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Carbon and nitrogen allocation of juvenile and adult beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees under contrasting ozone exposure and competition: a $^{13}C/^{12}C$ and $^{15}N/^{14}N$ labeling approach

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Summary

The present study examined the impact of chronically elevated tropospheric ozone (O_3) and interspecific competition on the carbon (C) and nitrogen (N) allocation of juvenile and adult European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) trees through phytotron and field experimentation. Both tree species represent functional groups (deciduous angiosperm tree *vs.* evergreen conifer) with distinct growth dynamics and are of major economical and ecological importance in Central Europe. The experimental approach allowed to cross-compare sapling with adult tree performance. Investigations were conducted as subjects of the interdisciplinary research program SFB 607 (*Sonderforschungsbereich 607 of the Deutsche Forschungsgemeinschaft, DFG*) entitled "Growth and Parasite Defence - Competition for Resources in Economic Plants from Agronomy and Forestry" (SFB 607 - Project B5).

The following hypotheses were tested: (I) Elevated O_3 and interspecific competition affect the whole-tree partitioning of newly acquired C and N in juvenile beech and spruce, (II) elevated O_3 reduces the allocation of recently fixed C to stem CO_2 efflux of juvenile and adult beech and spruce, and (III) juvenile trees reflect higher O_3 sensitivity of the allocation of recently fixed C to stem CO_2 efflux as compared with adult trees.

In a 1-year phytotron study, 4-year-old beech and 5-year-old spruce saplings were analyzed under field-relevant conditions as grown in competitive settings of mono- or mixed cultures. Trees were exposed to either an ambient ($1xO_3$ as control) or experimentally enhanced twice-ambient O_3 regime ($2xO_3$; restricted to < 150 nL L^{-1}). Hypotheses I-III were addressed by means of dual $^{13}CO_2/^{12}CO_2$ ($\delta^{13}C_{Air}$: +111.4 ‰) and $^{15}N/^{14}N$ labeling ($^{15}NH_4{}^{15}NO_3$ solution, ≥ 98 atom %) in late summer (August/September 2005) for 5 and 9 days, respectively. According to approaches in herbaceous plants, a compartmental analysis of the respiratory tracer kinetics in the stem CO_2 efflux was employed to characterize functional properties (i.e. number, size, half-time and relative contribution) of C substrate pools that feed stem respiration. To this end, bias from other CO_2 sources (i.e. soil- and root respired CO_2) but stem respiration was considered to be negligibly small, i.e. stem CO_2 efflux was regarded to represent "stem respiration".

Two years of exposure to elevated O_3 and interspecific competition did not significantly affect the above- and belowground biomass of beech and spruce saplings. Aboveground competition was low in the mono- and mixed cultures before canopy closure.

Hypothesis I was supported in juvenile trees to the extent that elevated O_3 significantly favoured the investment of "new C" into the fine root biomass of beech, along with an increased fine root biomass development. In the absence of O_3 effects, interspecific competition caused spruce to invest significantly more "new" C and N into needles. Overall, spruce displayed lower C investment into the root biomass than beech (root to shoot biomass of *c*. 0.47 and 1.02 in spruce and beech, respectively) which was compensated by increased belowground C allocation to associated mycorrhizae and soil microorganisms. The C

allocation of spruce represented the functional basis of increased competitiveness for belowground resources in comparison with beech, in particular under elevated O_3. Interspecific competition significantly stimulated the ^{15}N uptake of spruce (on a whole tree, total root and fine root biomass basis), but had no effect on the ^{15}N acquisition in beech. Hence, significant tree responses to either $2xO_3$ (beech) or interspecific competition (spruce) were observed in the C allocation to stem respiration in the absence of biomass effects. Carbon allocation to stem respiration was significantly lowered under $2xO_3$ in beech (supporting hypothesis II), whereas significant increase was found in spruce when grown in mixture with beech. In both species, the compartmental analysis of the respiratory tracer kinetics in the stem CO_2 efflux indicated the existence of a faster turned-over pool Q_1 (half-time: 1.3 to 2.7 days), and a slower turned-over pool Q_2 (half-time: 1.0 to 13.7 days). Stem respiration of spruce was predominantly supplied (> 63 to 99 %) by currently photosynthesized assimilates, whereas C reserves were a major source (50 to > 77 %) of respiratory substrate in beech stems. The difference in substrate supply to stem respiration between beech and spruce relates to stem anatomy: angiosperm stems contain a higher proportion of living parenchyma cells serving as storage tissue for starch in the secondary phloem and the xylem than encountered in gymnosperms.

Investigations on adult trees were carried out in a *c.* 60-year-old and 25 m high mixed beech/spruce stand (study site "Kranzberger Forst") near Freising/Bavaria. In order to address hypotheses II and III, $^{13}CO_2/^{12}CO_2$ labeling was performed in late summer (August/September 2006) and sustained for 19 and 18 days in beech and spruce, respectively. ^{13}C-depleted CO_2 ($\delta^{13}C$ of -46.9 ‰) was continuously released into the canopy atmosphere of six beech and spruce trees, respectively, using a free-air CO_2 exposure system ("isoFACE"). Half of the labeled trees (i.e. three beech and spruce, each) had been subjected to free-air twice-ambient O_3 exposure ($2xO_3$) since seven consecutive years.

Relative to the unlabeled control, the CO_2 concentration of the labeled canopy air was elevated by 110 µmol mol^{-1} (beech) and 75 µmol mol^{-1} (spruce), while $\delta^{13}C$ of the air decreased by 8 ‰ and 6 ‰, respectively. Xylem sap flow density of both species was not altered by the CO_2 enrichment, suggesting an unchanged leaf stomatal conductance. The C label was incorporated by the beech and spruce trees. The change in the C isotopic signal (i.e. $\delta^{13}C$ shift) resulting from labeling was pursued in the leaf and fine root tissue, the phloem sugars in the stem as well as in the stem and coarse root CO_2 efflux.

The low $\delta^{13}C$ shift in beech leaves (< 1.7 ‰) and spruce needles (< 0.7 ‰) indicated a strong background signal of "old C" compounds in the leaf tissue. The $\delta^{13}C$ in phloem sugars was lowered by *c.* 3-4 ‰ (beech) and 2-3 ‰ (spruce), suggesting that only 40-50% (beech) and 40-60% (spruce) of C were turned over after 19 and 18 labeling days, respectively. Unlabeled C in phloem sugars may derive from "old C" atoms in C skeletons of currently synthesized sucrose as a consequence of slow turnover of precursor molecules or from remobilized storage compounds.

Similar to the phloem sugars, the $\delta^{13}C$ in the stem CO_2 efflux was lowered by *c.* 3-4 ‰ (beech) and 2-3 ‰ (spruce), indicating that about half of the sampled C originated from C fixed during labeling. This result also suggested that phloem sugars represented the major C

source for stem CO_2 efflux and that xylem-transported CO_2 deriving from soil- and/or root respiration had negligible influence on the CO_2 diffusing out of the stem tissue. In accordance with beech saplings, the allocation of recently fixed C to the stem CO_2 efflux of adult beech was significantly reduced under $2xO_3$ (support of hypothesis II but rejection of hypothesis III), indicating that a substantial proportion of substrate C was derived from C storage pools.
In contrast with beech, the drop in $\delta^{13}C$ in the stem CO_2 efflux of adult spruce was significantly increased under $2xO_3$, indicating an O_3-induced stimulation in C allocation to stem respiration (rejection of hypothesis II and III). In parallel, elevated O_3 significantly stimulated the stem CO_2 efflux rates of spruce.
In both species, chronic elevated O_3 exposure had no impact on the C allocation to coarse root CO_2 efflux and belowground respiratory pools. The fast transfer of C label to the stem and coarse root CO_2 efflux of adult beech and spruce (within 2 to 4 days), and to fine root respiration of adult beech (within 2 to 3 days) confirmed that respiratory processes were tightly linked to canopy photosynthesis in late summer. CO_2 in the soil air around beech consisted of *c.* 25% of labeled C, suggesting that considerable amounts of assimilates were rapidly returned back into the atmosphere. Conversely, spruce appeared to favour the allocation of labeled C to storage and/or structural pools rather than to respiratory pools in the fine roots, since the C label was not detectable in the CO_2 of soil air.

In conclusion, the fast transfer of C label from adult beech and spruce crowns to the stem and coarse root CO_2 efflux confirmed that stem and coarse root respiration were tightly linked to canopy photosynthesis during late summer. Elevated O_3 affected the substrate supply to stem respiration of beech and spruce in different ways: the conifer significantly increased the consumption of current assimilates, whereas the allocation of recently fixed C to stems was restricted in the deciduous tree species.
Differing from saplings, adult beech and spruce trees indicated reduced ^{15}N acquisition under $2xO_3$ suggesting enhanced consumption of N storage pools. Elevated O_3 influenced the allocation of newly acquired N in adult beech and spruce in opposite ways: spruce tended to increase the allocation into aboveground organs (particularly the needles), indicating O_3-induced promotion of the shoot at the expense of roots/mycorrhiza; conversely, beech at $2xO_3$ tended to allocate more newly acquired N into belowground organs, accompanied by a significant stimulation in fine-root and ectomycorrhizal biomass develompent.
Adult beech was similarly sensitive to $2xO_3$ as were beech saplings in terms of a reduced allocation of recently fixed C to stem CO_2 efflux. In consistency with model predictions, decreased C allocation to the stem, together with declining annual stem production (as observed in adult beech) indicated that chronic ozone impact may substantially mitigate the C sink strength of trees. Different reactions in C and N allocation of beech and spruce to $2xO_3$ indicated that interspecific competition may change in mixed stands. Cumulative reductions in C and N storage pools under elevated O_3 impact may weaken the nutrient equilibrium in the long-term, with implications to the trees' growth performance and competitiveness.

Zusammenfassung

Die vorliegende Studie untersuchte den Einfluss chronisch erhöhter troposphärischer Ozon (O_3)-Konzentrationen sowie interspezifischer Konkurrenz auf die Kohlenstoff (C)- und Stickstoff (N)-Allokation von jungen und adulten Rotbuchen (*Fagus sylvatica* L.) und Fichten (*Picea abies* [L.] Karst.) im Phytotron und im Freiland. Die beiden Baumarten repräsentieren funktionelle Gruppen (wechselgrüner Laubbaum *vs.* immergrüne Konifere) mit unterschiedlichen Wachstumsdynamiken und sind von größter ökonomischer und ökologischer Bedeutung innerhalb Mitteleuropas. Der experimentelle Ansatz ermöglichte einen physiologischen Vergleich zwischen den Jung- und Altbäumen. Die Untersuchungen wurden innerhalb des Sonderforschungsbereichs „SFB 607 - Wachstum und Parasitenabwehr - Wettbewerb um Ressourcen in Nutzpflanzen aus Land- und Forstwirtschaft“ (SFB 607 - Projekt B5) durchgeführt.

Folgende Hypothesen wurden geprüft: (I) Erhöhte O_3-Konzentrationen und interspezifische Konkurrenz beeinträchtigen die pflanzeninterne Verteilung (Partitioning) des neu aufgenommenen C und N von jungen Buchen und Fichten, (II) erhöhte O_3-Konzentrationen reduzieren die Allokation des neu fixierten C in den Stamm-CO_2-Efflux junger und adulter Buchen und Fichten, und (III) verglichen mit Altbäumen weisen Jungbäume eine erhöhte O_3-Empfindlichkeit hinsichtlich der Allokation des neu fixierten C in den Stamm-CO_2-Efflux auf.

In einer Phytotronstudie wurden 4-jährige Buchen und 5-jährige Fichten in Mono- und Mischkultur über eine Vegetationsperiode unter freilandähnlichen Bedingungen untersucht. Die Bäume waren entweder ambienten ($1xO_3$ als Kontrolle) oder experimentell zweifach erhöhten O_3-Konzentrationen ($2xO_3$; maximal 150 nL L^{-1}) ausgesetzt. Zur Überprüfung der Hypothesen I-III wurden im Spätsommer (August/September 2005) ein $^{13}CO_2/^{12}CO_2$-($\delta^{13}C_{Luft}$: +111.4 ‰) und $^{15}N/^{14}N$-Markierungsexperiment ($^{15}NH_4{}^{15}NO_3$-Lösung, ≥ 98 Atom %) über 5 bzw. 9 Tage durchgeführt. Um die funktionellen Eigenschaften (d.h. Anzahl, Größe, Halbwertszeit und relativer Beitrag) der an der Stammatmung beteiligen C-Substratpools zu charakterisieren wurde die Kinetik des markierten CO_2 im Stamm-CO_2-Efflux mittels Kompartimentmodellierung (in Anlehnung an Studien mit krautigen Pflanzen) analysiert. Da der Einfluss weiterer CO_2-Quellen (d.h. CO_2 aus Wurzel- bzw. Bodenatmung) auf den Stamm-CO_2-Efflux als sehr klein eingeschätzt wurde, konnte er vernachlässigt bzw. der Stamm-CO_2-Efflux der „Stammatmung“ gleichgesetzt werden.
Nach zwei Vegetationsperioden konnte kein signifikanter Ozon- bzw. Konkurrenzeffekt auf die ober- und unterirdische Biomasse der jungen Buchen und Fichten festgestellt werden. In den Mono- und Mischkulturen herrschte angesichts des unvollständigen Kronenschlusses ein geringer oberirdischer Konkurrenzdruck.
Hypothese I konnte für die Jungbäume insofern bestätigt werden als die erhöhten O_3-Konzentrationen zu einer signifikant erhöhten Investition des „neuen C“ in die Feinwurzelbiomasse von Buche führten und gleichzeitig die Feinwurzelbiomasse signifikant erhöht war. Unabhängig von O_3 begünstigte die interspezifische Konkurrenz bei Fichte die Investition von

„neuem“ C und N in die Nadeln. Über alle Behandlungen hinweg investierte Fichte im Vergleich zu Buche weniger C in die Wurzelbiomasse (Wurzel-Spross-Verhältnis ca. 0.47 in Fichte *vs.* 1.02 in Buche), welche durch eine erhöhte unterirdische C-Allokation in assoziierte Mykorrhizapilze und Bodenmikroorganismen ausgeglichen wurde. Die C-Allokation der Fichte bildete die funktionelle Grundlage für die gesteigerte Konkurrenzfähigkeit um unterirdische Ressourcen im Vergleich zur Buche, insbesondere unter erhöhten O_3-Konzentrationen. Die interspezifische Konkurrenz steigerte die ^{15}N-Aufnahme bei Fichte signifikant (bezogen auf die Gesamtbaum-, Gesamtwurzel- und Feinwurzelbiomasse), hatte jedoch keinen Effekt auf die ^{15}N-Aufnahme von Buche.
Bei fehlendem Einfluss auf die Biomasse wurden jedoch signifikante Effekte auf die Behandlung mit $2xO_3$ (Buche) sowie bei der interspezifischen Konkurrenz (Fichte) hinsichtlich der C-Allokation in die Stammatmung beobachtet. Letztere war bei der Buche unter $2xO_3$ signifikant reduziert (Bestätigung von Hypothese II), in Fichte in Mischkultur mit Buche jedoch signifikant erhöht.
Die Analyse der Kinetik des markierten, stammrespirierten CO_2 durch Kompartiment-modellierung erlaubte in beiden Baumarten die Charakterisierung von zwei an der Stammatmung beteiligten C-Substratpools: einen „Transportpool“ Q_1 mit schnellem C-Umsatz (Halbwertszeit: 1.3 bis 2.7 Tage) und einen „Speicherpool“ Q_2 mit langsamerem C-Umsatz (Halbwertszeit: 1.0 bis 13.7 Tage).
Die Stammatmung von Fichte wurde überwiegend aus aktuellen Photosyntheseprodukten gespeist (> 63 bis 99 %), während ältere C-Reserven einen Großteil (50 bis > 77%) des veratmeten Substrates der Buchenstämme darstellten. Der Unterschied zwischen Buche und Fichte in der Bereitstellung von Substrat für die Stammatmung steht in Zusammenhang mit der Stammanatomie: die Stämme der Angiospermen weisen verhältnismäßig mehr Stärke speichernde lebende Parenchymzellen im sekundären Phloem und Xylem auf als die der Gymnospermen.

Die Untersuchungen an den Altbäumen fanden in einem ca. 60 Jahre alten und 25 m hohen Buchen-Fichten-Mischbestand (Versuchsfläche „Kranzberger Forst“) nahe Freising/Bayern statt. Zur Beantwortung der Hypothesen II und III wurde im Spätsommer (August/September 2006) ein $^{13}CO_2/^{12}CO_2$-Markierungsexperiment durchgeführt. ^{13}C-abgereichertes CO_2 ($\delta^{13}C$ von -46.9 ‰) wurde mittels eines Freiluft-CO_2-Begasungssytems („isoFACE“) in den Kronenraum von jeweils sechs Buchen und Fichten geleitet (19 bzw. 18 Tage lang). Die Hälfte der markierten Bäume (d.h. jeweils drei Buchen und Fichten) war seit sieben Jahren in Folge zweifach erhöhten O_3-Konzentrationen ($2xO_3$) ausgesetzt (mittels einer „free-air“-Ozonbegasungsanlage).
Im Vergleich zur nicht markierten Kontrolle wurde die CO_2-Konzentration der markierten Kronenluft um etwa 110 µmol mol^{-1} (Buche) und 75 µmol mol^{-1} (Fichte) erhöht und das $\delta^{13}C$-Signal der Luft gleichzeitig um 8 ‰ bzw. 6 ‰ erniedrigt.
Die Xylemsaftflussdichte beider Baumarten war unbeeinflusst von der Erhöhung der CO_2-Konzentration, weshalb von einer unveränderten stomatären Leitfähigkeit der Blätter und Nadeln ausgegangen werden konnte. Der markierte Kohlenstoff wurde von den Buchen und Fichten aufgenommen. Die Änderung des C-Isotopensignals (d.h. $\delta^{13}C$ shift) wurde in der

Blatt- und Wurzelbiomasse, in den Phloemzuckern des Stammes sowie im Stamm- und Grobwurzel-CO_2-Efflux verfolgt.

Der geringe $\delta^{13}C$ shift in Buchenblättern (<1.7 ‰) und Fichtennadeln (< 0.7 ‰) wies auf ein starkes „Hintergrundsignal" im Blattgewebe (vermutlich in Form „alter" C-Verbindungen) hin. Das $\delta^{13}C$-Signal in den Phloemzuckern war um ca. 3-4 ‰ (Buche) und 2-3 ‰ (Fichte) erniedrigt, was darauf hindeutete, dass lediglich 40-50 % (Buche) und 40-60 % (Fichte) des vorhandenen C umgesetzt worden waren. Der Anteil an nicht markiertem C in den Phloemzuckern war möglicherweise auf „alte" C-Atome im C-Skelett neu synthetisierter Saccharose durch langsame Umsetzung von Vorläufer-Molekülen (precursors) oder aus Speichern mobilisierten C-Verbindungen zurückzuführen.

Ähnlich wie in den Phloemzuckern war das $\delta^{13}C$-Signal im Stamm-CO_2-Efflux um ca. 3-4 ‰ (Buche) und 2-3 ‰ (Fichte) erniedrigt, was darauf hindeutete, dass lediglich etwa die Hälfte des beprobten C aus C bestand, der während der Markierung fixiert worden war. Dieses Ergebnis deutete darauf hin, dass die Phloemzucker die wichtigste Substratquelle für den Stamm-CO_2-Efflux darstellten und dass der Einfluss von im Xylem transportierten Boden- bzw. Wurzelatmungs-CO_2 auf den Stamm-CO_2-Efflux vernachlässigbar war. Übereinstimmend mit den Jungbuchen war die Allokation des neu fixierten C in den Stamm-CO_2-Efflux der Altbuchen unter $2xO_3$ signifikant reduziert (was Hypothese II bestätigt, jedoch nicht Hypothese III). Dies spricht dafür, dass ein beträchtlicher Anteil des Substrates aus C-Speicherpools stammte.

Im Gegensatz zu Buche war der $\delta^{13}C$ shift im Stamm-CO_2-Efflux von Fichte unter $2xO_3$ signifikant erhöht, was auf eine O_3-induzierte Stimulierung der C-Allokation hin zur Stammatmung schließen ließ (Ablehnung von Hypothese II und III). Gleichzeitig waren die Stamm-CO_2-Effluxraten von Fichte unter $2xO_3$ signifikant erhöht.

In beiden Baumarten zeigte die chronisch erhöhte O_3-Exposition keinen Effekt auf die C-Allokation in den Grobwurzel-CO_2-Efflux sowie in andere unterirdische, für respiratorische Prozesse relevante Pools. Der schnelle Transfer des markierten C in den Stamm- und Grobwurzel-CO_2-Efflux der adulten Buchen und Fichten (innerhalb von 2 bis 4 Tagen) sowie in die Feinwurzelatmung von Buche (innerhalb von 2 bis 3 Tagen) zeigte eine enge Koppelung der Atmungsprozesse an die photosynthetische Aktivität der Baumkronen im Spätsommer. Der markierte C hatte einen Anteil von etwa 25% im Bodenatmungs-CO_2 unter Buche, was auf eine schnelle Rückführung eines beträchtlichen Assimilatanteiles in die Atmosphäre hindeutet. Im Gegensatz zu Buche zog Fichte die Allokation des neu fixierten C in Speicherpools bzw. in strukturelle C-Pools in den Feinwurzeln der Allokation in die Feinwurzelatmung vor; der markierte C wurde nicht im Bodenatmungs-CO_2 wiedergefunden.

Zusammenfassend lässt sich festhalten, dass der schnelle Transfer des neu fixierten C zum Stamm- und Grobwurzel-CO_2-Efflux der adulten Buchen und Fichten auf eine enge Koppelung der Stamm- und Grobwurzelatmung an die photosynthetische Aktivität der Baumkronen im Spätsommer hindeutete. Erhöhtes O_3 beeinflusste die Substratversorgung der Stammatmung von Altbuche und -fichte auf unterschiedliche Art und Weise: die immergrüne Fichte zeigte einen gesteigerten Verbrauch an aktuellen Photosyntheseprodukten, während die

wechselgrüne Buche eine reduzierte Allokation des neu fixierten C in den Stamm-CO_2-Efflux aufwies.

Anders als die jungen Buchen und Fichten wiesen die Altbäume eine tendenziell reduzierte ^{15}N-Aufnahme unter $2xO_3$ auf, was auf einen erhöhten Verbrauch von N-Speicherpools schließen lässt. Erhöhtes O_3 beeinflusste die Allokation des neu aufgenommenen N in den adulten Buchen und Fichten auf unterschiedliche Weise: Fichte verstärkte die Allokation in die oberirdischen Organe (insbesondere die Nadeln) tendenziell, was auf eine O_3-induzierte Förderung der Sprossentwicklung zu Lasten von Wurzeln und Mykorrhiza hindeutete. Im Gegensatz dazu alloziierte Buche unter $2xO_3$ tendenziell mehr neu aufgenommenen N in die unterirdischen Organe, begleitet von einer signifikant erhöhten Feinwurzel- und Ektomykorrhizabiomasse.

Die Altbuchen reagierten auf $2xO_3$ ähnlich empfindlich wie die Jungbuchen im Sinne einer reduzierten Allokation des neu fixierten C in den Stamm-CO_2-Efflux. In Übereinstimmung mit aus Modellen hergeleiteten Ergebnissen deutet die reduzierte C-Allokation in den Stamm, zusammen mit einem verminderten Stammvolumenzuwachs (wie in Altbuchen beobachtet), auf eine wesentlich Abschwächung der C-Aufnahmekapazität von Bäumen durch chronische Ozonbelastung hin. Die unterschiedlichen Reaktionsmuster in der C- und N-Allokation von Buche und Fichte unter $2xO_3$ lassen zudem eine Änderung der interspezifischen Konkurrenz in Mischbeständen auf lange Sicht vermuten. Eine stetig zunehmende Ausschöpfung der C- und N-Speicherpools unter erhöhtem O_3 kann langfristig betrachtet das Nährstoffgleichgewicht der Bäume beeinträchtigen, mit negativen Folgen für Wachstum und Konkurrenzfähigkeit.

Publications

Parts of results and data of this PhD thesis are incorporated in the following publications:

ANDERSEN C.P., **RITTER W.**, GREGG J., MATYSSEK R., GRAMS T.E.E. (2010) Below-ground carbon allocation in mature beech and spruce trees following long-term, experimentally enhanced O_3 exposure in Southern Germany. Environmental Pollution. doi:10.1016/ j.envpol.2010.05.008.

GRAMS T.E.E., WERNER H., KUPTZ D., **RITTER W.**, FLEISCHMANN F., ANDERSEN C.P., MATYSSEK R. (2010) A free-air system for long-term stable carbon isotope labeling of adult forest trees. Trees (submitted).

RITTER W., LEHMEIER C.A., MATYSSEK R., WINKLER J.B., GRAMS T.E.E "Contrasting responses in carbon allocation of juvenile European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies*) to competition and disturbance by ozone". New Phytologist (scheduled for submission 07.2010).

