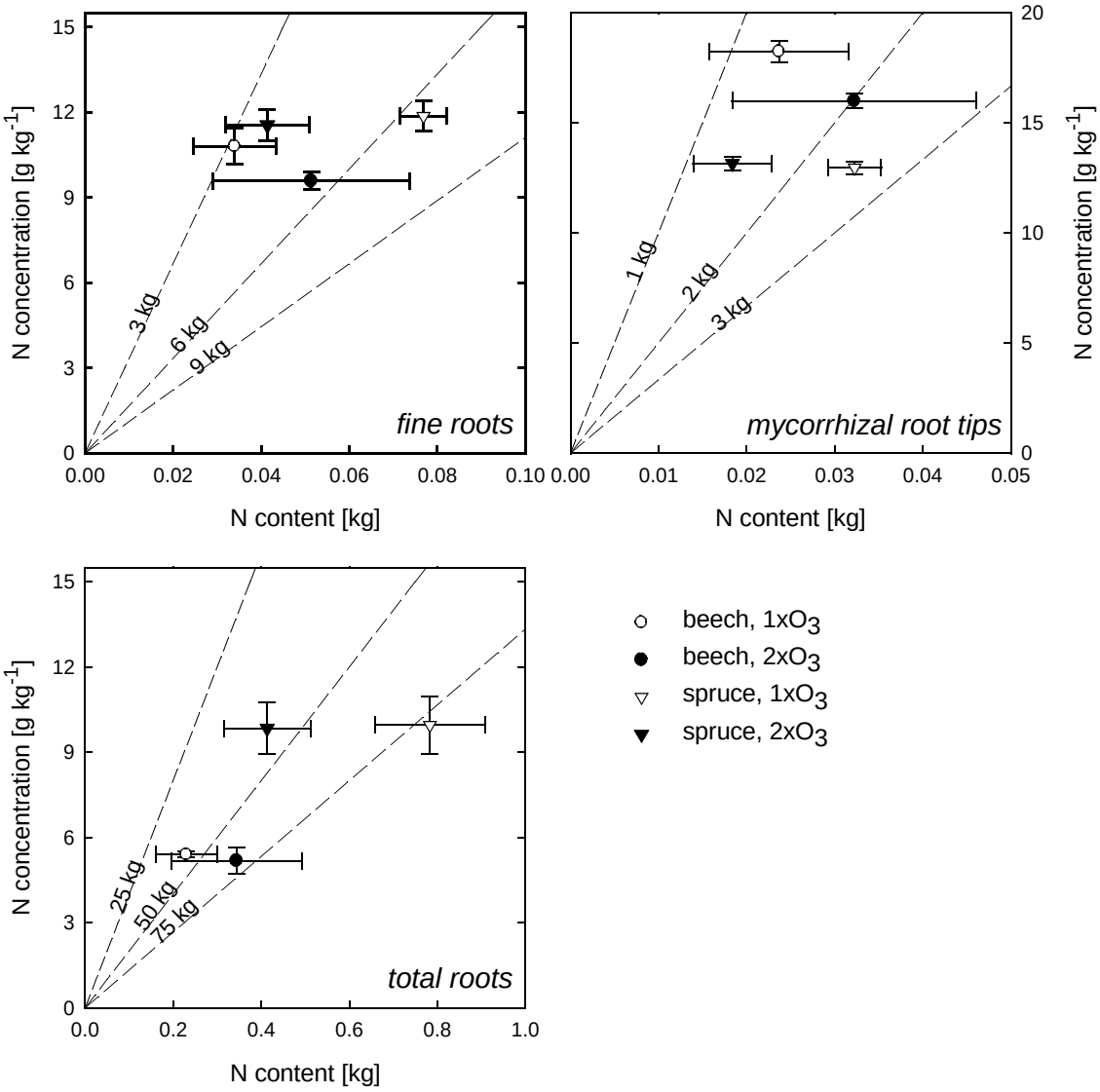

Figure 31: N_{total} concentration versus N_{total} content in fine roots, mycorrhizal root tips and total roots per tree in winter, as means ± SE, with n=5 trees. Biomass of organ or root class per tree is indicated by dashed lines.

Nitrogen dynamics in foliage

Total N content per tree foliage accounted for up to 123 g (beech) and 300 g (spruce) in the mature trees within a whole-tree N content of 1.2 and 1.7 kg per tree, respectively, averaged over both O_3 treatments. N content in beech foliage showed seasonal variation with high levels in summer and decrease over late summer to autumn (*Fig. 32;* compare *Fig. 9,* chapter *1 Nitrogen in above-ground organs*). About 30 % of autumnal leaf N (based on sampling in September) was not retranslocated into storage tissue and got lost via litter fall. N content in buds remained stable over summer, autumn and winter (no measurements in spring). In beech, half of the yearly N demand of the foliage is already stored in the buds. In contrast, foliage of evergreen spruce trees, containing in total *ca.* 2.4 to 4fold the amount of N in beech foliage, remained rather stable throughout the season (*Fig. 32;* compare *Fig. 10* and *11,* chapter *1 Nitrogen in above-ground organs*). The N content of buds per tree, however, was in the same range as for the buds of beech.

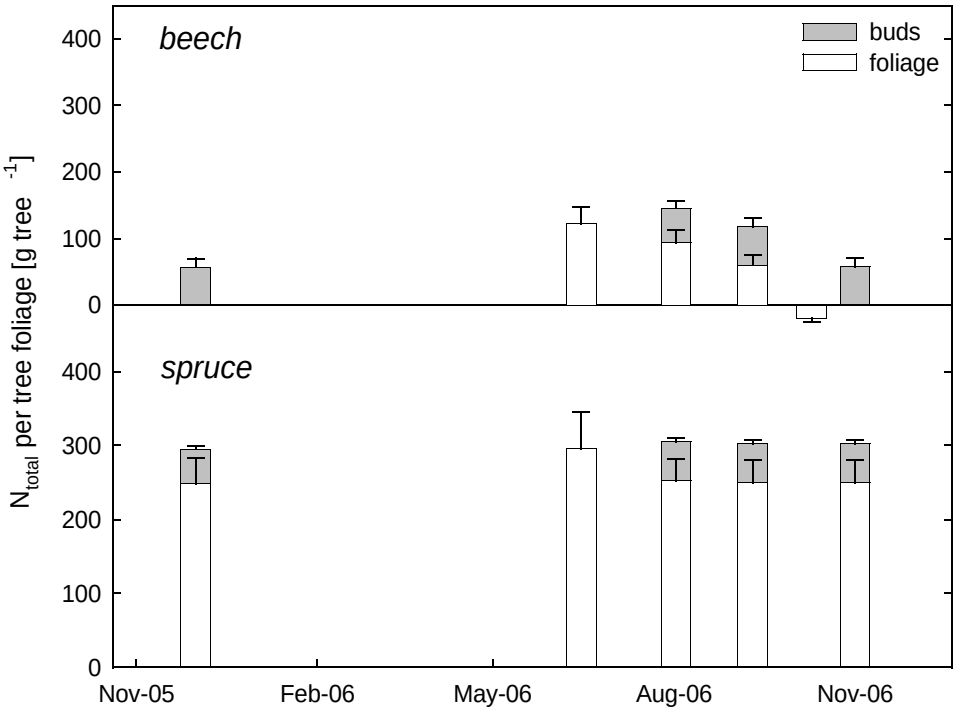

Figure 32: Total N content in tree foliage over one growing season. Negative numbers represent nitrogen loss via leaf litter, as means ± SE, with n=10 trees, averaged over both O_3 treatments.

Recovery of labelled N at the whole-tree level

Sixteen months upon application, around 72 % of the labelled N was recovered in trees and soil. On average, 23 % was recovered in the target trees and 29 % in the soil across both species and treatments (*Fig. 33*). Recovery in neighbouring trees within a distance of up to 2 m accounted for *ca.* 14 %. About 5 % of the labelled N was leached out, particularly under beech (*Fig. 33, Tab. 8*). Although recovery per total tree was similar between species, buds and mycorrhizal root tips accumulated significantly higher amounts of labelled N in beech than in spruce (*Fig. 33, Tab. 8)*. Under $2\times O_3$, slightly more labelled N remained in the soil ($p = 0.0855$) and the total recovery in trees tended to be lower than under $1\times O_3$. Despite higher standing biomass of the 62 yrs old trees under $2\times O_3$, beech thus seemed to take up less of the labelled (i.e. new) N, whereas in spruce, the lower N uptake under $2\times O_3$ was more likely related to the lower tree biomass. Note that recovery of labelled N in soil and roots is based on the labelled plots, but as horizontal diffusion of the applied label was small (see *Fig. 18*) it reflects closely the complete rooting area of an individual tree.

The recovery of labelled N in nitrate leaching was assessed on the basis of comparable plots treated with non-labelled NH_4NO_3 (see *Material and Methods*). The additional supply of water with the ^{15}N label (dissolved in 70 l water), leading to a slightly higher leaching underneath N-treated compared to control plots ($p = 0.0984$, *Fig. 34*) was taken into account. Underneath beech, the recovery of labelled N was higher than under spruce, since total N leaching measured under beech was higher than under spruce ($p = 0.0023$, *Fig. 34*).

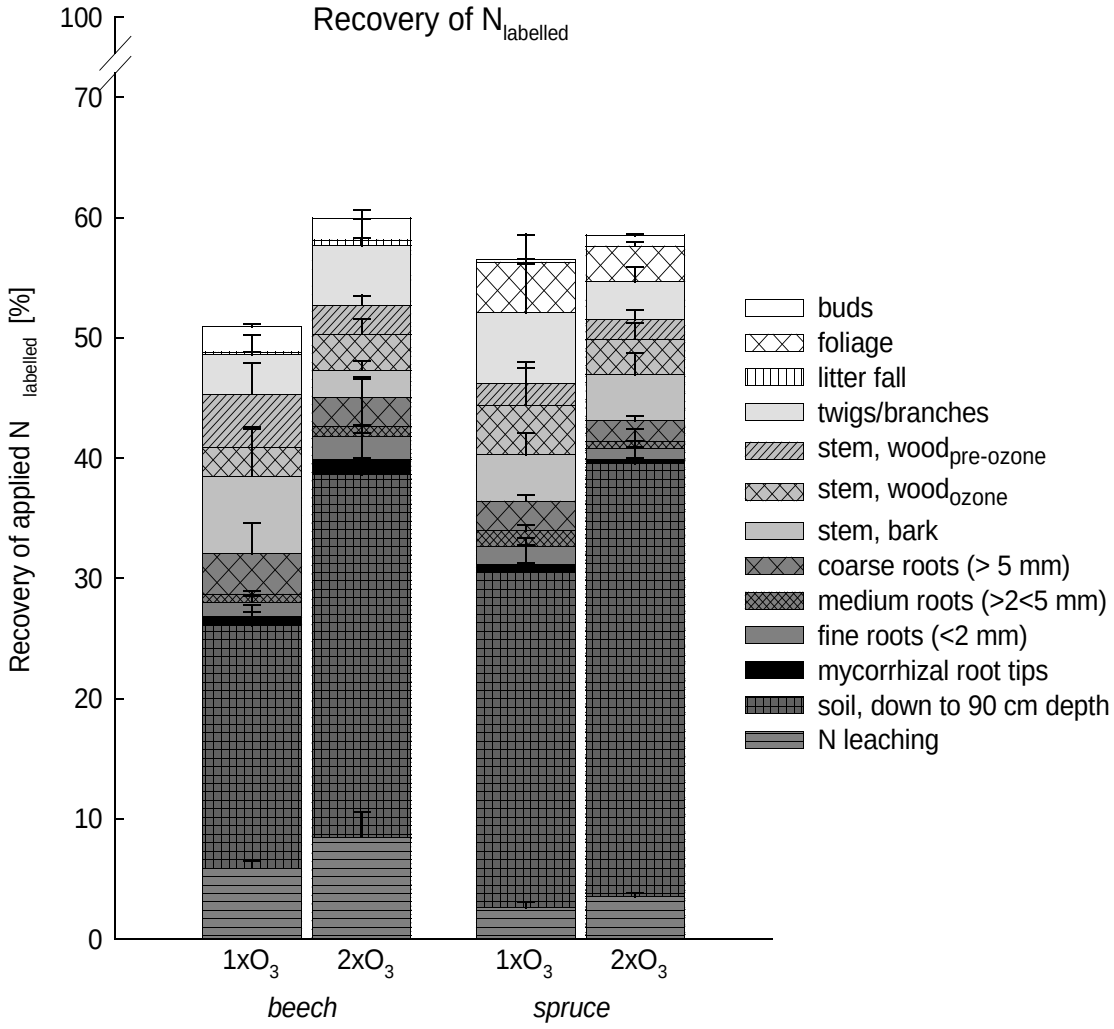

Figure 33: Recovery of $N_{labelled}$ in tree and soil compartments at 16 months upon ^{15}N-label application, as means ± SE, with n=3 trees. Note that recovery in below-ground compartments is based on the 1 m² plots down to 90 cm depth. Recovery assessment in soil includes an additional area surrounding the plots of up to 30 cm distance from plot borderline and down to 60 cm depth.

*Table 8: Statistical analysis of $N_{labelled}$ recovery in tree and soil compartments at 16 months upon ^{15}N label application. Effects of species and ozone treatments according to two-way ANOVA (*p*-values), n.s. = not significant, non-significant trends in parentheses, b = beech, sp = spruce.*

	Species	**Ozone**	**Species × Ozone**
tree total	n.s.	n.s.	n.s.
buds	0.0041	n.s.	n.s.
foliage	-	n.s. (spruce only)	-
litter fall	-	n.s. (beech only)	-
twigs/branches	n.s.	n.s.	n.s.
stem total	n.s.	n.s.	n.s.
stem, $wood_{pre\text{-}ozone}$	n.s.	n.s.	n.s.
stem, $wood_{ozone}$	n.s.	n.s.	n.s.
stem, bark	n.s.	n.s.	n.s.
roots total	n.s.	n.s.	n.s.
coarse roots	n.s.	n.s.	n.s.
medium roots	n.s.	n.s.	n.s.
fine roots	n.s.	n.s.	n.s.
mycorrhizal root tips	0.0490	n.s.	n.s.
soil (plot, down to 0.9 m)	n.s.	(0.0855)	n.s.
N leaching	0.0057	n.s.	n.s.

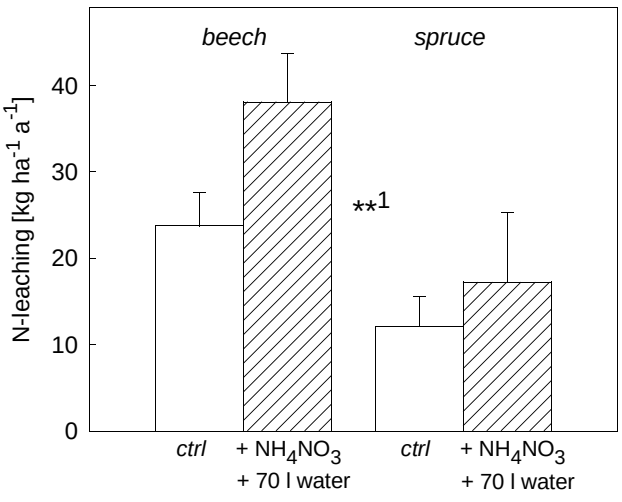

Figure 34: N-leaching underneath 1 m²-plots applied with 10 g NH_4NO_3 in 70 l water and underneath non-treated control (ctrl) plots, as means ± SE, with n=5 trees. Asterisks indicate significant differences either between species ([1]) or treatment ([2]) as main effects at p < 0.05 (*), 0.01 (**) or 0.001 (***).

Partitioning of new N within trees

Although the partitioning of biomass as well as total nitrogen differed considerably between beech and spruce trees (see *Fig. 24* and *25*), the partitioning of labelled (i.e. new) nitrogen within the trees was similar in both species, except for buds and medium-sized roots: Buds received 2.6fold more new N per tree in beech than in spruce although this was not significant (*Fig. 35, Tab. 9*). Additionally, beech had allocated less newly acquired N to medium-sized roots compared to spruce.

$2\times O_3$ significantly altered the partitioning of new N in below-ground organs: In beech, a higher proportion of the newly acquired N remained in mycorrhizal root tips under $2\times O_3$ trees, as a result of a slightly higher biomass as well as recovery of $N_{labelled}$, showing a trend similar to fine roots. In contrast, in spruce, new N allocated to roots was lower under $2\times O_3$, particularly in medium-sized roots, because of a significantly lower biomass (*Fig. 24, Tab. 6*) and slightly lower recovery of $N_{labelled}$. Above-ground, the partitioning of new N was not significantly affected by ozone in both species. However, a tendency of less N allocated to stem bark and foliage was observed in beech, whereas in spruce, a slightly higher proportion of new N was allocated to the stem.