1 Introduction

Living in highly variable environments, plants are constrained in growth due to competition with neighbours and defence against biotic and abiotic stressors (HERMS 1999, ZANGERL & BAZZAZ 1992, BAZZAZ & GRACE 1997, RAI *et al.* 2006). Although altered precipitation, temperature, CO_2 concentrations and N deposition have received attention with respect to impact of global change on terrestrial ecosystems (GEIDER *et al.* 2001, PRENTICE *et al.* 2001, DENMAN *et al.* 2007), enhanced tropospheric ozone (O_3) is now considered at the global scale to be the most important air pollutant affecting vegetation in both rural and urban areas (ASHMORE 2005, KARNOSKY *et al.* 2007, MATYSSEK *et al.* 2007b, PAOLETTI *et al.* 2007). Ozone is a naturally occuring greenhouse gas formed under the action of sunlight from oxygen and nitrogen oxides (NO_x), the latter being released mainly from automobiles and biomass burning, in the presence of volatile organic compounds (VOCs) of both natural and industrial origin (SITCH *et al*. 2007, FOWLER *et al.* 1999, STOCKWELL *et al.* 1997). On the basis of limited observations and modeling, tropospheric ozone has globally increased by about 35% since pre-industrial times, reaching highest levels in the northern hemisphere (PRATHER *et al.* 2001). This trend is estimated to continue over the next 50 years, by up to 50% by 2100 (PRATHER *et al.* 2001, FOWLER *et al.* 1999, 2008), so that ozone levels might exceed internationally accepted levels for vegetation (VINGARZAN 2004). Currently about 25% of the global forests are exposed to O_3 levels > 60 nl L^{-1} during the growing season, being at risk of adverse effects on growth and productivity (FOWLER *et al.* 1999).

In forest trees, tropospheric O_3 has turned out to be a key air pollutant responsible for visible leaf injury, premature leaf loss, photosynthetic decline and growth limitation (MATYSSEK *et al.* 2010, BROADMEADOW 1998, SKÄRBY *et al.* 1998, KARNOSKY *et al.* 2007). The primary site of O_3 impact within plants is the leaf mesophyll (MATYSSEK & INNES 1999, REICH 1987). After passage through the stomata, O_3 rapidly dissolves into the wet surface of the exposed cell walls (HABERER *et al.* 2006) and induces the formation of aggressive radicals (reactive oxygen species, ROS) (OKSANEN *et al.* 2003). Both O_3 and ROS induce lipid peroxidation, damaging the plasmalemma and other membranes of the leaf mesophyll cells (PODILA *et al.* 2001, FOYER *et al*. 1994).

As a consequence of the direct impact on the aboveground plant organs, O_3 can alter the timing and quantitiy of the carbon flux into the soil (ANDERSEN 2003). Ozone stress can reduce the C uptake and alter the C partitioning between metabolic pathways and plant organs (cf. ANDERSEN 2003, DIZENGREMEL 2001, MATYSSEK & SANDERMANN 2003). For instance, photosynthetic decline as well as impaired phloem loading and carbon transport to the root was observed in *Pinus taeda* seedlings (SPENCE *et al.* 1990) and juvenile trees of *Betula pendula* (MATYSSEK *et al.* 1992) in response to ozone. *Pinus echinata* Mill. seedlings responded to ozone by reducing the partitioning of C to sucrose and increasing C retention in the needles (PAYNTER *et al.* 1992). Often, the limitation on carbohydrate export from ozone stressed leaves is indicated by increased soluble sugar concentrations or starch accumulation along the leaf veins (BRAUN 2004, GRANTZ & FARRAR 1999, RENNENBERG *et al.* 1996, GÜNTHARDT-GOERG *et al.* 1993). Given increased assimilate demand in leaves for

detoxification and membrane repair versus an inhibited translocation, other tree organs became carbon-limited under O_3 stress (cf. MATYSSEK *et al.* 2010). Often, the root system was affected most, and in stems radial rather than longitudinal growth was limited (MATYSSEK *et al.* 1992, 1993a,b). For instance, beech saplings attempted to compensate for the O_3-related loss of photosynthetic capacity through an increase of shoot growth by 10 to 20% (GRAMS *et al.* 2002), resulting in an overall decrease of the root/shoot biomass ratio (KOZOVITS *et al.* 2005b). Ozone-induced alterations in belowground C sinks (i.e. reduced root growth and longevity or restricted mycorrhizal networks) may affect the nutrient acquisition, e.g. of nitrogen (HABERER *et al*. 2007, ANDERSEN 2003, LUEDEMANN *et al.* 2005, 2009). Altered C flux may eventually curtail fructification and weaken the whole-tree defense status and competitiveness (cf. MATYSSEK & SANDERMANN 2003).

Plant-plant interactions are known to modify the outcome of studies on abiotic stressors (POORTER & NAVAS 2003), since competition between plants for resources (energy, carbon, water, nutrients) has a profound impact on plant development and allocation (MOONEY & WINNER 1991, GRACE & TILMAN 1990). However, it is uncertain to what extent perturbations in resource allocation in response to multiple stress, as imposed e.g. by O_3 and competition, are expressed as changes in biomass development.

In a 2-year phytotron approach, KOZOVITS *et al.* (2005a,b) investigated the effects of intra- and interspecific competition on tree sensitivity to combined O_3/CO_2 regimes with saplings of European beech (*Fagus sylvatica*) and Norway spruce (*Picea abies)*. When beech grew in mixed culture, the whole-plant biomass increment was about two to three times lower as compared with growth in mixed culture with spruce, irrespective of the gas regime. The effect of the mixed culture was exacerbated in beech by elevated ozone. The competitive disadvantage of beech in mixture was accompanied by a significant reduction in the stem respiration rate, which reflected the aboveground growth. Belowground, beech was also less effective in the mixture in competing for nitrogen, in particular, under elevated O_3, as reflected by decreases in N content and concentration at the whole-plant level (cf. KOZOVITS *et al.* 2005b). The growth of spruce contrasted by being higher in mixed than monoculture, profiting from the weakness of beech under elevated ozone. Plant competition governed the productivity of both beech and spruce, an outcome similar to that of competition studies under elevated CO_2 or O_3 (KÖRNER 2006, BARBO *et al.* 1998, 2002, MCDONALD *et al.* 2002).

In a 2-year phytotron study, LUEDEMANN *et al.* (2005, 2009) focused on mixed cultures of juvenile beech and spruce. Trees were exposed to elevated O_3 concentrations in combination with controlled infection with the root rot pathogen *Phytophthora citricola*. Beech displayed a lower biomass production and captured less ^{15}N per unit of fine-root biomass both under elevated O_3 and *P. citricola* infection, whereas spruce appeared to profit from the lower resource acquisition of beech in these treatments. In the combined treatment, the pathogen infection apparently enhanced the N acquisition capacity of spruce, which was substantiated by an increased dry-mass related N concentration at the whole-plant level. This effect perhaps indicated enhanced N demand of spruce for stress defence (MATYSSEK *et al.* 2005a, GRAMS & MATYSSEK 2010).

The O_3 responses of plants can vary strongly by species, genotype, age of leaf and plants and are altered by environmental factors (KARNOSKY *et al.* 2007, KOZOVITS *et al.* 2005b). However, most information on responses of trees to elevated O_3 has been gained from juvenile trees in chamber studies and during limited periods of time so that the validity for up-scaling O_3 effects to mature forest trees is limited (MATYSSEK *et al.* 2007a, NUNN *et al.* 2005b, KOLB & MATYSSEK 2001). Findings from juvenile trees are frequently used to develop hypotheses about mature trees, which differ in physiological terms, for instance stomatal conductance, ozone sensitivity or resource allocation patterns (KOLB *et al*. 1997, KOLB & MATYSSEK 2001). For instance, GRULKE & MILLER (1994) reported greater foliar photosynthetic sensitivity to ozone in two-year-old *Sequoidendron giganteum* seedlings than in 125-year-old trees. Exposure to twice-ambient concentrations of ozone induced a 50% reduction in light-saturated photosynthesis along with declines in carboxylation efficiency and quantum yield of leaves of mature northern red oak trees (*Quercus rubra* L.), whereas leaf physiology of seedlings was hardly affected (SAMUELSON & EDWARDS 1993, HANSON *et al*. 1994). Despite greater ozone uptake and photosynthetic decline of mature northern red oak trees, ozone did not reduce aboveground growth (SAMUELSON *et al.* 1996). Perhaps, changes in allocation in the mature red oak trees prevented or postponed growth reduction. In mature beech (*Fagus sylvatica*), chronically elevated ozone exposure reduced both sucrose and starch concentrations in the sun leaves (BLUMENRÖTHER *et al.* 2007). In contrast, elevated ozone did not affect leaf sugar and starch levels of *Fagus sylvatica* saplings growing in climate chambers (LIU *et al.* 2005).

Up to date, knowledge about the potential effects of elevated O_3 and interspecific competition on the whole-tree C and N partitioning of juvenile beech and spruce is scarce. Clarification is required about O_3 effects on the allocation of newly assimilated C to stem CO_2 efflux, and, hence, stem diameter growth of juvenile and adult beech and spruce. Since stem respiration of forest trees is strongly driven by current assimilate supply (13% to 42% of gross primary production; CAREY *et al*. 1997), ozone may limit the activity of respiring stem tissues, and hence, stem diameter growth (cf. GÜNTHARDT-GOERG *et al.* 1993, MATYSSEK *et al.* 2002).
The present study examined the impact of chronically elevated tropospheric ozone (O_3) and interspecific competition on the carbon and nitrogen allocation of juvenile and adult European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) trees through phytotron and field experimentation. The experimental approach allowed to cross-compare sapling with adult tree performance.

The following hypotheses were examined:

I. Elevated O_3 and interspecific competition affect the whole-tree partitioning of newly acquired C and N in juvenile beech and spruce.
II. Elevated O_3 reduces the allocation of recently fixed C to stem CO_2 efflux of juvenile and adult beech and spruce.
III. Juvenile trees reflect higher O_3 sensitivity of the allocation of recently fixed C to stem CO_2 efflux as compared with adult trees.

In a 1-year phytotron study, beech and spruce saplings were analyzed under field-relevant conditions as grown in competitive settings of mono- and mixed cultures (cf. KOZOVITS *et al.* 2005a,b, LUEDEMANN *et al.* 2005, 2009). Trees were exposed to either an ambient ($1xO_3$ as control) or experimentally enhanced twice-ambient O_3 regime ($2xO_3$; restricted to < 150 nL L^{-1}). At the end of the study, beech and spruce saplings were 4 and 5 years old, respectively. Since stable isotopes are ideal tools for quantifying element fluxes and pool sizes in plants (SCHNYDER *et al.* 2003, LATTANZI *et al.* 2005, DYCKMANS & FLESSA 2001), the hypotheses I-III were addressed by means of dual $^{13}CO_2/^{12}CO_2$ and $^{15}N/^{14}N$ isotope labeling (5 and 9 days, respectively) in late summer (August/ September 2005). According to approaches in herbaceous plants, a compartmental analysis of the respiratory tracer kinetics in the stem CO_2 efflux was employed to characterize functional properties (i.e. number, size, half-time and relative contribution) of C substrate pools that feed stem respiration (LEHMEIER *et al.* 2008). To this end, bias from xylem-transported CO_2 deriving from soil- and root respiration (TESKEY *et al.* 2008) was considered to be small, i.e. stem CO_2 efflux was regarded to represent "stem respiration".

In parallel, investigations on adult trees were carried out in a *c*. 60-year-old and 25 m high mixed beech/spruce stand (study site "Kranzberger Forst") near Freising/Bavaria. In order to address the hypotheses II and III, $^{13}CO_2/^{12}CO_2$ labeling was performed in late summer (August/September 2006) and sustained for 19 and 18 days in beech and spruce, respectively. ^{13}C-depleted CO_2 ($\delta^{13}C$ of -46.9 ‰) was continuously released into the canopy atmosphere of six beech and spruce trees, respectively, using the free-air CO_2 exposure system "isoFACE" (GRAMS *et al*. 2010b). Half of the labeled trees (i.e. three beech and spruce, each) had been subjected to free-air twice-ambient O_3 exposure ($2xO_3$) since seven consecutive years.

Investigations were conducted as subjects of the interdisciplinary research program SFB 607 (*Sonderforschungsbereich 607 of the Deutsche Forschungsgemeinschaft, DFG*) entitled "Growth and Parasite Defence - Competition for Resources in Economic Plants from Agronomy and Forestry" (SFB 607 - Project B5).

2 Investigations on juvenile beech and spruce

2.1 Materials and Methods

2.1.1 Plants and treatments

Individuals of European beech (*Fagus sylvatica* L., seed source 810-24 Bad Griesbach) and Norway spruce (*Picea abies* [L.] Karst., seed source 840-27 Altötting) were grown as either monoculture or "one-by-one" beech/spruce mixture and exposed to ambient ($1xO_3$) or twice-ambient ($2xO_3$) ozone regimes. The study was carried out in the phytotrons of the „Helmholtz Zentrum München - German Research Center for Environmental Health" in Neuherberg, Germany. The phytotron system has been described by PAYER *et al.* (1993) and THIEL *et al.* (1996) and allows for a site-relevant simulation of environmental conditions, as climatic events and/or exposure to gaseous pollutans can be reproduced realistically. The experimental sequence is shown in Tab. 2.1 In spring 2004, 2-year-old beech and 3-year-old spruce trees were planted into a total of 42 containers with a size of 0.058 m³ each (i.e. area and soil depth 0.55 x 0.35 m and 0.30 m, respectively). Containers had been filled with natural forest soil (dystric cambisol, Ah-B horizon), taken from the study site "Höglwald" (540 m a.s.l.), a beech stand, near Augsburg, Germany (KREUTZER *et al.* 1991). In each container, 20 trees were arranged by rows of 4 x 5 individuals. During the growing season of 2004, i.e. one year prior to the phytotron study, plants had been kept in climate-controlled greenhouse chambers, programmed to track outside climate conditions under ambient CO_2 as well as $1xO_3$ and $2xO_3$ regimes, respectively. During the winter months 2004/2005, plants were kept outside.

Tab. 2.1 Experimental sequence.

Date	Description	Number of containers					
Apr 2004	Plantation of 2-year-old beech and 3-year-old spruce in 48 containers filled with natural forest soil (20 trees/container)	48					
Apr - Oct 2004	Trees growing inside a climate-controlled glasshouse under ambient ($1xO_3$) and twice-ambient ($2xO_3$) ozone regime	$1xO_3$			$2xO_3$		
		8	8	8	8	8	8
Nov 2004 - Mar 30 2005	Plants kept outdoors						
Mar 31, 2005	Containers transferred into glasshouse						
Apr 15, 2005	36 containers were selected for uniform tree height	6	6	6	6	6	6
Apr 18 – 20, 2005	1st harvest at start of experiment: 12 containers used for initial biomass assessment (2 per treatment)						
May 10, 2005	24 containers transferred into phytotrons (4 per treatment)	4	4	4	4	4	4
May 11 - Jun 3, 2005	Replacing of *C. destructans* infested beech trees						
Aug 29, 2005	Assessment of biomass ^{15}N atom% and $\delta^{13}C$ prior labeling (2 trees per species and treatment used)						
Aug 30 - Sep 10, 2005	^{15}N labeling with double-labeled $^{15}NH_4{}^{15}NO_3$						
Sep 03 -20, 2005	5-day $^{13}CO_2/^{12}CO_2$ labeling and subsequent final harvest of 24 containers	4	4	4	4	4	4
	Treatments	**$1xO_3$ mono beech**	**$1xO_3$ mono spruce**	**$1xO_3$ mixed (1:1)**	**$2xO_3$ mono beech**	**$2xO_3$ mono spruce**	**$2xO_3$ mixed (1:1)**

At the beginning of the growing season of 2005 the 42 containers were repositioned into the greenhouse chambers. On May 10, a total of 24 containers was selected for uniform tree height and stem base diameter and transferred into four phytotrons (size 2.8 x 3.4 m), comprising four plexiglas-sub-chambers with temperature-controlled soil compartments (Fig. 2.1). Each treatment was replicated by four containers, and in mixed cultures measurements were performed on three trees and in monocultures on six trees per container. Soil moisture of each container was monitored continuously by tensiometers (Model T5, UMS, Munich, Germany) installed at a soil depth of 11 cm, which triggered irrigation with deionized water whenever soil water tension reached 400 hPa. Plants were fertilized two and four times during the growing season of 2004 and 2005, respectively, each time with 1 L of a double-concentrated Hoagland solution (HOAGLAND & ARNON 1950) to provide soil and tissue nutrient level similar to those in natural soils and trees of Bavarian forests (KREUTZER *et al*. 1991).

2.1.2 Climatic conditions and O_3 regimes

Climatic conditions and O_3 regimes recorded in 1999 at the study site "Kranzberger Forst" (near Freising in Bavaria/Germany, 490 m a.s.l., see PRETZSCH *et al.* 1998) were reproduced in the phytotrons on an hourly basis. $1xO_3$ provided the basis for the experimental $2xO_3$ regime, being restricted to ≤ 150 nL O_3 L^{-1}. Ozone concentrations applied were typical for southern Germany (NUNN *et al*. 2002). Tab. 2.2 displays the monthly means of air temperature, irradiance, relative air humidity, CO_2 and O_3 concentrations during daylight hours and night O_3 regimes were further expressed as "Accumulated exposure over a threshold of 40 nl L^{-1} (AOT40) (FUHRER & ACHERMANN 1999) and "Sum of all O_3 concentrations" (SUM0).

Tab. 2.2 Monthly means of air temperature (T_{air}), photosynthetic photon flux density (PPFD), relative air humidity (RH), CO_2 concentration, $1xO_3$ and $2xO_3$ concentrations, AOT 40 as well as SUM0 values recorded throughout the growing season of 2005 in the phytotrons.

Month	Day/ night	T_{air} (°C)	PPFD (µmol $m^{-2}s^{-1}$)	RH (%)	CO_2 (µL L^{-1})	$1xO_3$ (nL L^{-1})	$2xO_3$ (nL L^{-1})	AOT 40 $1xO_3$ (µL L^{-1} h)	AOT 40 $2xO_3$ (µL L^{-1} h)	SUM0 $1xO_3$ (µL L^{-1} h)	SUM0 $2xO_3$ (µL L^{-1} h)
May	day	17.4	435.2	61.0	378.3	33.2	63.6	0.9	8.2	12.6	20.5
	night	13.0	0	75.5	409.7	19.6	38.2	-	-		
Jun	day	17.5	451.9	61.1	376.7	35.8	76.7	2.8	17.4	21.8	37.2
	night	12.9	0	76.3	402.0	23.1	43.2	-	-		
Jul	day	20.8	407.0	63.1	374.0	38.3	74.6	4.9	18.0	27.8	45.4
	night	16.4	0	80.8	404.5	25.6	55.1	-	-		
Aug	day	20.6	401.7	61.4	371.0	36.5	67.9	3.0	13.2	24.0	38.6
	night	16.2	0	76.9	390.1	22.5	41.4	-	-		
Sep	day	18.5	375.6	68.8	377.4	31.2	59.4	2.7	6.2	11.8	18.3
	night	14.6	0	76.8	390.6	19.9	53.7	-	-		

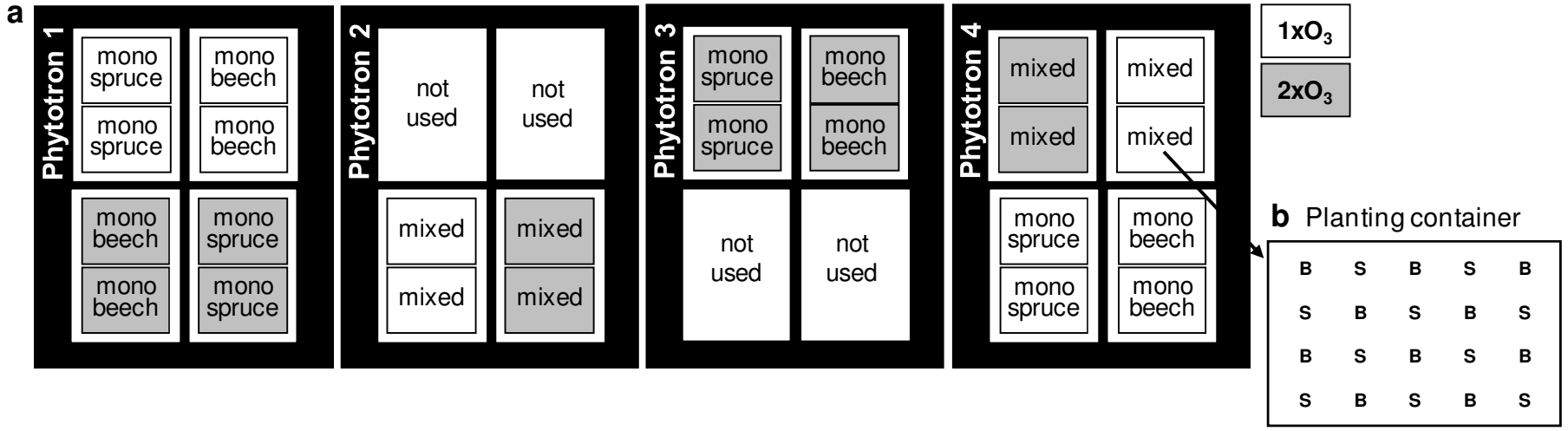

Fig. 2.1 (a) Experimental set-up in the phytotrons of the "Helmholtz Zentrum München - German Research Center for Environmental Health". Each phytotron (size 2.8 x 3.4 m) contains four plexiglas sub-chambers, for individual O_3 fumigation. White and shaded boxes draft $1xO_3$ and $2xO_3$ treatment, respectively. Each treatment was represented with four containers. In mixed cultures measurements were performed on three trees and in monocultures on six trees per container and species. The experimental design considered that one container of each treatment was replicated once in the same sub-chamber. In addition, this set-up was replicated independently in a second phytotron. (b) Spacing of beech (B) and spruce (S) trees in a mixed planting container.

2.1.3 Phenology of shoots and senescence of beech foliage

To monitor seasonal plant development, phenology of beech and spruce was observed 2 to 3 times per month between April 07 and June 17 of the growing season of 2005. Tab. 2.3 shows the classification of leaves and needles by six developmental stages according to KOCH (2005). Senescence of beech foliage was assessed by counting the total number of leaves at the beginning of July, when all leaves were still attached to the tree, and during the final harvest in September. Senescence was expressed as the percentage of attached leaves in September per total number of leaves recorded in July.

Tab. 2.3 Developmental stages of leaf flushing in beech and spruce.

Species	Stage	Definition
Beech	0	buds closed, unswollen
	1	buds swollen and elongated, leaf tip not visible
	2	green leaf tip at bud visible
	3	first leaf visible, folded
	4	leaves unfolded but hanging
	5	leaves horizontally positioned, advanced length growth, axes still hanging
	6	leaf and shoot development completed
Spruce	0	buds closed, unswollen
	I	buds swollen, green needles shining through
	II	bud break, bud sheath partially thrown off
	III	beginning of axis length growth
	IV	advanced length growth, new axis > 3 cm
	V	needles of new shoots slightly spread
	VI	length growth completed

2.1.4 Visual O_3-induced leaf injury symptoms

During the growing season, beech and spruce trees were checked regularly for appearance of visual O_3 injury symptoms on leaves and needles. Beech injury induced by O_3 appears as bronze leaf discoloration ("bronzing"), chlorotic and necrotic dots or small areas of necrosis which occur in isolation or spread across the leaf lamina. Ozone-induced injury symptoms on beech leaves were quantified as percentage of affected in total foliage area. In spruce needles, O_3 injury appears as yellowish chlorotic mottling and banding and was expressed as percentage of symptomatic individuals within all spruce trees in each container.

2.1.5 Assessment of plant biomass

The initial tree biomass was assessed during mid-April in two containers per treatment by a subsample of six or three trees per species in mono- or mixed culture each, respectively (1st

harvest; cf. Tab. 2.1). Final harvest was performed in four containers per treatment. Dry mass of shoot (buds, foliage, axes, stem) and root organs (fine roots ≤ 2 mm, coarse roots > 2 mm in diameter) was assessed after drying for 72 h at 65 °C to constant weight (Tab. 2.4).

Tab. 2.4 Above- and belowground biomass fractions of beech and spruce trees as assessed in April and September of the growing season of 2005.

Aboveground	Belowground
buds	fine roots (Ø ≤ 2 mm)
beech leaves (only in Sep)	coarse roots (Ø > 2mm)
spruce current-year needles	
spruce older needles	
current-year axes	
older axes	
stem	

Between May 11 and June 3, 2005 a portion of beech trees suffered from an infestation by the root pathogen *Cylindrocarpon destructans*. Beech individuals, showing wilting symptoms were substituted by trees with similar stem base diameter and shoot length. About 35 % of infested beech trees were replaced in the mono- and mixed culture under $1xO_3$ (Fig. 2.2). The proportion of beech trees replaced under $2xO_3$ was 40 % (monoculture) and 52 % (mixed culture). The substitutes were excluded from measurements. A semi-quantitative PCR-based assay was employed to coarse root material of harvested study trees (HAMELIN *et al.* 1996). DNA extraction and purification was performed as described in Luedemann *et al.* (2005) using the Plant DNeasy Minikit (Qiagen GmbH, Hilden, Germany) and the Wizard DNA Clean up System (Promega GmbH, Mannheim, Germany). Except for one beech tree, which was eliminated from statistical evaluations, no infestation was observed.

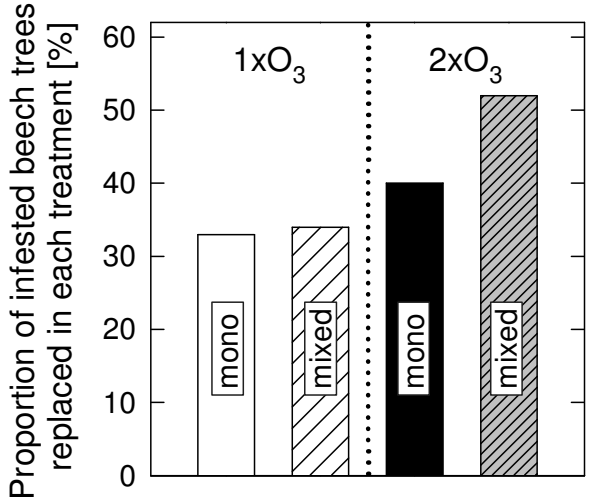

Fig. 2.2 Proportion of infested beech trees replaced in each treatment (n = 4 containers). Data were assessed on June 3 of the growing season of 2005. Plants were growing under the $1xO_3$ and $2xO_3$ regime. Open and solid bars denote monocultures, and hatched bars the mixed cultures.

2.1.6 Relative annual shoot axes biomass increment

The relative annual shoot axes biomass increment (RBI) was calculated after Eqn. 2.1, where Bio_{2004} and Bio_{2005} are the shoot axes biomass at the end of the growing season of 2004 and 2005, respectively (cf. KOZOVITS *et al.* 2005b). Using the basal stem diameter (x) as input data, Bio_{2004} was calculated *via* an allometric relationship in beech ($y = 0.015 * x^{3.0578}$; GAYLER *et al.* 2009), and a linear function in spruce ($y = 1.292 * x - 3.742$; GAYLER, unpublished).

$$RBI = \frac{Bio_{2005} - Bio_{2004}}{Bio_{2004}} * 100 \quad [\%] \qquad \text{Eqn. 2.1}$$

2.1.7 Relative annual cross-sectional stem area increment

Relative annual cross-sectional stem area increment of the growing season of 2005 was calculated from the basal cross-sectional stem area as determined at the end of the growing season of 2004 and 2005, respectively. Calculation was performed in analogy to Eqn. 2.1.

2.1.8 Foliage area and specific leaf area (SLA)

Total foliage of each tree was scanned immediately after the harvest with fresh plant material. Scanning was performed with CanonScan LiDE 35 software with a resolution of 300 dpi. For spruce, current-year and older foliage was scanned separately. Projected foliage area of each tree was calculated using Image J 1.37v software. The specific leaf area (SLA) is defined as the ratio of projected leaf area versus leaf dry mass, expressed as $m^2\ kg^{-1}$. Foliar dry mass was assessed after drying leaves and needles for 72 h at 65 °C to constant weight.

2.1.9 Leaf chlorophyll fluorescence

The operating efficiency of photosystem II (PS II) of leaves ($F_q'/F_m' = (F_m'-F)/F_m'$; cf. BAKER 2008) was assessed at about monthly intervals from May 30 through August 22 using a portable pulse-amplitude modulation fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). During measurements on light exposed leaves, PPFD in the phytotrons was kept constant. Noncyclic electron transport rate through PS II (ETR) was calculated according to (F_q'/F_m') * PPFD * *a* * *f*, as the light absorptivity factor *a* was assigned to 0.84 and the light partitioning factor *f*, distributing energy between PS I and II, was set to 0.5.

2.1.10 Assessment of leaf gas exchange

Measurements of leaf gas exchange were conducted by means of a programmable gas exchange equipment (HCM-1000, open flow CO_2/H_2O porometer equipped with infrared gas analysers, H. Walz GmbH, Effeltrich, Germany) on sun leaves (performed by Dr. J. B.

WINKLER, Helmholtz Zentrum München, Neuherberg). CO_2 response of the net CO_2 uptake rate (i.e. A/c_i curves) was measured under standardized temperature (20 °C cuvette temperature), 50-60 % air humidity and saturating PPFD (1200 µmol m^{-2} s^{-1}). Net CO_2 uptake rate measured at 380 µmol CO_2 mol^{-1} of the ambient air (A_{max}) was derived from the A/c_i curves and was based on the one-sided surface area and the projected area of beech leaves and spruce needles, respectively. In the case of beech measurements were taken during August 03 through September 07, and in the case of spruce during July 05 through July 29.

2.1.10.1 Modeling of seasonal net C gain and transpiration

Maximum carboxylation rate of ribulose-1,5-bis-phosphate-carboxylase/oxygenase ($V_{C,max}$) and maximum rate of electron transport driving the regeneration of ribulose-1,5-bisphosphate (J_{max}) were calculated from the A/c_i curves (VON CAEMMERER & FARQUHAR 1981). Quantum yield of CO_2 assimilation (ΦCO_2) was derived from the initial slope of the photosynthetic light response curves at saturating CO_2 concentration. The derived parameters were used in beech and spruce to parametrise the "PSN6 - leaf gas exchange model for trees" (FALGE *et al.* 1996). Using data on PPFD, relative air humidity, air temperature and foliage area as input values, the photosynthesis model was employed to calculate the seasonal (May 11 - September 07) net C gain and transpiration of the trees.

2.1.11 $^{15}N/^{14}N$ labeling

A 9-day $^{15}N/^{14}N$ labeling experiment was applied prior to the final harvest (cf. Tab. 2.1). Each container was irrigated with 1 liter of a 1.6 mM double-labeled NH_4NO_3 solution (≥ 98 % $^{15}NH_4{}^{15}NO_3$, Cambridge Isotope Laboratories) on a Hoagland basis. In this way 253.5 mg ^{15}N per m² were added and each container received 48.8 mg of ^{15}N.

2.1.12 $^{13}CO_2/^{12}CO_2$ labeling

$^{13}CO_2/^{12}CO_2$ labeling was applied to the trees in parallel to the $^{15}N/^{14}N$ labeling (section 2.1.11). During the 14h-light period of five subsequent days CO_2 of ≥ 99 atom % ^{13}C (Campro Scientific, Berlin, Germany) was continuously supplied at a flow rate of 2 mL min^{-1} into the phytotron chambers and mixed with ambient chamber air. In this way, $\delta^{13}C$ of chamber air ($\delta^{13}C_{Air}$) was raised from -9.8 ± 0.1 ‰ to + 111.4 ± 2.5 ‰ (mean ± SE; n = 4) (Fig. 2.3). In one phytotron $\delta^{13}C_{Air}$ was increased only by 22.6 ± 8.9 ‰ during labeling days 1 to 3. This was compensated for by doubling of the flow rate during day 4 and 5. By this, the same amount of ^{13}C was taken up by the trees across the four chambers ($p > 0.05$, data not shown). On day 0 and during the 5-day labeling period, climatic conditions of a typical day in August were replicated in the phytotrons (PPFD of 581 ± 3 µmol m^{-2} s^{-1}, day-time and night-time means of T_{air} of 22.2 ± 0.1 and 15.2 ± 0.1 °C and of RH of 63 ± 0 and 78 ± 0 %, respectively). Photosynthetic photon flux density, relative air humidity and air temperature data were recorded every 5 minutes. After the 5-day labeling period, the chamber air temperature was

decreased to + 5 °C and light was switched off to suppress plant physiological processes for up to two days until harvest was completed.

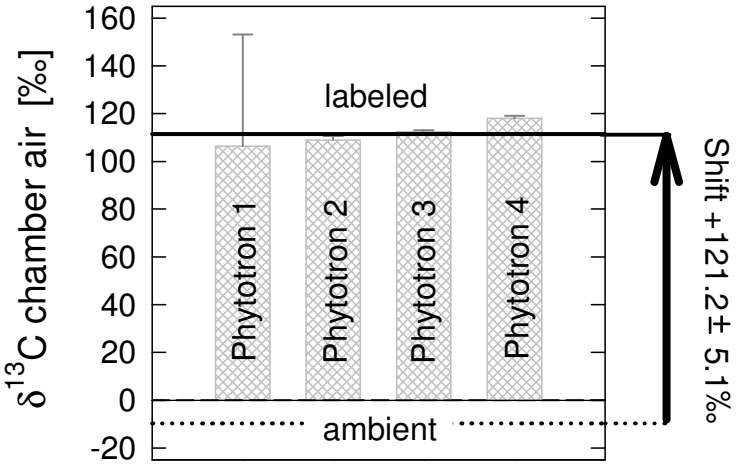

Fig. 2.3 $\delta^{13}C$ of ambient (-9.8 ± 0.2 ‰) and labeled chamber air (+111.4 ± 2.5 ‰). On average, the $\delta^{13}C$ shift in the air was +121.2 ± 5.1 ‰ (mean ± SE; n = 4 phytotrons). Gray bars: $\delta^{13}C$ of labeled chamber air (means ± SE; n = 5 days).

2.1.13 Assessment of stem CO_2 efflux

CO_2 efflux from beech and spruce stems was sampled from June 20 through September 18 with a custom-made open gas exchange system (GRAMS *et al.* 2010b; Fig. A-1, Appendix). Tank CO_2 with a $\delta^{13}C$ of -3.2 ‰ was added to CO_2-free air (Zander KEN 3100, Essen, Germany) to maintain a constant reference CO_2 concentration of 360 µmol mol^{-1} inside the system. Absolute CO_2 concentration was measured by means of an infrared gas analyzer operated in absolute mode (IRGA Binos 4b.1, Rosemount AG, Hanau, Germany). Air was moisturized to prevent stems from drying out. To avoid condensation of water vapor in tubes the dew point was set to + 5 to 10 °C. Two manifolds splitted the air stream into 18 sub-air streams which were pushed through 30 m long PVC tubes to two empty cuvettes and 16 stem cuvettes, where surplus air was blown off. In each planting container two or three trees were supplied with stem cuvettes (Plexiglas®, Röhm GmbH, Darmstadt, Germany) with a length of either 8 or 10 cm and 5 cm in diameter. From each cuvette, gas was continuously sucked by a series of membrane pumps (ASF Thomas, Wisa, Wuppertal, Germany) achieving an almost ambient air pressure inside the cuvettes. Flow rates of air were adjusted to 0.2 L min^{-1} in stem cuvettes and to 0.4 L min^{-1} in empty cuvettes. The 18 sub-air streams from the 18 cuvettes were sequentially conveyed by computer programmed solenoids for six minutes, each, to CO_2 analysis (IRGA, Binos 100 4P, Rosemount AG, Hanau, Germany). Measurements were made at the end of each six-minute interval upon reaching stable CO_2 levels in the IRGA. The stem CO_2 efflux was assessed as the difference in CO_2 concentration between the sample gas passing through a stem cuvette ($[CO_2]_{sample}$ in µmol mol^{-1}) and the reference gas passing through an empty cuvette ($[CO_2]_{reference}$ in µmol mol^{-1}). Gas deriving from a stem cuvette or an empty cuvette was sampled using a programmable Gilson 221 XL Autosampler device (Gilson Inc. Middleton, USA), interfaced to the open stem gas exchange system. The sampling device was implemented with five racks and a total of 220 storing positions for 12 mL glass vials (Exetainer, Labco Limited, High Wycombe, UK) which were flushed for six minutes, each, at a flow rate of 0.15 L min^{-1} with sample gas.

2.1.13.1 Stem CO_2 efflux rate

The rate of stem CO_2 efflux (E_s in µmol m^{-3} s^{-1}) was based on the total stem volume enclosed by a stem chamber (V_s in m³) and calculated as follows:

$$E_s = \frac{Flow * (CO_{2sample} - CO_{2reference})}{22.414 * V_s * 60} \quad \text{Eqn. 2.2}$$

where,

Flow = flow rate of air through the cuvette [L min^{-1}]
22.414 = molar volume of an ideal gas (at standard temperature, T_0 = 273.15 K and standard pressure, P_0 = 1.013 bar) [L mol^{-1}]

2.1.13.2 $\delta^{13}C$ of stem CO_2 efflux

$\delta^{13}C$ of CO_2 deriving from stem efflux ($\delta^{13}C_{Es}$ in ‰) was calculated using a 2-pool mixing model (FRY 2006):

$$\delta^{13}C_{Es} = \frac{(CO_{2sample} * \delta^{13}C_{sample}) - (CO_{2reference} * \delta^{13}C_{reference})}{(CO_{2sample} - CO_{2reference})} \quad \text{Eqn. 2.3}$$

where,

$\delta^{13}C_{sample}$ = $\delta^{13}C$ signature of sample gas from a stem cuvette [‰]
$\delta^{13}C_{reference}$ = $\delta^{13}C$ signature of sample gas from an empty cuvette [‰]

2.1.14 $\delta^{13}C$ analysis of gas samples

Gas samples were analyzed within 48 hours on an isotope-ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany) interconnected to a gas autosampler. Carbon isotope ratios were expressed in δ-notation using Vienna PeeDee Belemnite (VPDB) as the standard (Eqn. 2.4). Based on iterated measurements of a laboratory working standard, the precision of $\delta^{13}C$ measurements was < 0.1‰ (SD, n=10).

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{VPDB}} - 1 \right) * 1000 \quad [‰] \quad \text{Eqn. 2.4}$$

where R_{sample} and R_{VPDB} represent the isotopic ratios of $^{13}C/^{12}C$ of CO_2 in gas samples and VPDB, respectively.

2.1.15 Fraction of new C in stem CO_2 efflux

The fraction of C assimilated during $^{13}CO_2/^{12}CO_2$ labeling and detected in the stem CO_2 efflux is hereafter refered to as "fraction of new C in stem CO_2 efflux ($f_{E,new}$)". $f_{E,new}$ was calculated (LÖTSCHER & GAYLER 2005) as follows:

$$f_{E,new} = (\delta^{13}C_{Es} - \delta^{13}C_{Ec}) / (\delta^{13}C_{El} - \delta^{13}C_{Ec}) \quad \text{Eqn. 2.5}$$

$$\delta^{13}C_{El} = ([\delta^{13}C_{Airl} - \Delta^{13}C] / [1000 + \Delta^{13}C]) * 1000\ [‰] \quad \text{Eqn. 2.6}$$

$$\Delta^{13}C = ([\delta^{13}C_{Airc} - \delta^{13}C_{Ec}] / [1000 + \delta^{13}C_{Ec}]) * 1000\ [‰] \quad \text{Eqn. 2.7}$$

where,

$\delta^{13}C_{El}$ = $\delta^{13}C$ of stem CO_2 efflux of a tree grown continuously with labeled CO_2 [‰] (calculated after Eqn. 2.6 and 2.7)
$\delta^{13}C_{Es}$ = $\delta^{13}C$ of stem CO_2 efflux during labeling [‰]
$\delta^{13}C_{Ec}$ = $\delta^{13}C$ of stem CO_2 efflux before labeling [‰]
$\Delta^{13}C$ = ^{13}C discrimination during plant uptake [‰]
$\delta^{13}C_{Airl}$ = $\delta^{13}C$ of chamber air during labeling [‰]
$\delta^{13}C_{Airc}$ = $\delta^{13}C$ of chamber air before labeling [‰]

$\delta^{13}C_{Es}$, $\delta^{13}C_{Ec}$, $\delta^{13}C_{Airl}$ and $\delta^{13}C_{Airc}$ were calculated as 24h-flux-weighted means:

$$\overline{\delta^{13}C_{E_x}} = \frac{\sum_1^n (E_s * \delta^{13}C_E * \Delta_t)}{\sum_1^n (E_s * \Delta_t)} \quad \text{Eqn. 2.8}$$

$$\overline{\delta^{13}C_{Air_x}} = \frac{\sum_1^n (A * \delta^{13}C_{Air} * \Delta_t)}{\sum_1^n (A * \Delta_t)} \quad \text{Eqn. 2.9}$$

where,

$\delta^{13}C_{Ex}$ = $\delta^{13}C$ of stem CO_2 efflux before or during labeling [‰]
$\delta^{13}C_{Airx}$ = $\delta^{13}C$ of chamber air before or during labeling [‰]
A = modeled net CO_2 assimilation rate [µmol m^{-2} s^{-1}]
Δ_t = time interval between two measurements [min]
n = $\frac{\Delta_t}{1440}$

2.1.16 Compartmental modeling of C pools supplying stem respiration

The time course of label incorporation into the respiration of the beech and spruce stems was analyzed with compartmental modeling (performed by Dr. C. LEHMEIER, Grassland Science, Technische Universität München). A "two-pool model" was tested on its ability to reproduce the carbon labeling kinetics in the stem respiration of the trees. For this purpose, the assumption was made that the total stem CO_2 efflux of the trees exclusively consists of stem-respired CO_2. The model was translated into a set of mathematical equations under the assumptions that the system was in a steady-state (i.e. pool sizes and fluxes do not change with time) and that fluxes obey first-order kinetics (i.e. fluxes are the product of pool sizes times a rate constant). The fraction of unlabeled C ($f_{\text{unlabeled-C}} = 1 - f_{\text{E,new}}$) in each pool with respect to time is given by:

(2.10 a) $$f_{\text{unlabeled-C-Q1}} = (Q_1 * f_{\text{unlabeled-C-Q1}} + F_{in} * f_{\text{labeled-C}} - F_{12} * f_{\text{unlabeled-C-Q1}} + F_{21} * f_{\text{unlabeled-C-Q2}} - F_{10} * f_{\text{unlabeled-C-Q1}}) / Q_1$$

(2.10 b) $$f_{\text{unlabeled-C-Q2}} = (Q_2 * f_{\text{unlabeled-C-Q2}} + F_{12} * f_{\text{unlabeled-C-Q1}} - F_{21} * f_{\text{unlabeled-C-Q2}}) / Q_2$$

(2.10 c) $$f_{\text{unlabeled-C}} = f_{\text{unlabeled-C-Q1,}}$$

where Q_1 and Q_2 are the pool sizes, and F_{in} is the flux of carbon assimilated in photosynthesis that enters the pool system in Q_1. Since the system is in steady-state, F_{in} equals F_{10} (which is the specific respiration rate of the plant expressed in mg stem-respired C g^{-1} plant-C h^{-1}) and F_{12} equals F_{21}. Indices refer to donor and receptor pools, respectively; index 0 represents the environment. The measured parameter which the model prediction is compared to, is $f_{\text{unlabeled-C}}$, i.e. the fraction of unlabeled C in stem-respired CO_2. $f_{\text{unlabeled-C-Qi}}$ is the fraction of unlabeled C in a pool Q_i, and $f_{\text{labeled-C}}$ is the constant fraction of fully labeled C entering the plant after the onset of labeling. Equations 2.10 a-c were transferred into a custom-made program using the software R (R Development Core Team, 2007) and the model optimization was carried out as described by LEHMEIER *et al.* (2008).

Optimized fluxes and pool-sizes served to determine the half-times of the pools ($t_{1/2}$):

(2.10 d) $$t_{1/2}(Q_1) = \ln(2) / ((F_{10} + F_{12}) / Q_1)$$

(2.10 e) $$t_{1/2}(Q_2) = \ln(2) / (F_{21} / Q_1)$$

and the contribution of a pool Q_i (C_{Qi}) to stem respiration, which is defined here as the probability of label moving in a certain flux of the system:

(2.10 f) $$C_{Q1} = F_{10} / (F_{10} + F_{12})$$

(2.10 g) $$C_{Q2} = F_{12} / (F_{10} + F_{12})$$

2.1.17 C and N isotope and element concentration analysis of organic samples

Dried plant samples were ground to a fine powder with a steel ball mill (Retsch MM 2000, Haan, Germany) and 2 mg were weighted into tin capsules for carbon and nitrogen isotope ratio analysis. All biomass fractions were analyzed on an EA 3000 Elemental Analyzer (Euro Vector Instruments and Software, Milan, Italy) coupled with an isotope-ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany) for both carbon and nitrogen isotope and element concentration analysis. The isotopic signal for C was expressed as $\delta^{13}C$ in ‰ (cf. Eqn. 2.4) and that for N was expressed as an absolute proportion atom % ^{15}N (Eqn. 2.11). The precision of measurement for $\delta^{13}C$ was < 0.1 ‰ and for $\delta^{15}N$ < 0.2 ‰ based on repeated measurements of a laboratory working standard (SD; n = 10).

$$\text{Atom\% } ^{15}N = \frac{^{15}N}{^{15}N + ^{14}N} * 100 \qquad \text{Eqn. 2.11}$$

2.1.18 Calculation of whole-tree C and N partitioning

The carbon and nitrogen partitioning of the trees was assessed by performing a $^{13}CO_2/^{12}CO_2$ and $^{15}N/^{14}N$ labeling (sections 2.1.11 and 2.1.12). Partitioning of a labeled element was defined as the proportion of newly incorporated element into a plant organ relative to the total labeled element in the whole plant (DELÉENS *et al*. 1994). The partitioning (P_E in %) of the "new" C and N was calculated according to DYCKMANNS & FLESSA (2001, 2002):

$$P_E = \frac{E_{\text{organ}} * f_{\text{new organ}}}{E_{\text{plant}} * f_{\text{new plant}}} * 100 \qquad \text{Eqn. 2.12}$$

$$f_{\text{new}} = \frac{E_p - E_c}{E_l - E_c} * 100 \qquad \text{Eqn. 2.13}$$

where,

E_{organ} = amount of C or N in the specific plant organ
E_{plant} = amount of C or N in the whole plant
f_{new} = fraction of newly incorporated C or N in organ or whole plant tissue
E_p = $\delta^{13}C$ or atom % ^{15}N of a plant sample after labeling
E_c = $\delta^{13}C$ or atom % ^{15}N of a plant sample before labeling
E_l = $\delta^{13}C$ of a plant sample grown continuously with labeled CO_2 or atom % ^{15}N of the labeled nutrient solution (calculated after Eqn. 2.6 and 2.7)

2.1.19 Soil CO_2 efflux rate

Soil CO_2 efflux rates were measured by means of a soil CO_2 efflux chamber which was connected to an EGM-3 Environmental Gas Monitor (PP-Systems, Hitchin, UK). The chamber was internally equipped with a fan for adequate mixing of chamber air before measurement. The chamber was attached to 1 cm depth in the soil, along with a portable temperature probe (STP-1, PP-Systems, Hitchin, UK) to determine soil temperature at 8 cm depth. When a measurement was started, air was continuously circulated through the closed system and CO_2 concentration was recorded by 8 sec intervals. The rate of soil CO_2 efflux was calculated from the increase in CO_2 concentration (60 µmol mol^{-1} at maximum) over time (120 sec at maximum), the chamber volume (550 cm^3) and the enclosed soil surface area (38.5 cm^2). Measurements were made at three randomly selected and marked positions within each container. For each container an average of three measurements was calculated as soil CO_2 efflux rate.

2.1.20 Statistical analyses

Data were analysed using the SPSS 16.0. software package (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) was performed to test main effects of ozone, type of competition and interactions between the two factors on investigated parameters at a significance level of $p < 0.05$. Containers were employed as experimental units. All data were checked for skewed distribution.

2.2 Results

2.2.1 Phenology of shoots

In beech, leaf flushing started earlier and evolved faster in the monoculture under $1xO_3$ than in the other treatments (Fig. 2.4). Leaf flushing in beech was during progressive flushing stages significantly retarded by $2xO_3$. Likewise, interspecific competition tended to delay leaf flushing, which became significant only towards completion of flushing by mid-June. In spruce, leaf flushing started in the monoculture under $2xO_3$ almost two weeks later than in the other treatments. Since the beginning of axis extension growth, interspecific competition tended to delay leaf flushing. Elevated O_3 affected shoot phenology in spruce significantly only towards the end of flushing. Overall, differences in shoot phenology were more pronounced in beech than in spruce.

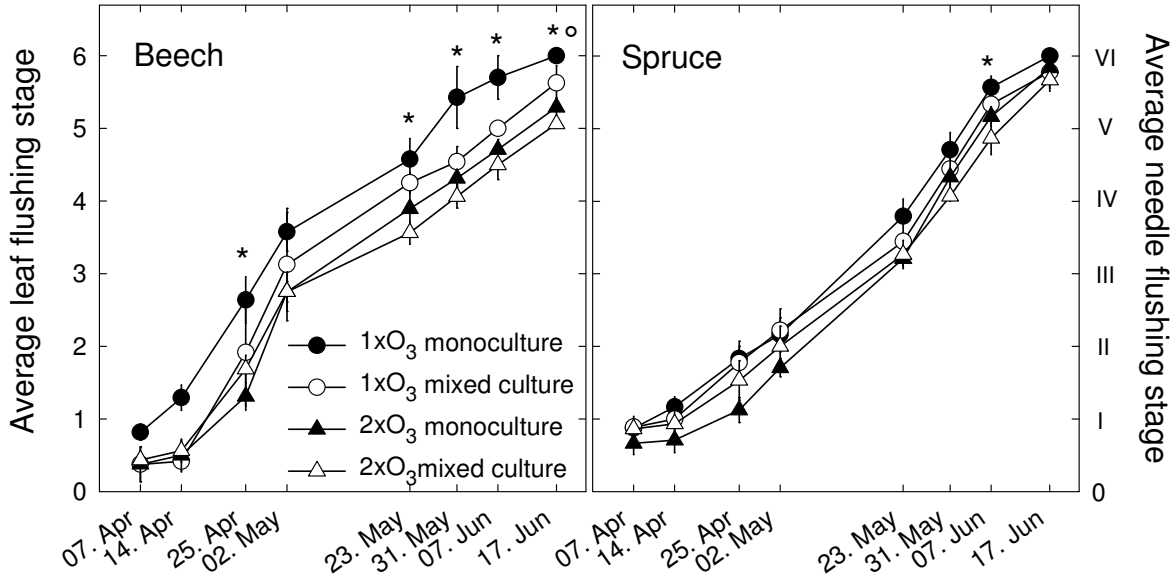

Fig. 2.4 Flushing of beech and spruce recorded throughout the growing season of 2005. Circles denote $1xO_3$ and triangels $2xO_3$ regimes. Monocultures are given as solid and mixed cultures as open symbols. Data are presented as means ± SE (n = 4 containers). Significant main effects by O_3 and type of competition are indicated by * and °, respectively. Stages of beech: (0) buds closed and unswollen (1) buds swollen and elongated, non-green, (2) green leaf tip at bud visible, (3) first leaf visible, folded, (4) leaves unfolded but hanging, (5) leaves horizontally positioned, (6) leaf and shoot development completed. Stages of spruce: (0) buds closed, unswollen, (I) buds swollen, green needles shining through, (II) bud break and bud sheath partly thrown off, (III) beginning of axis length growth, (IV) advanced length growth, new axis > 3 cm, (V) needles of new shoots slightly spread, (VI) length growth completed.