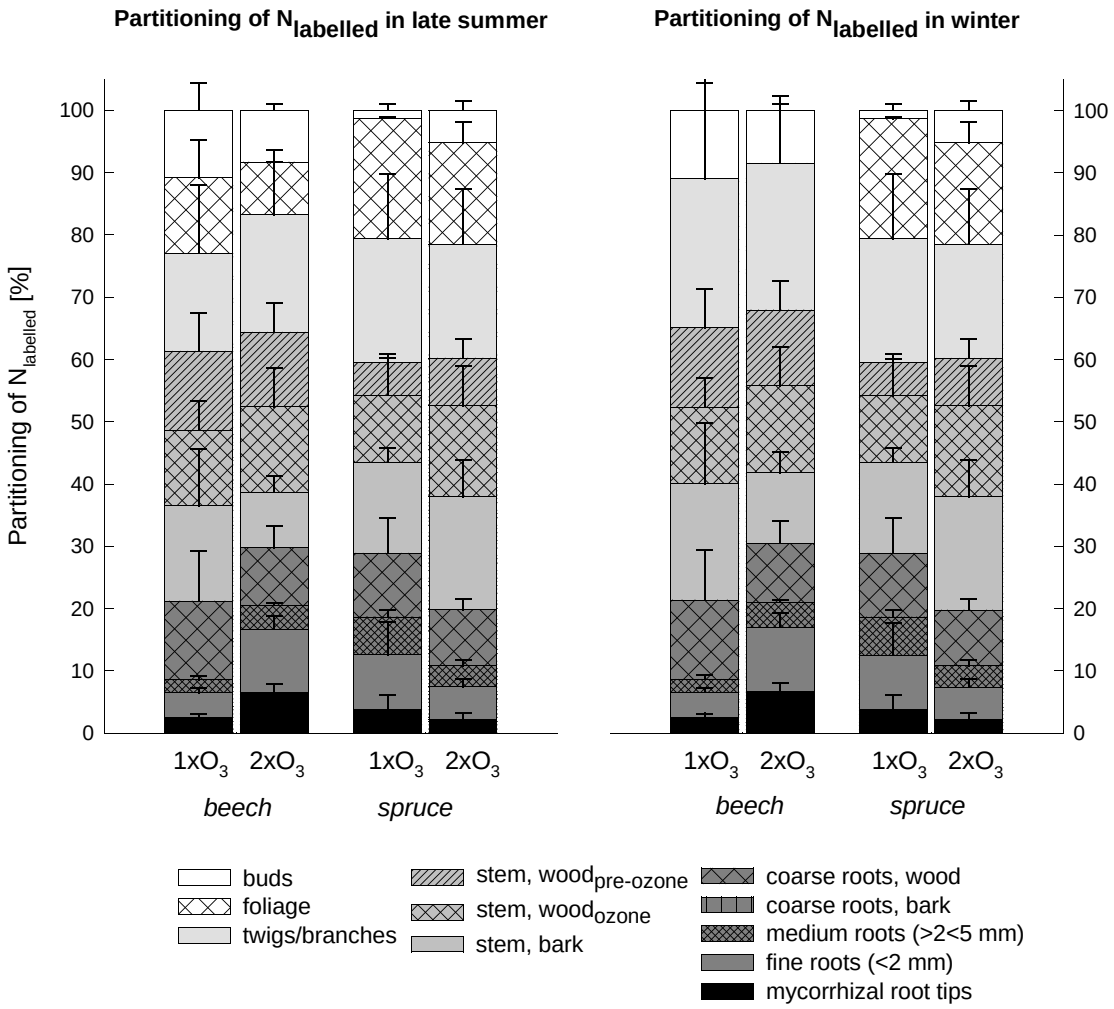

Figure 35: Partitioning of $N_{labelled}$ in trees before (left) and after (right) beech leaf fall, as means ± SE, with n=3 trees.

*Table 9: Statistical analysis of $N_{labelled}$ partitioning within trees in late summer and winter. Effects of species and ozone treatments according to two-way ANOVA (*p*-values), n.s. = not significant, non-significant trends in parentheses. Within interacting factors, species effects under one O_3 treatment or ozone effects of only one species are detected as significant contrasts; b = beech, sp = spruce.*

	Species	**Ozone**	**Species × Ozone**	**Contrast Species**	**Contrast Ozone**
Late summer					
buds	(0.0561)	n.s.	(0.2745)	0.0498 (1×O_3)	n.s.
foliage	n.s.	n.s.	n.s.	n.s.	n.s.
twigs/branches	n.s.	n.s.	n.s.	n.s.	n.s.
stem total	n.s.	n.s.	n.s.	n.s.	n.s.
stem, $wood_{pre\text{-}ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, $wood_{ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, bark	n.s.	n.s.	n.s.	n.s.	n.s.
roots total	n.s.	n.s.	n.s.	n.s.	n.s.
coarse roots	n.s.	n.s.	n.s.	n.s.	n.s.
medium roots	0.0478	n.s.	0.0214	0.0089 (1×O_3)	0.0459 (sp)
fine roots	n.s.	n.s.	(0.0674)	n.s.	0.077 (b)
mycorrhizal root tips	n.s.	n.s.	(0.0639)	0.0384 (1×O_3)	(0.0516, b)
Winter					
buds	0.0502	n.s.	(0.2872)	0.0506 (1×O_3)	-
foliage	-	n.s. (spruce only)	-	-	-
twigs/branches	n.s.	n.s.	n.s.	n.s.	n.s.
stem total	n.s.	n.s.	n.s.	n.s.	n.s.
stem, $wood_{pre\text{-}ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, $wood_{ozone}$	n.s.	n.s.	n.s.	n.s.	n.s.
stem, bark	n.s.	n.s.	n.s.	n.s.	n.s.
roots total	n.s.	n.s.	n.s.	n.s.	n.s.
coarse roots	n.s.	n.s.	n.s.	n.s.	n.s.
medium roots	(0.0533)	n.s.	0.0201	0.0091 (1×O_3)	0.0461 (sp)
fine roots	n.s.	n.s.	(0.0655)	n.s.	(0.0723, b)
mycorrhizal root tips	n.s.	n.s.	(0.0624)	0.0357 (1×O_3)	0.0487 (b)

N cycle at stand level

The N balance was assessed at the study site for the mixed stand of beech and spruce trees (*Fig. 36*). Beech accounted for 27 % and spruce for 73 % of the basal tree area on a hectare basis, whereas the proportion in view of tree number was 35 % (beech) compared to 65 % (spruce).

Based on the basal area, the tree N pool represented about 10 % of the total N storage in the stand, with dead-wood material and understorey vegetation not being included here. N input as wet and dry deposition was similar to the output (leaching and denitrification), indicating a low demand of external nitrogen of this old-growth stand and a possible high contribution of stand-internal or tree internal cycling to cover the N demand. This is confirmed in beech by a high proportion of N retranslocated from foliage before leaf fall which accounted for 84 % of the total foliage maximum N level in summer (or 70 % of the N level in September).

Beech contributed to the total tree N pool of the stand by about 31 % (as based on the basal area). However, the proportion of yearly N uptake between beech and spruce (as based on yearly growth increment) was slightly different with 38 % (beech) and 62 % (spruce). Thus, relative to the proportion of the total tree N pool, the N demand tended to be higher in beech than in spruce, as also shown in comparison of stands composed of just one single species, beech or spruce, respectively (*Fig. 37)*.

Under $2\times O_3$, the tree N pool tended to be reduced, especially in spruce (29 % of total tree N) but also in beech (16 % of total tree N). These findings were consistent with a slightly reduced N uptake in both species as well as a slightly reduced retranslocation of N from beech leaves under $2\times O_3$. A reduced demand of or capability to acquire N by the trees may therefore alter the amount of available N in the soil through accumulation or higher loss from the system. Under spruce, the microbial N pool was significantly higher under $2\times O_3$ compared to $1\times O_3$ ($p = 0.0123$). A change in soil N may in turn affect mineralisation of organic matter in the long term. Under $2\times O_3$, the N cycle seems to be affected in a similar way through beech and spruce, but the effect was mediated to a different extend.

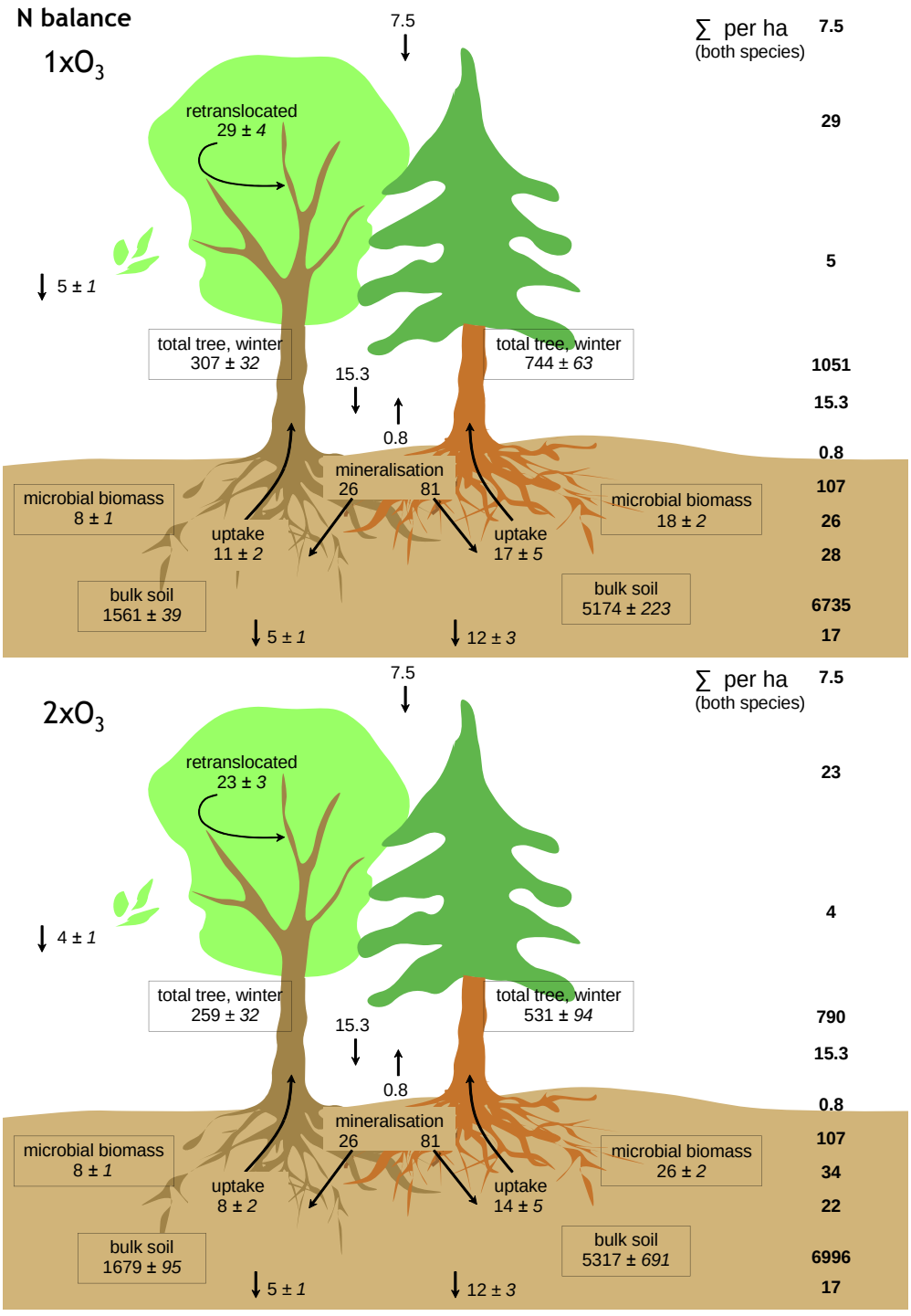

Figure 36: N balance and cycle of the study site [kg N ha^{-1}] as a mixed stand of beech and spruce under 1×O_3 (above) and 2×O_3 (below). Arrows indicate fluxes [kg N ha^{-1} a^{-1}], boxes indicate pools [kg N ha^{-1}], means ± SE, with n=3-5 trees. N pools are divided between beech and spruce according to their proportion in total basal wood area of the study site (sum per ha as given on the right). N-pools in bulk soil and microbial biomass referred to soil depths down to 90 cm each.

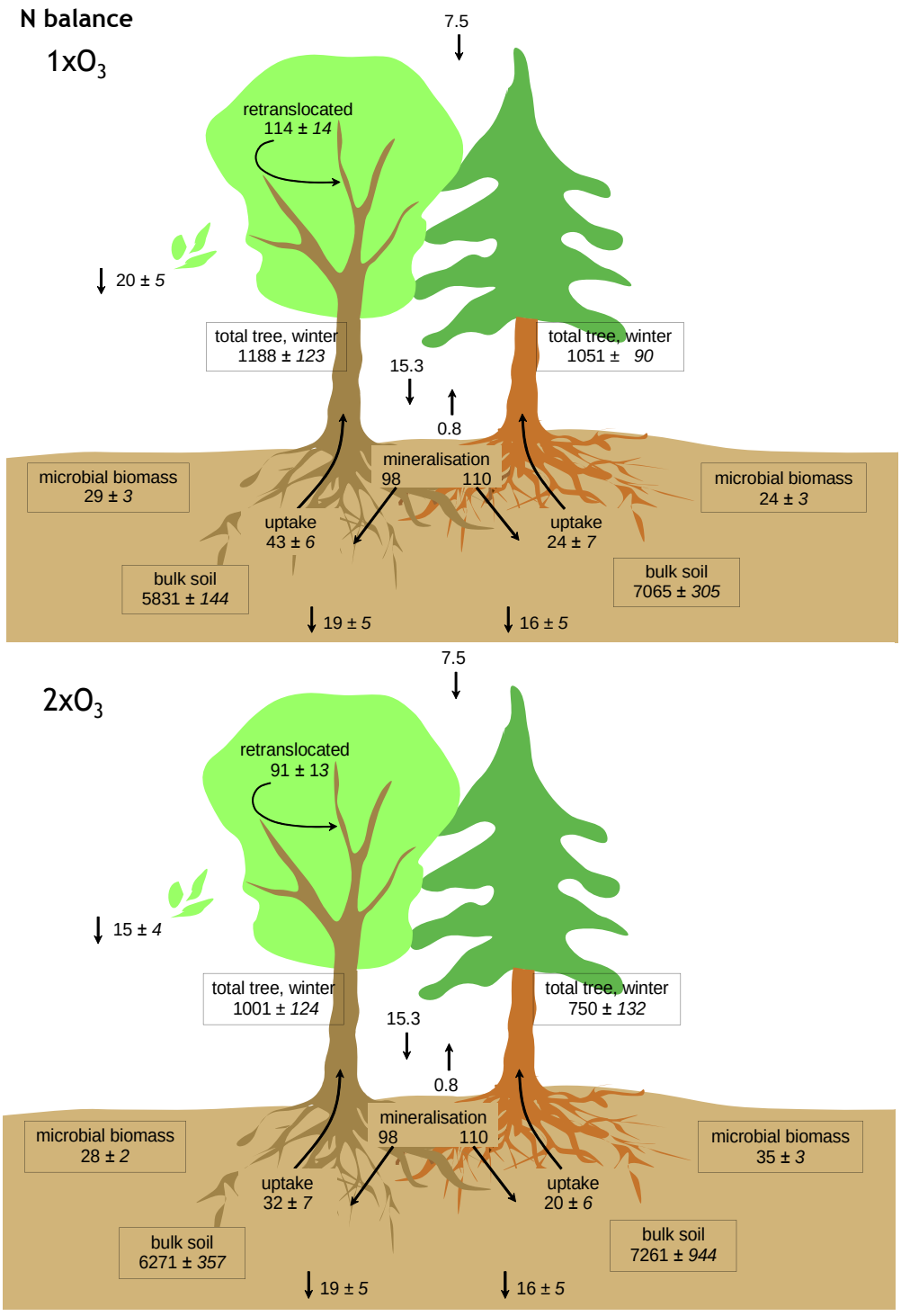

Figure 37: N balance and cycle of the study site [kg N ha^{-1}] assuming stands composed of one single species, beech or spruce, respectively, under 1×O_3 (above) and 2×O_3 (below). Arrows indicate fluxes [kg N ha^{-1} a^{-1}], boxes indicate pools [kg N ha^{-1}], means ± SE, with n=3-5 trees. N-pools in bulk soil and microbial biomass referred to soil depths down to 90 cm each.

Discussion

1. Nitrogen concentrations in above-ground organs

Leaves and buds: Above-ground, the incorporation of nitrogen under elevated ozone was studied with focus on the foliage. In both species, incorporation of new N (= $N_{labelled}$) into foliage increased from the year of labelling (2005) towards the following growing season (2006). As leaves and needles had already reached maturity by the time of labelling, the tracer taken up in the first year most likely contributed to the soluble nitrogen fraction which is mainly represented by proteins such as Rubisco. The latter enzyme can make up about 30 % of total leaf N (SCHULZE *et al.* 2002). Conversely, in the second year of the study, the new N could also be incorporated into structural tissue of the newly built foliage, thus reflecting N uptake over the total growing season. The increase in $N_{labelled}$ incorporation further implies that the tracer N was plant-available from the soil also in the second year.

Under 2×O_3, beech leaves had lower concentrations of the newly acquired $N_{labelled}$ than under 1×O_3, which was the case particularly in the sun crown (significant in July and August 2006), whereas $N_{labelled}$ was not altered in the leaf litter (*Tab. 10*, cf. *Fig. 9*). As cumulative transpiration in 2005 through 2006 was significantly reduced by 42 % (cf. *Fig. 16*, data supplied by METZGER, pers. comm.), and was linearly correlated to the foliage $N_{labelled}$ concentration, this indicated impaired transport and incorporation of N in beech under 2×O_3. Similar to leaves, buds of beech exhibited lowered $N_{labelled}$ concentrations under 2×O_3 (significant in the sun crown in 2006, cf. *Fig. 12*), indicating a manifested chronic O_3 effect. The relationship between reduced $N_{labelled}$ uptake in foliage and transpiration was supported by the (non-significant) reduction in average monthly transpiration by 12 % during the growing season 2006 in the same beech trees under 2×O_3 (METZGER, pers. comm.). This lowered transpiration can be explained by reduced stomatal conductance as observed in these trees during several years in response to 2×O_3 (NUNN *et al.* 2005*a*, LÖW *et al.* 2006, KITAO *et al.* 2009).

Table 10: Summary of ozone effects on the tree N status of beech. Arrows mark differences between ozone treatments: black arrows indicate significant differences at p < 0.05; arrows in parentheses indicate trends at p < 0.08; arrows in double parentheses indicate consistency to N concentrations at p < 0.2; tildes indicate no ozone effect, empty cells indicate missing values.