2.2.2 Senescence of beech leaves

Senescence of beech leaves was quantified as percentage of attached leaves in early September relative to the total leaf number as assessed in July (Fig. 2.5). Growth in the mixed culture significantly stimulated leaf senescence in beech. The relative fraction of attached leaves was diminished by 9.5%, irrespective of the O_3 treatment.

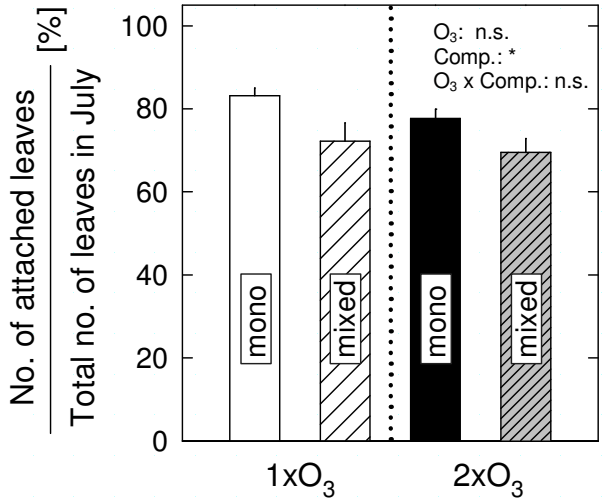

Fig. 2.5 Percentage of attached leaves in early September relative to total leaf number in July (means ± SE, n = 4 containers). Open and solid bars denote monocultures, and hatched bars the mixed cultures under $1xO_3$ and $2xO_3$ regime, respectively. Significant differences between mono- and mixed cultures are denoted by * ($p < 0.05$).

2.2.3 Visual O_3-induced injury symptoms

Leaf injury symptoms appeared in both species primarily in June and reached maximal expression in August (Fig. 2.6). From July on, ozone and competition had a significant effect on the formation of leaf chloroses in beech (Fig. 2.6 A). In August, the percentage of leaf chloroses was 1.9 times higher under the $2xO_3$ than the $1xO_3$ regime. Chlorotic leaf injury in the mixed cultures exceeded that of the monocultures by a factor of 1.4 at that time. At the end of August leaf necrotic injury in beech was significantly enhanced by elevated O_3 (Fig. 2.6 B). In spruce, the percentage of individuals showing chlorotic mottling symptoms was significantly increased by elevated O_3 only in August (Fig. 2.7). Under the $2xO_3$ regime chlorotic mottling was more than twice as high as compared with the extent under $1xO_3$, irrespective of the plantation types.

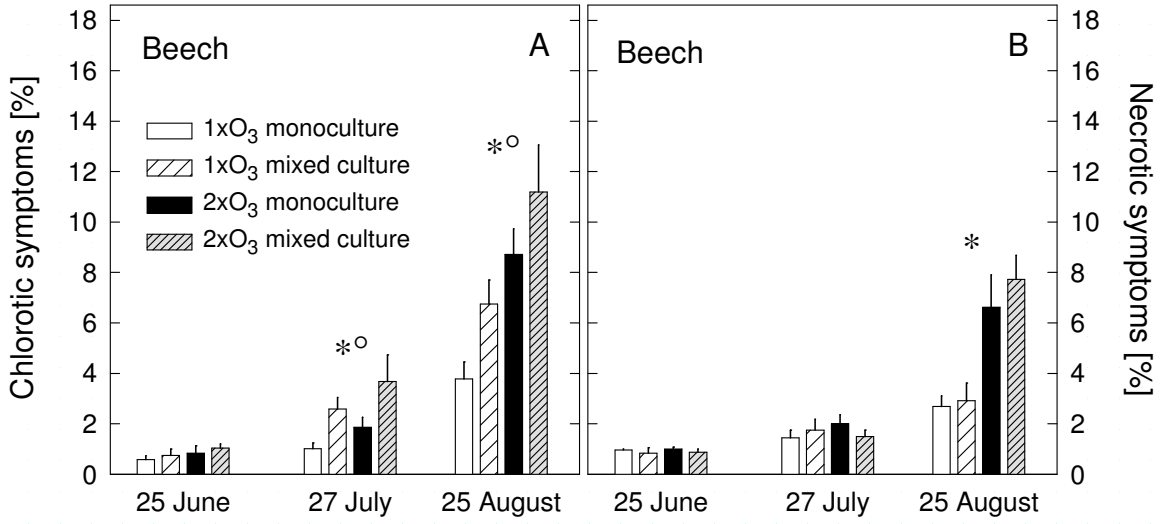

Fig. 2.6 Percentage of total beech foliage area showing O_3-induced chlorotic (A) and necrotic (B) injury symptoms (means ± SE, n = 4 containers). Open and solid bars denote monocultures and hatched bars represent mixed cultures under $1xO_3$ and $2xO_3$ regime. Significant differences between O_3 regimes and types of competition are denoted by * and °, respectively ($p < 0.05$).

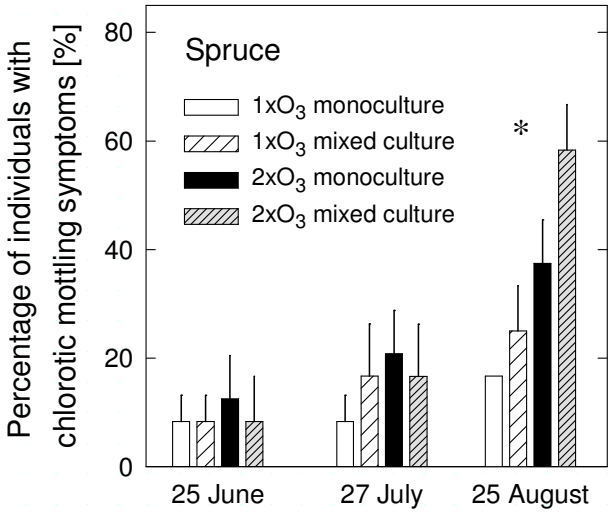

Fig. 2.7 Perercentage of spruce individuals showing chlorotic mottling on current-year needles (means ± SE, n = 4 containers). Open and solid bars denote monocultures, and hatched bars represent mixed cultures. Significant differences between the $1xO_3$ and $2xO_3$ regime are denoted by * ($p < 0.05$).

2.2.4 Total aboveground and belowground biomass

After two growing seasons under elevated O_3 exposure and interspecific competition, total shoot and root biomass, as well as the root to shoot biomass ratio, were neither affected by O_3 nor the type of competition within each species (Fig. 2.8). Elevated O_3 increased the fine root biomass in beech significantly. On average, spruce established 72% more aboveground and 30% less belowground biomass than beech ($p < 0.05$). The root to shoot biomass ratio in beech was about 58% higher compared with spruce ($p < 0.05$). The fine to coarse root biomass ratio amounted to 2.1 in beech and to 1.2 in spruce.

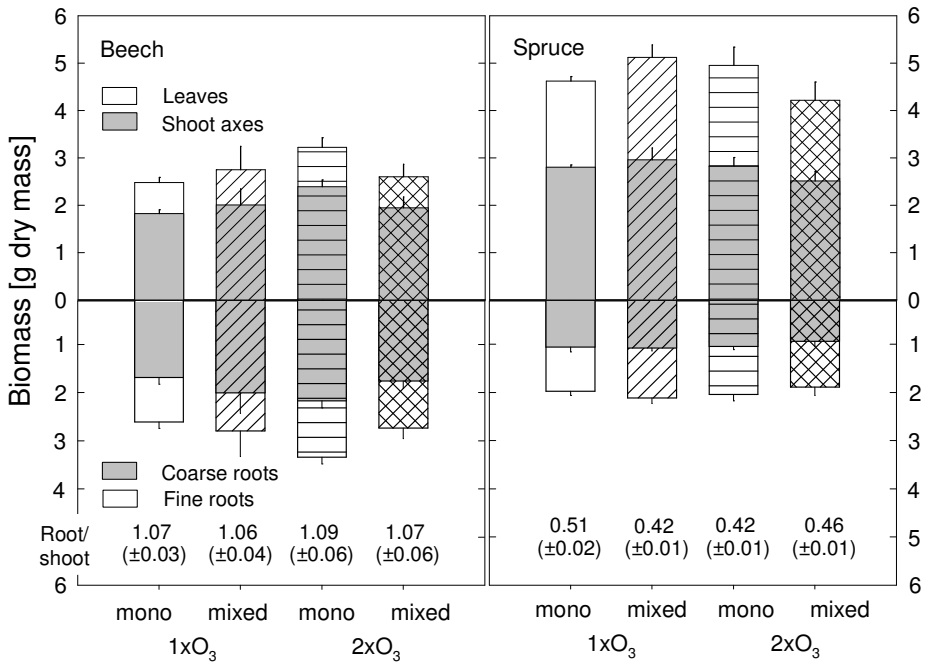

Fig. 2.8 Final biomass of beech and spruce, growing in mono- or mixed cultures under $1xO_3$ or $2xO_3$ regime. Above- and belowground biomass is given above and below the zero line. White bars represent leaves and fine root biomass whereas gray bars represent shoot axes and coarse roots. Root to shoot biomass ratio is inserted below the bar. Elevated O_3 increased the fine root biomass in beech significantly ($p < 0.05$). Data are means ± SE (n = 4 containers).

2.2.5 Development of shoot axes biomass

The development of the shoot axes biomass as determined during two growing seasons under elevated O_3 and interspecific competition is presented in Fig. 2.9 In April 2005, no significant main effect by O_3 or type of competition was observed in beech. In spruce, the shoot axes biomass was significantly diminished by 37% when growing in mixture with beech at that time. However, in September 2005, no significant main effect by O_3 or type of competition was observed in either species.

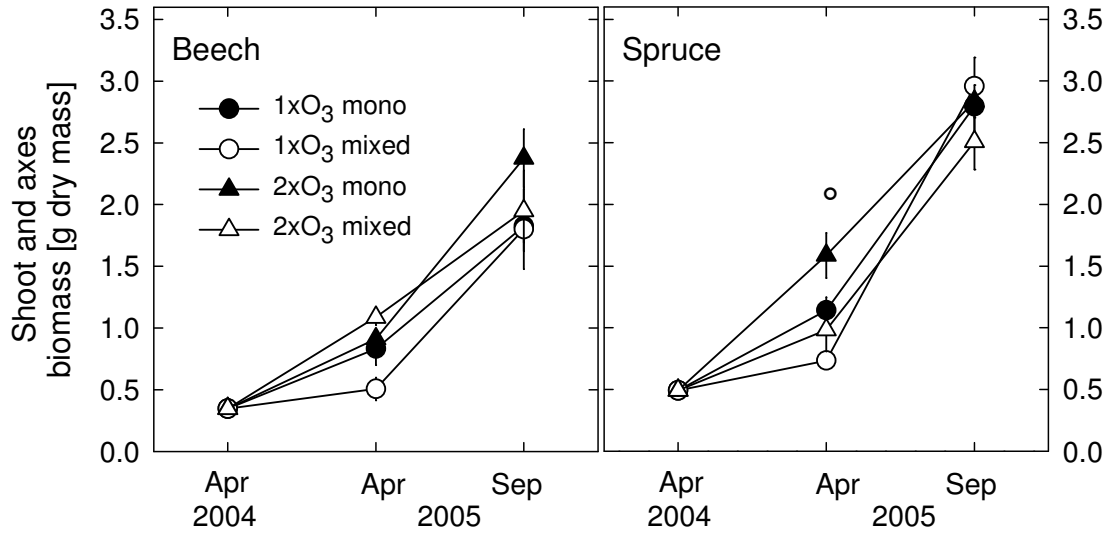

Fig. 2.9 Shoot axes biomass of beech and spruce measured in April 2004 (means ± SE, n = 10 trees, each species) and in April and September 2005 (means ± SE, n = 4 containers). Monocultures are given as solid and mixed cultures as open symbols. Circles denote the $1xO_3$ and triangles the $2xO_3$ regime. In spruce, significant differences between mono- and mixed cultures are indicated by ° ($p < 0.05$).

2.2.6 Relative annual shoot axes biomass increment (RBI) and cross-sectional stem area increment

The relative annual shoot axes biomass increment (RBI) and cross-sectional stem area increment in beech and spruce are compared in Fig. 2.10. The RBI in spruce exceeded that in beech more than twice in the monoculture and more than 5 times in the mixed culture ($p < 0.05$). The relative annual cross-sectional stem area increment in spruce was 1.8 and 1.6 times higher ($p < 0.05$) in the mono- and mixed cultures compared with beech. The largest difference in both increment parameters between beech and spruce was observed under $1xO_3$ regime in the mixed culture. In beech, growth in the mixed culture significantly reduced RBI and cross-sectional stem area increment. At the end of the second growing season under elevated O_3, the air pollutant had no impact on the biomass increments in both species.

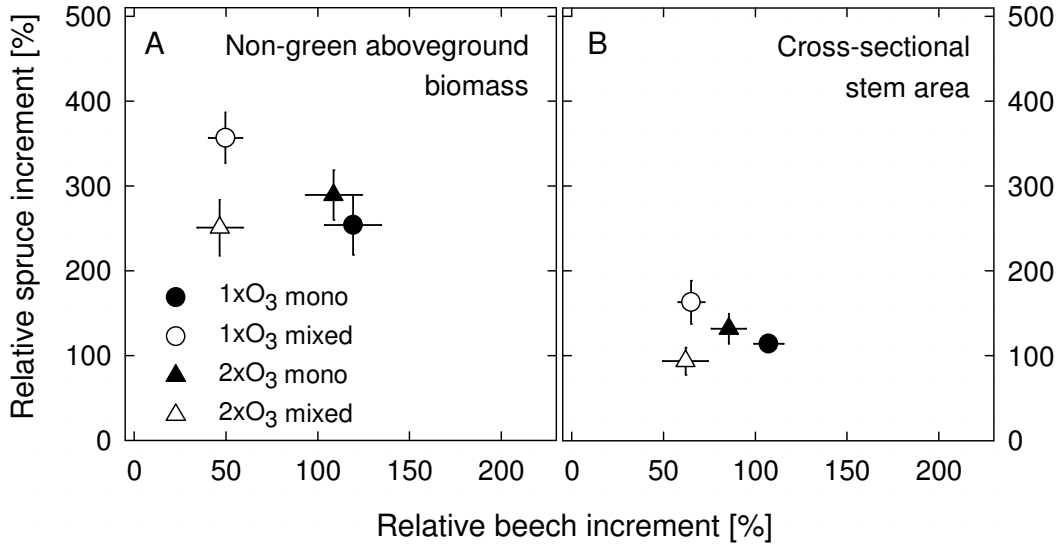

Fig. 2.10 Annual shoot axes biomass increment (RBI) (A) and cross-sectional stem area increment (B) of beech (x-axis) and spruce (y-axis) during the growing season of 2005. Increment parameters were calculated relative to the respective initial values at the end of the preceding growing season. Monocultures are given as solid and mixed cultures as open symbols. Circles denote the $1xO_3$ and triangles the $2xO_3$ regime (means ± SE, n = 4 containers). In beech, significant differences in RBI and cross-sectional stem area appeared between mono- and mixed cultures ($p < 0.05$).

2.2.7 Total leaf area

Overall, spruce displayed a significantly higher total projected leaf area than beech (Tab. 2.5). Growth in the mixture significantly diminished the total leaf area of beech by 12% ($1xO_3$) and by 27% ($2xO_3$). In spruce, total leaf area was not affected by the applied treatments.

Tab. 2.5 Total leaf area in beech and spruce exposed to $1xO_3$ or $2xO_3$ regime in early September (means ± SE, n = 4 containers). Significant differences between mono- and mixed cultures are indicated by ° ($p < 0.05$).

Total leaf area [cm²]	Beech	Spruce
$1xO_3$ monoculture	147.3 ± 5.87	172.5 ± 10.7
$1xO_3$ mixed culture	130.2 ± 31.4 °	185.8 ± 10.8
$2xO_3$ monoculture	167.5 ± 10.3	206.4 ± 16.2
$2xO_3$ mixed culture	122.8 ± 17.8 °	186.7 ± 21.5

2.2.8 Specific leaf area

In beech, the SLA was significantly reduced, both by interspecific competition and elevated O_3 (Fig. 2.11). Reductions in the SLA of beech caused by competition (- 1.6 m^2 kg^{-1}) were more than twice as high as those caused by O_3 (- 0.7 m^2 kg^{-1}). Beech monoculture exposed to $1xO_3$ concentrations reflected the highest SLA (significant interaction of O_3 x competition). The SLA in spruce increased significantly under elevated O_3 by 0.8 m^2 kg^{-1}, whereas competition had no impact.

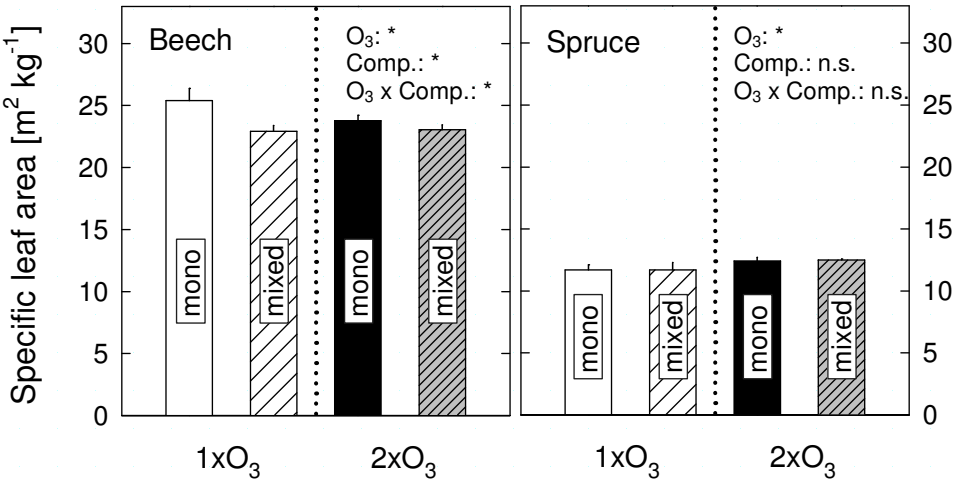

Fig. 2.11 Specific leaf area (SLA) of beech and spruce mono- and mixed cultures growing under $1xO_3$ and $2xO_3$ regime in early September (means ± SE, n = 4 containers). Open and solid bars denote monocultures, and hatched bars represent mixed cultures. Significant effects of the factors O_3 and competition as well as their interaction (O_3 x comp.) are indicated by * ($p < 0.05$).

2.2.9 Leaf chlorophyll fluorescence

Photosystem II operating efficiency (Fq'/Fm') and apparent electron transport rate (ETR) in beech and spruce leaves are compared in Fig. 2.12. Overall, spruce displayed 2 to 3 times higher levels at Fq'/Fm' and ETR than beech, with the largest differences between both species occuring in August. Except for the decline in Fq'/Fm' and ETR in beech in August, no pronounced seasonal variation was observed in either species. Neither O_3 nor competition had a significant effect on leaf chlorophyll fluorescence in each species.

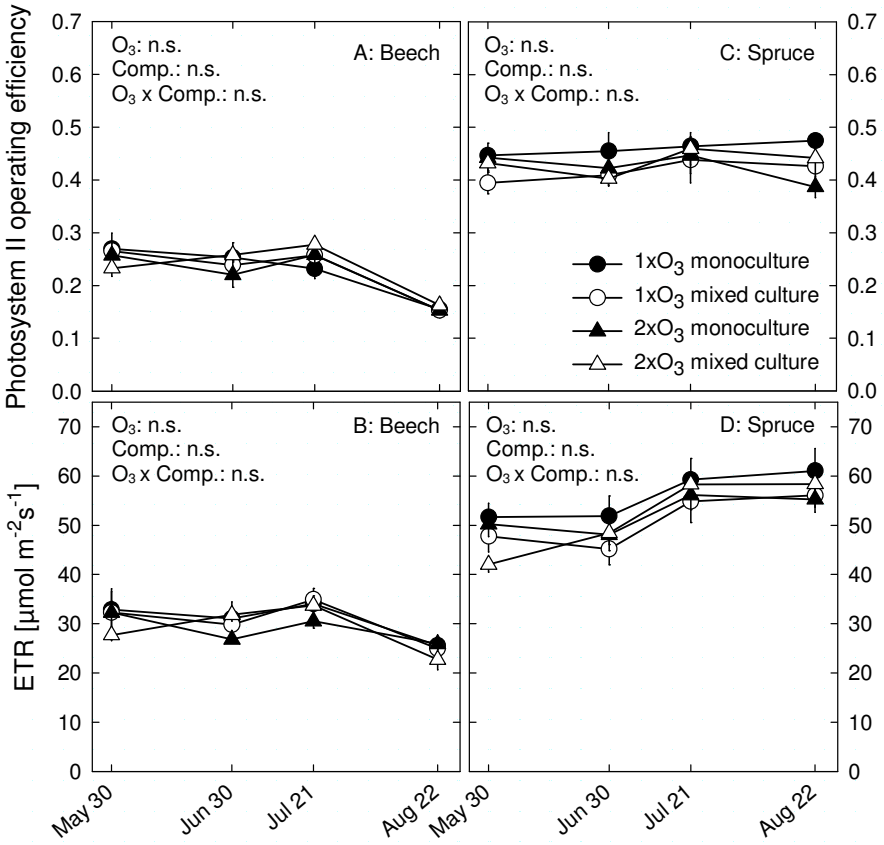

Fig. 2.12 Apparent electron transport rate (ETR) and photosystem II operating efficiency (Fq'/Fm') in beech (A, B) and spruce leaves (C, D) as assessed through the growing period of 2005. Monocultures are given as solid and mixed cultures as open symbols. Circles denote the $1xO_3$ and triangles the $2xO_3$ regime (means ± SE, n = 4 containers). No significant main effect by O_3 or type of competition was found.

2.2.10 Net CO_2 assimilation rate (A_{max})

The net CO_2 assimilation rate (A_{max}) of beech and spruce leaves is compared in Fig. 2.13. In beech, A_{max} levels were found to be about 2 to 4 times lower ($p < 0.05$) compared with spruce. In spruce, A_{max} was significantly reduced by about 25% under elevated O_3. A significant interaction between O_3 x competition increased A_{max} of spruce strongly in the mixed culture under $1xO_3$, relative to the other treatments. No significant effect by competition on either species was observed.

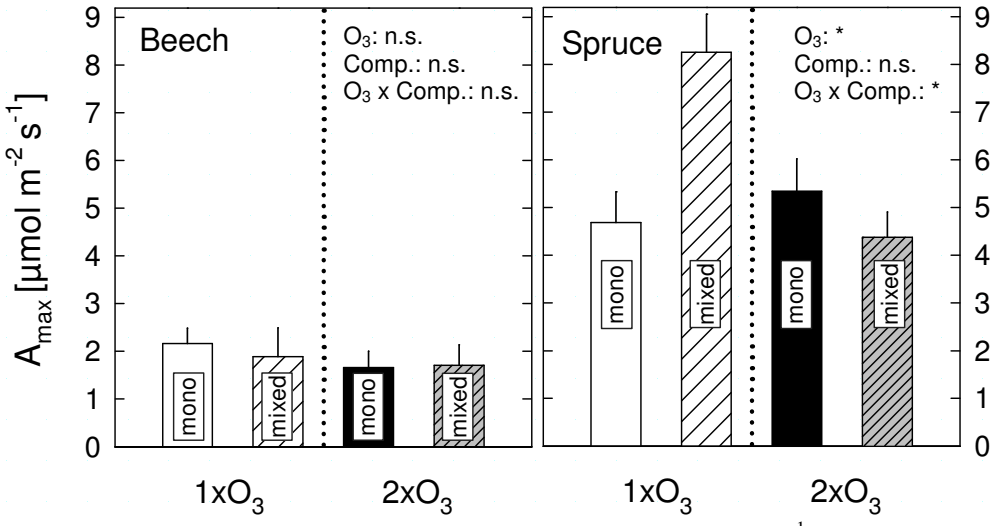

Fig. 2.13 Net CO_2 assimilation rate (A_{max}) measured at 380 µmol mol^{-1} CO_2 of the ambient air and saturating light conditions (1200 µmol m^{-2} s^{-1} PPFD) throughout July (spruce) and August (beech) of the growing season of 2005. Open and solid bars denote monocultures, and hatched bars the mixed cultures. Significant effects of the factor O_3 as well as the interaction O_3 x competition are indicated by * ($p < 0.05$).

2.2.11 Maximum rates of RuBP carboxylation ($V_{C,max}$) and electron transport (J_{max})

Maximum carboxylation rates of ribulose-1,5-bis-phosphate-carboxylase/oxygenase ($V_{C,max}$) and maximum rates of electron transport driving the regeneration of ribulose-1,5-bisphosphate (J_{max}) of beech and spruce leaves are presented in Fig. 2.14. The $V_{C,max}$ levels of spruce were about 3 to 5 times higher ($p < 0.05$) in comparison with beech. The J_{max} levels of spruce exceeded that of beech by about 50% ($p < 0.05$). In beech, irrespective of the treatment, J_{max} was found to be about three times as high as $V_{C,max}$ during measurements in August. During July, J_{max} of spruce was found to be about 1.2 times as high as $V_{C,max}$ under $1xO_3$ and about 2.0 times under $2xO_3$, respectively. In spruce, the levels of J_{max} and $V_{C,max}$ tended to be enhanced in the mixed culture, however, they did not differ significantly from the monoculture. No significant effect by O_3 or competition on either species was observed.

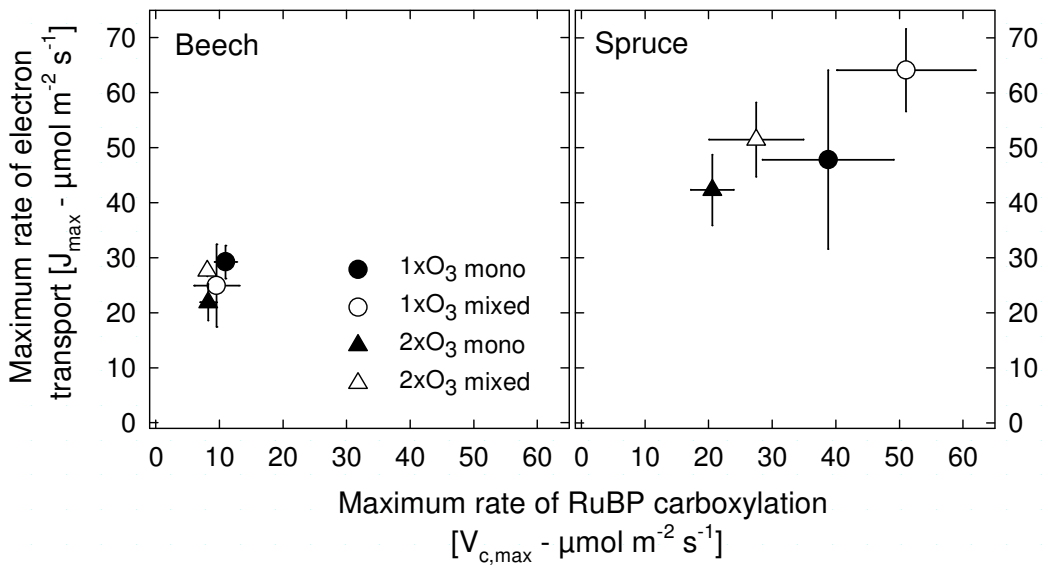

Fig. 2.14 Maximum rates of RuBP carboxylation ($V_{C,max}$) and maximum rates of electron transport (J_{max}) of beech and spruce leaves. Data were assessed throughout July (spruce) and August (beech) of the growing season of 2005 (means ± SE, n = 2 to 4 containers). Monocultures are given as solid and mixed cultures as open symbols. Circles denote $1xO_3$ and triangles $2xO_3$.

2.2.12 Modeled seasonal net C gain and transpiration

The seasonal net C gain and transpiration of beech and spruce trees, modeled for the whole growing season of 2005, are compared in Fig. 2.15. Ozone and competition had no significant impact on the net C gain on either species. Compared with beech, the C gain of the spruce trees was irrespective of the treatments about 1.8 times higher.

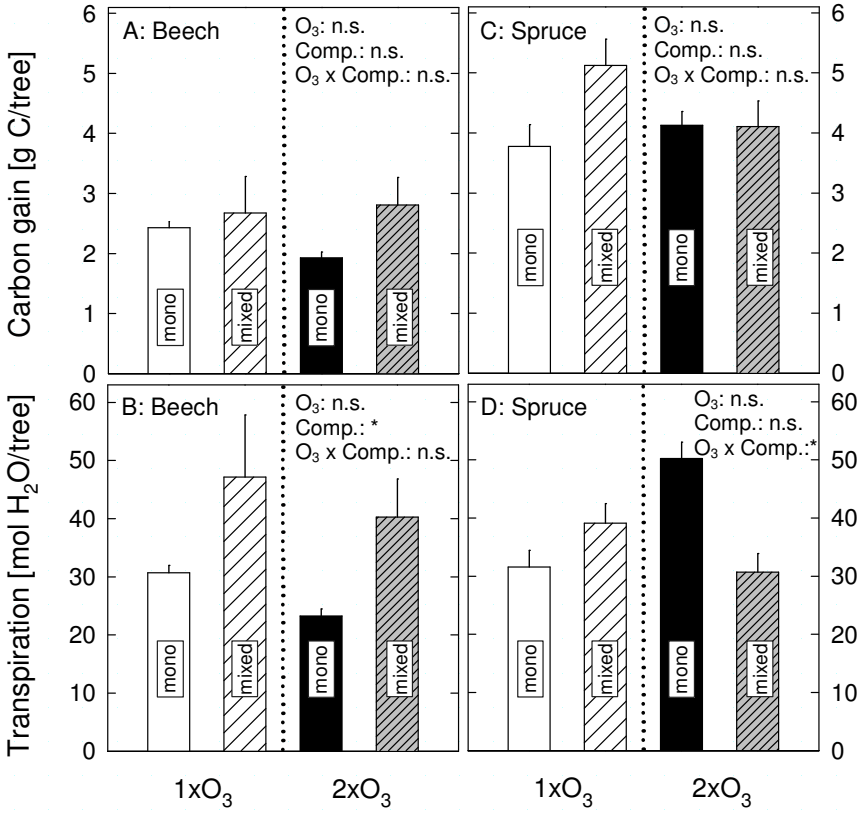

Fig. 2.15 Modeled seasonal net C gain and transpiration of beech and spruce (May 11 to September 07). Data are presented as means ± SE (n = 4 containers). Open and solid bars denote the monocultures and hatching bars the mixed cultures. Significant effects of the factor competition and the interaction O_3 x competition are indicated by * ($p < 0.05$).

In beech, the C gain tended to be enhanced in the mixed culture by 26% (Fig. 2.15 A). The lowest C gain was observed in beech monoculture under $2xO_3$ regime. In spruce, interspecific competition tended to increase the C gain by 17% (Fig. 2.15 C). Transpiration of beech trees growing in the mixed culture exceeded that of the monoculture significantly by 62% (Fig. 2.15 B). Under elevated O_3, beech transpired about 18% less water, however, the O_3 impact was not significant. In spruce, the growth in the mixed culture tended to increase the transpiration under $1xO_3$ by 8%, however, diminished it by 20% under $2xO_3$ (Fig. 2.15 D). As single main factors, O_3 and competition had no significant impact on the transpiration of spruce. However, a significant interaction between O_3 x competition increased the transpiration in the spruce monoculture under $2xO_3$ strongly, relative to the other treatments.

2.2.13 Stem CO_2 efflux rate

The stem CO_2 efflux rates of beech and spruce trees as observed in early September, are compared in Fig. 2.16. In spruce monocultures, the rates were found to be 1.8 times ($1xO_3$) and 3.0 times ($2xO_3$) higher than in beech monocultures ($p < 0.05$). In the mixed culture, the CO_2 efflux rates of spruce stems were about 3.6 times higher as compared to beech ($p < 0.05$). Spruce displayed significantly increased rates in the mixed culture, however, O_3 had no significant impact. In beech, the stem CO_2 efflux rates were significantly reduced by elevated O_3, whereas the type of competition had no impact.

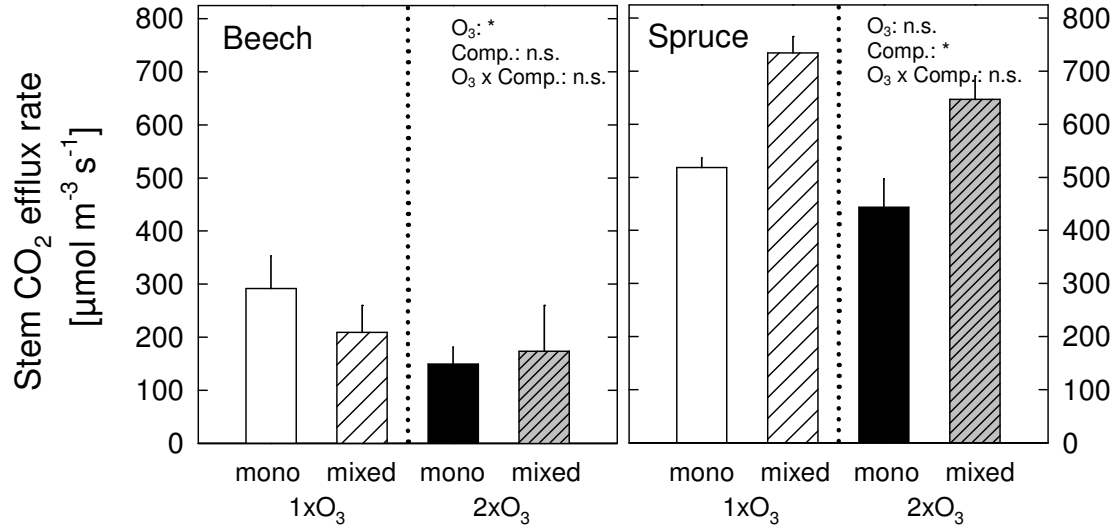

Fig. 2.16 Stem CO_2 efflux rates of beech and spruce trees during early September (means ± SE, n = 5 days). Open and solid bars denote monocultures, and bars with wide and narrow hatching represent mixed cultures. Significant effects of the factors O_3 and competition are indicated by * ($p < 0.05$).

2.2.14 Fraction of new C in stem CO_2 efflux

In beech, the fraction of labeled C in the stem CO_2 efflux ($f_{E,new}$) was significantly diminished under $2xO_3$ from day 4 on (Fig. 2.17). At the end of labeling, $f_{E,new}$ in beech reached 54% ($1xO_3$) and 29% ($2xO_3$) in the monoculture and 45% ($1xO_3$) and 40% ($2xO_3$) in the mixture. In spruce, $f_{E,new}$ was significantly enhanced in the mixture from day 3 on. On day 5, $f_{E,new}$

reached 62% ($1xO_3$) and 54% ($2xO_3$) in the monoculture and 75% ($1xO_3$) and 72% ($2xO_3$) in the mixture. These results indicate that the stem respiration of spruce was supplied to a greater extent from current photosynthates instead of C stores, in comparison with beech.

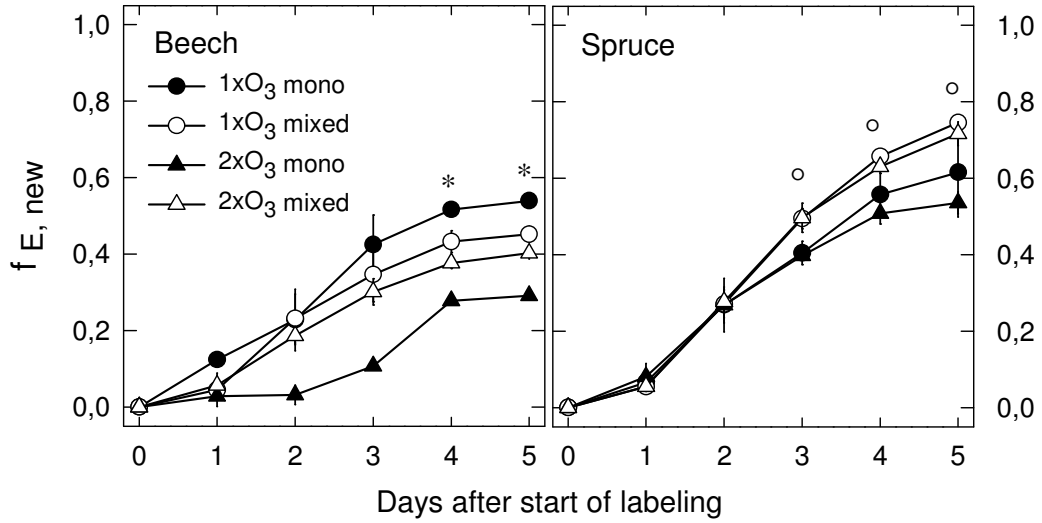

Fig. 2.17 Fraction of labeled, i.e. recently fixed, C in stem CO_2 efflux ($f_{E,new}$) of beech and spruce. Data are presented as 24h-means ± SE (n = 2 to 4 containers). Monocultures are given as solid and mixed cultures as open symbols. Circles denote $1xO_3$ and triangles $2xO_3$ regime. Significant main effects by O_3 and type of competition are indicated by * and °, respectively ($p < 0.05$).

2.2.15 Compartmental modeling of C pools supplying stem respiration

The structure of a "two-pool model" exhibiting the substrate pools Q_1 and Q_2, supplying C to the stem respiration of beech and spruce is drafted in Fig. 2.18. Here, it was assumed that the total stem CO_2 efflux exclusively consists of stem-respired CO_2 (see section 2.1.16).

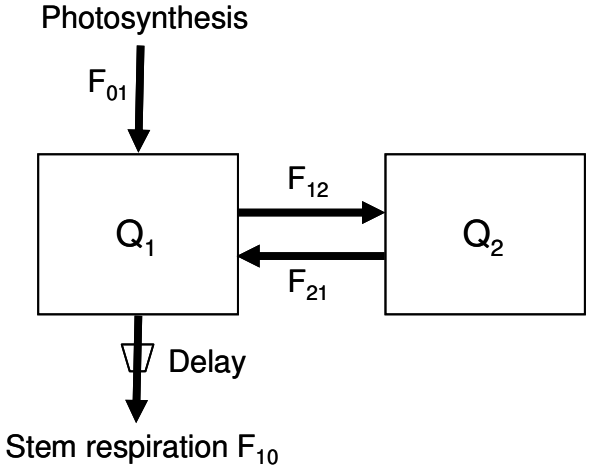

Fig. 2.18 Structure of a "two-pool model" of substrate supply to stem respiration of beech and spruce. Newly acquired (i.e. labeled) C enters *via* "transport" pool Q_1. From there, C is either released directly (F_{10}) or transferred to "storage" pool Q_2, where it cycles through, before being released from Q_1 in F_{10}. A lag time ("delay") between label acquisition (i.e. incorporation in Q_1) and subsequent release in F_{10} was included at the CO_2 release side of Q_1. The magnitude of pool sizes (i.e. box sizes) varied among both species and the different treatments.

Under all treatments, the label appeared in the stem respiration after a time lag ("delay") of half a day to almost two days (Tab. 2.6). In each treatment, stem respiration was supplied by one rapidly turned over pool Q_1 (i.e. "transport" pool) with a half-time between 1.3 and 2.7 days and a slower turned over pool Q_2 (i.e. "storage" pool) with a half-time ranging between 1.0 and 13.7 days. In both species, the estimated size of Q_1, was smaller in the mixed than monocultures. In beech, Q_1 contributed between 50 % and > 77 % and Q_2 between < 23 % and 50 % to stem respiration. In spruce, Q_1 contributed to a higher degree to stem respiration (> 63 to 99 %), whereas contribution of Q_2 was diminished (1 to > 37 %). As the CO_2 released by the stems was labeled to a degree of about 50 % to 70 % after 5 days (Fig. 2.17), characterization of pool Q_2 was difficult. In some cases, sizes and half-lives of Q_2 are interpreted with caution, or not given (Tab. 2.6; Fig. 2.19). The sensitivity of the goodness of the model fits was expressed as root mean squared error (RMSE) of the fits (cf. LEHMEIER *et al.* 2008). The specific stem respiration rate (SRR) of spruce was in general higher, compared with beech, and varied hardly among the treatments (Tab. 2.6). The SRR of beech growing in mixture was 25 % ($1xO_3$) and 11 % ($2xO_3$) lower than in the monoculture. Overall, the substrate supply to stem respiration was turned over faster in spruce than in beech.

Tab. 2.6 Results of two-pool model optimization in beech and spruce regarding the model parameters "size", "half-time" and "contribution to stem respiration" of substrate pools Q_1 and Q_2; n.a. denotes failure in parameter characterization; SRR = specific stem respiration rate; delay = delay for label release; RMSE = root mean squared error.

	Poolsize [mg C g^{-1} total plant C]		**Half-time [days]**		**Contribution to stem respiration [%]**		**SRR [mg stem respired C g^{-1} plant-C h^{-1}]**	**Delay [days]**	**RMSE**
	Q_1	**Q_2**	**Q_1**	**Q_2**	**Q_1**	**Q_2**			
Beech									
$1xO_3$ mono beech	29	n.a.	2.5	n.a.	>77	<23	0.26	0.4	0.026
$1xO_3$ mixed beech	18	≥94	1.3	≥ 13.7	50	50	0.20	0.8	0.008
$2xO_3$ mono beech	41	≥8	2.5	≥ 1.0	50	50	0.24	1.8	0.028
$2xO_3$ mixed beech	28	≥70	1.9	≥ 9.5	50	50	0.21	0.6	0.01
Spruce									
$1xO_3$ mono spruce	26	n.a.	2.7	n.a.	93	7	0.26	0.7	0.011
$1xO_3$ mixed spruce	21	≤10	2.1	n.a.	99	1	0.28	0.8	0.019
$2xO_3$ mono spruce	25	≥48	1.7	≥8.3	>63	>37	0.28	0.6	0.01
$2xO_3$ mixed spruce	21	<8	2.1	n.a.	96	4	0.28	0.8	0.012

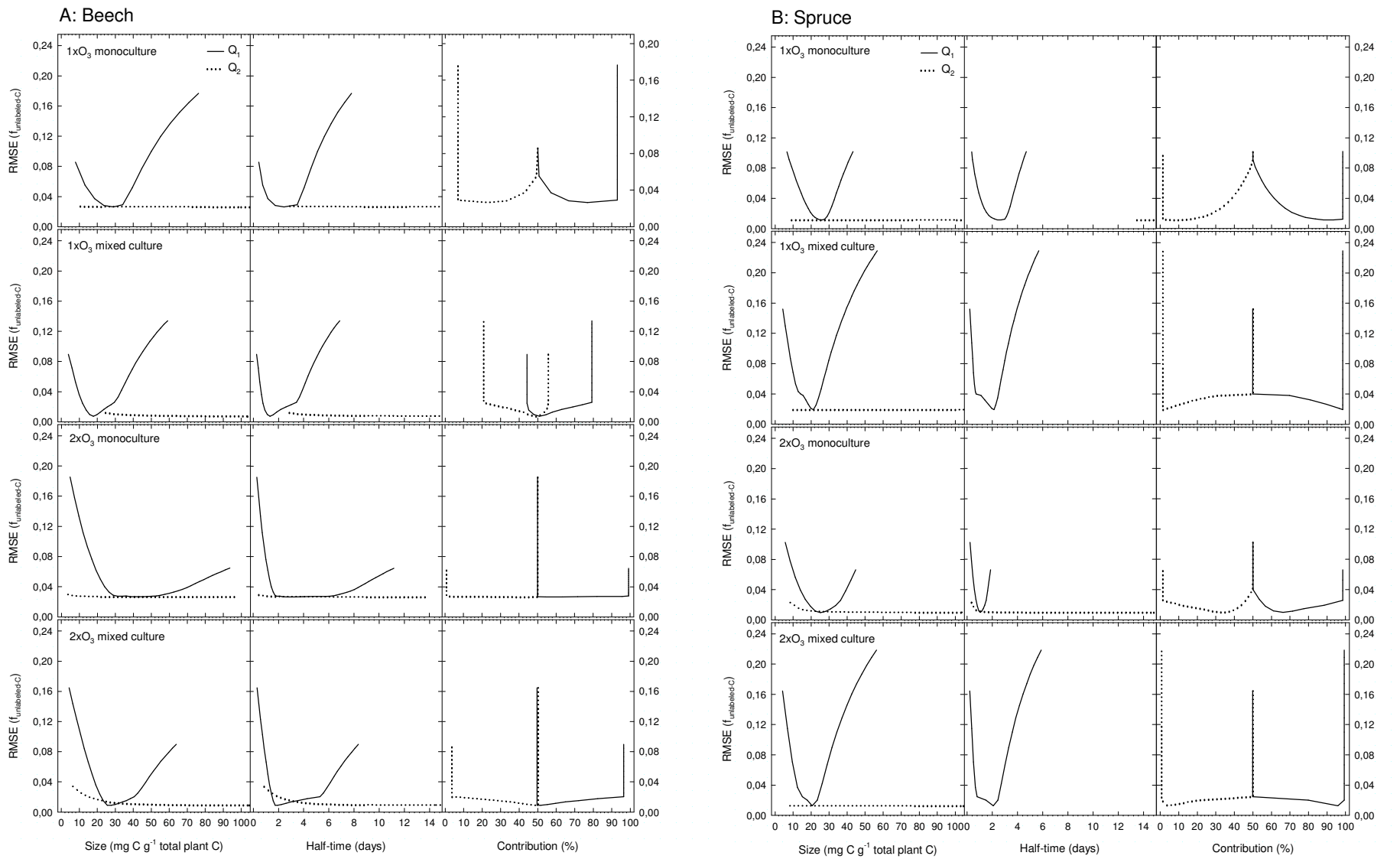

Fig 2.19 Sensitivity of the goodness of the model fits for pool size, half-time and contribution to stem respiration of beech (A) and spruce (B). Mono- and mixed cultures were exposed to $1xO_3$ or $2xO_3$ regimes, respectively. The sensitivity to changes in parameter values is expressed as the root mean squared error (RMSE) of the fit. Minimum RMSE reflects the optimized value of a model parameter. The solid line denotes pool Q_1, the dotted line pool Q_2.

2.2.16 Daily ^{13}C uptake

In beech, both O_3 and competition had no significant impact on the daily ^{13}C uptake (Fig. 2.20 A, B). In spruce, the daily ^{13}C uptake per tree (Fig. 2.20 C) and per unit of total foliage dry mass (Fig. 2.20 D) was significantly enhanced by 50% to 60%, respectively, due to interspecific competition. The daily ^{13}C uptake per spruce tree exceeded that of beech significantly ($p < 0.05$).

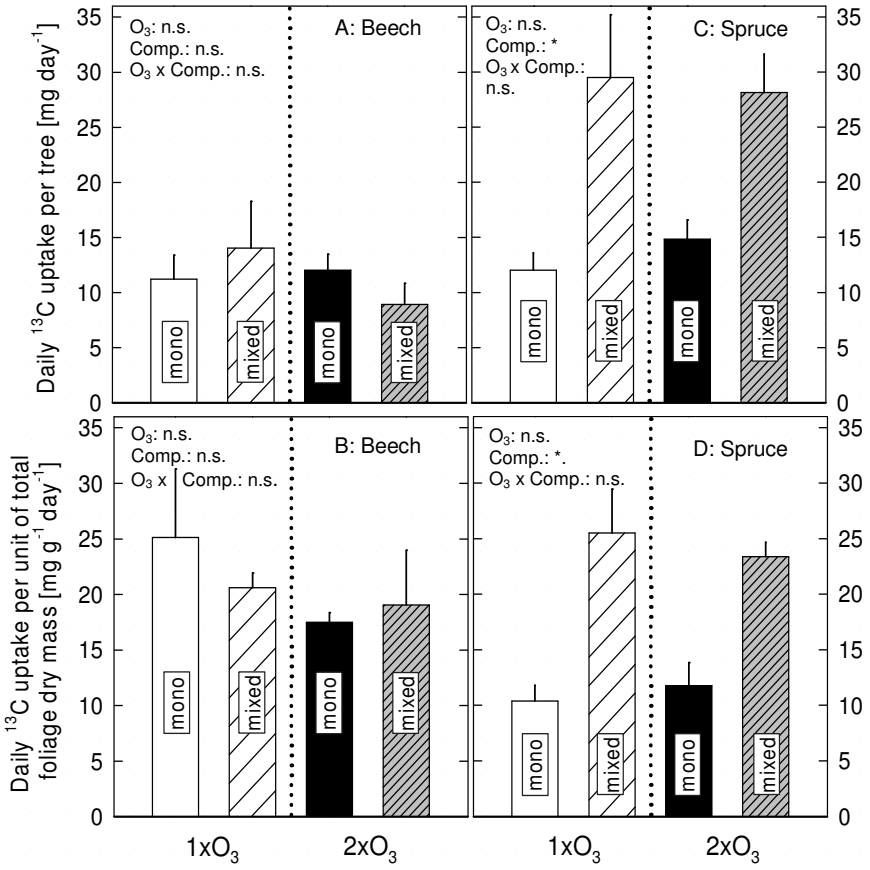

Fig. 2.20 Daily ^{13}C uptake per tree (A, C) and per unit of biomass of beech and spruce leaves (B, D) in early September (means ± SE, n = 4 containers). Open and solid bars denote monocultures, and hatching bars the mixed cultures. In spruce, the significant effect of the factor competition is indicated by * ($p < 0.05$).