	N_{new} [µg g^{-1}]		N_{total} [mg g^{-1}]		N_{new}/N_{total}		N_{new} partitioning within tree [%]		N_{total} partitioning within tree [%]		C/N	
	B	Sp	B	Sp	B	Sp	B	Sp	B	Sp	B	Sp
Buds	↓[1]	~	↑[1]	(↑)	(↓)[1]	~	~	~	~	↑	↓[1]	
Leaves/Needles	↓[1]	~	~	↓[3]	↓[1]	(↑)[4]	~	~		~	(↓)[5]	↑
Litter	~		↓		~						↓[5]↑[6]	
Twigs total	~	~	~	~	~	~	~	~	~	(↓)	~	~
Twigs wood	~	~	~	↓	~	~					(↑)	(↑)
Twigs bark	~	~	~	~	~	~					~	~
Stem wood$_{pre\text{-}ozone}$	~	~	~	~	~	~	~	~	~	(↑)	~	~
Stem wood$_{ozone}$	~	~	↑	~	~	~	~	~	((↑))	~	(↓)	~
Stem bark	(↓)	~	~	~	~	~	~	~	~	(↑)	~	~
Phloem	↓	~	(↓)	~	~	~						
Coarse roots	~	~	~	~	~	~	~	~	~	~	↑[2]	~
Medium roots	~	~	~	~	~	~	((↑))	↓	~	~	~	((↑))
Fine roots	~	~	~	~	(↑)[2]	~	(↑)	~	~	~	↑[2]	~
Mycorrhizal root tips	~	~	↓	~	~	~	↑	~	~	~	((↑))	~
Soil	↑[2]	↑[2]	↑[2]	↑[2]	(↑)	↑					(↓)	(↓)

[1] in 2006

[2] within B-horizon

[3] current-year needles in 2005 and one-year-old needles in both years

[4] one-year-old needles in sun crown

[5] sun crown

[6] shade crown

N_{total} concentrations in beech leaves did not differ between both ozone treatments over the two growing seasons, being consistent with measurements on these trees during the same and the previous years (Haberer *et al.* 2007, Göttlein *et al.* 2009, Kitao *et al.*

2009). As this contrasts to the findings of $N_{labelled}$, two explanations are imaginable: the effect of diminished N transport via sapflow, as detected by N labelling, is small relative to the total leaf N content and overruled by N-cycling. Or, transpiration over the growing season is high enough to maintain the N_{total} concentration of leaves, although new N uptake from soil is reduced under elevated O_3. The latter possibility would suggest the tree-internal storage pools to serve as enhanced N sources relative to the demand of the leaves (see also below).

In the leaf litter, N_{total} concentrations were significantly lower under $2\times O_3$ in shade crown leaves along with enhanced C/N ratio, whereas litter of the sun crown had a significantly lowered C/N. This may suggest O_3-induced alterations in N retranslocation processes. Since the beech trees of the present study had shown a tendency of accelerated senescence under $2\times O_3$ in sun and shade crown (NUNN *et al.* 2002, 2005*b*, GIELEN *et al.* 2007) by up to 9 days days (NUNN *et al.* 2002), insufficient N retranslocation might explain the lowered C/N ratio in sun crown litter.

In contrast to leaves, N_{total} concentration in buds tended to be increased under $2\times O_3$ over all sampling dates, being significant in September 2006 in the sun crown. However, this latter effect can not be explained by a difference in bud mass, which was similar under both ozone regimes (data by FARNADY, pers. comm.). Reduced numbers and size of buds due to reduced carbohydrate reserves under elevated ozone (OKSANEN 2003*a, b*, RIIKONEN *et al.* 2008) may also lead to lowered N concentrations. Despite a rather reduced proportion of new N ($=N_{labelled}$) in the N_{total} of buds under $2\times O_3$, it may be assumed that N allocation to the buds was slightly increased under $2\times O_3$ in order to maintain a high photosynthetic capacity in the following growing season even if N supply is reduced. This would suggest a higher proportion of N originating from storage.

Reduced leaf N concentrations in mature trees under elevated ozone were reported from northern red oak trees (SAMUELSON *et al.* 1996), sugar maple (SAGER *et al.* 2005), and sycamore maple (CALATAYUD *et al.* 2007), possibly as a consequence of altered retranslocation (SAMUELSON *et al.* 1996). In trembling aspen and paper birch, O_3-induced reductions in leaf N concentrations were only marginal (LINDROTH *et al.* 2001), and in ponderosa pine seedlings, lowered needle N concentrations in response to ozone were found only in combination with species competition (ANDERSEN *et al.* 2001). In other studies foliage N concentration did not respond to elevated O_3 (e.g. REICH *et al.* 1988 on white pine (*Pinus strobus* L.), SKÄRBY *et al.* 1995 on Norway spruce, MAURER *&* MATYSSEK 1997 on European birch).

Accelerated leaf senescence with the consequence of altered N translocation in response to elevated O_3 has also been reported from other studies. FENN (1991) and FENN *&* DUNN (1989) found enhanced N concentrations in foliage and litter in response to high O_3 concentrations, however, site N fertility was also high in these studies. Enhanced N concentrations in the leaf litter due to early leaf abscission in response to elevated O_3 may lead to an increased N loss in deciduous trees (FINDLAY *&* JONES 1990, UDDLING *et al.* 2005). Decreased nitrogen resorption efficiencies in response to O_3 were reported from paper birch leaves (LINDROTH *et al.* 2001) as well as from seedlings of European white birch (UDDLING *et al.* 2005). In contrast, increased C/N and decreased N concentration of leaf litter were found in young trembling aspen and paper birch trees as a result of increased soluble sugars, soluble phenolics and tannins under elevated O_3 (LIU *et al.* 2005, 2009). European white birch as well as ponderosa pine seedlings also showed decreased N concentrations in senescent leaves in response to elevated ozone (OKSANEN 2003*a*, TEMPLE *&* RIECHERS 1995). For ponderosa pine, these authors suggested a higher resorption of N from senescing foliage that increases the N supply to the current-year foliage (see below).

In spruce needles, the concentrations of newly acquired N (= $N_{labelled}$) were not different between $1\times O_3$ and $2\times O_3$, which was the case also in buds (*Tab. 10,* cf. *Fig. 10, 11, 13*). However, the N_{total} concentration was significantly reduced under $2\times O_3$ in both measured age classes (current-year and one-year-old needles) at most sampling dates. This was consistent with a significantly higher C/N ratio under $2\times O_3$, particularly in one-year-old needles. In contrast in the buds, similar to those of beech, N_{total} concentrations were enhanced under $2\times O_3$ over all sampling dates, significantly in December 2005 in the shade crown, at an unchanged bud dry mass.
The lowered N_{total} concentration in needles under $2\times O_3$ might reflect a long-term effect that was not observable in the uptake of new N within the two-year study. Continuous monitoring of the nutritional status over the years of the O_3 fumigation showed that the $2\times O_3$ spruce trees of the study site already had lower N concentrations in needles before the onset of the ozone treatment in 2000 (GÖTTLEIN, pers. comm.). Until 2005, after six years of $2\times O_3$ fumigation, no change in N concentration due to elevated O_3 was observed in needles of these trees (GÖTTLEIN *et al.* 2009). Therefore, it is assumed that access to nutrients may have been impaired in the $2\times O_3$ spruce group, e.g. via lower root biomass as shown by the modelled biomasses (see section *Nitrogen pools and*

partitioning at the whole-tree level), although it remains unclear whether this impairment was enhanced by the ozone treatment. In contrast, the proportion of new N on total needle N – i.e. the quotient $N_{labelled}/N_{total}$ – tended to be higher under $2\times O_3$ in the sun crown, particularly in one-year-old needles (*Tab. 10*). This suggests that the needles remained a high sink for newly acquired N, even if total N supply seemed to be restricted in long-term. An increased N demand in needles in response to $2\times O_3$, as indicated also by a trend of increased concentration of non-structural proteins in the same study trees (ALEXOU *et al.* 2007) may result from increased defence processes (SCHMITZ *et al.* 1993). A similar tendency of increased proportion of new N in the N_{total} was found in the buds under $2\times O_3$. Including the higher N_{total} concentration, it is assumed, as for beech buds, that N allocation to the buds was slightly increased under $2\times O_3$. This may be interpreted as a compensation for the lowered N supply of needles under $2\times O_3$, but also as response for reduced C assimilation, since stomatal conductance was reduced on the same study trees (measured during the first three years of $2\times O_3$ exposure), in particular in the shade crown (NUNN *et al.* 2006)

Increased N concentrations in response to elevated ozone were found in needles of ponderosa pine seedlings as a consequence of altered N allocation (TEMPLE *&* RIECHERS 1995) and in loblolly pine seedlings in association with a reduced biomass (TJOELKER *&* LUXMOORE 1991, EDWARDS *et al.* 1992, KELLY *et al.* 1993, BAKER *et al.* 1994). According to TEMPLE *&* RIECHERS (1995) retranslocation of N from older to current-year needles can be increased in response to ozone, suggesting a compensation for the O_3-induced accelerated loss of older needles by increased photosynthesis.

O_3-induced reduction of photosynthesis may lower leaf N concentrations e.g. by reduced concentration and/or activity of Rubisco (PELL *et al.* 1994, 1997). In both beech and spruce at the study site, photosynthesis was reduced in response to $2\times O_3$ (NUNN *et al.* 2005*b*), but varied with time and crown position (MATYSSEK *et al.* 2007*a, b*, NUNN *et al. 2005b*, 2006). Decrease was most pronounced when water availability was not limited (LÖW *et al.* 2006, 2007).

In beech trees of the study site no difference in Rubisco activity between ozone treatments was observed (MATYSSEK *et al.* 2007*b*). Nevertheless, reduced levels of Rubisco as well as downregulation of transcripts involved in photosynthesis, such as Rubisco precursors were observed in beech as response to $2\times O_3$ (OLBRICH *et al.* 2009). Also at the same study site, reduced content of soluble proteins was found in beech

leaves after five years of 2×O_3 exposure, whereas no such effect was observed in spruce (ALEXOU *et al.* 2007, ALEXOU 2007).

Reduced photosynthesis reflects lowered demand for CO_2 in the substomatal chamber (VON CAEMMERER & FARQUHAR 1981). This may cause stomatal closure and reduced stomatal conductance as an indirect effect of elevated O_3 concentrations (TEMPLE 1986, WIESER & HAVRANEK 1993, REILING & DAVISON 1995, PLEIJEL *et al.* 2002). Reduced stomatal conductance in response to O_3 lowers transpiration (HILL & LITTLEFIELD 1969, SKÄRBY *et al.* 1987, KOLB & MATYSSEK 2001, MAURER & MATYSSEK 1997) as a prerequisite for nutrient transport (LARCHER 1994). Conversely, chronic O_3 exposure may disturb the stomatal guard cells and disrupt the stomatal control, sometimes leading to enhanced transpiration (KELLER & HÄSLER 1984, SKÄRBY *et al.* 1987, MILLS *et al.* 2009, WILKINSON & DAVIES 2009). Although transpiration of both beech and spruce at the study site tended to be reduced under 2×O_3 (METZGER, pers. comm.), this reduction in relation to N uptake was more severe in beech, with its higher water consumption than spruce (see below). Additional measurements of the N concentrations in the xylem sap of sun crown twigs, carried out once in July 2006 showed no difference between the O_3 treatments in both species. Nevertheless, the concentration of amino compounds in the xylem sap, measured over three years (2002-2004), was reduced at some dates in beech and spruce at the same study site (ALEXOU 2007), indicating a reduced supply of nitrogen or N-assimilates.

O_3-induced stress responses, e.g. higher lignin content, thicker cell walls as defence mechanisms, or accumulation of starch and carbohydrates for repair processes (MATYSSEK *et al.* 1992, GÜNTHARDT-GOERG *et al.* 1997, MATYSSEK & SANDERMANN 2003, BRAUN *et al.* 2004) may increase leaf mass and thus indirectly result in reduced leaf N concentrations. This was not observed in the study beech trees, as leaf mass per area (LMA) did not differ between the ozone treatments over the years 2003 through 2006 (data supplied by BLUMENRÖTHER and METZGER, pers. comm.), confirming measurements at the same study site in 2006 (KITAO *et al.* 2009). Rather, reduced sucrose and starch concentrations in the sun crown foliage under 2×O_3 (BLUMENRÖTHER *et al.* 2007) indicated reduced carbon assimilation or high consumption of carbohydrates in detoxification (BLUMENRÖTHER *et al.* 2007). In contrast in spruce, LMA of needles of different age classes (current-year to five-year-old) were significantly increased in the sun crown under 2×O_3, although in the shade crown, LMA was significantly reduced

(data supplied by METZGER, pers. comm.). Changes in epicuticular wax characteristics in the same study trees under 2×O_3 including an increase in fatty acids (PERCY *et al.* 2009) further indicated defence-related processes. This suggests that carbon allocation to needles may have been increased under 2×O_3, particular in the sun crown, although no O_3-induced changes in C investment in a variety of compounds such as lignins was found in the study trees (HÄBERLE *et al.* 2009). Since, repair and defence processes at the leaf level (e.g. production of antioxidants) may also increase enzyme production and turnover (ANDERSEN 2003), defence-related processes could explain the increased demand of new N in the needles.

A stronger response of sun crown foliage to elevated ozone, as found for N concentrations in both species, might be explained by a higher cumulative ozone uptake in the sun compared to the shade crown (BLUMENRÖTHER *et al.* 2007) due to higher stomatal conductance. Stronger reaction of sun crown foliage, particularly in beech, has been reported also from other studies (ALEXOU *et al.* 2007, WINWOOD *et al.* 2007), although various physiological parameters generally showed a similar sensitivity to ozone in sun and shade crown foliage (MATYSSEK *et al.* 2007*b*, NUNN *et al.* 2005*b*, KITAO *et al.* 2009). Taking into account the proportion of shade crown on total foliage of at least 50 %, stronger ozone effects in the sun crown may be mitigated at the whole-tree level.

In summary, these findings in leaves and buds indicate that the transport of newly acquired nitrogen into beech leaves and buds is reduced under elevated ozone as a consequence of reduced stomatal conductance and hence transpiration rates. Whereas in spruce, incorporation of new nitrogen into foliage (and to lower extent into buds) appeared to be increased under 2×O_3, even at a concurrently lowered N_{total} status in needles, which may result in a decrease in N storage in long term.

Stem and woody axes: In young twigs, beech showed no difference in the $N_{labelled}$ and N_{total} concentration between the O_3 treatments, whereas in spruce, similar to foliage, the N_{total} concentration was significantly reduced under 2×O_3 in the wood of sun crown twigs. In the stem, a trend of lowered $N_{labelled}$ concentration in the bark, and conversely, significantly higher N_{total} concentration in $wood_{ozone}$ of beech under 2×O_3, suggested an

influence of O_3 on N translocation processes in beech. Similarly, in seedlings and young trees of loblolly pine, increased N concentrations were reported in stem tissue under elevated O_3 (WRIGHT *et al.* 1991, BAKER *et al.* 1994). These authors suggested that such an increase was associated with O_3-induced premature leaf senescence promoting internal N translocation to the stem (see also leaf litter above), and also with reduced tree growth. This is in line with the findings in the mature beech trees, as they showed a tendency of accelerated leaf senescence (see above) and decreased N_{total} concentration in shade leaf litter. Furthermore, stem growth of the study trees was also affected by $2\times O_3$. Taking into account alteration of stem shape, i.e. diameter-height relationships in the course of the tree growth, PRETZSCH *et al.* (2009) found a significant reduction in stem volume increment of beech by 44 % after 8 years of $2\times O_3$ exposure but no significant change for spruce. For beech, this would explain a higher proportion of N_{total} in the $wood_{ozone}$. Decreased C allocation to stem respiration in beech and, conversely, increased C allocation in spruce at the same study site (RITTER, pers. comm.) further support the observed changes in stem growth. In addition, both, beech and spruce showed an altered stem shape (PRETZSCH *et al.* 2009). Based only on the breast height diameter, radial growth was reduced under $2\times O_3$ in both species (WIPFLER *et al.* 2005, 2009, PRETZSCH *et al.* (2009).

The trend of lowered $N_{labelled}$ concentration in the bark of beech under $2\times O_3$ is consistent with the reduced $N_{labelled}$ concentrations indicated in leaves, buds, and also in the phloem. Therefore, reduced supply of new N to the stem under $2\times O_3$ was indicated for beech. The significantly shortened growing season observed under $2\times O_3$ during four out of eight years of O_3 fumigation (NUNN *et al.* 2002) further supports the assumption that N uptake is reduced in beech. Thus, internal N cycling seem to be affected by elevated O_3.

Species characteristics: At the leaf level, differences between deciduous beech and evergreen spruce were obvious in the concentration of newly acquired N ($=N_{labelled}$) as well as of N_{total}, being on average twice as high each in beech leaves and buds as compared to those of spruce across both ozone treatments. The C/N of about 20 and 40 mg mg^{-1} in beech leaves and buds, respectively, versus about 40 and 100 mg mg^{-1} in spruce needles and buds, respectively, underlines the xeromorphic characteristics of spruce needles. Higher foliar N concentration also indicate higher SLA and

photosynthetic capacity of deciduous trees compared to evergreen coniferous trees (SCHULZE *et al.* 1994, MATYSSEK 1986). High photosynthetic capacity is correlated with high stomatal conductance, leading to a higher water demand and thus transpiration rate in deciduous versus coniferous trees (MATYSSEK 1986). Hence, the transpiration rate was about twice as high in beech compared to spruce (*Fig. 16*, and METZGER, pers. comm.). The increased incorporation of new N per unit of N_{total} in beech leaves and buds compared to spruce (significant in the sun crown under $1\times O_3$), underlines the importance of beech foliage as a N sink, where a major proportion of nitrate assimilation takes place (GEBAUER *&* SCHULZE 1997). Deciduous beech also needs to cover the tree's demand for assimilates at a smaller foliage biomass fraction per tree and within a shorter growing season compared to evergreen spruce. Measurements of phloem and xylem sap in late summer in 2006 further indicated a high cycling rate of nitrogen in beech, as particularly in the phloem, $N_{labelled}$ and N_{total} concentrations were significantly higher in beech than in spruce (cf. *Fig. 15*). Thus, the supply of N assimilates from leaves to other organs is higher in beech as compared to spruce. In the present study, at the end of the growing season, about 70 % of the total leaf N was retranslocated before leaf fall, which is similar to other findings in beech (STAAF 1982, SANTA REGINA *et al.* 1997), although lower retranslocation has also been reported, depending on site conditions or genetic variability (OLSEN 1948 cited in: CHAPIN *&* KEDROWSKI 1983, STAAF *&* STJERNQUIST 1986).

In contrast to deciduous trees, the long-living coniferous foliage plays an important role in plant internal nutrient cycling, serving as important storage from which nutrients such as nitrogen are translocated to newly built tissue (e.g. FIFE *&* NAMBIAR 1984, MILLARD *&* PROE 1992). Current-year needles exhibited significantly higher $N_{labelled}$ concentrations than one-year-old needles although N_{total} concentrations were similar. This confirms the newly built needles to be the stronger sink for new N. Needles built in 2005 – the year of N labelling – increased their $N_{labelled}$ concentration in 2006, when they were one year old, indicating further demand for ongoing growth and contribution to C assimilation (SCHULZE *et al.* 1977).

In contrast to foliage, the wood of sun crown twigs showed lower concentrations of $N_{labelled}$ (as a trend only) and N_{total} in beech than in spruce, and consistently a slightly lower ratio of $N_{labelled}/N_{total}$ ($p = 0.1070$). This suggests that supply of new N to these woody tissues was slightly lower in beech than in spruce. Conversely, in shade crown twigs, stem wood and living bark, $N_{labelled}$ concentration was similar between species,

whereas N_{total} concentration was higher and C/N lower in beech than in spruce. The higher N_{total} concentration in woody tissues of beech most likely reflects the important storage function, particularly of the stem, with a higher proportion of parenchyma tissue compared to spruce/coniferous trees (KIENITZ & KLAUDITZ 1940). Compared to other studies on the same species (ELLENBERG *et al.* 1986, BAUER *et al.* 2000), N_{total} concentration was similar in the woody compartments (including bark), although somewhat higher in twigs, indicating a relatively high N availability at the study site.

2. Nitrogen concentrations in below-ground organs and soil

Roots and mycorrhiza: In both species, concentrations of $N_{labelled}$ and N_{total} in the three root classes did not significantly differ between the O_3 treatments, but there was a tendency of lowered N concentrations under $2\times O_3$ and in some cases increased C/N ratio. Increasing N_{total} concentrations in fine roots with increasing soil N_{total} concentrations (across the soil horizons), as also shown by BURKE & RAYNAL (1994), were assumed to be an indicator for N uptake capacity. This capacity of N uptake tended to be saturated at lower N concentrations in fine roots under $2\times O_3$ compared to $1\times O_3$ in both species (cf. *Fig. 23*). These findings indicated that, despite high variation in $N_{labelled}$ and N_{total} concentrations within soil and roots each, elevated O_3 concentrations may lead to reduced N acquisition.

This pattern of slightly lowered N concentrations in roots confirms previous measurements on beech at the same study site by HABERER *et al.* (2007), who found reduced N_{total} concentration in beech fine roots under $2\times O_3$ over two growing seasons. ALEXOU (2007) reported consistently that total amino compounds were partly reduced in fine roots of both beech and spruce at the same study site, however, the concentration of total soluble proteins was increased at some sampling dates. Based on short-term studies using nutrient solutions, N uptake rates of fine roots were reduced under $2\times O_3$ in the same beech trees in two out of three years (ALEXOU 2007, HABERER *et al.* 2007), whereas no difference in N uptake was observed in spruce (ALEXOU 2007). In contrast, studies on mature northern red oak showed an increase in N concentration in fine and coarse roots in response to elevated ozone (SAMUELSON *et al.* 1996).

In ectomycorrhizal root tips within the A horizon, the concentration of $N_{labelled}$ did not

differ between the ozone treatments, whereas the concentration of N_{total} was significantly reduced in beech under $2\times O_3$. While biomasses were similar the C/N ratio consequently tended to be higher. Such effects probably result from changes in mycorrhizal species composition as well as increased number of ectomycorrhizal root tips as reported at the study site by GREBENC & KRAIGHER (2007) and HABERER *et al.* (2007). Relative to the low N_{total} pool in beech mycorrhizae under $2\times O_3$, new N tended to be increased, suggesting mycorrhizae to be a higher sink for the new N in response to $2\times O_3$.

Reduced N concentrations in roots and mycorrhizae under elevated ozone may have been induced by altered C allocation and biomass production. Standing root biomass per unit ground area (and down to 0.5 m of depth) did not differ between ozone treatments within each root class (diameter < 2 mm, ≥ 2 < 5 mm and > 5 mm) in both species. This was consistent to previous findings on fine root biomass as reported by NIKOLOVA *et al.* (2010). When distinguishing root biomass by soil horizon, a shift in the vertical root distribution was indicated in both species: Under $2\times O_3$, area-related biomass as well as volume-related root density of fine roots (< 2 mm) were increased in the A horizon, particularly in beech, while both parameters were reduced in the B horizon at a depth of 20-30 cm (cf. *Fig.19, 20*). Within the organic layers, fine root density of spruce under $2\times O_3$ was decreased in the upper organic layer (O_i) but increased in the lower organic layer (O_a). This indicated restriction of spruce fine roots under $2\times O_3$ to more favourable growth conditions with high nutrient and water availability. As late summer and autumn prior to root sampling were marked by low precipitation, the reduced root biomass of fine roots (< 2 mm and ≥ 2 < 5 mm) in the upper organic layer perhaps also reflected a higher susceptibility of $2\times O_3$ spruce trees to drought conditions than under $1\times O_3$. However, underneath the $2\times O_3$ spruce trees, thickness of the O_i layer was significantly higher while the O_a layer was less pronounced than underneath the $1\times O_3$ trees. Both layers together resulted in a similar root density under both ozone treatments.

At the same study site, NIKOLOVA *et al.* (2010) found enhanced soil respiration rates in response to $2\times O_3$ underneath beech and spruce as well as increased annual fine root (< 2 mm) production in beech in the upper soil down to 20 cm depth. However, the turn over rate, i.e. the ratio between root growth and mortality, of finest roots (< 1 mm) of beech at the same site, was not altered in response to $2\times O_3$ (MAINIERO *et al.* 2009). According to RITTER (pers. comm.) the C allocation to coarse root respiration of the same trees as in the present study was not different in beech but tended to be increased in spruce during late summer in response to $2\times O_3$, whereas no difference in C allocation

was observed in phloem sugars.
Similarly, KING *et al.* (2001) did not find differences in fine root biomass of young paper birch and trembling aspen trees each in response to ozone, although in each species the proportion of dead-root biomass tended to be increased. However, stimulation of root growth by elevated CO_2 was decreased, when O_3 was enhanced in parallel (KING *et al.* 2001, KARNOSKY *et al.* 2003). In response to long-term (10 yr) ozone exposure, production as well as mortality of fine roots of trembling aspen were increased while root turnover stayed unaffected (PREGITZER *et al.* 2008). In contrast, a decrease in net fine root production and turnover under elevated ozone was reported from mature red oak trees (KELTING *et al.* 1995, SAMUELSON *&* KELLY 2001).

Although C allocation to below-ground organs can be reduced in response to above-ground ozone impact, the C sink strength of roots may be increased as well in order to compensate for reduced nitrogen levels in the shoot (ANDERSEN 2003). Regarding the Kranzberg study site here, below-ground C allocation appeared in total to be slightly enhanced under $2\times O_3$ in both species, although to a different extend depending on organ. While the standing root biomass across the whole rooting zone was not altered (this study), the costs of growth and maintenance appeared to be increased under $2\times O_3$ as indicated by enhanced soil respiration underneath both species (NIKOLOVA *et al.* 2010), increased fine root production and number of mycorrhizal root tips in beech (GREBENC *&* KRAIGHER 2007, HABERER *et al.* 2007, NIKOLOVA *et al.* 2010) as well as slightly enhanced coarse root respiration in spruce (RITTER, pers. comm.). Therefore, it may be suggested that the shift in root distribution under $2\times O_3$, particularly of fine roots, may enhance in the long term the susceptibility of the trees to drought.

Soil: The concentrations of $N_{labelled}$ and N_{total} in the bulk soil tended to be higher under trees exposed to $2\times O_3$ than to $1\times O_3$, although this effect was significant only in the B horizon down to 10 cm depth (B_{-10}). Conversely, the C/N ratio tended to be reduced, which was significant in the O_a layer under spruce.
In contrast, ZAK *et al.* (2007*a*) did not find any O_3-induced change in the N concentration of soil and young trees of trembling aspen and paper birch, whereas reduced plant growth under elevated ozone resulted in reduced N demand and in turn in a lowered N input to the soil via leaf litter. O_3-induced decrease in litter production and

increase in C/N ratio of leaf litter due to altered litter chemistry (LIU *et al.* 2005, 2009) was found to decrease N mineralisation rates, but did not change N immobilization, thus leading to a possible decrease of N input into the soil (HOLMES *et al.* 2006, LIU *et al.* 2007).
At the study site, N availability is not regarded as a limiting factor (STMLF 2007). Therefore, the observed increase of C/N in roots and the partly reduced N concentrations in roots, mycorrhizae and above-ground organs of both species under $2\times O_3$ indicate an impaired capacity of N acquisition under chronically enhanced ozone stress.

Species characteristics: The investigated root diameter classes showed partly lower $N_{labelled}$ and N_{total} concentrations in beech as compared to spruce, being significant for $N_{labelled}$ in medium-sized roots within the A horizon, and for N_{total} in all root diameter classes within the B horizon (cf. *Fig. 21*). Similarly, in bark and wood of coarse roots, $N_{labelled}$ concentration was about three to five times lower and N_{total} concentration about 50 % lower in beech than in spruce (cf. *Fig. 22*). These findings indicate the roots across all size classes as a smaller N pool in beech than in spruce at the end of the growing season (see section *Nitrogen pools and partitioning at the whole-tree level*). The reduced proportion of new N in the N_{total} pool in beech, particularly in medium-sized roots and, in tendency, also in the coarse root bark, further suggest that beech roots represent a smaller sink for N over the year than do spruce roots. The lower $N_{labelled}$ concentration in beech roots may also reflect a low residence time of N in beech roots before being transported to other sink organs, whereas in spruce, roots are the predominant organs of N assimilation (e.g. high nitrate reductase activity, GEBAUER & SCHULZE 1997). However, also the different root structure and morphology can explain the lower N concentration and consequently higher C/N in beech compared to spruce: E.g. a higher lignification of beech fine roots (HELLER, pers. comm.), and increased starch accumulation in roots at the end of the growing season (LARCHER 1994).
In contrast to roots, both $N_{labelled}$ and N_{total} concentrations were enhanced in the mycorrhizal root tips of beech rather than spruce, although being significant only in the case of N_{total}. Compared to the corresponding fine roots, the $N_{labelled}$ concentration of mycorrhizal root tips of beech was 1.5× higher ($p = 0.012$), whereas in spruce, the difference was marginal. However, in both species, N_{total} concentrations were

significantly higher in mycorrhizal root tips than in the corresponding fine roots. This indicated that in autumn (at the time of sampling), mycorrhizae represented an important sink for N in beech and spruce, having a higher N demand in beech. As the biomass ratio of mycorrhizal root tips to fine roots was similar in both species, the higher N concentrations in beech further implies a strong control of - or high dependency on - mycorrhizae in terms of N acquisition (ELLENBERG *et al.* 1986). Spruce, in contrast, is known to take up high amounts of N also via non-mycorrhizal roots (ELTROP *&* MARSCHNER 1996). Studies on mycorrhizal root systems growing in rhizotrons at the Kranzberg Forest also showed the tendency of higher mycorrhization of beech than spruce root tips (WEIGT, unpublished). A higher content of tannins and other secondary compounds, such as phenylpropanoids, in ectomycorrhizae of conifers (WEISS *et al.* 1999) may also result in a lower N concentration in the mycorrhizae of spruce than of beech. In addition, the different mycorrhizal fungi associations in beech and spruce at the study site (GREBENC *&* KRAIGHER 2007, RAIDL pers. comm.) with possibly differential preferences for nutrients may also result in differences in N concentration. The mycorrhization of spruce fine roots within the thick organic layers was not considered in the present study, as mycorrhiza was investigated only in the A horizon, so that in total the mycorrhizal N pool in spruce probably was higher.

Soil $N_{labelled}$ and N_{total} concentrations underneath beech were significantly lower in the upper organic layer and in the A horizon as compared to spruce. This suggests that, under beech, nitrogen might be available for roots more readily than under spruce due to higher mineralization and nitrification (PERSSON *et al.* 2000*b*). Conversely under spruce, high amounts of N accumulate in the upper layers of more slowly degradable organic matter, consisting of thick packages of needles litter. As mineral N uptake and total N pool were not greater in beech than in spruce, the lower N concentrations in the soil under beech probably do not reflect a higher N consumption by the trees compared to spruce. Rather, higher leaching under beech was indicated (but see section *Stand level*).

3. Nitrogen pools and partitioning at the whole-tree level

N pools, recovery and partitioning of new N: At the whole-tree level, recovery of $N_{labelled}$ (=new N) tended to be lower under $2\times O_3$ than under $1\times O_3$ in both beech and spruce, with more $N_{labelled}$ remaining in the soil under $2\times O_3$ (cf. *Fig. 33*). The uptake of new N per unit of whole-tree biomass indicated a (non-significant) reduction under $2\times O_3$ by about 15 % in beech and 17 % in spruce (*Tab. 11*). Root mass (<5 mm) -related uptake of new N in beech tended towards a reduction of 46 % under $2\times O_3$, but no reduction was found in spruce (*Tab. 11*), as root mass was decreased in parallel (cf. *Fig. 24).* However, relative to the N_{total} pool, the recovery of $N_{labelled}$ in spruce was increased by about 42 % under $2\times O_3$ although not being significant, suggesting a tendency of increased N demand under $2\times O_3$. Thus, at the whole-tree level, N uptake and/or demand seemed to be impaired in beech in response to high ozone impact. Conversely, in spruce, the slightly reduced N uptake may be related to the smaller root system, while the N demand may be even higher under elevated ozone.

The higher recovery of $N_{labelled}$ as a whole in the soil (down to 90 cm) under $2\times O_3$ compared to $1\times O_3$, being consistent with the higher $N_{labelled}$ concentration observed in some horizons (see section *Nitrogen concentrations in below-ground organs and soil*), further indicated reduced N uptake under $2\times O_3$ in both species.

The recovery of $N_{labelled}$ was highest in the target trees (23 % per tree) as compared to other surrounding trees (about 14 % in four to five neighbouring trees), showing the ^{15}N-labelled plots to be dominated by the target trees. About 28 % of the label was not recovered in the analysed stand compartments. As roots were investigated only within the plots, the distribution of $N_{labelled}$ within the entire root system was not considered. In other field studies with young or mature trees, recovery of labelled N - applied directly to the soil or as wet deposition - ranged between 56 and 100 % for the total system and between 2 and 57 % in trees (e.g. FEIGENBAUM *et al.* 1987, BUCHMANN *et al.* 1996, WEINBAUM *&* VAN KESSEL 1998, SCHLEPPI *et al.* 1999, GEBAUER *et al.* 2000, NADELHOFFER *et al.* 2004).

Decreased N uptake under elevated ozone was also reported from young beech trees, as a result of reduced plant biomass after two years of $2\times O_3$ fumigation, whereas spruce was less affected in its N uptake under elevated ozone (LUEDEMANN *et al.* 2005). However, increased whole-tree N_{total} concentration, as observed in young beech and

spruce trees under elevated ozone, indicated a higher demand of N presumably for stress defence (LUEDEMANN *et al.* 2005). Reduced amount of N under elevated ozone was also observed in other tree species as a result of reductions in photosynthesis and plant growth (ZAK *et al.* 2007*a*). On the other hand, N acquisition under elevated O_3 also depends on the sensitivity of the genotype, and may therefore be decreased, increased or not altered (ZAK *et al.* 2007*b*). BIELENBERG *et al.* (2002) found no effect of ozone on N uptake in O_3-sensitive poplar hybrid (*Populus trichocarpa*×*maximowizii*).

The partitioning of new N also differed between the ozone treatments: In beech, a higher proportion of the new N remained in roots under $2\times O_3$ compared to $1\times O_3$ (*Tab. 12*), being significant for mycorrhizal root tips and a trend in fine roots (*Tab. 10*), whereas in spruce, allocation of new N to roots was decreased under $2\times O_3$ (*Tab. 12*), significantly in medium-sized roots (*Tab. 10*).
As the underlying growth model did not include an algorithm for O_3 impact, possible changes in biomass under chronically enhanced ozone were not taken into account apart from the first three years of ozone treatment (biomasses were modelled as based on measurements of breast height diameter, tree height and crown size after three years of ozone fumigation, in 2002). However, tree height and breast height diameter in 2006 as well as increment of stem $wood_{ozone}$ did not differ between ozone treatments for the studied trees of both species. In spruce, the tree internal partitioning of biomass was significantly different between the ozone treatments: Above-ground, the proportion of shoot axes biomass was reduced and that of stem biomass was increased under $2\times O_3$, whereas below-ground, total root biomass was reduced. Based on the modelling approach, this led to a significantly decreased root-shoot ratio of spruce under $2\times O_3$ as compared to $1\times O_3$ ($p = 0.0466$). Although reduced stem growth under $2\times O_3$ was reported from both species, particularly from beech at the study site, as discussed previously (see section *Nitrogen concentrations in above-ground organs*), the reduction was small compared to the total standing stem biomass and the remaining uncertainties of modelling.
Similar to the findings in beech, SAMUELSON *et al.* (1996) suggested an influence of ozone on translocation processes as based on their findings of reduced foliage N concentration and increased root N concentration in mature northern red oak trees under ozone at the end of the growing season. Conversely, a change in partitioning of newly acquired nitrogen in response to ozone towards increased allocation to leaves and roots,

was reported from poplar trees in relation to O_3-induced accelerated leaf senescence (BIELENBERG *et al.* 2002). When taking into account the increased leaf shedding, a slightly increased N allocation to foliage under the influence of ozone was also observed in young birch trees (MAURER & MATYSSEK 1997).

In summary, the indicated trend of reduced uptake of new N in beech trees under $2\times O_3$ as compared to $1\times O_3$ is in consistency with the reduced $N_{labelled}$ concentration found in leaves, buds and, as a trend in stem bark. As foliage $N_{labelled}$ concentration was correlated with reduced transpiration under $2\times O_3$, an impaired coverage of leaf N demand is suggested. At the same time, a higher proportion of the newly acquired N was allocated to, or remained in the roots, although such an effect was not observed in the N concentrations (both $N_{labelled}$ and N_{total}). The latter rather tended to be reduced under $2\times O_3$, significantly in mycorrhizal root tips, while the C/N was increased (see above), suggesting a slight increase in below-ground C allocation.
In spruce trees, the slightly reduced uptake of new N under $2\times O_3$ was not reflected in the $N_{labelled}$ concentration at the organ level. The N uptake may be related to the lowered root/shoot biomass ratio, as uptake per root biomass was not different between the ozone treatments. However, it remains open, whether the altered root/shoot ratio is due to elevated ozone. The slightly increased uptake of new N relative to the N_{total} pool under $2\times O_3$ rather indicated an increased N demand. This view is supported by the increased allocation of newly acquired N into the shoot of spruce, particularly pronounced by the increased proportion of new N in N_{total} of needles, while roots received less new N under $2\times O_3$. It is therefore suggested, that the demand of N at the whole-tree level can not be fully covered under $2\times O_3$ or may be even increased, e.g. under a stronger need for defence and repair mechanisms.

Table 11: Uptake of newly acquired N (=$N_{labelled}$) at the whole-tree level under 2×O_3 relative to the uptake under 1×O_3. Differences between ozone treatments are not statistically significant (black arrows indicate consistency to N concentrations at p < 0.2, grey arrows indicate no significance).