2.2.17 Daily ^{15}N uptake

Overall, the ^{15}N uptake in spruce (Fig. 2.21 D-F) was higher than in beech (Fig. 2.21 A-C), in particular in the mixed culture. In beech, both O_3 and competition had no impact on the daily ^{15}N uptake. In spruce, the daily ^{15}N uptake per tree (Fig. 2.21 D) and per unit of fine root biomass (Fig. 2.21 F) was significantly increased by interspecific competition. A significant interaction between O_3 x competition increased the ^{15}N uptake per unit of total spruce root biomass strongly in the mixture under $1xO_3$, relative to the other treatments (Fig. 2.21 E).

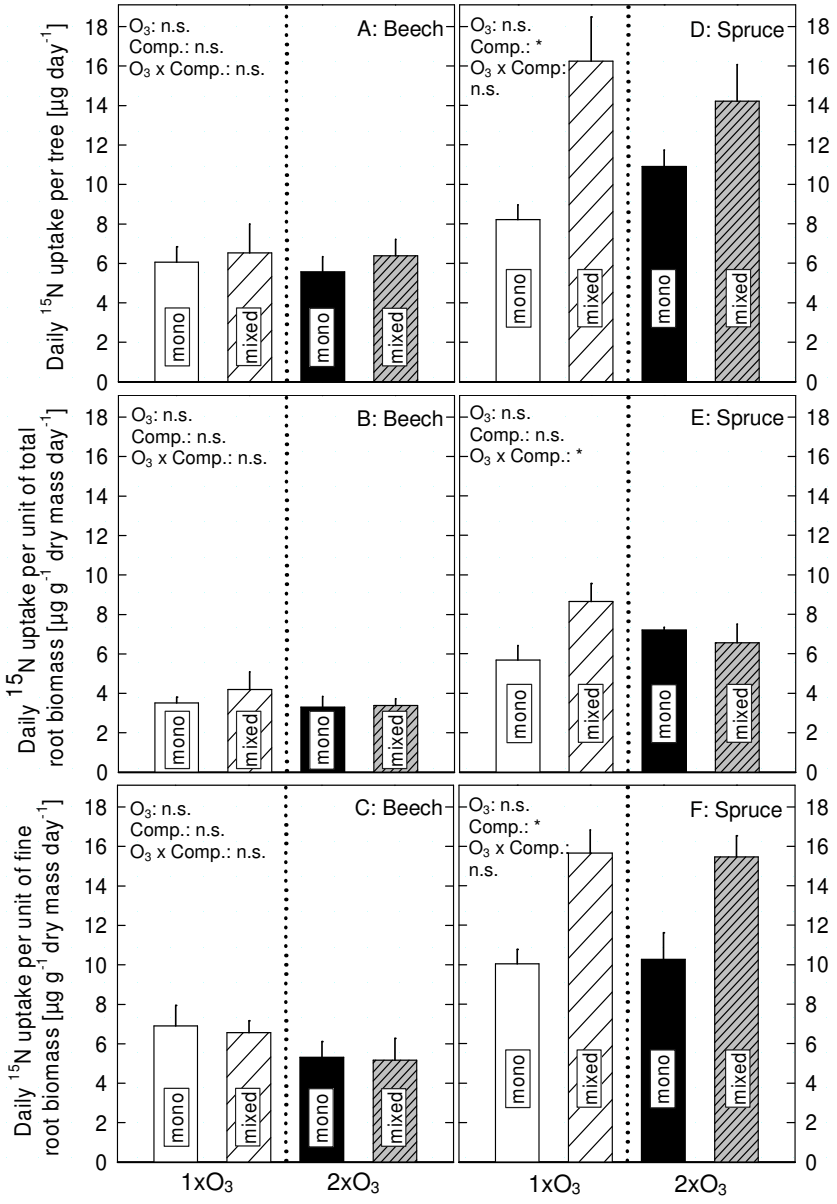

Fig. 2.21 Daily whole tree ^{15}N uptake (A, D), daily ^{15}N uptake per unit of total biomass (B, E) and per unit of fine root biomass (C, F) of beech and spruce in early September (means ± SE, n = 4 containers). Open and solid bars represent the monocultures, and hatched bars the mixed cultures. Significant effects of the factor competition and the interaction O_3 x competition are indicated by * ($p < 0.05$).

2.2.18 Whole-tree partitioning of new C and N

In early September, beech invested 56% to 59% "new C" into the shoot and 41% to 44% into the roots (Fig. 2.22 A). The magnitude of „new C" partitioning in beech increased in the following order: old axes < current-year axes < buds < fine roots < leaves < stem < coarse roots (irrespective of treatments). Exposure to $2xO_3$ favoured the investment of „new C" into fine roots significantly by 8%. Beech invested between 35% to 42% „new N" into the shoot and between 58% to 65% into the roots (Fig. 2.22 B). The magnitude of „new N" partitioning in beech increased in the following order: old axes < buds < current-year axes < leaves < stem < fine roots < coarse roots (irrespective of treatments).

Spruce invested 64% to 69% „new C" into the shoot and 31% to 38% into the roots (Fig. 2.22 C). The magnitude of „new C" partitioning increased in the following order: buds < old axes < current-year axes < leaves < fine roots < coarse roots < stem (irrespective of treatments). Interspecific competition reduced the „new C" partitioning into the buds significantly by

0.4%, but, enhanced it in the leaves (+ 13%) and coarse roots (+ 6%). Spruce invested 39% to 54% „new N“ into the shoot and 46% to 61% into the roots (Fig. 2.22 D). The magnitude of „new N“ partitioning in spruce increased in the following order: old axes < buds < current-year axes < leaves < stem < fine roots < coarse roots (irrespective of treatments). Interspecific competition stimulated the investment of „new N“ into spruce needles significantly by 15%, but, in contrast, decreased it in the coarse roots by 8%.

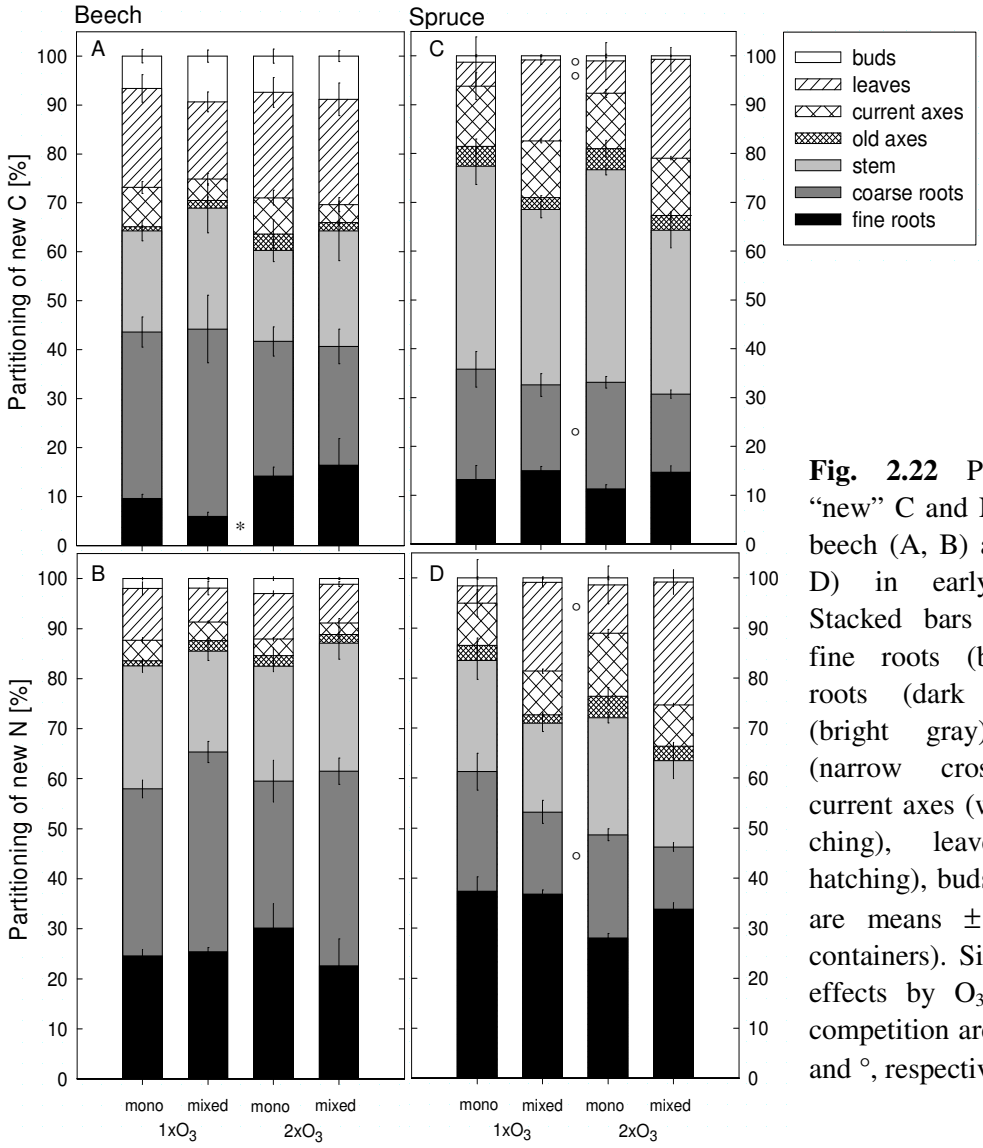

Fig. 2.22 Partitioning of “new” C and N in organs of beech (A, B) and spruce (C, D) in early September. Stacked bars represent the fine roots (black), coarse roots (dark grey), stem (bright gray), old axes (narrow cross hatching), current axes (wide cross hatching), leaves (diagonal hatching), buds (white). Data are means ± SE (n = 4 containers). Significant main effects by O_3 and type of competition are denoted by * and °, respectively ($p < 0.05$).

2.2.19 Total amount of new C and N

Overall, beech invested 29.6 ± 6.3 to 35.8 ± 9.3 mg of „new C“ into the shoot and 20.5 ± 4.5 to 23.1 ± 3.9 mg into the roots. The total amount of „new C“ in the buds of beech was significantly enhanced (+ 60 %) by interspecific competition (Tab. 2.7 A).

The deciduous tree species invested 17.8 ± 4.0 to 21.2 ± 3.8 µg of „new N“ into the shoot and 29.5 ± 2.6 to 37.8 ± 8.0 µg into the roots. A significant interaction between O_3 x competition increased the amount of „new N“ by 9 % in beech fine roots in the monoculture under $1xO_3$ (Tab. 2.7 B).

Tab. 2.7 Amount of "new" C and N in plant organs of beech (A, B) and spruce (C, D) after application of dual $^{13}CO_2/^{12}CO_2$ and $^{15}N/^{14}N$ labeling (5 and 9 days, respectively) in early September (means ± SE; n = 4 containers). * denotes significant effects of O_3 and competition as well as their interaction (O_3 x comp.) ($p < 0.05$); n.s. states "not significant".

Beech

A: New C [mg]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	5.1 ± 0.8	8.4 ± 1.9	4.9 ± 1.3	7.5 ± 1.7	n.s.	*	n.s.
Leaves	13.5 ± 2.4	11.0 ± 3.7	13.8 ± 1.4	18.0 ± 2.8	n.s.	n.s.	n.s.
Current axes	4.3 ± 0.4	4.4 ± 1.6	5.3 ± 1.0	4.7 ± 0.8	n.s.	n.s.	n.s.
Old axes	1.4 ± 0.3	2.5 ± 0.1	4.3 ± 1.7	2.7 ± 1.7	n.s.	n.s.	n.s.
Stem	15.0±2.7	13.8 ± 3.7	13.0 ± 2.4	11.1 ± 5.0	n.s.	n.s.	n.s.
Coarse roots	21.9±4.5	21.7 ± 5.7	21.2 ± 3.5	17.5 ± 3.9	n.s.	n.s.	n.s.
Fine roots	6.0±1.5	5.4 ± 2.2	7.1 ± 1.1	7.8 ± 1.4	*	n.s.	n.s.
Total tree	48.1±7.5	57.7 ± 13.6	52.3 ± 6.3	58.4 ± 7.7	n.s.	n.s.	n.s.

B: New N [µg]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	1.4 ± 0.2	1.2 ± 0.3	1.4 ± 0.5	1.0 ± 0.5	n.s.	n.s.	n.s.
Leaves	6.3 ± 1.2	4.2 ± 1.6	5.8 ± 1.0	6.1 ± 1.6	n.s.	n.s.	n.s.
Current axes	2.9 ± 0.5	2.9 ± 0.3	2.6 ± 0.5	2.9 ± 0.8	n.s.	n.s.	n.s.
Old axes	0.4 ± 0.2	1.1 ± 0.3	0.8 ± 0.3	1.3 ± 0.7	n.s.	n.s.	n.s.
Stem	13.5 ± 2.7	13.1 ± 1.9	14.3 ± 2.0	14.7 ± 4.2	n.s.	n.s.	n.s.
Coarse roots	18.7 ± 1.0	22.5 ± 4.8	20.8 ± 3.2	18.5 ± 5.5	n.s.	n.s.	n.s.
Fine roots	16.4 ± 1.1	15.3 ± 3.7	14.6 ± 1.8	15.1 ± 0.8	n.s.	n.s.	*
Total tree	45.3 ± 3.0	55.6 ± 11.9	51.4 ± 5.3	54.8 ± 9.1	n.s.	n.s.	n.s.

Spruce

C: New C [mg]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	1.2 ± 0.2	1.4 ± 0.5	1.2 ± 0.5	1.3 ± 0.4	n.s.	n.s.	n.s.
Leaves	11.0 ± 3.1	25.0 ± 4.4	12.8 ± 9.0	27.7 ± 6.6	n.s.	*	n.s.
Current axes	9.4 ± 1.8	16.9 ± 3.6	9.7 ± 0.8	15.5 ± 2.0	n.s.	*	n.s.
Old axes	3.4 ± 0.9	4.1 ± 0.9	4.1 ± 1.4	3.9 ± 0.9	n.s.	n.s.	n.s.
Stem	37.2 ± 7.8	39.7 ± 7.1	40.9 ± 5.0	44.8 ± 5.7	n.s.	n.s.	n.s.
Coarse roots	14.5 ± 2.7	24.1 ± 2.0	19.1 ± 2.3	24.9 ± 3.0	n.s.	*	n.s.
Fine roots	8.5 ± 2.0	23.1 ± 4.9	10.8 ± 0.8	19.9 ± 3.5	n.s.	*	n.s.
Total tree	68.0 ± 4.9	119.8 ± 13.0	64.3 ± 7.4	131.3 ± 11.3	n.s.	*	n.s.

D: New N [µg]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	1.4 ± 0.2	1.5 ± 0.4	1.4 ± 0.2	1.4 ± 0.4	n.s.	n.s.	n.s.
Leaves	12.8 ± 5.5	27.9 ± 5.6	27.6 ± 8.0	32.4 ± 5.8	n.s.	n.s.	n.s.
Current axes	10.6 ± 4.1	13.5 ± 2.4	15.2 ± 2.6	10.7 ± 2.3	n.s.	n.s.	n.s.
Old axes	3.1 ± 0.4	4.0 ± 0.6	6.1 ± 2.8	4.2 ± 0.7	n.s.	n.s.	n.s.
Stem	23.5 ± 4.4	26.6 ± 2.5	29.9 ± 2.8	25.7 ± 3.2	n.s.	n.s.	n.s.
Coarse roots	23.9 ± 1.9	24.0 ± 2.9	32.6 ± 3.5	17.2 ± 2.9	n.s.	*	n.s.
Fine roots	37.1 ± 6.1	53.8 ± 5.7	60.8 ± 6.9	41.9 ± 5.5	n.s.	n.s.	n.s.
Total tree	96.5 ± 6.6	144.1 ± 18.4	129.1 ± 15.8	134.8 ± 16.8	n.s.	*	n.s.

Spruce invested 46.0 ± 1.2 to 64.8 ± 8.4 mg (monoculture) and 72.6 ± 7.3 to 85.2 ± 7.2 mg of „new C“ (mixed culture) into the shoot. In the roots, the total amount of „new C“ ranged from 22.0 ± 3.8 to 28.8 ± 1.6 mg (monoculture) and 46.1 ± 5.4 to 47.2 ± 6.9 mg (mixed culture). Interspecific competition increased the total amount of „new C“ significantly in leaves (+ 121 %), current axes (+ 69 %), coarse roots (+ 46 %), fine roots (+ 122 %) and in the whole tree (+ 90 %) (Tab. 2.7 C).

The conifer invested 33.4 ± 2.5 to 55.6 ± 4.6 µg (monoculture) and 66.2 ± 10.8 to 74.9 ± 10.2 µg of „new N“ (mixed culture) into the shoot. In the roots, the amount of „new N“ ranged from 61.2 ± 6.1 to 85.6 ± 9.8 µg (monoculture) and 59.9 ± 8.1 to 77.9 ± 8.2 µg (mixed culture). In spruce, interspecific competition decreased the total amount of „new N“ in the coarse roots significantly (- 27 %), but, increased it in the whole tree (+ 24 %) (Tab. 2.7 D).

2.2.20 Soil CO_2 efflux rate

On June 15, the soil CO_2 efflux rates were significantly lowered by elevated ozone in both species (Fig. 2.23). On July 17 and August 21, beech monocultures displayed the lowest soil CO_2 efflux rates among all treatments. On August 21, the soil CO_2 efflux rates in spruce monocultures were significantly increased compared with the other treatments. The rates observed in the mixed cultures ranged between those of the beech and spruce monocultures.

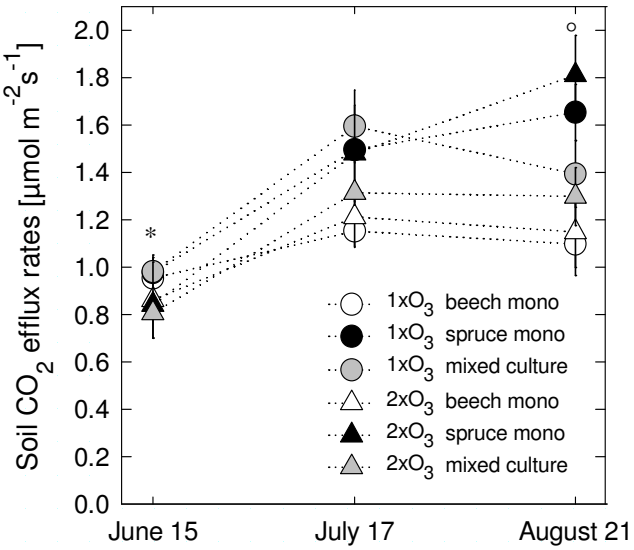

Fig. 2.23 Soil CO_2 efflux rates in beech and spruce mono- and mixed cultures, exposed to $1xO_3$ or $2xO_3$ regime (means ± SE, n = 4 containers). White and black symbols denote the monocultures, and gray symbols the mixed cultures. Significant main effects by O_3 and type of competition are denoted by * and °, respectively ($p < 0.05$).

2.3 Discussion

In the following, the findings on juvenile beech and spruce trees are discussed. The focus was on the C and N allocation under the impact of ozone ($1xO_3$ *vs.* $2xO_3$ regime) and plant competition (intra- *vs*. interspecific), with beech and spruce trees being 4 and 5 years old, at the end of the study, respectively.

The hypotheses tested were:

I. Elevated O_3 and interspecific competition affect the whole-tree partitioning of newly acquired C and N in juvenile beech and spruce.
II. Elevated O_3 reduces the allocation of recently fixed C to stem CO_2 efflux of juvenile beech and spruce.

In both chamber and field experiments O_3 effects have often caused delayed shoot development, visible leaf injuries, as well as inhibition of photosynthesis and growth (MATYSSEK *et al*. 2007b, KARNOSKY *et al*. 2007, NOVAK *et al*. 2007, PELL *et al*. 1999). Plant competition can modify the O_3 response of trees, i.e. their O_3 sensitivity (cf. KOZOVITS *et al*. 2005a,b, LIU *et al*. 2004, MC DONALD *et al*. 2002, ANDERSEN *et al*. 2001).

In the present work, elevated O_3 significantly retarded the leaf flushing in beech, whereas in spruce, O_3 significantly affected the leaf development only at one stage during flushing (Fig. 2.4). The delay in shoot development of trees, as caused by O_3, agrees with most of the published literature (ORENDOVICI-BEST *et al*. 2008, PRITSCH *et al*. 2008, KARNOSKY *et al*. 2005, RANFORD & REILING 2007). Nevertheless, the onset of leaf senescence in beech (assessed in early September) was not accelerated under $2xO_3$ (Fig. 2.5), which may be related to the antioxidant status in the foliage and/or a low cumulative O_3 uptake (cf. MATYSSEK & SANDERMANN 2003, PELL *et al*. 1999).

Since O_3 acts primarily in the leaf tissue after uptake *via* stomata (cf. MATYSSEK & SANDERMANN 2003), its primary visible injury symptoms are located on the leaf surface (LEIPNER *et al*. 2001). In the present study, elevated O_3 significantly increased the percentage of visible leaf injury symptoms in beech and spruce (Fig. 2.6 and 2.7). The high soil moisture favoured stomatal opening of the leaves, and thus O_3 influx, even under $1xO_3$. This is in line with other studies detecting visible foliar injuries at ambient O_3 concentrations under similar conditions (SCHAUB *et al*. 2003, NOVAK *et al*. 2005, ORENDOVICI *et al*. 2003). The expression of O_3-induced visible leaf injuries does not necessarily coincide with physiological disturbances that lead to reduced growth (FELZER *et al*. 2007).

The ability of beech and spruce to maintain photosynthetic perfomance under $2xO_3$ indicates that, at the leaf level, both species were able to compensate for O_3 injury (cf. SAMUELSON & KELLY 1996, ANDERSEN *et al*. 1997). Nevertheless, beech at $2xO_3$ supported new leaf formation during the growing season, and significantly increased the number of leaves (Tab

A-1, Appendix). Through new leaf formation beech may have compensated for foliar O_3 injury (OKSANEN *et al.* 2003). Likewise, KOZOVITS *et al*. (2005b) observed such a stimulating O_3 effect in beech monocultures. In contrast with KOZOVITS *et al*. (2005b), the SLA of O_3 exposed beech leaves in the present study was significantly decreased, resulting perhaps from reduced cell extension, stimulated lignin biosynthesis (cf. MATYSSEK & SANDERMANN 2003) or increased sugar accumulation (RIIKONEN *et al*. 2008). The latter was supported by the significant increase in the foliar C/N ratio (Tab. 2.8), along with an enhancement in total C concentration (Tab. A-2, Appendix). Increased foliar pool sizes of soluble sugars/starch may be related to impaired phloem loading, reduced assimilate translocation from O_3 stressed leaves (GRANTZ & FARRAR 1999, LANDOLT *et al*. 1994, LUX *et al.* 1997) or injury compensation through repair processes (TOPA *et al*. 2001, COOLEY & MANNING 1987).

After two years of elevated O_3 exposure and interspecific competition, the total shoot and root biomass as well as the root to shoot biomass ratio of beech and spruce were not affected by the applied treatments (Fig. 2.8). Significant differences became only apparent between the two species, with spruce showing *c.* 70% higher shoot and *c.* 30% less root biomass, as compared to beech. Since initial growth conditions, in particular stand density, strongly influence the outcome of competition (GRAMS & ANDERSEN 2007, GAYLER *et al*. 2006, WENT 1973), the low canopy density in the mono- and mixed plantations (including an incomplete canopy closure up to the final harvest) may explain the lack of competition effects on the standing biomass of beech and spruce trees. Lack of O_3 effects on the biomass of deciduous and coniferous saplings also occured in other chamber studies (BORTIER *et al*. 2001, 2000, SAMUELSON *et al*. 1996). Despite some treatment effects on the shoot phenology of both species (Fig. 2.4), the relationship between biomass production and C and N concentrations and pools in the whole tree (Fig. A-3, Appendix) was not affected, confirming C and N balanced growth.

Dual $^{13}CO_2/^{12}CO_2$ and $^{15}N/^{14}N$ isotope labeling was applied in the late growing season, when leaves were fully mature and roots and stems likely acted as strong sink for C (SAMUELSON & KELLY 1996, ADAMS *et al*. 1990, LUX *et al*. 1997). Hypothesis I was supported in beech, depending on the scenario: the partitioning of "new" C and N was unchanged by interspecific competition, whereas elevated O_3 significantly favoured the investment of "new C" into fine root biomass (+ 8 %) (Fig. 2.22, Tab. 2.8). In consistency with „new C" partitioning, beech significantly stimulated its fine root growth in response to $2xO_3$ (Fig. 2.8), which contrasts with many observations in juvenile trees (cf. LUEDEMANN *et al*. 2005, 2009, ANDERSEN *et al*. 1997, MATYSSEK *et al*. 1995, 2002). Such O_3-induced stimulatory effects on growth processes are seen as a strategy of trees to compensate for the O_3 stress (OKSANEN *et al.* 2001, KARNOSKY *et al*. 1996, NIKULA *et al*. 2009). For instance, adult beech trees (*Fagus sylvatica*), growing in a mixed beech/spruce stand, increased the fine root production during the third year of $2xO_3$ exposure to meet increased foliar demand for nutrients in support of growth and/or repair processes (NIKOLOVA *et al*. 2010). Likewise, PREGITZER *et al*. (2008) reported on an increased fine root production for trembling aspen (*Populus tremuloides*) after 10 years

Tab. 2.8 Main effects of O_3 and plant competition on parameters as observed in shoot and root of juvenile beech (A) and spruce (B). Arrows indicate an increase (↑) or decrease (↓) of each parameter in response to elevated ozone ($2xO_3$ *vs.* $1xO_3$ regime) or interspecific competition (mixed *vs.* monoculture) (* $p < 0.05$, ° $p < 0.1$, not significant differences are denoted as "-").

(A) Beech	**O_3 main effect**					**Competition main effect**				
	Partitioning of new C [%]	Amount of new C [mg]	Partitioning of new N [%]	Amount of new N [µg]	C/N	Partitioning of new C [%]	Amount of new C [mg]	Partitioning of new N [%]	Amount of new N [µg]	C/N
buds	-	-	-	-	-	-	↑*	-	-	↑*
leaves	-	-	-	-	↑*	-	-	-	-	-
current axes	-	-	-	-	-	-	-	-	-	↑°
old axes	-	-	-	-	-	-	-	-	-	-
stem	-	-	-	-	-	-	-	-	-	-
coarse roots	-	-	-	-	-	-	-	-	-	↓*
fine roots	↑*	-	-	-	↓°	-	-	-	-	-
total tree		-		-	-		↑*		-	-

(B) Spruce	**O_3 main effect**					**Competition main effect**				
	Partitioning of new C [%]	Amount of new C [mg]	Partitioning of new N [%]	Amount of new N [µg]	C/N	Partitioning of new C [%]	Amount of new C [mg]	Partitioning of new N [%]	Amount of new N [µg]	C/N
buds	-	-	-	-	-	↓*	-	-	-	-
leaves	-	-	-	-	↓*	↑*	↑*	↑*	-	↓*
current axes	-	-	-	-	-	-	↑*	-	-	-
old axes	-	-	-	-	-	-	-	-	-	-
stem	-	-	-	-	-	-	-	-	-	-
coarse roots	-	-	-	-	-	↓*	↑*	↓*	↓*	-
fine roots	-	-	-	-	-	-	↑*	-	-	-
total tree		-		-	↓*		↑*		↑*	↓*

of elevated O_3-fumigation, however, the fine root turnover was not affected.

Since the $^{13}CO_2/^{12}CO_2$ labeling was applied towards the end of the growing season, only a minor portion of the total ^{13}C incorporation was invested into spruce needles (monoculture *c.* 6 %, mixed culture *c.* 18 %) (Fig. 2.22). Hence, foliar ^{13}C uptake (on a whole-tree and foliage basis) of the conifer was significantly increased when growing in mixture with beech (Fig. 2.20). Hypothesis I was supported in spruce, depending on the scenario: in the absence of O_3 effects, interspecific competition caused significant changes in the partitioning of „new" C and N in buds (only „new C"), needles and coarse roots (Fig. 2.22, Tab. 2.8). Spruce invested significantly more recently assimilated C and N in the needles under mixed than monoculture, stressing their importance as „new" C an N sink under interspecific competition. This kind of competition caused spruce trees to significantly increase the amount of recently assimilated "new" C and N in the whole tree (Tab. 2.8).

Spruce saplings displayed a significantly higher photosynthetic performance (Fig. 2.13 and 2.14) and annual C gain (Fig. 2.15) than beech saplings, although parameters were not significantly affected by $2xO_3$ or interspecific competition in either species. Nevertheless, elevated O_3 significantly diminished the volume-related CO_2 efflux from stems in beech (Fig. 2.16), indicating an ozone-driven disturbance in C allocation between shoot and root (KOZOVITS 2003). In view of the O_3-induced foliar injuries (see above), an inhibited assimilate transport from the leaves might have limited the respiratory activity of the stem tissues (MATYSSEK *et al.* 2002, AMTHOR 2000, THORNLEY & CANNEL 2000). In young O_3-exposed birch trees (*Betula pendula*), reduced stem respiration was related to the size reduction and structural decline of cortical and phloem parenchyma cells, which dominated the respiratory CO_2 release (GÜNTHARDT-GOERG *et al*. 1993, MATYSSEK *et al.* 1992).

In cultures with intense aboveground competition, the lowered stem CO_2 efflux rates of beech trees in the mixed culture (cf. KOZOVITS 2003) reflected the lowered aboveground growth, in particular under $2xO_3$ (cf. KOZOVITS *et al.* 2005a,b). In consistency with the photosynthetic performance, spruce displayed a significantly increased volume-based stem CO_2 efflux, as compared to beech, irrespective of the treatments (Fig. 2.16). The relatively higher CO_2 efflux from spruce stems might have been associated with proceeding lignin synthesis in the stem tissues (KOZLOWSKI 1992). In spruce, interspecific competition significantly stimulated the stem CO_2 efflux rates, as compared to intraspecific competition. Slightly enhanced $\delta^{13}C$ in the CO_2 efflux from spruce stems in the mixed culture indicated increased lignification *vs.* spruce stems in the monoculture (BENNER *et al*. 1987; Fig. A-2, Appendix).

Since the stem CO_2 efflux of woody species is strongly driven by current assimilate supply (between 13 to 42% of gross primary production; CAREY *et al*. 1997), beech and spruce trees were expected to show a high responsivenss to elevated O_3 in the allocation of recently fixed C to stem CO_2 efflux (see hypothesis II). The application of continuous $^{13}CO_2/^{12}CO_2$ labeling allowed to follow the labeling kinetics of CO_2 in the stem CO_2 efflux of the saplings.

CO_2 sampled from stem CO_2 efflux may not exclusively originate from local tissue respiration, but be biased by xylem-transported CO_2 deriving from soil and root respiration (cf. TESKEY *et al*. 2008). Referring to recent literature (UBIERNA *et al*. 2009, KODAMA *et al.* 2008), however, bias from other CO_2 sources but stem respiration was concluded to be small. Therefore, in the present study, stem CO_2 efflux was regarded to represent "stem respiration".

Beech and spruce saplings showed a high responsiveness in allocation of recently fixed C to stem respiration to the treatments, although the response differed between the species. Interspecific competition significantly increased $f_{E,new}$ in spruce, whereas elevated O_3 significantly lowered $f_{E,new}$ in beech (support of hypothesis II; RITTER *et al*., unpublished), suggesting a physiological imbalance in the latter species. Shifts in carbon allocation may reflect plant injury and are the predominant way in which plants compensate for O_3 stress (RANFORD & REILING 2007, PELL *et al*. 1994). Ozone-induced readjustments in C allocation to stem respiration might favour C allocation to defence/repair at the leaf level at the expense of stem growth. In the long-term, cummulative reductions in C storage pools (TOPA *et al*. 2001, OKSANEN *et al*. 2003) of beech may weaken the whole-tree defense status (cf. MATYSSEK & SANDERMANN 2003), with implications to competitiveness in mixture with spruce.

In both species, the compartmental analysis indicated the existence of a relatively faster turned-over pool Q_1, being exchanged within within 1.3 to 2.7 days, and a relatively slower turned-over pool Q_2, having a half-time between 1.0 to 13.7 days (Tab. 2.6). According to its half-time, Q_1 was defined as "transport" pool, containing substrates that are known to be transported (such as sucrose) in trees. Pool Q_2 was considered to act as "storage" pool containing C reserves such as starch (SCHÄDEL *et al*. 2009, HOCH 2007, KOZLOWSKI & PALLARDY 1991). In both species, Q_1 was presumed to be located in the leaves/phloem, and Q_2 in plant parts other than the foliage (i.e. axes, stem, coarse roots, fine roots). As the CO_2 released by the stems was only labeled to a degree of about 50% to 70% after 5 days (Fig. 2.17), characterization of Q_2 was difficult. In some cases, sizes and half-times of Q_2 were less accurately or not estimated (Tab. 2.6), and should therefore be interpreted with caution.

Beech and spruce displayed a fundamental disparity in accessing the substrate pools Q_1 and Q_2, related to stem respiration. In beech, about half of the respired C cycled through "storage" pool Q_2 before being released *via* the stem (Tab. 2.6). That means, that beech relied to a great extent on C reserves to supply its stem respiration. Consistently, starch concentration of shoot axes and coarse roots of beech saplings were distinctly diminished under elevated O_3 (LIU *et al.* 2004, SPENCE *et al.* 1990, LUX *et al.* 1997). Reduced assimilate storage in stems and roots at the end of the growing season (MC LAUGHLIN *et al.* 1982) and, hence, reduced C for regrowth and bud development in spring (OKSANEN & SALEEM 1999, OKSANEN *et al.* 2001, TOPA *et al.* 2001) was observed in several tree species under chronic O_3 exposure. In spruce, the "transport" pool Q_1 had an important role in stem respiration: > 63 to 99 % of the respired C cycled only through Q_1, whereas a minor proportion cycled through "storage" pool Q_2 before being released *via* the stem (Tab. 2.6). The size of Q_1 in spruce tended to be decreased under interspecific competition (*c.* 4 to 5 mg C g^{-1} total plant C). Conversely, after two years

of interspecific competition, LIU *et al*. (2005) observed higher sugar (significant) and starch levels (trend) in needles of spruce saplings, when growing in mixture with beech.

Growth in the mixed culture significantly diminished the shoot axes biomass increment (RBI) of beech, and, slightly increased it in spruce (Fig. 2.10). The response in RBI of the saplings was comparable to that in the phytotron study of KOZOVITS *et al.* (2005a,b), examining the effects of intra- and interspecific competition on tree sensitivity to combined ambient and twice-ambient O_3/CO_2 regimes. At the dense canopies of the beech/spruce mixed cultures, competitiveness of individuals was based on a higher investment-related efficiency in aboveground space occupation (cf. KOZOVITS *et al.* 2005b, GRAMS *et al.* 2002). The competitive success of spruce in mixture was based on the ability to enlarge crown volume at low structural costs (i.e. higher crown volume per unit of shoot biomass). Conversely, interspecific competition lowered the space occupation per unit of shoot biomass in beech, resulting from lowered size-independent investment into foliage per unit of shoot biomass.

In agreement with comparable phytotron experiments (cf. LUEDEMANN *et al*. 2005, 2009, KOZOVITS *et al*. 2005a,b) spruce had a distinctly lower root to shoot biomass ratio than beech (*c*. 0.47 and 1.02 in spruce and beech, respectively; Fig. 2.8). Nevertheless, spruce increased belowground C allocation to associated mycorrhizae, as well as to soil microorganisms (GRAMS *et al*., unpublished), to compensate for the lower C investment into the root biomass, relative to beech. The annual C gain per tree was twice as high in spruce compared to beech (Fig. 2.15), representing the basis for higher C investment of spruce into the mycorrhizosphere. Consistently, the soil CO_2 efflux rates under spruce were raised by c. 20 to 50%, as compared to the CO_2 efflux from beech soils (Fig. 2.23). It is likely that the C allocation strategy of spruce represented the mechanistic basis for increased competitiveness for belowground resources, as compared to beech. Earlier observations demonstrated that spruce appeared to be the "stronger competitor" for belowground resources such as water and nitrogen, in particular under the elevated O_3 regime (GRAMS & ANDERSEN 2007, LUEDEMANN *et al*. 2005, 2009, KOZOVITS *et al*. 2005b). In the present study, interspecific competition had no effect on the ^{15}N acquisition in beech, however, significantly stimulated the ^{15}N uptake of spruce saplings on a whole tree, as well as on a total root and fine root basis (Fig. 2.21). The presence of the "weaker competitor" beech likely favoured the N availability to spruce, resulting in an increased ^{15}N acquisition in mixture. However, the presence of the "stronger competitor" spruce might limit, on the long-run, the availability of belowground resources to beech (KOZOVITS *et al*. 2005b, HAGEDORN *et al*. 2002, WANG *et al.* 2001).

In conclusion, two years of exposure to elevated O_3 and interspecific competition were not sufficient to significantly affect the standing biomass of beech and spruce saplings. Consideration has to be given to the low aboveground competition in the mono- and mixed cultures before canopy closure. Nevertheless, data on C allocation to stem respiration indicated incipient tree responses to elevated O_3 (beech) and interspecific competition (spruce) providing the mechanistic basis for biomass partitioning in plantations under elevated O_3 and with intense aboveground competition (cf. KOZOVITS *et al*. 2005a,b,

LUEDEMANN *et al*. 2005, 2009). Angiosperm stems comprise a higher proportion of living parenchyma cells in the secondary phloem and the xylem than encountered in gymnosperms, serving as storage tissue for starch (KOZLOWSKI & PALLARDY 1997, HÖLL 2000). The fundamental anatomical disparity between beech and spruce relates to observed differences in substrate supply to stem respiration: the conifer primarily consumed currently formed photosynthates, whereas the broadleaved tree species largely relied on C reserves.

3 Investigations on adult beech and spruce

3.1 Materials and Methods

3.1.1 Site description

The study was carried out in the season of 2006 in a mixed beech/spruce stand at the "Kranzberger Forst" in southern Bavaria, near Freising, Germany (Tertiary Hill Region, elevation 485 m a.s.l., 48°25'N, 11°39'E; PRETZSCH *et al.* 1998). Adult European beech (*Fagus sylvatica* [L.]) and Norway spruce (*Picea abies* [L.] Karst.), about 50 to 70 years old and 25 m high, were exposed to either ambient or twice-ambient O_3 concentrations ($1xO_3$ as control and $2xO_3$, respectively), the latter regime being experimentally enhanced since 2000, using a free-air O_3 exposure system (NUNN *et al.* 2002, WERNER & FABIAN 2002). To prevent risk of acute O_3 injury in the $2xO_3$ regime, maximum O_3 concentrations were restricted to 150 nl L^{-1} (REICH 1987). Ozone concentrations were monitored by O_3 analyzers (Model 8811 UV analyzers, Teledyne Monitor Labs, USA) within the canopy at 10-min intervals (for details see LÖW *et al*. 2006). Seasonal O_3 concentrations, AOT 40 and SUM 0 (both parameters representing external O_3 exposure) of $1xO_3$ and $2xO_3$ regime are shown in Tab. 3.1 Seasonal radial stem growth of study trees was monitored with dendrometer bands (Model D1, UMS, München, Germany) at 1.3 m breast height between February 20 through October 24 (by Chair of Forest Yield Science, Technische Universität München). The forest is situated on a luvisol soil derived from loess over tertiary sediments. Long-term mean annual air temperature (1970-2000) and rainfall were 7.8 °C and 786 mm, respectively (monitored by Deutscher Wetterdienst at climate station "Weihenstephan", at 4 km distance from the research site; DWD Offenbach, Germany) (MATYSSEK *et al.* 2007a). Highest O_3 concentrations occurred in July and AOT40 exceeded the critical level of 5 µL O_3 L^{-1} h for forest trees (LRTAP Mapping Manual 2004, NUNN *et al.* 2005a) under the $1xO_3$ regime. The seasonal O_3 concentration of the experimentally imposed $2xO_3$ regime was enhancend by a factor of 1.6 instead of 2 relative to the $1xO_3$ regime. Climatic conditions during the growing season of 2006 are presented in Tab. 3.2 After a warm and dry period in July mean air temperature decreased conspicuously in August (seasonal rainfall maximum) and remained between 11.5 °C and 16.2 °C in the following months.

Tab. 3.1 Seasonal O_3 concentrations (means ± SE, n = 30 to 31 days), AOT40 and SUM0 of the $1xO_3$ and $2xO_3$ regime measured at the study site "Kranzberger Forst".

Season 2006	May	June	July	Aug	Sep	Oct
$1xO_3$ concentration [nl L^{-1}]	47.5±2.8	45.3±1.8	53.0±1.7	29.5±1.5	26.0±1.6	15.5±1.4
$2xO_3$ concentration [nl L^{-1}]	67.0±3.3	72.6±3.7	86.2±3.6	47.9±2.3	44.1±2.9	23.5±2.2
AOT40 $1xO_3$ [µL L^{-1} h]	5.7	4.7	7.4	0.8	0.6	0.0
AOT40 $2xO_3$ [µL L^{-1} h]	13.0	17.1	23.2	6.7	5.1	1.0
SUM0 $1xO_3$ [µL L^{-1} h]	33.0	30.1	36.8	21.6	18.6	8.7
SUM0 $2xO_3$ [µL L^{-1} h]	47.7	52.2	64.1	35.6	31.7	13.4

Photosynthetic photon flux density (PPFD) above the canopy, air temperature, relative air humidity, rainfall and vapor pressure deficit (VPD) within the canopy were recorded at 10-minute intervals (LÖW *et al*. 2006). Soil moisture was monitored in different soil depths between 5 and 140 cm at 30-min intervals. Scaffolding and a 45 m stationary research crane, equipped with a 50 m boom and a working gondola allowed access to the canopies (MATYSSEK & HÄBERLE 2002).

Tab. 3.2 Climatic conditions at the "Kranzberger Forst" during the growing season of 2006. Monthly means are given for photosynthetic photon flux density (PPFD), relative air humidity (RH), air temperature (T_{air}), rainfall, vapor pressure deficit (VPD) and soil moisture (means ± SE; n=30 to 31).

Season 2006	May	June	July	Aug	Sep	Oct
PPFD [µmol $m^{-2}s^{-1}$]	458.6±29.7	565.3±28.7	601.1±23.7	345.6±20.4	363.4±23.1	217.7±12.4
RH [%]	69.8±2.2	68.5±2.0	66.2±2.3	80.7±1.2	77.7±1.4	80.7±0.6
T_{air} [°C]	12.8±0.5	16.9±0.9	21.4±0.4	14.5±0.4	16.2±0.4	11.5±0.5
Rainfall [mm]	82.4±0.7	92.1±1.3	29.0±0.4	113.8±0.9	12.6±0.3	35.6±0.8
VPD [hPa]	5.1±0.5	7.1±0.7	10.2±0.8	3.5±0.4	4.7±0.4	2.8±0.2
Soil moisture [vol %][1]						
5 cm depth	30.7±0.2	28.4±0.5	22.5±0.8	21.4±0.4	17.5±0.2	17.1±0.1
30 cm depth	34.1±0.2	32.3±0.4	27.9±0.4	26.1±0.1	24.7±0.1	25.4±0.1
70-140 cm depth	29.6±0.2	27.9±0.2	25.0±0.3	22.9±0.1	21.5±0.1	21.7±0.1

[1] Data were provided by LWF - Bayerische Landesanstalt für Wald und Forstwirtschaft, Freising, Germany.

3.1.2 $^{13}CO_2/^{12}CO_2$ labeling

In August and September of 2006, the crowns of six beeches and spruces, each, were labeled *via* the free-air CO_2 exposure system "isoFACE" designed for continuous $^{13}CO_2/^{12}CO_2$ labeling of adult forest trees (cf. GRAMS *et al*. 2010b; Fig. 3.1). Each tree crown was supplied, depending on its size, with eight to twelve 30 m long vertically hanging PVC tubes (Øout/in 15/9 mm), extending about 1 m into the canopies of the surrounding trees. To ensure a homogeneous release of CO_2 into the canopy (7 a.m. to 7 p.m.), each tube was micro-perforated by ca. 400 glass capillaries (Øout/in 360/75 µm) at a distance of 2 cm each across the upper eight meters of the tube. The CO_2 used for the $^{13}CO_2/^{12}CO_2$ labeling of the canopy atmosphere, stored at the site in a 10.000 kg CO_2 tank, was distinctly depleted in ^{13}C compared to CO_2 in ambient air (-46.9 ‰ vs. -8.6 ‰, respectively). Typically 75-100 L CO_2 per tree and day have been used. Beech was labeled over the course of 19 days (Aug 18 through Sept 5) and spruce over the course of 18 days (Aug 26 through Sept 12). Half of the labeled trees, i.e. three beeches and spruces, each, were exposed to either $1xO_3$ or $2xO_3$ regime. Daily means of O_3 concentrations and weather conditions during the whole labeling period are presented in Fig. 3.2. Mean O_3 concentrations of the $1xO_3$ and $2xO_3$ regime (± SE) amounted to 29.7 ± 6.9 and 49.3 ± 11.9 nl L^{-1}, respectively (Fig. 3.2 A).

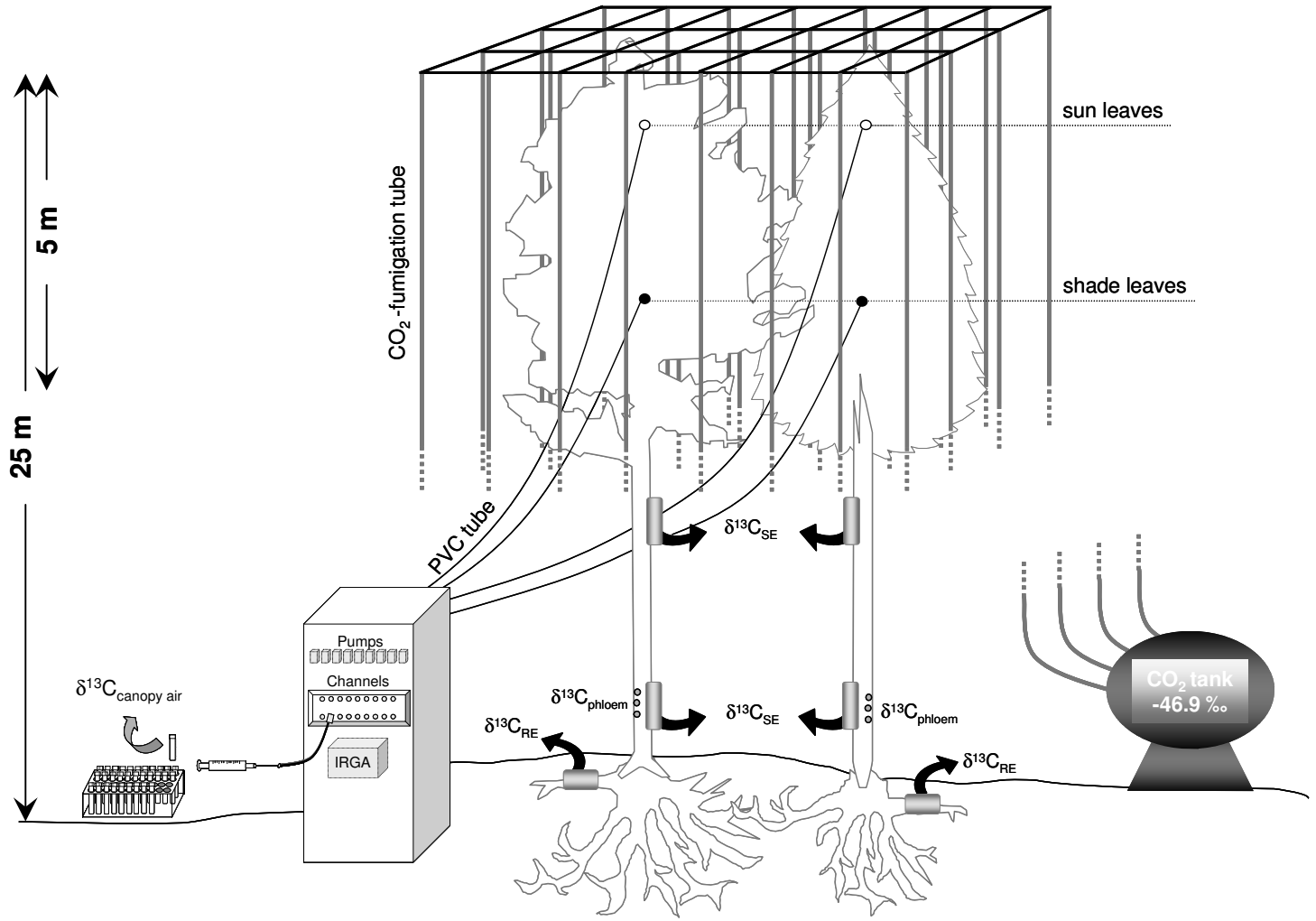

Fig. 3.1 Scheme of the free-air CO_2 exposure system "isoFACE", combined with a multiplex sampling system (cf. GRAMS *et al*. 2010b). Continuous $^{13}CO_2/^{12}CO_2$ labeling was conducted during daytime (7 a.m. to 7 p.m.) by addition of pure CO_2 ($\delta^{13}C$ of -46.9 ‰) into the canopies of six beeches (Aug 18 through Sept 5) and spruces (Aug 26 through Sept 12), each, using 30 m long vertically hanging micro-perforated CO_2-fumigation tubes. Each tube was connected with the lower end to the 10.000 kg CO_2 tank from which labeling-CO_2 was delivered. Canopy air was sampled at two heights (1 and 5 m within the canopy, corresponding to sun and shade leaves) using 30 m long PVC tubes. Stem CO_2 efflux was assessed from a lower (1.3 m breast height) and upper stem position (6 to 17 m), together with coarse root CO_2 efflux. Ploem sap was sampled from the lower stem position nearby the stem cuvette.