	Beech [% of 1×O_3]	*Spruce* [% of 1×O_3]
Uptake of new N per kg tree biomass	-15 ↓	-17 ↓
Uptake of new N per kg roots (<5 mm)	-46 ↓	+3
Uptake of new N per kg N_{total}	-12 ↓	+42 ↑

Table 12: Partitioning of newly acquired N (=$N_{labelled}$) at the whole-tree level under 2×O_3 relative to the partitioning under 1×O_3. Differences between ozone treatments are not statistically significant.

Partitioning of new N	*Beech* [% of 1×O_3]	*Spruce* [% of 1×O_3]
Shoot	-11 ↓	+13 ↑
Root	+40 ↑	-32 ↓

<u>*Species characteristics:*</u> At the whole-tree level, the lower tree N pool as well as the lower tree N concentration in beech than in spruce (cf. *Fig. 25, 26*) suggest either a lower demand or lower capacity in uptake of beech compared to spruce. This higher N pool in spruce was mainly dominated by the high N status of foliage and woody axes (cf. *Fig. 25, 28*). In contrast, when scaled up to the stand level and taking into account the different tree sizes, the tree N pools were similar in both species. N uptake as

mineral N form (NH_4NO_3), indicated by $N_{labelled}$ recovery, was similar between both species (cf. *Fig. 33*), being comparable to other findings at different sites by WALLENDA *et al.* (2000). At similar root density and biomass in both species (per unit ground area or soil volume), N uptake per unit of occupied space is suggested to be similar also. However, uptake calculated from yearly growth increment tended to be higher in beech than in spruce (cf. *Fig. 37*). Studies on single rootlets at the same study site indicated a higher capacity in mineral N uptake by beech compared to spruce, mediated by higher specific root length (WEIGT *et al.* unpublished). At a whole-tree basis, the different root distribution across the soil profile has also to be taken into account for N uptake, with beech displaying deeper rooting compared to spruce, particularly in mixed stands (this study; SCHMID 2002, SCHMID *&* KAZDA 2002, BOLTE *&* VILLANUEVA 2006).

The discrepancy in tree N pool and N uptake may result from a high contribution of organic N forms as well as above-ground N uptake via crown interception in spruce that may contribute up to > 30 % of the annual tree N demand (BRUCKNER *et al.* 1993 cited in: GEBAUER *&* SCHULZE 1997, HARRISON *et al.* 2000, IGNATOVA *&* DAMBRINE 2000).

The tree internal N partitioning between tree organs, following partly the differences in biomass partitioning between beech and spruce, revealed differences in N sink and storage organs between both species: Buds and stem tissues represented a greater N pool in beech, whereas roots served as a minor N pool in beech as compared to spruce (see above). In contrast, spruce roots represent an important N pool (apart from the foliage). Foliage comprised up to about 9 % of total tree N in beech (during summer) compared to about 19 % in spruce. The root-shoot biomass ratio at the end of the growing season of 0.11 (beech) and 0.15 (spruce) versus the root-shoot ratio in terms of N_{total} pool of 0.3 (beech) and 0.6 (spruce) further emphasize the importance of the above-ground N pools of beech and the below-ground N pools in spruce. In buds of beech, the N pool provided about 50 % of the total leaf N, showing that foliage N supply depends to a large extent on the N uptake and bud formation during the previous year (MILLARD 1996). The remaining half of the yearly foliage N demand was thus remobilized from storage tissues. According to SCHULZE *et al.* (2002), about 60 % of leaf N is derived from stem storage in beech.

The partitioning of newly acquired N was less different between the two species as was the partitioning of the N_{total} pool. The most striking difference was found in buds, with beech buds receiving about 11 % of the new N, whereas spruce buds received only 1 %. New N in other above-ground organs was similarly distributed in both species in late

summer and winter (cf. *Fig. 35*). Similarly to N concentrations, the partitioning (and total recovery) of new N in above-ground woody tissues did not differ between the species, although the proportion of the stem in the whole-tree biomass and whole-tree N_{total} pool was higher in beech than in spruce. This may indicate that the stem as an important N pool, although much larger in beech than in spruce, is not supplied by newly acquired N to a higher extent in beech as one might expect. Therefore, the proportion of new N in the foliage of beech should be allocated to other N sinks after leaf shedding, e.g. below-ground organs.

Below-ground, less new N was allocated to roots in beech than in spruce, being most pronounced in medium-sized roots (2 % in beech versus 6 % in spruce). This pattern confirmed that the buds and roots had different importance as N sinks in beech and in spruce. Other organs, mainly stem and coarse roots, receiving similar amounts of new N in beech and spruce, therefore most likely represent long-term N storage pools with a larger proportion of old nitrogen. The present study did not allow to quantify the extent to which the storage of needles, branches, roots and stem may contribute to the N supply to new tissue. In mature trees, remobilisation of this plant-internal N can account for 50 to 80 % of N in new tissue (MILLER 1984).

4. Species-specific ozone sensitivity in relation to nitrogen status

The study showed that beech may be affected more strongly in N uptake under $2\times O_3$ as compared to spruce, possibly through pronounced impacts on stomatal conductance and thus, C assimilation (KITAO *et al.* 2009). Despite reduced stomatal conductance under $2\times O_3$ in spruce (NUNN *et al.* 2006, see section *Nitrogen concentrations in above-ground organs*), the latter species showed a higher investment of N and probably also of C in foliage as compared to beech, possibly as defence strategy. Such C investment is more important for long-living spruce needles than for deciduous beech leaves, although no O_3-induced changes in C investment in a variety of compounds such as lignins was found in the study trees (HÄBERLE *et al.* 2009). In addition to the physiological differences at the leaf level between both species, beech may have to cope with ozone stress (e.g. impairment of stomatal conductance and photosynthesis) almost during the whole leaf lifespan, whereas spruce needles may compensate negative effects during the

winter months, when O_3 concentrations are low in the temperate zone. Therefore, O_3-induced reactions that affect the foliage N pool seem to be more severe in deciduous beech as compared to coniferous spruce and thus may have greater importance for internal N cycling in beech.

Regarding below-ground compartments, both species showed a similar tendency of reduced N concentrations under $2\times O_3$, although in mycorrhizal root tips, the N_{total} concentration was reduced only in beech (see section *Nitrogen concentrations in below-ground organs and soil*). However, the allocation of new N to the N pool of roots and mycorrhizae was reduced under $2\times O_3$ in spruce, whereas it was increased in beech (see section *Nitrogen pools and partitioning at the whole-tree level*). In response to ozone stress, reduced N concentrations in roots and mycorrhizae in beech but simultaneously increased allocation of new N to these organs suggest an increased C investment to below-ground (see above). It is known from other studies that beech responds to stress, such as drought or ozone, by increasing fine root production and root tip density (Leuschner *et al.* 2001, 2004, Grebenc & Kraigher 2007, Nikolova *et al.* 2009). These stress-induced responses at the root level may be advantageous to ensure nutrient and water access on the long term, also in view of the higher soil water consumption of beech compared to spruce.

If ozone stress in beech leads to an increased consumption of the tree N storage to maintain high leaf N concentration, while N resorption from senescing leaves is not clearly changed (see section *Nitrogen concentrations in above-ground organs*), then litter N might in the long term represent a more substantial loss than in the absence of ozone stress.

In contrast, stress-induced responses in spruce included a higher investment of new N and C in foliage (see above) while below-ground organs did not benefit. However, increased C allocation to coarse root respiration and increased soil respiration under ozone stress indicated that maintenance costs of below-ground compartments were high in spruce. The increased allocation of new N - relative to the N_{total} pool - to needles (see section *Nitrogen concentrations in above-ground organs*) suggests that N loss via needle litter may be enhanced under $2\times O_3$. Thus, spruce seems to rely more on its above-ground defence strategies compared to beech, which may be advantageous when water and nutrient availability (either above-ground or via the soil) is not limiting.

5. Stand level

The site of the present study is characterized by a high N availability, indicated by the foliage and whole-tree N_{total} concentrations that were within a range comparable to other forest stands in Central Europe (e.g. BAUER *et al.* 1997, 2000, MELLERT *et al.* 2004). As N deposition is moderate (*Fig. 36, 37*, STMLF 2007), the high N status of the trees is predominantly due to high mineralisation (*Fig. 36, 37*). Similar N depositions are found throughout Central Europe (PERSSON *et al.* 2000*a*) and similar mineralisation has been reported from other sites with comparable soil types (PERSSON *et al.* 2000*b*). The high soil N availability lead to moderate to high N leaching. In a neighbouring beech/oak stand (~1 km distance), N leaching was lower than at the study site (SCHULZ, pers. comm.). However, the sites may not necessarily be comparable, as the study site was dominated by spruce which has a higher N interception as compared to deciduous beech and oak (NIHLGARD 1970, KREUZER *et al.* 1986). In addition, N input may have been underestimated, as the study site is located near the forest edge with surrounding agricultural land, whereas the neighbouring forest ecosystem monitoring station was located in the centre of the forest (compare SPANGENBERG *&* KÖLLING 2004). Moreover, high variability of soil N dynamics within a stand as well as different approaches to assess the N loss (area-based method in this study versus concentration-based at the neighbouring site) have to be taken into account. Overall, the high N availability and high water capacity (cf. *Tab. 4*) promotes stand growth at the study site (PRETZSCH *et al.* 1998).

Under elevated ozone, the indicated trend of reduced N uptake in both species and, conversely, higher N concentration in the soil, indicated increased soil N availability. Particularly in beech, where reduced stem volume increment was reported under $2\times O_3$ at the study site (PRETZSCH *et al.* 2009, see above), the high soil N availability apparently was not used for tree growth. Under spruce, the observed trend in reduced N uptake resulted in a significantly greater N pool of the soil microbial biomass in response to elevated ozone. As reduced N uptake and tree growth under elevated O_3 was also observed in young trees, particularly in beech (KOZOVITS *et al.* 2005*b*, LUEDEMANN *et al.* 2005, and see above), less N would be accumulated in the plant biomass of growing stands, which increases soil N with increasing stand age. Young spruce trees, however,

are less affected in N uptake under elevated ozone, and profit from growing in mixed culture with beech (KOZOVITS *et al.* 2005*b*, LUEDEMANN *et al.* 2005).

The role of mature trees in the N cycle of stands does not differ greatly between deciduous and evergreen species regarding N demand or N uptake, and is mainly influenced by soil N availability and tree growth (MILLER 1984). However, the plants themselves influence soil properties and nutrient availability e.g. via litter (e.g. VITOUSEK 1982, MCCLAUGHERTY *et al.* 1985). Litter chemistry influences soil N availability and N cycling e.g. through a changed C/N ratio. In the present study, it remains open how litter quality may be altered in the long term in response to elevated ozone. Lowered C/N in the litter of sun foliage but increased C/N in the shade foliage of beech (see above), resulted in a slight (not significant) reduction in N loss at the whole-tree level, although resorption of N was also slightly reduced. Spruce litter was not investigated here. However, the increased allocation of newly acquired N in needles may suggest an increased loss of N via litter under $2\times O_3$. However, litter biomass has also to be taken into account, and may be reduced in the long term under elevated O_3, along with reduced plant growth.

Reduced input and altered biochemistry of leaf litter under elevated ozone may lead to a change in fungal community composition and fungal metabolism, which may in turn affect carbon and nutrient cycling (CHUNG *et al.* 2006). Changes in soil microbial community as well as decreased soil enzyme activities were reported from young beech trees exposed to elevated ozone, possibly reflecting increases in stress-induced compounds transferred from the plants (PRITSCH *et al.* 2009). Altered C allocation to below-ground compartments was also indicated by increased microbial biomass under young beech trees in response to ozone (ESPERSCHÜTZ *et al.* 2009), whereas no such effect was observed under mature trees at the study site.

6. Possible ozone effects under different site conditions

Different climatic and edaphic conditions are likely to influence the above outlined effects of elevated ozone on the long term. Possible scenarios are displayed in *Fig. 38* in comparison to the findings of the present study (shown as first arrow within rows).

Elevated ground-level O_3 concentrations are often associated with increased N

deposition, e.g. through emission of NO_x as a main precursor for O_3 formation. Therefore, ozone impacts on forests mostly may occur at sites of relatively high N availability or even N saturation. The effect of high N deposition on forest stands in terms of growth, productivity and susceptibility to further stress such as elevated O_3, thus has to be taken into account (BRAUN *et al.* 1999). For instance, decline in root biomass (e.g. ABER *et al.* 1989, MATZNER *&* MURACH 1995, NADELHOFFER 2000) as well as mycorrhizal species diversity (WALLENDA *&* KOTTKE 1998, TAYLOR *et al.* 2000) under high N input/soil fertility may weaken trees when facing additional stress (ERISMAN *et al.* 1998, ERISMAN *&* DE VRIES, 2000). Root decline in combination with reduced capacity of N uptake under elevated O_3 may lower the root:shoot ratio, with presumably negative effects on physical tree stability and resource uptake. The reduced root:shoot ratio of spruce under $2\times O_3$ in the present study indicated such effects, although at the given site, the altered root:shoot ratio probably is not due to excessive N input.

At nutrient poor sites with low N availability, tree responses to elevated O_3 may cause more severe N shortage and thus a stronger impact on growth as compared to sites of high N availability (*Fig. 38:* second arrow within rows). Low water availability, at shallow soil depths or under drought conditions, may additionally enhance the impacts of elevated O_3 on N uptake (*Fig. 38:* third arrow within rows), e.g. through decreased fine root production in spruce (NIKOLOVA *et al.* 2009, 2010). Due to its potential for deep rooting, particularly in mixed stands (see above), its high plasticity in root growth (e.g. HARLEY 1940, CURT *&* PRÉVOSTO 2003), as well as with better water capturing via stem flow along the smooth bark (e.g. REYNOLDS *&* HENDERSON 1967, BENECKE 1984,), beech can better cope with water shortage compared to spruce. However, beech is also sensitive to long-term drought (ELLENBERG 1996, LEUSCHNER *et al.* 2001, PEUKE *et al.* 2002) which may result in reduced fine root growth (LEUSCHNER *et* HERTEL 2003, LEUSCHNER *et al.* 2004) and reduced nutrient uptake (FOTELLI *et al.* 2002, GESSLER *et al.* 2004*a*). Consequently, tree growth decreases under these constraints, and the capacity for stress defence may be lowered in both species (WOLFENDEN *&* MANSFIELD 1991). The shift in root distribution under elevated ozone, as observed in the present study, particularly in fine roots, may promote drought susceptibility on the long term.

In addition to elevated O_3 and N input, increasing atmospheric CO_2 may partially compensate for negative ozone impact on trees (ANDERSEN 2003, KING *et al.* 2005; *Fig. 38:* forth arrow within rows). Although CO_2 may be regarded as a non-limiting factor for trees especially under increasing atmospheric CO_2 (KÖRNER 2003, MILLARD *et al.*

2007), growth increment under simultaneously elevated CO_2 and O_3 is likely to be reduced or remains unaffected in trees (McDonald *et al.* 2002, Karnosky *et al.* 2003, 2005, Kozovits *et al.* 2005*b*).

Under such scenarios, N surplus of ecosystems that can not be used for plant growth, may alter the soil N status and, in the long term, increase N leaching.

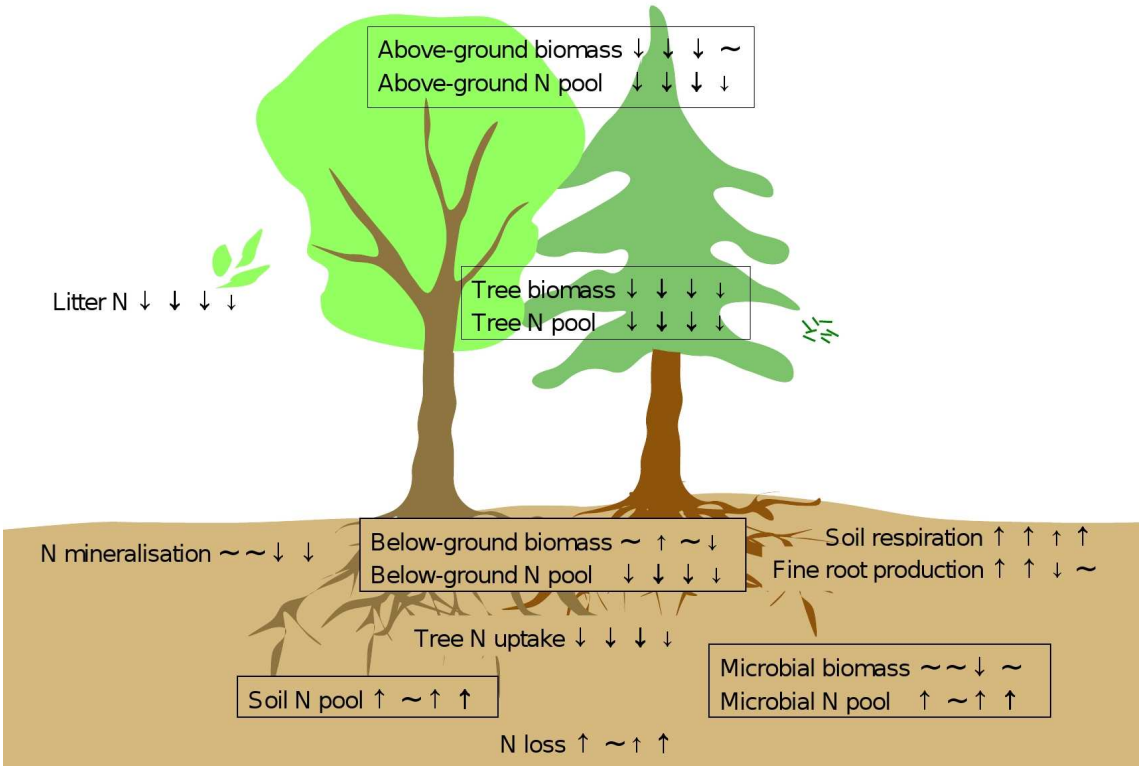

Figure 38: Possible long-term effects of chronic O_3 exposure on N cycling and related pools and processes, in a mixed stand of beech and spruce, and under four different site conditions. Boxes indicate pools; arrows indicate increase (upward) or decrease (downward) of a pool or process, tilde indicates no change; pools are marked by boxes. Arrow size displays qualitative effect strength of different site conditions in comparison to the present study site. Site conditions are: study site conditions, as described in the text (first arrow in line), nutrient poor sites (second arrow), sites of low water availability/drought conditions (third arrow), elevated CO_2 and N input (forth arrow).

Conclusions

The present study revealed effects of elevated O_3 concentrations on the N status in mature beech and spruce trees, as there are:

- reduced N acquisition (both beech and spruce) along with a slight increase in N demand (particularly in spruce), leading to the assumption that consumption of N storage pools may be enhanced, which may weaken the nutrient equilibrium in the long term; in spruce, the lower N uptake appeared to be related to the lower root mass under $2\times O_3$
- stronger reduction in N uptake in beech compared to spruce, in relation to reduced stomatal conductance and transpiration, confirming the findings of reduced stem growth (Pretzsch *et al.* 2009)
- change in resource allocation in opposite directions in beech and spruce: Increased allocation of new N into roots and mycorrhizae in beech as a possible compensation for tree growth reduction; conversely, increased allocation of new N to above-ground organs, particularly foliage, in spruce, indicating stress-induced promotion of above-ground growth and defence at the expense of lower investment in below-ground organs
- increased soil N availability with N not being used for tree growth, leading to possibly enhanced N loss from the ecosystem on the long term, e.g. via leaching, particularly at moderate to high N deposition.

References

Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. BioScience, 39 (6): 378-386.

AG Bodenkunde (1982) Bodenkundliche Kartieranleitung. 3rd edition, Schweizerbart'sche Verlagsbuchhandlung, Stuttgart.

Alexou M (2007) Impact of free-air ozone fumigation on the seasonal allocation of C, N, S, in adult beech and spruce in a mixed stand. PhD Thesis, 294 p.

Alexou M, Hofer N, Liu X, Rennenberg H, Haberer K (2007) Significance of ozone exposure for inter-annual differences in primary metabolites of old-growth beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* L.) trees in a mixed forest stand. Plant Biology, 9: 227-241.

Andersen CP, Hogsett WE, Plocher M, Rodecap K, Lee EH (2001) Blue wild-rye grass competition increases the effect of ozone on ponderosa pine seedlings. Tree Physiology, 21 (5): 319-327.

Andersen CP (2003) Source-sink balance and carbon allocation below ground in plants exposed to ozone. New Phytologist, 157: 213-228.

Ashmore MR (2005) Assessing the future global impacts of ozone on vegetation. Plant, Cell and Environment, 28: 949-964.

Baker TR, Allen HL, Schoeneberger MM, Kress LW (1994) Nutritional response of loblolly pine exposed to ozone and simulated acid rain. Canadian Journal of Forest Research, 24: 453–461.

Bauer GA, Schulze ED, Mund M (1997) Nutrient contents and concentrations in relation to growth of *Picea abies* and *Fagus sylvatica* along a European transect. Tree Physiology, 17: 777—786.

Bauer GA, Persson H, Persson T, Mund M, Hein M, Kummetz E, Matteucci G, van Oene H, Scarascia-Mugnozza G, Schulze ED (2000) Linking plant nutrition and ecosystem processes. *In:* Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Springer, Heidelberg, pp. 63–98.

Benecke P (1984) Der Wasserumsatz eines Buchen- und eines Fichtenwaldökosystems im Hochsolling. Schriften aus der Forstlichen Fakultät der Universität Göttingen und der Niedersächsischen Forstlichen Versuchsanstalt, 77, 158 p.

Bielenberg DG, Lynch JP, Pell EJ (2002) Nitrogen dynamics during O_3-induced accelerated senescence in hybrid poplar. Plant, Cell and Environment, 25: 501-512.

Bischoff WA, Kaupenjohann M (1998) Passivsammler als neue Methode zur in situ Bestimmung von Pestizidversickerung. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz, Sonderheft XVI: 779-786.

Bischoff WA (2007) Entwicklung und Anwendung der Selbst-Integrierenden Akkumulatoren: Eine Methode zur Erfassung der Sickerfrachten umweltrelevanter Stoffe. PhD Thesis, TU Berlin, 172 p.

Blumenröther MC, Löw M, Matyssek R, Oßwald W (2007) Flux-based response of sucrose and starch in leaves of adult beech trees (*Fagus sylvatica* L.) under chronic free-air O_3 fumigation. Plant Biology, 9: 207-214.

Bolte A, Villanueva I (2006) Interspecific competition impacts on the morphology and distribution of fine roots in European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* (L.) Karst.). European Journal of Forest Research, 125 (1): 15-26.

Braun S, Rihm B, Schindler C, Flückiger W (1999) Growth of mature beech in relation to ozone and nitrogen deposition: an epidemiological approach. Water, Air, and Soil Pollution 116: 357-364.

Braun S, Zugmaier U, Thomas V, Flückiger W (2004) Carbohydrate concentrations in different plant parts of young beech and spruce along a gradient of ozone pollution. Atmospheric Environment, 38 (15): 2399-2407.

Braun S, Schindler C, Rihm B, Flückiger W (2007) Shoot growth of mature *Fagus sylvatica* and *Picea abies* in relation to ozone. Environmental Pollution 146: 624-628.

Buchmann N, Gebauer G, Schulze ED (1996) Partitioning of ^{15}N-labeled ammonium and nitrate among soil, litter, below- and above-ground biomass of trees and understorey in a 15-year-old *Picea abies* plantation. Biogeochemistry, 33:1-23.

Burke MK, Raynal DJ (1994) Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. Plant and Soil, 162: 135-146.

Calatayud V, Cerveró J, Sanz MJ (2007) Foliar, physiologial and growth responses of four maple species exposed to ozone. Water, Air, & Soil Pollution, 185: 239-254.

Çepel N (1967) Interzeption (= Niederschlagsverdunstung im Kronenraum) in einem Buchen-, einem Eichen- und einem Kiefernbestand des Belgrader Waldes bei Istanbul. Forstwissenschaftliches Centralblatt, 86 (5): 301-314.

Cermak J (1998) Leaf distribution in large trees and stands of the floodplain forest in southern Moravia. Tree Physiology, 18: 727-737.

Chapin FS III, Kedrowski RA (1983) Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous Taiga trees. Ecology, 64 (2): 376-391.

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytologist, 169: 27-34.

Chappelka AH, Samuelson LJ (1998) Ambient ozone effects on forest trees of the eastern United States: a review. New Phytologist, 139: 91-108.

Chung H, Zak DR, Lilleskov EA (2006) Fungal community composition and metabolism under elevated CO_2 and O_3. Oecologia, 147: 143–154.

Coleman MD, Dickson RE, Isebrands JG, Karnosky DF (1996) Root growth and physiology of potted and field-grown trembling aspen exposed to tropospheric ozone. Tree Physiology, 16: 145-152.

Curt T, Prévosto B (2003) Rooting strategy of naturally regenerated beech in Silver birch and Scots pine woodlands. Plant and Soil, 255 (1): 265-279.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. Annual Review of Ecology and Systematics, 33: 507-559.

Dietz P (1975) Dichte und Rindengehalt von Industrieholz. Holz als Roh- und Werkstoff, 33: 135-141.

Edwards NT (1991) Root and soil respiration responses to ozone in *Pinus taeda* L. seedlings. New Phytologist, 118: 315-321.

Edwards GS, Kelly JM, Mays PA (1992) Ozone, acidic precipitation, and soil Mg impacts on soil and loblolly pine seedling nutrient status after three growing seasons. Water, Air, and Soil Pollution, 63: 281-294.

Ellenberg H, Mayer R, Schauermann J (eds.) (1986) Ökosystemforschung – Ergebnisse des Sollingprojekts. Verlag Eugen Ulmer, Stuttgart, 507 p.

Ellenberg H (1996) Vegetation Mitteleuropas mit den Alpen. Verlag Eugen Ulmer, Stuttgart, 5th ed., 1095 p.

Eltrop L, Marschner H (1996) Growth and mineral nutrition of non-mycorrhizal and mycorrhizal Norway spruce (*Picea abies*) seedlings grown in semi-hydroponic sand culture. I. Growth and mineral nutrient uptake in plants supplied with different forms of nitrogen. New Phytologist, 133: 469-478.

Emberson LD, Ashmore MR, Murray F, Kuylenstierna JCI, Percy KE, Izuta T, Zheng Y, Shimizu H, Sheu BH, Liu CP, Agrawal M, Wahid A, Abdel-Latif NM, van Tienhoven M, de Bauer LI, Domingos M (2001) Impacts of air pollutants on vegetation in developing countries. Water, Air, and Soil Pollution, 130: 107-118.

Erisman JW, Draaijers GPJ, Steingröver E, van Dijk H, Boxman A, de Vries W (1998) Assessment of the exposure and loads of acidifying and eutrophying pollutants and ozone, as well as their harmful influence on the vitality of the trees and the Speulder forest ecosystem as a whole. Water, Air, & Soil Pollution, 105: 539-571.

Erisman JW, Vries, de W (2000) Nitrogen deposition and effects on European forests. Environmental Reviews, 8: 65-93.

Esperschütz J, Gattinger A, Mäder P, Schloter M, Fließbach A (2007) Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiology Ecology, 61: 26-37.

Esperschütz J, Pritsch K, Gattinger A, Welzl G, Haesler F, Buegger F, Winkler JB, Munch JC, Schloter M (2009) Influence of chronic ozone stress on carbon translocation pattern into rhizosphere microbial communities of beech trees (*Fagus sylvatica* L.) during a growing season. Plant and Soil, 323: 85-95.

Falkengren-Grerup U (1998) Effect of stemflow on beech forest soils and vegetation in southern Sweden. Journal of Applied Ecology, 26 (1): 341-352.

FAO (2006) Guidelines for soil description. Food and Agriculture Organization of the United Nations, Rome, 4th ed., 97 p.

Farnady DJ (2008) Einfluss von Ozon und Licht auf die Astarchitektur von Buche (*Fagus sylvatica*) und Fichte (*Picea abies*). Diploma thesis, 101 p.

Field CH, Mooney HA (1986) The photosynthesis-nitrogen relationship in wild plants. *In:* Givnish TJ (ed.) On the Economy of Plant Form and Function. Cambridge University Press, New York, pp. 25-55.

Fife DN, Nambiar EKS (1984) Movement of nutrients in radiata pine needles in relation to the growth of shoots. Annals of Botany, 54: 303-314.

Findlay S, Jones CG (1990) Exposure of cottonwood plants to ozone alters subsequent leaf decomposition. Oecologia, 82 (2): 248-250.

Feigenbaum S, Bielorai H, Erner Y, Dasberg S (1987) The fate of ^{15}N labeled nitrogen applied to mature citrus trees. Plant and Soil, 97: 179-187.

Felzer BS, Cronin T, Reilly JM, Melillo JM, Wang X (2007) Impacts of ozone on trees and crops. Comptes Rendus Geosciences, 339: 784-798.

Fenn ME, Dunn PH (1989) Litter Decomposition across an air-pollution gradient in the San Bernardino Mountains. Soil Science Society of America Journal, 53 (5): 1560-1567.

Fenn ME (1991) Increased site fertility and litter decomposition rate in high-pollution sites in the San Bernardino Mountains. Forest Science, 37 (4): 1163-1181.

Fotelli MN, Rennenberg H, Geßler A (2002) Effects of drought on the competitive interference of an early successional species (*Rubus fruticosus*) on *Fagus sylvatica* L. seedlings: ^{15}N uptake and partitioning, responses of amino acids and other N compounds. Plant Biology, 4 (3): 311-320.

Fowler D, Cape NJ, Coyle M, Flechard C, Kuylenstierna J, Hicks K, Derwent D, Johnson C, Stevenson D (1999) The global exposure of forests to air pollutants. Water, Air, and Soil Pollution, 116: 5-32.

Galsterer E (1979) Der Weiler Oberberghausen vor und nach dem Ankauf durch das "Königlich Bayerische Forstaerar". Diploma Thesis.

Gebauer G, Schulze ED (1997) Nitrate Nutrition of Central European Forest Trees. *In:* Rennenberg, H, Eschrich W, Ziegler H (eds.) Trees - contributions to modern tree physiology. Backhuys Publishers, Leiden, pp. 273-291

Gebauer G, Zeller B, Schmidt G, May C, Buchmann N, Colin-Belgrand M, Dambrine E, Marin F, Schulze ED, Bottner P (2000) The fate of ^{15}N-labelled nitrogen inputs to coniferous and broadleaf forests. *In*: Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies 142, Springer Verlag, Berlin, Heidelberg, pp. 144-170.

Geßler A, Schneider S, Weber P, Hanemann U, Rennenberg H (1998) Soluble N compounds in trees exposed to high loads of N: a comparison between the roots of Norway spruce (*Picea abies*) and beech (*Fagus sylvatica*) trees grown under field conditions. New Phytologist, 138: 385-399.

Geßler A, Keitel C, Nahm N, Rennenberg H (2004*a*) Water shortage affects the water and nitrogen balance in Central European beech forests. Plant Biology, 6: 289-298.

Geßler A, Kopriva S, Rennenberg H (2004*b*) Regulation of nitrate uptake at the whole-tree level: interaction between nitrogen compounds, cytokinins and carbon metabolism. Tree Physiology, 24: 1313-1321.

Gielen B, Löw M, Deckmyn G, Metzger U, Franck F, Heerdt C, Matyssek R, Valcke R, Ceulemans R (2007) Chronic ozone exposure affects leaf senescence of adult beech trees: a chlorophyll fluorescence approach. Journal of Experimental Botany, 58 (4): 785-795.

Göttlein A, Rodenkirchen H, Häberle KH, Matyssek R (2009) Nutritional effects triggered by the extreme summer 2003 in the free air ozone fumigation experiment at the Kranzberger Forst. European Journal of Forest Research, 128: 129-134.

Grebenc T, Kraigher H (2007) Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentrations. Plant Biology, 9: 279-287.

Günthardt-Goerg MS, McQuattie CJ, Scheidegger C, Rhiner C, Matyssek R (1997) Ozone-induced cytochemical and ultrastructural changes in leaf mesophyll cell walls. Canadian Journal of Forest Research 27: 453–463.

Haberer K, Grebenc T, Alexou M, Geßler A, Kraigher H, Rennenberg H (2007) Effects of long-term free-air ozone fumigation on $\delta^{15}N$ and total N in *Fagus sylvatica* and associated mycorrhizal fungi. Plant Biology, 9: 242-252.

Häberle KH, Nunn AJ, Reiter IM, Werner H, Heller W, Bahnweg G, Gayler S, Lütz C, Matyssek R (2009) Variation of defence-related metabolites in the foliage of adult beech and spruce: a conceptual approach to approximating traded-off carbon. European Journal of Forest Research, 128: 99–108.

Harley JL (1940) A Study of the root system of the Beech in woodland soils, with especial reference to mycorrhizal infection. Journal of Ecology, 28 (1): 107-117.

Harrison AF, Schulze ED, Gebauer G, Bruckner G (2000) Canopy uptake and utilization of atmospheric pollutant nitrogen. *In*: Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Ecological Studies 142, Springer Verlag, Berlin, Heidelberg, pp. 171-188.

Heagle AS (1973) Interactions between air pollutants and plant parasites. Annual Review of Phytopathology, 11: 365-388.

Heck WW, Taylor OC, Tingey DT (eds.) (1988) Assessment of crop loss from air pollutants. Elsevier Applied Sciences, London, New York, 552 p.

Heerdt C (2007) Methodenentwicklung zum kleinräumigen Ozonmonitoring in einem 60-jährigen Buchen / Fichten Mischbestand unter doppelt ambienter Free-Air-Ozonexposition. PhD Thesis, 217 p.

Heller W, Rosemann D, Oßwald WF, Benz B, Schönwitz R, Lohwasser K, Kloos M, Sandermann jr. H (1990) Biochemical response of Norway spruce (*Picea abies* (L.) Karst.) towards 14-month exposure to ozone and acid mist: Part I – Effects on polyphenol and monoterpene metabolism. Environmental Pollution, 64: 353-366.

Hill AC, Littlefield N (1969) Ozone. Effect on apparent photosynthesis, rate of transpiration, and stomatal closure in plants. Environmental Science and Technology, 3 (1): 52-56.

Hobbie SE (1992) Effects of plant species on nutrient cycling. Trends in Ecology & Evolution, 7 (10): 336-339.

Holmes WE, Zak DR, Pregitzer KS, King JS (2006) Elevated CO_2 and O_3 alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. Ecosystems, 9: 1345-1363.

Ignatova N, Dambrine E (2000) Canopy uptake of N deposition in spruce (*Picea abies* L. Karst) stands. Annals of Forest Science, 57 (2): 113-120.

IPCC (2007) Climate Change 2007: Impacts, Adaptations and Vulnerability. Contribution of the Working Group II for the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Parry ML, Canziani OF, Palutikof JP, Van der Linden PJ, Hanson CE (eds.), Cambridge University Press, UK, 976 p.

ISRIC (International Soil Reference and Information Centre) (1992) Procedures for soil analysis. Reeuwijk LP Van (ed.) Wageningen, NL, 3rd ed.

IUPAC (2002) Isotope-abundance variations of selected elements. International Union of Pure and Applied Chemistry, IUPAC Technical Report. Pure and Applied Chemistry, 74 (10): 1987-2017.

Jäggi M, Siegwolf R, Genenger M, Hallenbarter D, Brunner I, Fuhrer J (2004) Wood ash treatment affects seasonal N fluctuations in needles of adult *Picea abies* trees: a ^{15}N-tracer study. Trees, 18: 54-60.

Joergensen RG, Mueller T (1996) The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EN) value. Soil Biology and Biochemistry, 28 (1): 33-37.

Karlsson E, Örlander G, Langvall O, Uddling J, Hjorth U, Wiklander K, Areskoug B, Grennfelt P (2006) Negative impact of ozone on the stem basal area increment of mature Norway spruce in south Sweden. Forest Ecology and Management, 232 (1-3): 146-151.

Karnosky DF, Zak DR, Pregitzer KS, Awmack CS, Bockheim JG, Dickson RE, Hendrey GR, Host GE, King JS, Kopper BJ, Kruger EL, Kubiske ME, Lindroth RL, Mattson WJ, Mcdonald EP, Noormets A, Oksanen E, Parsons WFJ, Percy KE, Podila GK, Riemenschneider DE, Sharma P, Thakur R, Sôber A, Sôber J, Jones WS, Anttonen S, Vapaavuori E, Mankovska B, Heilman W, Isebrands JG (2003) Tropospheric O_3 moderates responses of temperate hardwood forests to elevated CO_2: a synthesis of molecular to ecosystem results from the Aspen FACE project. Functional Ecology, 17: 289-304.

Karnosky DF, Pregitzer KS, Zak DR, Kubiske ME, Hendrey GR, Weinstein D, Nosal M, Percy KE (2005) Scaling ozone responses of forest trees to the ecosystem level in a changing climate. Plant, Cell and Environment, 28: 965-981.

Keller T, Häsler R (1984) The influence of a fall fumigation with ozone on the stomatal behavior of spruce and fir. Oecologia, 64 (2): 284-286.

Kelly JM, Taylor Jr. GE, Edwards NT, Adams MB, Edwards GS, Friend AL (1993) Growth, physiology, and nutrition of loblolly pine seedlings stressed by ozone and acidic precipitation: A summary of the ropis-south project. Water, Air, and Soil Pollution, 69: 363-391.

Kelting DL, Burger JA, Edwards GS (1995) The effects of ozone on the root dynamics of seedlings and mature red oak (*Quercus rubra* L.). Forest Ecology and Management, 79 (3): 197-206.

Kienitz GA, Klauditz W (1940) Über die anatomisch bedingte unterschiedliche chemische Zusammensetzung des Buchen-Sulfitzellstoffes. European Journal of Wood and Wood Products, 3 (11): 353-357.

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF (2001) Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO_2 and tropospheric O_3. Oecologia, 128 (2): 237-250.

King JS, Kubiske ME, Pregitzer KS, Hendrey GR, McDonald, EP, Giardina CP, Quinn VS, Karnosky DF (2005) Tropospheric O_3 compromises net primary production in young stands of trembling aspen, paper birch and sugar maple in response to elevated atmospheric CO_2. New Phytologist, 168: 623-636.

Kitao M, Löw M, Heerdt C, Grams TEE, Häberle KH, Matyssek R (2009) Effects of chronic elevated ozone exposure on gas exchange responses of adult beech trees (*Fagus sylvatica*) as related to the within-canopy light gradient. Environmental Pollution, 157: 537-544.

Koch AS, Matzner E (1993) Heterogeneity of soil and soil solution chemistry under Norway spruce (*Picea abies* Karst.) and European beech (*Fagus silvatica* L.) as influenced by distance from the stem basis. Plant and Soil 151: 227-237.

Körner C (2003) Carbon limitation in trees. Journal of Ecology, 91 (1): 4 – 17.

Kolb TE, Matyssek R (2001) Limitations and perspectives about scaling ozone impacts in trees. Environmental Pollution 115 (3): 373-393.

Kozovits AR, Matyssek R, Winkler JB, Goettlein A, Blaschke H, Grams TEE (2005*a*) Above-ground space sequestration determines competitive success in juvenile beech and spruce trees. New Phytologist, 167: 181-196.

Kozovits AR, Matyssek R, Blaschke H, Goettlein A, Grams TEE (2005*b*) Competition increasingly dominates the responsiveness of juvenile beech and spruce to elevated CO_2 and/or O_3 concentrations throughout two subsequent growing seasons. Global Change Biology, 11: 1387-1401.

Larcher W (1994) Ökophysiologie der Pflanzen. Verlag Eugen Ulmer, Stuttgart, 5th ed., 394 p.

Lefohn AS (1992) Surface level ozone exposures and their effects on vegetation. Chelsea, Lewis Publishers, 366 p.

Leuschner C, Backes K, Hertel D, Schipka F, Schmitt U, Terborg O, Runge M (2001) Drought responses at leaf, stem and fine root levels of competitive *Fagus sylvatica* L. and Quercus petraea (Matt.) Liebl. trees in dry and wet years. Forest Ecology and Management, 149: 33-46.

Leuschner C, Hertel D (2003) Fine root biomass of temperate forests in relation to soil acidity and fertility, climate, age and species. Progress in botany, 64: 405-438.

Leuschner C, Hertel D, Schmid I, Koch O, Muhs A, Hölscher D (2004) Stand fine root biomass and fine root morphology in old-growth beech forests as a function of precipitation and soil fertility. Plant and Soil, 258 (1): 43-56.

Lindroth RL, Kopper BJ, Parsons WFJ, Bockheim JG, Karnosky DF, Hendrey GR, Pregitzer KS, Isebrands JG, Sober J (2001) Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). Environmental Pollution, 115 (3): 395-404.

Liu L, King JS, Giardina CP (2005) Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiology, 25: 1511–1522.

Liu L, King JS, Giardina CP (2007) Effects of elevated atmospheric CO_2 and tropospheric O_3 on nutrient dynamics: decomposition of leaf litter in trembling aspen and paper birch communities. Plant Soil, 299: 65–82.

Liu L, King JS, Giardina CP, Booker FL (2009) The influence of chemistry, production and community composition on leaf litter decomposition under elevated atmospheric CO_2 and tropospheric O_3 in a northern hardwood ecosystem. Ecosystems, 12: 401–416.

Löw M, Herbinger K, Nunn AJ, Häberle KH, Leuchner M, Heerdt C, Werner H, Wipfler P, Pretzsch H, Tausz M, Matyssek R (2006) Extraordinary drought of 2003 overrules ozone impact on adult beech trees (*Fagus sylvatica*). Trees - Structure and Function, 20 (5): 539-548.

Löw M, Häberle KH, Warren CR, Matyssek R (2007) O_3 flux-related responsiveness of photosynthesis, respiration, and stomatal conductance of adult *Fagus sylvatica* to experimentally enhanced free-air O_3 exposure. Plant Biology, 9: 197-206.

Luedemann G, Matyssek R, Fleischmann F, Grams TEE (2005) Acclimation to ozone affects host/pathogen interaction and competitiveness for nitrogen in juvenile *Fagus sylvatica* and *Picea abies* trees infected with *Phytophthora citricola*. Plant Biology, 7: 640-649.

McDonald EP, Kruger EL, Riemenschneider DE, Isebrands JG (2002) Competitive status influences tree-growth responses to elevated CO_2 and O_3 in aggrading Aspen stands. Functional Ecology, 16 (6): 792-801.

Mainiero R, Kazda M, Häberle KH, Nikolova PS, Matyssek R (2009) Fine root dynamics of mature European beech (*Fagus sylvatica* L.) as influenced by elevated ozone concentrations. Environmental Pollution, 157 (10): 2638-2644.

Manderscheid R, Jaeger HJ, Kress LW (1992) Effects of ozone on foliar nitrogen metabolism of *Pinus taeda* L. and implications for carbohydrate metabolism. New Phytology, 121: 623-633.

Manning WJ, Keane KD (1988) Effects of air pollutants on interactions between plants, insects, and pathogens. *In:* Heck WW, Taylor OC, Tingey DT (eds.) Assessment of crop loss from air pollutants. Elsevier Applied Sciences, London, New York, pp. 365-386.

Manning WJ, von Tiedemann A (1995) Climate change: potential effects of increased atmospheric carbon dioxide (CO_2), ozone (O_3), and ultraviolet-B (UV-B) radiation on plant diseases. Environmental Pollution: 219-245.

Mariotti A (1983) Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. Nature 303, 685 – 687.

Matyssek R (1986) Carbon, water and nitrogen relations in evergreen and deciduous conifers. Tree Physiology, 2: 177-187.

Matyssek R, Günthardt-Goerg MS, Saurer M, Keller T (1992) Seasonal growth, $\delta^{13}C$ in leaves and stem, and phloem structure of birch (*Betula pendula*) under low ozone concentrations. Trees, Structure and Function, 6: 69-76.

Matyssek R, Günthardt-Goerg MS, Landolt W, Keller T (1993) Whole-plant growth and leaf formation in ozonated hybrid poplar (*Populus* × *euramericana*). Environmental Pollution 81 (3): 207-212.

Matyssek R, Innes JL (1999) Ozone – a risk factor for trees and forests in Europe? Water, Air and Soil Pollution, 116: 199-226.

Matyssek R, Schnyder H, Elstner EF, Munch JC, Pretzsch H, Sandermann H (2002) Growth and parasite defence in plants; the balance between resource sequestration and retention: in lieu of a guest editorial. Plant Biology, 4: 133-136.

Matyssek R, Sandermann H (2003) Impact of ozone on trees: an ecophysiological perspective. Progress in Botany, 64: 349-404.

Matyssek R, Agerer R, Ernst D, Munch JC, Oßwald W, Pretzsch H, Priesack E, Schnyder H, Treutter D (2005) The plant`s capacity in regulating resource demand. Plant Biology, 7: 560-580.

Matyssek R, Bytnerowicz A, Karlsson PE, Paoletti E, Sanz M, Schaub M, Wieser G (2007*a*) Promoting the O_3 flux concept for European forest trees. Environmental Pollution, 146 (3): 587-607.

Matyssek R, Bahnweg G, Ceulemans R, Fabian P, Grill D, Hanke DE, Kraigher H, Oβwald W, Rennenberg H, Sandermann H, Tausz M, Wieser G (2007*b*) Synopsis of the CASIROZ Case Study: *Ca*rbon *Si*nk Strength of *Fagus sylvatica* L. in a Changing Environment - Experimental *R*isk Assessment of Mitigation by Chronic *Oz*one Impact. Plant Biology, 9 (2): 163-180.

Matzner E, Murach D (1995) Soil changes induced by air pollutant deposition and their implication for forests in Central Europe. Water, Air and Soil Pollution, 85: 63-76.

Maurer S, Matyssek R (1997) Nutrition and the ozone sensitivity of birch (*Betula pendula*). II. Carbon balance, water-use efficiency and nutritional status of the whole plant. Trees, 12: 11-20.

McClaugherty CA, Pastor J, Aber JD, Melillo JM (1985) Forest litter decomposition in relation to soil nitrogen dynamics and litter quality. Ecology, 66 (1): 266-275.

Millard P, Neilsen GH (1989) The influence of nitrogen supply on the uptake and remobilisation of stored N for the seasonal growth of apple trees. Annals of Botany, 63: 301-309.

Millard P, Proe MF (1992) Storage and internal cycling of nitrogen in relation to seasonal growth of Sitka spruce. Tree Physiology, 10 (1): 33-43.

Millard P (1996) Ecophysiology of the internal cycling of nitrogen for tree growth. Journal of plant nutrition and soil science / Zeitschrift fuer Pflanzenernaehrung und Bodenkunde, 159: 1-10.

Millard P, Sommerkorn M, Grelet GA (2007) Environmental change and carbon limitation in trees: a biochemical, ecophysiological and ecosystem appraisal. New Phytologist, 175: 11–28.

Miller HG (1984) Dynamic of nutrient cycling in plantation ecosystems. *In:* Bowen GD, Nambiar EKS (eds.) Nutrition of plantation forests. Academic Press, London, pp. 53-78.

Mills G, Hayes F, Wilkinson S, Davies WJ (2009) Chronic exposure to increasing background ozone impairs stomatal functioning in grassland species. Global Change Biology, 15: 1522-1533.

Mellert KH, Prietzel J, Straussberger R, Rehfuess KE (2004) Long-term nutritional trends of conifer stands in Europe:results from the RECOGNITION project. European Journal of Forest Research, 123: 305–319.

Morgan PB, Mies TA, Bollero GA, Nelson RL, Long SP (2006) Season-long elevation of ozone concentration to projected 2050 levels under fully open-air conditions substantially decreases the growth and production of soybean. New Phytologist, 170: 333-343.

Nadelhoffer KJ, Fry B (1994) Nitrogen isotope studies in forest ecosystems. *In:* Lajtha K, Michener RH (eds.) Stable isotopes in ecology and environmental science. Blackwell Scientific, Oxford, pp. 22-44.

Nadelhoffer KJ, Downs MR, Fry B, Aber JD, Magill AH, Melillo JM (1995) The fate of ^{15}N-labelled nitrate additions to a northern hardwood forest in eastern Maine, USA. Oecologia, 103: 292-301.

Nadelhoffer KJ, Downs MR, Fry B (1999) Sinks for ^{15}N-enriched additions to an oak forest and a red pine plantation. Ecological Applications, 9 (1): 72-86.

Nadelhoffer KJ (2000) The potential effects of nitrogen deposition on fine-root production in forest ecosystems. New Phytologist, 147: 131-139.

Nadelhoffer KJ, Colman BP, Currie WS, Magill A, Aber JD (2004) Decadal-scale fates of ^{15}N tracers added to oak and pine stands under ambient and elevated N inputs at the Harvard Forest (USA) Forest Ecology and Management, 196: 89-107.

Nambiar EKS, Fife DN (1991) Nutrient retranslocation in temperate conifers. Tree Physiology, 9: 185-207.

Nihlgard B (1970) Precipitation, its chemical composition and effect on soil water in a beech and a spruce forest in South Sweden. Oikos, 21 (2): 208-217.

Nikolova PS, Raspe S, Andersen CP, Mainiero R, Blaschke H, Matyssek R, Häberle KH (2009) Effects of the extreme drought in 2003 on soil respiration in a mixed forest. European Journal of Forest Research, 128 (2): 87-98.

Nikolova PS, Andersen CP, Blaschke H, Matyssek R, Häberle KH (2010) Belowground effects of enhanced tropospheric ozone and drought in a beech/spruce forest (*Fagus sylvatica* L./*Picea abies* [L.] Karst). Environmental Pollution, 158 (4): 1071-1078.

Nunn AJ, Reiter IM, Häberle KH, Werner H, Langebartels C, Sandermann H, Heerdt C, Fabian P, Matyssek R (2002) „Free-air“ ozone canopy fumigation in an old-growth mixed forest: concept and observations in beech. Phyton, 42: 105-119.

Nunn AJ, Kozovits AR, Reiter IM, Heerdt C, Leuchner M, Luetz C, Liu X, Löw M, Winkler JB, Grams TEE, Häberle KH, Werner H, Fabian P, Rennenberg H, Matyssek R (2005*a*) Comparison of ozone uptake and sensitivity between a phytotron study with young beech and a field experiment with adult beech (*Fagus sylvatica*). Environmental Pollution 137: 494-506.

Nunn AJ, Reiter IM, Häberle KH, Langebartels C, Bahnweg G, Pretzsch H, Sandermann H, Matyssek R (2005*b*) Response patterns in adult forest trees to chronic ozone stress: identification of variations and consistencies. Environmental Pollution 136: 365-369.

Nunn AJ, Wieser G, Reiter IM, Häberle KH, Grote R, Havranek WM, Matyssek R (2006) Testing the unifying theory of ozone sensitivity with mature trees of *Fagus sylvatica* and *Picea abies*. Tree Physiology, 26: 1391-1403.

Ollinger SV, Aber JD, Reich PB (1997) Simulating ozone effects on forest productivity: interactions among leaf-, canopy-, and stand-level processes. Ecological Applications, 7 (4): 1237-1251.

Oksanen E (2003*a*) Physiological responses of birch (*Betula pendula*) to ozone: a comparison between open-soil-grown trees exposed for six growing seasons and potted seedlings exposed for one season. Tree Physiology, 23: 603-614.

Oksanen E (2003*b*) Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. Plant, Cell and Environment, 26: 875-886.

Patzner KM (2004) Die Transpiration von Waldbäumen als Grundlage der Validierung und Modellierung der Bestandestranspiration in einem Wassereinzugsgebiet des Flusses “Ammer”. PhD thesis, 168 p.

Pell EJ, Eckardt NA, Glick RE (1994) Biochemical and molecular basis for impairment of photosynthetic potential. Photosynthesis Research, 39 (3): 453-462.

Pell EJ, Schlagnhaufer CD, Arteca RN (1997) Ozone-induced oxidative stress: mechanisms of action and reaction. Physiologia Plantarum 100: 264–273.

Percy KE, Manninen S, Häberle KH, Heerdt C, Werner H, Henderson GW, Matyssek R (2009) Effect of 3 years' free-air exposure to elevated ozone on mature Norway spruce (*Picea abies* (L.) Karst.) needle epicuticular wax physicochemical characteristics. Environmental Pollution, 157 (5): 1657-1665.

Peuke AD, Schraml C, Hartung W, Rennenberg H (2002) Identification of drought-sensitive beech ecotypes by physiological parameters. New Phytologist, 154 (2): 373-387.

Persson T, van Oene H, Harrison AF, Karlsson PS, Bauer GA, Cerny J, Coûteaux MM, Dambrine E, Högberg P, Kjøller A, Matteucci G, Rudebeck A, Schulze ED, Paces T (2000*a*) Experimental sites in the NIPHYS/CANIF project. *In:* Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Springer, Heidelberg, pp. 14–46.

Persson T, Rudebeck A, Jussy JH, Colin-Belgrand M, Prieme A, Dambrine E, Karlsson PS, Sjöberg RM (2000*b*) Soil nitrogen turnover – mineralisation, nitrification and denitrification in European forest soils. *In:* Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Springer, Heidelberg, pp. 298–331.

Pleijel H, Danielssona H, Vandermeirenc K, Blum C, Colls J, Ojanperä K (2002) Stomatal conductance and ozone exposure in relation to potato tuber yield—results from the European CHIP programme. European Journal of Agronomy, 17 (4): 303-317.

Prather M, Gauss M, Berntsen T, Isaksen I, Sundet J, Bey I, Brasseur G, Dentener F, Derwent R, Stevenson D, Grenfell L, Hauglustaine D, Horowitz L, Jacob D, Mickley L, Lawrence M, von Kuhlmann R, Muller JF, Pitari G, Rogers H, Johnson M, Pyle J, Law K, van Weele M, Wild O (2003) Fresh air in the 21st century? Geophysical Research Letters, 30 (2): 1100, doi:10.1029/2002GL016285.

Pregitzer KS, Burton AJ, King JS, Zak DR (2008) Soil respiration, root biomass, and root turnover following long-term exposure of northern forests to elevated atmospheric CO_2 and tropospheric O_3. New Phytologist, 180 (1): 153 – 161.

Pretzsch H, Kahn M, Grote R (1998) Die Fichten-Buchen-Mischbestände des Sonderforschungsbereiches „Wachstum oder Parasitenabwehr?“ im Kranzberger Forst – The mixed spruce-beech forest stands of the „Sonderforschungsbereich“ „Growth or Parasite Defense?“ in the forest district Kranzberger Forst. Forstwissenschaftliches Centralblatt, Berlin, 117: 241-257.

Pretzsch H, Grote R, Reineking B, Rötzer TH, Seifert ST (2008) Models for forest ecosystem management: a European perspective. Annals of Botany, 101: 1065-1087.

Pretzsch H, Dieler J, Matyssek R, Wipfler P (2009) Tree and stand growth of mature Norway spruce and European beech under long-term ozone fumigation. Environmental Pollution, doi:10.1016/j.envpol.2009.07.035.

Pritsch K, Esperschuetz J, Haesler F, Raidl S, Winkler B, Schloter M (2009) Structure and activities of ectomycorrhizal and microbial communities in the rhizosphere of *Fagus sylvatica* under ozone and pathogen stress in a lysimeter study. Plant and Soil, 323: 97–109.

Reich PB, Amundson RG (1985) Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science, 230: 566-570.

Reich PB (1987) Quantifying plant response to ozone: a unifying theory. Tree Physiology, 3: 63-91.

Reich PB, Schoettle AW, Stroo HF, Amundson RG (1988) Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. III. Nutrient relations. Canadian Journal of Botany, 66 (8): 1517–1531.

Reiling K, Davison AW (1995) Effects of ozone on stomatal conductance and photosynthesis in populations of Plantago major L. New Phytologist, 129 (4): 587 – 594.

Reiter IM, Häberle KH, Nunn AJ, Heerdt C, Reitmayer H, Grote R, Matyssek R (2005) Competitive strategies in adult beech and spruce: space-related foliar carbon investment versus carbon gain. Oecologia, 146: 337-349.

Riikonen J, Kets K, Darbah J, Oksanen E, Sober A, Vapaavuori E, Kubiske ME, Nelson N, Karnosky DF (2008) Carbon gain and bud physiology in *Populus tremuloides* and *Betula papyrifera* grown under long-term exposure to elevated concentrations of CO_2 and O_3. Tree Physiology, 28: 243-254.

Rötzer T, Seifert T, Pretzsch H (2009) Modelling above and below ground carbon dynamics in a mixed beech and spruce stand influenced by climate. European Journal of Forest Research, 128: 171-182.

Runge M (1970) Untersuchung und Bestimmung der Stickstoffnachlieferung am Standort. Flora, 159: 233-257.

Sager EPS, Hutchinson TC, Croley TR (2005) Foliar phenolics in sugar maple (*Acer saccharum*) as a potential indicator of tropospheric ozone pollution. Environmental Monitoring and Assessment, 105: 419-430.

Samuelson LJ, Kelly JM, Mays PA, Edwards GS (1996) Growth and nutrition of *Quercus rubra* L. seedlings and mature trees after three seasons of ozone exposure. Environmental Pollution, 91 (3): 317-323.

Samuelson LJ, Kelly JM (2001) Scaling ozone effects from seedlings to forest trees. New Phytologist, 149: 21-41.

Santa Regina I, Tarazona T, Calvo R (1997) Nitrogen as a limiting factor in the development of beech forests in the Sierra de la Demanda (Spain). Plant Ecology, 133 (1): 49-56.

Schimel JP, Bennett J (2004) Nitrogen mineralization: Challenges of a changing paradigm. Ecology, 85 (3): 591-602.

Schleppi P, Bucher-Wallin L, Siegwolf R, Saurer M, Muller N, Bucher JB (1999) Simulation of increased nitrogen deposition to a montane forest ecosystem: Partitioning of the added ^{15}N. Water, Air, and Soil Pollution, 116: 129-134.

Schlesinger WH (1997) Biogeochemistry: An analysis of global change. 2nd ed., Academic Press, San Diego, California, 588 p.

Schmid I (2002) The influence of soil type and interspecific competition on the fine root system of Norway spruce and European beech. Basic and Applied Ecology, 3 (4): 339-346.

Schmid I, Kazda M (2002) Root distribution of Norway spruce in monospecific and mixed stands on different soils. Forest Ecology and Management, 159: 37-47.

Schmitz H, Löffel U, Weidner M (1993) The rate of protein synthesis in needles of Norway spruce (*Picea abies*): Light stimulation, regulation through photophosphorylation, stress enhancement. Physiologia Plantarum, 87 (2): 237–247.

Schroeder D (1972) Bodenkunde in Stichworten. Verlag Ferdinand Hirt, Kiel, 2nd ed., 144 p.

Schuhbäck T (2004) Nährelementstatus und Bodenzustand an der Bestandesgrenze Buche-Fichte im Mischbestand Kranzberger Forst. Diploma Thesis, Technische Universität München.

Schulze ED, Fuchs M, Fuchs MI (1977) Spacial distribution of photosynthetic capacity and performance in a mountain spruce forest of Northern Germany. III. The significance of the evergreen habit. Oecologia 30: 239-248.

Schulze ED, Oren R, Lange OL (1989) Nutrient relations of trees in healthy and declining Norway spruce stands. *In:* Schulze ED, Lange OL, Oren R (eds.) Forest decline and air pollution – A study of spruce (*Picea abies*) on acid soils. Ecological Studies 77, Springer Verlag, Berlin, Heidelberg, pp. 392-417.

Schulze ED, Kelliher FM, Körner C, Lloyd J, Leuning R (1994) Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition: A global ecology scaling exercise. Annual Review of Ecology and Systematics, 25: 629-662.

Schulze ED, Beck E, Müller-Hohenstein K (2002) Pflanzenökologie. Spektrum Akademischer Verlag, Heidelberg, Berlin, 846 p.

Skärby L, Troeng E, Bostrom CA (1987) Ozone uptake and effects on transpiration, net photosynthesis, and dark respiration in Scots pine. Forest Science, 33(3): 801-808.

Skärby L, Wallin G, Selldén G, Karlsson PE, Ottosson S, Sutinen S, Grennfelt P (1995) Tropospheric Ozone - A Stress Factor for Norway Spruce in Sweden. Ecological Bulletins, 44: 133-146.

Spangenberg A, Kölling C (2004) Nitrogen deposition and nitrate leaching at forest edges exposed to high ammonia emissions in Southern Bavaria. Water, Air, & Soil Pollution, 152: 233-255.

Staaf H (1982) Plant nutrient changes in beech leaves during senescence as influenced by site characteristics. Acta Oecologia / Oecologia Plantarum, 3 (2): 161-170.

Staaf H, Stjernquist I (1986) Seasonal dynamics, especially autumnal retranslocation, of nitrogen and phosphorus in foliage of dominant and suppressed trees of beech, *Fagus sylvatica*. Scandinavian Journal of Forest Research, 1: 333-342.

StMLF (2007) Waldzustandsbericht 2007. Bayerisches Staatsministerium für Landwirtschaft und Forsten, München

Taylor AFS, Martin F, Read DJ (2000) Fungal diversity in ectomyccorhizal communities of Norway spruce [*Picea abies* (L.) Karst.] and beech (*Fagus sylvatica* L.) along North-South transects in Europe. *In:* Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Springer, Heidelberg, pp. 343-365.

Temple PJ (1986) Stomatal conductance and transpirational responses of field-grown cotton to ozone. Plant, Cell and Environment, 9 (4): 315-321.

Temple PJ, Riechers GH (1995) Nitrogen allocation in ponderosa pine seedlings exposed to interacting ozone and drought stress. New Phytologist, 130: 97-104.

Timmer VR, Stone EL (1978) Comparative foliar analysis of young balsam fir fertilized with nitrogen, phosphorus and lime, Soil Science Society of America Journal, 42: 125–130.

Timmer VR, Morrow LD (1984) Predicting fertilizer growth response and nutrient status of jack pine by foliar diagnosis. *In:* Stone, EL (ed.) Forest soils and treatment impacts. Proceedings of the 6th North American Forest Soils Conference, University of Tennessee, Knoxville, pp. 335–351.

Tjoelker MG, Luxmoore, RJ (1991) Soil nitrogen and chronic ozone stress influence physiology, growth and nutrient status of *Pinus taeda* L. and *Liriodendron tulipifera* seedlings. New Phytologist, 119 (1): 69-81.

Uddling J, Karlsson PE, Glorvigen A, Sellden G (2005) Ozone impairs autumnal resorption of nitrogen from birch (*Betula pendula*) leaves, causing an increase in whole-tree nitrogen loss through litter fall. Tree Physiology, 26: 113-120.

Vitousek P (1982) Nutrient cycling and nutrient use efficiency. The American Naturalist, 119 (4): 553-572.

Vingarzan R (2004) A review of surface ozone background levels and trends. Atmospheric Environment, 38: 3431-3442.

von Caemmerer S, Farquhar GD (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153, 376-387.

Wallenda T, Kottke I (1998) Nitrogen deposition and ectomycorrhizas. New Phytologist, 139 (1): 169-187.

Wallenda T, Stober C, Hogbom L, Schinkel H, George E, Högberg P, Read DJ (2000) Nitrogen Uptake Processes in Roots and Mycorrhizas. *In:* Schulze ED (ed.) Carbon and nitrogen cycling in European forest ecosystems. Springer, Heidelberg, pp. 122–143.

Weinbaum SA, Muraoka TT, Plant RE (1994) Intracanopy variation in nitrogen cycling through leaves is influenced by irradiance and proximity to developing fruit in mature walnut trees. Trees, 9: 6-11.

Weinbaum SA, van Kessel C (1998) Quantitative estimates of uptake and internal cycling of ^{14}N-labeled fertilizer in mature walnut trees. Tree Physiology, 18: 795-801.

Weiss M, Schmidt J, Neumann D, Wray V, Christ R, Strack D (1999) Phenylpropanoids in mycorrhizas of the Pinaceae. Planta, 208 (4): 491-502.

Werner H, Fabian P (2002) Free-air fumigation of mature trees. Environmental Science and Pollution Research, 9: 117-121.

Wieser G, Havranek WM (1993) Ozone uptake in the sun and shade crown of spruce: quantifying the physiological effects of ozone exposure. Trees - Structure and Function, 7 (4): 227-232.

Wilkinson S, Davies WJ (2009) Ozone suppresses soil drying- and abscisic acid (ABA)-induced stomatal closure via an ethylene-dependent mechanism. Plant, Cell and Environment, Volume 32 (8): 949-959.

Winwood J, Pate AE, Price AJ, Hanke DE (2007) Effects of long-term, free-air ozone fumigation on the cytokinin content of mature beech trees. Plant Biology, 9: 265–278.

Wipfler P, Seifert T, Heerdt C, Werner H, Pretzsch H (2005) Growth of adult Norway spruce (*Picea abies* [L.] Karst.) and European beech (*Fagus sylvatica* L.) under free-air ozone fumigation. Plant Biology, 7: 611-618.

Wipfler P, Seifert T, Biber P, Pretzsch H (2009) Intra-annual growth response of adult Norway spruce (*Picea abies* [L.] KARST.) and European beech (*Fagus sylvatica* L.) to an experimentally enhanced, free-air ozone regime. European Journal of Forest Research, 128: 135–144.

Wolfenden J, Mansfield TA (1991) Physiological disturbances in plants caused by air pollutants. Proceedings of the Royal Society of Edinburgh, 97B: 117-138.

Wright LM, Lockaby BG, Meldahl R, Thornton F, Chappelka AH (1991) The influence of acid precipitation and ozone on nitrogen nutrition of young loblolly pine. Water, Air, and Soil Pollution, 54: 135-142.

Zak DR, Holmes WE, Pregitzer KS (2007*a*) Atmospheric CO_2 and O_3 alter the flow of ^{15}N in developing forest ecosystems. Ecology, 88 (10): 2630-2639.

Zak DR, Holmes WE, Pregitzer KS, King JS, Ellsworth DS, Kubiske ME (2007*b*) Belowground competition and the response of developing forest communities to atmospheric CO_2 and O_3. Global Change Biology, 13: 2230-2238.

Appendix

Table 13: Number of samples (i) for statistical analysis of $N_{labelled}$ concentration, N_{total} concentration and C/N ratio of tree compartments and soil.

$N_{labelled}$ *(n = 3 trees)*	***N_{total}*** *(n = 5 trees)*	***C/N ratio*** *(n = 5 trees)*
	Leaves (beech)	
^{15}N-labelled trees; one sample per position (sun/shade), tree and sampling date, chosen by highest concentration of $N_{labelled}$ within tree crown *i* = 70	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), tree and sampling date *i* = 257	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), tree and sampling date *i* = 235
	Needles (spruce)	
^{15}N-labelled trees; one sample per position (sun/shade), needle age (0 yr/1 yr), tree and sampling date, chosen by highest concentration of $N_{labelled}$ within tree crown 0 yr: *i* = 71; 1 yr: *i* = 72	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), needle age (0 yr/1 yr), tree and sampling date 0 yr: *i* = 264; 1 yr: *i* = 263	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), needle age (0 yr/1 yr), tree and sampling date 0 yr: *i* = 220; 1 yr: *i* = 219
	Buds (beech & spruce)	
^{15}N-labelled trees; one sample per position (sun/shade), tree and sampling date, chosen by highest concentration of $N_{labelled}$ within tree crown; beech: *i* = 34; spruce: *i* = 36	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), tree and sampling date; beech: *i* = 103; spruce: *i* = 69	^{15}N-labelled and non-labelled control trees; 1 to 4 orientations within tree (N/E/S/W) per position (sun/shade), tree and sampling date; beech: *i* = 103; spruce: *i* = 68
	Current-year twigs (beech & spruce)	
^{15}N-labelled trees; one sample per position (sun/shade) and tree; sun: bark and wood separated; beech: *i* = 18; spruce: *i* = 16	^{15}N-labelled and non-labelled control trees; one sample per position (sun/shade) and tree; sun: bark and wood separated; beech: *i* = 30; spruce: *i* = 18	^{15}N-labelled and non-labelled control trees; one sample per position (sun/shade) and tree; sun: bark and wood separated; beech: *i* = 30; spruce: *i* = 18

Table 13, continued.

$N_{labelled}$ *(n = 3 trees)*	N_{total} *(n = 5 trees)*	***C/N ratio*** *(n = 5 trees)*
Tree stem (beech & spruce)		
^{15}N-labelled trees; three samples per tree: bark, $wood_{ozone}$, $wood_{pre\text{-}ozone}$; bark: beech: *i* = 3; spruce: *i* = 5 $wood_{ozone}$: beech: *i* = 6; spruce: *i* = 5 $wood_{pre\text{-}ozone}$; beech: *i* = 1; spruce: *i* = 1	^{15}N-labelled and non-labelled control trees; three samples per tree: bark, $wood_{ozone}$, $wood_{pre\text{-}ozone}$; bark: beech: *i* = 5; spruce: *i* = 9 $wood_{ozone}$: beech: *i* = 9; spruce: *i* = 9 $wood_{pre\text{-}ozone}$; beech: *i* = 2; spruce: *i* = 2	^{15}N-labelled and non-labelled control trees; three samples per tree: bark, $wood_{ozone}$, $wood_{pre\text{-}ozone}$; bark: beech: *i* = 5; spruce: *i* = 9 $wood_{ozone}$: beech: *i* = 9; spruce: *i* = 9 $wood_{pre\text{-}ozone}$; beech: *i* = 2; spruce: *i* = 2
Roots (beech & spruce) and mycorrhizal root tips		
^{15}N-labelled trees; 1 to 3 samples per root class, m²-plot and horizon; beech: *i* = 200; spruce: *i* = 257	^{15}N-labelled and non-labelled control trees; 1-3 samples per root class, m²-plot and horizon; beech: *i* = 220; spruce: *i* = 285	^{15}N-labelled and non-labelled control trees; 1-3 samples per root class, m²-plot and horizon; beech: *i* = 220; spruce: *i* = 285
Soil (beech & spruce)		
^{15}N-labelled trees; 1 to 3 samples per m²-plot and horizon; beech: *i* = 123[6]; spruce: n = 144[6]	^{15}N-labelled and non-labelled control trees; 1 to 3 samples per m²-plot and horizon; beech: *i* = 183[6]; spruce: *i* = 209[6]	^{15}N-labelled and non-labelled control trees; 1 to 3 samples per m²-plot and horizon; beech: *i* = 183[6]; spruce: *i* = 209[6]
Xylem sap (beech & spruce)		
^{15}N-labelled trees; one twig per sun crown; beech: *i* = 6; spruce: *i* = 6	^{15}N-labelled and non-labelled control trees; one twig per sun crown; beech: *i* = 11; spruce: *i* = 9	^{15}N-labelled and non-labelled control trees; one twig per sun crown; beech: *i* = 11; spruce: *i* = 9
Phloem exudate (beech & spruce)		
^{15}N-labelled trees; three cores of stem bark per sampling date; beech: *i* = 33; spruce: *i* = 35	^{15}N-labelled trees; three cores of stem bark per sampling date; beech: *i* = 33; spruce: *i* = 35	-

6 Soil data of the layers $B_{30\text{-}50}$ and $B_{60\text{-}80}$ were excluded from the N and C analysis as they had been contaminated with N-rich upper soil during the soil core sampling.

Table 13, continued.

$N_{labelled}$ of leaves (beech) & current year needles (spruce)	***Transpiration***
^{15}N-labelled trees; one sample per tree at the last sampling date before autumnal leaf fall (Sept. `06), chosen by highest concentrations of $N_{labelled}$ within tree crown; beech: $i = 5$; spruce: $i = 5$	^{15}N-labelled trees; for spruce, 1×O_3: non-labelled trees; sum of transpiration per m² leaf area of sun crown from beginning of ^{15}N labelling through 2005, and, in 2006, from beginning of the growing season (May) until last sampling date before autumnal leaf fall (Sept. 2006); beech: $i = 5$; spruce: $i = 5$

Table 14: Ozone parameters: annual mean O_3 level, AOT40, and SUM0 measured at Kranzberger Forst at 20 m above ground under the 1xO_3 and 2xO_3 regime during the growing seasons 2005 and 2006. Cumulative ozone uptake (COU) as mean maxima of beech sun crown, and as whole canopy-based mean per growing season of beech and spruce; uptake related to projected area (beech) or total needle surface area (spruce). Data by Heerdt, Nunn, Metzger *(pers. comm.).*

	2005		**2006**	
	1×O_3	**2×O_3**	**1×O_3**	**2×O_3**
mean O_3 level [nl l^{-1}]	31.7	51.8	35.2	58
SUM0 [µl l^{-1} h]	128.6	205	148.8	245
AOT40 [µl l^{-1} h]	11.8	47.4	19.2	66.1
COU_{beech} [mmol m^{-2}] mean maxima of sun crown	16.2	22.1	21.7	33.1
COU_{beech} [mmol m^{-2}] whole-canopy	-	-	11.30	18.21
COU_{spruce} [mmol m^{-2}] whole-canopy	-	-	5.56	7.70