Compared with the last two weeks in August, higher air temperature (+3 %), photosynthetic photon flux density (+3 %) and vapor pressure deficit (+17 %) were recorded during the first half of September (Fig. 3.2 B). Abundant precipitation in August was followed by a conspicuous decrease in sum of rainfall in September (-52 %), accompanied by a decrease in relative air humidity (-6 %) and soil moisture (-3.5 %) (Fig. 3.2 C).

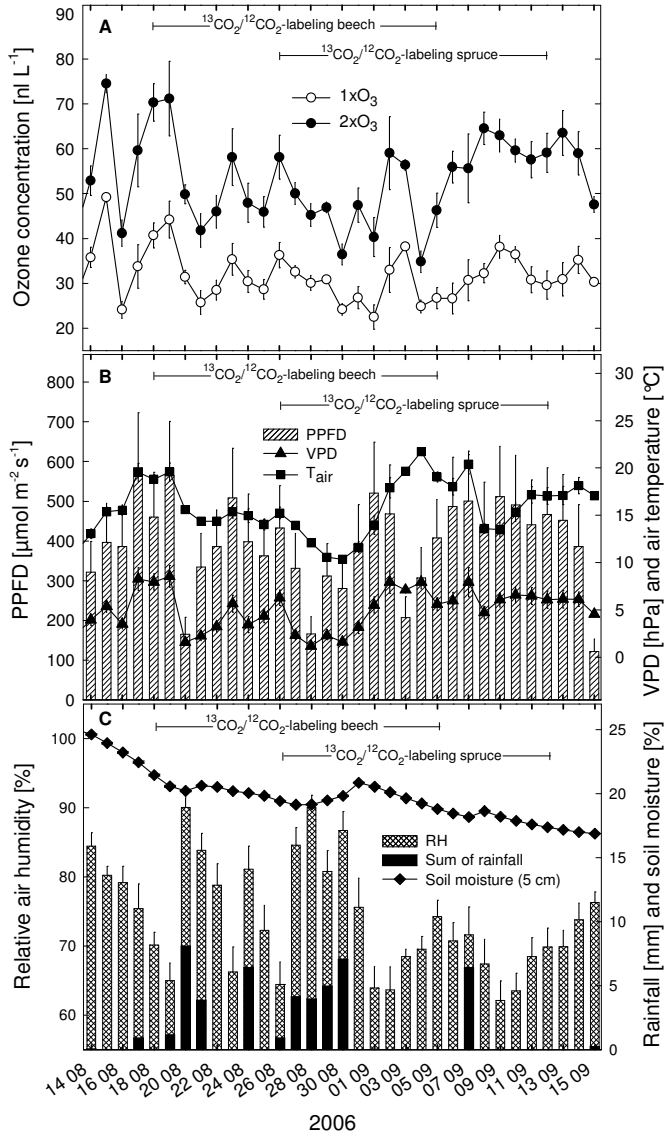

Fig. 3.2 Ambient ($1xO_3$) and twice-ambient O_3 concentrations ($2xO_3$) and weather conditions during continuous $^{13}CO_2/^{12}CO_2$ labeling at the study site "Kranzberger Forst". (A) $1xO_3$ (open circles) and $2xO_3$ (closed circles) (B) Daily sums of photosynthetic photon flux density (PPFD) (hatched bars), air temperature (squares), vapor pressure deficit (VPD) (triangles) and (C) relative air humidity (RH) (checked bars), sum of rainfall (black bars) and soil moisture (diamonds). Except for the sum of rainfall, data are daily means (± SE).

3.1.2.1 CO_2 concentration in canopy air

Canopy air was sampled at two heights: 1 and 5 m within the canopy, corresponding to sun and shade leaves, respectively. Utilizing a multiplex sampling system which was programmed and controled by DIAdem software (Version 8.10, National Insturments, Austin, USA) canopy air was sucked (Wisa membrane pumps, ASF Thomas, Wuppertal) at a flow rate of 1.4 L min^{-1} (Tylan FM360, Tylan GmbH, Eching) through 30 - 40 m long PVC tubes (cf. Fig. 3.1). A total of 14 air channels was sequentially sampled by computer programmed solenoids (AVS, Römer GmbH, Königsdorf-Wiesen, Germany) to conduct each air stream for three minutes to the infrared gas analyzer (Binos 4b.1, Rosemount AG, Hanau). Canopy air CO_2 concentration data were logged onto PC.

3.1.2.2 $\delta^{13}C$ of canopy air

Canopy air was assessed through the entire $^{13}CO_2/^{12}CO_2$ labeling period. $\delta^{13}C$ of canopy air ($\delta^{13}C_{sample}$ in ‰) was calculated using a 2-pool mixing model (PATAKI *et al.* 2003) as described in Eqn. 3.1. Daily means of $\delta^{13}C$ and CO_2 concentration in the canopy air of adult beech and spruce trees are described in section 3.2.1. Following the "Keeling plot approach" (KEELING 1958, 1961), the $\delta^{13}C$ of the source CO_2 utilized for labeling (-46.9 ± 1.1 ‰ SE) was estimated by the y-intercept of the linear regression, plotting $\delta^{13}C$ of canopy air against the inverse of CO_2 concentration ($1/[CO_2]$ mol µmol^{-1}; Fig. B-1, Appendix).

$$\delta^{13}C_{sample} = \frac{\delta^{13}C_{ambient} * [CO_2]_{ambient} + \delta^{13}C_{source} * [CO_2]_{increase}}{[CO_2]_{sample}} \qquad \text{Eqn. 3.1}$$

where,

$[CO_2]_{ambient}$ = CO_2 concentration in unlabeled canopy air [µmol mol^{-1}]
$\delta^{13}C_{ambient}$ = $\delta^{13}C$ of CO_2 in unlabeled canopy air [‰]
$\delta^{13}C_{source}$ = $\delta^{13}C$ of source CO_2 utilized for labeling [‰]
$[CO_2]_{increase}$ = Increase in CO_2 concentration in labeled canopy air (calculated from $[CO_2]_{sample} - [CO_2]_{ambient}$) [µmol mol^{-1}]
$[CO_2]_{sample}$ = CO_2 concentration in labeled canopy air [µmol mol^{-1}]

3.1.3 Assessment of stem CO_2 efflux

Stem CO_2 efflux of adult trees was assessed from July 25 through September 12 with a custom-made open stem gas exchange system (Fig. B-2, Appendix). The measuring principle is in analogy to the open system described in section 2.1.13. Stem CO_2 efflux was assessed from a lower (breast height) and an upper stem position (6 to 17 m) of each study tree by means of plexiglas cuvettes (Plexiglas®, Röhm GmbH, Darmstadt, Germany). Each cuvette was attached to the stem at a position cleaned from mosses, lichens or algae and sealed to the bark with rubber sealant (Terostat IX, Henkel, Düsseldorf, Germany) which subsequently was coated with acrylate (Lugato, Hamburg, Germany). Two strong tension belts were used to fasten the cuvette to the stem. To avoid refixation of efflux CO_2 by photosynthetic stem tissue, cuvettes were covered with aluminized polyester foil. Altogether, the system comprised 20 channels for sampling CO_2 from stem efflux and two channels for sampling reference gas with a constant $\delta^{13}C$ signature (Linde Gas, Pullach, Germany). Reference gas was pushed through 30 m long PVC tubes to stem cuvettes and empty cuvettes, where surplus air was blown off. From cuvettes, gas was continuously sucked by a series of membrane pumps (ASF Thomas, Wisa, Wuppertal, Germany), achieving an almost ambient air pressure inside each cuvette. The various components of the open system have been checked for $\delta^{13}C$ stability by manually taking gas samples using a 100 mL syringe at 11 positions along the gas

way of the system (Fig. B-2 and Tab. B-1, Appendix). Flow rates of air streams through the stem cuvettes were adjusted to between 0.3 and 1.0 L min^{-1} and in empty cuvettes to 0.3 L min^{-1}. Stem temperature was measured with calibrated NTC temperature sensors (SEMI 833 ET, Hygrotec Messtechnik GmbH, Titisee-Neustadt, Germany) which were mounted inside the cuvettes with rubber sealant to the stem. A voltage signal was electronically logged at 8-minute intervals onto PC and converted into a temperature signal (GEBHARDT 2008).

3.1.3.1 Stem CO_2 efflux rate

The calculation of the stem CO_2 efflux rate was based on the volume of the stem sector behind the stem cuvette (GEBHARDT 2008) as described in section 2.1.13.1.

3.1.3.2 $\delta^{13}C$ of stem CO_2 efflux

Gas deriving from a stem cuvette or an empty cuvette was sampled using an automated gas sampler (Gilson 221 XL, Gilson Inc. Middleton, USA). 12 mL glass vials (Exetainer, Labco Limited, High Wycombe, UK) were flushed for six minutes, each, at a flow rate of 0.15 L min^{-1} with sample gas. Calculation of $\delta^{13}C$ of CO_2 deriving from stem efflux ($\delta^{13}C_{Es}$ in ‰) is described in section 2.1.13.2.

3.1.4 Assessment of coarse root CO_2 efflux

An individual coarse root of each study tree was enclosed in a 10 or 20 cm long root cuvette (Plexiglas®, Rölm GmbH, Darmstadt, Germany). Root temperature was measured inside each cuvette with a calibrated NTC temperature sensor (SEMI 833 ET, Hygrotec Messtechnik GmbH, Titisee-Neustadt, Germany). CO_2 from coarse root efflux was sampled by means of a custom build closed respiration system (PRATER *et al*. 2006) controlled by DIAdem software (Version 8.10, National Instruments, Austin, USA) (Fig. B-3, Appendix). Individual root cuvettes were connected to the system being equipped with six parallel sampling channels, each flushing a sampling vial. Before the sampling, the system was flushed for 10 min with ambient air with a flow rate of approximately 0.5 L min^{-1} by means of a membrane pump (ASF Thomas, Wisa, Wuppertal, Germany). For gas sampling, the system was operated in a closed mode for 60 min, as the CO_2 concentration inside the system increased by CO_2 released from the coarse root. At each measurement, a total of six gas samples were collected in glass vials (12 mL, Exetainer, Labco, High Wycombe, UK) which were continuously flushed with cuvette air. Every 10 min one vial was isolated from the closed respiration system by simultaneously closing solenoids (AVS, Römer GmbH, Königsdorf-Wiesen, Germany) directly before and after the vial. Gas samples were analysed for $\delta^{13}C$ and CO_2 concentration on an IRMS (section 2.1.14).

3.1.4.1 Coarse root CO_2 efflux rate

CO_2 from coarse root efflux was sampled utilizing the open stem gas exchange system (section 2.1.13). The calculation of the coarse root CO_2 efflux rate was based on the coarse root volume enclosed by the root cuvette (see section 2.1.13.1).

3.1.4.2 $\delta^{13}C$ of coarse root CO_2 efflux

$\delta^{13}C$ of CO_2 deriving from coarse root efflux was calculated from six gas samples taken at each measurement according to the "Keeling Plot approach" (see Fig. B-1, Appendix).

3.1.5 Assessment of phloem sap

Phloem sap was sampled from the lower stem position nearby the stem chamber following the method of GESSLER *et al*. (2004). Small pieces of bark with adherent phloem tissue (∅ 5 mm) were cored from the stem and incubated (5 h at 4 °C) in 15 mM sodium polyphosphate buffer (Sigma-Aldrich, Munich, Germany). After centrifugation (12.500 rpm, 5 min), a subsample of the phloem sap extract was analyzed by means of HPLC to quantifying water soluble sugars (sum of sucrose, fructose and glucose, C_{PS} in mg). A further subsample was transferred into tin caps, freeze-dried and analyzed for carbon isotope ($\delta^{13}C_{sample}$ in ‰) and element composition (C_{sample} in mg).

3.1.5.1 Quantification of phloem sugars

An aliquot of the phloem sap extract was analyzed using HPLC (CARBOsep CHO-820 calcium column, Transgenomic, Glasgow, UK) with HPLC water (Aldrich, Taufkirchen, Germany) as mobile phase at a flow rate of 0.6 mL min^{-1} and a constant temperature of 85 °C. Concentration of water soluble sugars (sucrose, glucose and fructose) was quantified with a refractive index monitor (RI 2000, Schambeck SFD, Bad Honnef, Germany) on the basis of sucrose, glucose and fructose standards (performed by Dr. F. FLEISCHMANN, Pathology of Woody Plants, Technische Universität München).

3.1.5.2 $\delta^{13}C$ analysis of phloem sugars

A volume of the phloem sap extract containing 1 mg total sugar was transferred into 1.5 mL tubes (Eppendorf, Hamburg, Germany) and freeze-dried (Beta 1-8, Christ, Osterode, Germany) for 10 hours. Dried material was solved in 150 µL of aqua dest., transferred into 170 µL tin caps and freeze-dried for 10 hours for subsequent carbon isotope and element concentration analysis on an EA 3000 Elemental Analyzer (Euro Vector Instruments and Software, Milan, Italy) coupled with an isotope-ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany). $\delta^{13}C$ of sugar carbon in the phloem sap ($\delta^{13}C_{PS}$ in ‰) was calculated (cf. FRY 2006) using a 2-pool mixing model:

$$\delta^{13}C_{PS} = \frac{\delta^{13}C_{sample} * C_{sample} - \delta^{13}C_{NPS} * C_{NPS}}{C_{PS}} \quad [‰] \qquad \text{Eqn. 3.2}$$

where,

$\delta^{13}C_{NPS}$ = $\delta^{13}C$ of non-sugar C in phloem (assuming $\delta^{13}C_{NPS}$ corresponds to $\delta^{13}C_{sample}$ before labeling) [‰]

C_{NPS} = content of non-sugar C in phloem after labeling (calculated from $C_{sample} - C_{PS}$) [mg]

3.1.6 C isotope and element concentration analysis of leaves and fine roots

Leaves and recently grown fine roots (∅ ≤ 2 mm) were collected a few days prior to start and during the last day of $^{13}CO_2/^{12}CO_2$ labeling. Leaves were sampled from sun and shade canopy and fine roots from two or three different locations around the study tree. Fine roots were collected from organic soil horizons to 10 cm soil depth and cleaned from soil with distilled water. Samples were oven-dried for 72 h at 65 °C until constant weight and subsequently ground with a steel ball mill (Retsch MM, 2000, Haan, Germany) to a fine powder. Carbon isotope and element concentration analysis were performed on an EA 3000 Elemental Analyzer (Euro Vector Instruments and Software, Milan, Italy) coupled with an isotope-ratio mass spectrometer (IRMS, GVI-Isoprime, Elementar, Hanau, Germany).

3.1.7 Statistical analyses

Statistical analysis was performed using the SPSS 16.0. software package (SPSS Inc., Chicago, USA). The General Linear Model (GLM) approach was applied to identify significant ozone effects, whereas $p < 0.05$ was regarded as statistically significant. Individual study trees were regarded as experimental units. Datasets had been proved for normal distribution and homogeneity of variances (Levene`s test) within each ozone regime.

3.2 Results

3.2.1 CO_2 concentration and $\delta^{13}C$ of canopy air

Relative to the unlabeled control tree ($1xO_3$ plot), the CO_2 concentration in the canopy air of the labeled beech trees was elevated by 108 ± 6 µmol mol^{-1} ($1xO_3$) and 115 ± 7 µmol mol^{-1} ($2xO_3$) (Fig. 3.3 A). The $\delta^{13}C$ of the canopy air was lowered by 8.1 ± 0.4 ‰ and 8.5 ± 0.5 ‰, respectively. The CO_2 concentration in the canopy air of the labeled spruce trees was elevated by 73 ± 4 µmol mol^{-1} ($1xO_3$) and 77 ± 4 µmol mol^{-1} ($2xO_3$) (Fig. 3.3 B). The $\delta^{13}C$ was lowered by 6.0 ± 0.3 ‰ and 6.2 ± 0.3 ‰, respectively. In both species, the canopy air CO_2 concentration and $\delta^{13}C$ before labeling were similar to those after labeling. However, the change in CO_2 concentration and $\delta^{13}C$ in the labeled canopy air of beech exceeded that of spruce significantly ($p < 0.05$).

3.2.2 Stem and coarse root CO_2 efflux

3.2.2.1 $\delta^{13}C$ of stem and coarse root CO_2 efflux

The $\delta^{13}C$ in the stem CO_2 efflux of the unlabeled control beech tree varied from -28.7 ± 0.2 ‰ to -29.3 ± 0.2 ‰ (upper stem) and from -29.5 ± 0.3 ‰ to -30.4 ± 0.6 ‰ (lower stem) (Fig. 3.4 a). The $\delta^{13}C$ in the coarse root CO_2 efflux varied from -28.9 ‰ to -29.2 ‰. Before labeling, the stem CO_2 efflux of the labeled beech trees showed a $\delta^{13}C$ of -28.2 ± 0.1 ‰ (lower stem) and -27.9 ± 0.4 ‰ (upper stem) under $1xO_3$ and -27.9 ± 0.4 ‰ (lower stem) and -27.4 ± 0.4 ‰ (upper stem) under $2xO_3$ (Fig. 3.4 b). The initial $\delta^{13}C$ in the coarse root CO_2 efflux of beech was *c.* -28.5 ‰ under both O_3 regimes (Fig. 3.4 c).

The $\delta^{13}C$ in the stem CO_2 efflux of the unlabeled control spruce tree varied from -26.7 ± 0.1 ‰ to -27.2 ± 0.1 ‰, and in the coarse root CO_2 efflux from -26.7 ‰ to -27.3 ‰ (Fig. 3.4 g). The initial $\delta^{13}C$ in the stem CO_2 efflux of the labeled spruce trees was -26.6 ± 0.1 ‰ (lower stem) and -27.1 ± 0.1 ‰ (upper stem) under $1xO_3$, which was significantly higher compared to $2xO_3$, with -27.7 ± 0.3 ‰ (lower stem) and -28.2 ± 0.3 ‰ (upper stem) (Fig. 3.4 h). The initial $\delta^{13}C$ in the coarse root CO_2 efflux was -26.7 ± 0.4 ‰ ($1xO_3$) and -27.5 ± 0.9 ‰ ($2xO_3$) (Fig. 3.4 i).

After start of labeling, the $\delta^{13}C$ in the stem CO_2 efflux of the labeled beech trees declined from day 3 on (Fig. 3.4 b). The shift in $\delta^{13}C$, was significantly more pronounced under $1xO_3$ than under $2xO_3$. The $\delta^{13}C$ in the coarse root CO_2 efflux of beech declined from day 2 on (Fig. 3.4 c). Unlike the $\delta^{13}C$ in the stem CO_2 efflux, the shift was less pronounced.
As in beech, the $\delta^{13}C$ in the stem CO_2 efflux of the labeled spruce trees declined from day 3 on (Fig. 3.4 h). In contrast with beech, the shift was significantly higher under $2xO_3$ than under $1xO_3$. The shift in $\delta^{13}C$ of the upper stem CO_2 efflux of spruce was more pronounced (at several days significantly) than the lower one.

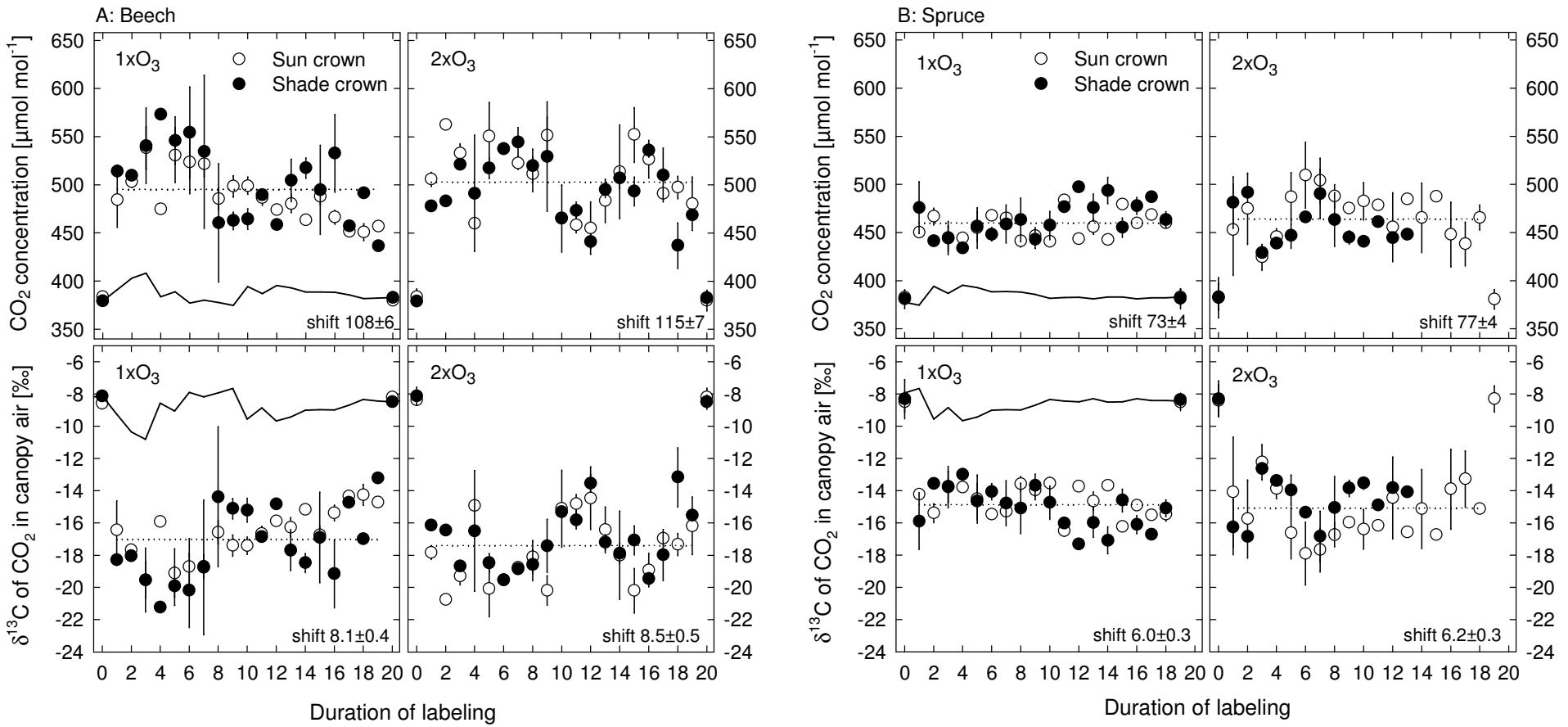

Fig. 3.3 Daytime CO_2 concentration and $\delta^{13}C$ in the canopy air of labeled beech (A) and spruce (B), exposed to ambient ($1xO_3$) and twice-ambient ($2xO_3$) ozone regime. Open and closed circles denote daytime data on canopy air in sun and shade crowns (means ± SE; n=12). Drawn line: unlabeled canopy air ($1xO_3$ control). Dashed line: 19-day (beech) and 18-day (spruce) mean value of labeled canopy air. The shift in CO_2 concentration and $\delta^{13}C$ (relative to the $1xO_3$ control) is denoted in the lower right of each graph (means ± SE; n = 18 to 19 days).

The $\delta^{13}C$ in the coarse root CO_2 efflux of spruce decreased from day 4 on (Fig. 3.4 i). Unlike the $\delta^{13}C$ in the stem CO_2 efflux, the shift was less pronounced.

3.2.2.2 Rates of stem and coarse root CO_2 efflux

The upper stem of the unlabeled control beech tree had *c.* 50% higher CO_2 efflux rates (11 to 14 µmol m^{-3} s^{-1}) than the lower stem (Fig. 3.4 d). The coarse root displayed 10 to 18 times higher CO_2 efflux rates than the stem. The stem CO_2 efflux rate of the unlabeled control spruce tree varied from 17 to 22 µmol m^{-3} s^{-1} (Fig. 3.4 j).

The labeled beech trees displayed the highest CO_2 efflux rates in the upper stem under $1xO_3$ (13 to 15 µmol m^{-3} s^{-1}) (Fig. 3.4 e). In comparison, the stem CO_2 efflux rates under the other treatments were about two-thirds lower. Throughout the entire labeling period, the stem position had a significant impact on the stem CO_2 efflux rates in beech. The coarse root CO_2 efflux rates of the labeled beech trees (Fig. 3.4 f) were 40 to 60 times higher compared with those of the lower stem. Under $2xO_3$, the coarse roots of the labeled beech trees displayed *c.* 0.6 times greater CO_2 efflux rates than under $1xO_3$, being not significant though.

In comparison with beech, the labeled spruce trees displayed overall 2 to 3 times higher stem and coarse root CO_2 efflux rates. The stem CO_2 efflux rates of spruce were significantly enhanced under $2xO_3$ (Fig. 3.4 k). The highest rates were observed at the upper stem (26 to 28 µmol m^{-3} s^{-1}). In comparison, the stem CO_2 efflux rates under the other treatments were about 1.5 to 2.5 times lower. At several days, the stem position influenced the CO_2 efflux rates of spruce significantly. The coarse root CO_2 efflux rates of the labeled spruce trees (Fig. 3.4 l) were 30 to 50 times higher compared with those of the lower stem. Under $1xO_3$, the spruce coarse roots displayed about 1.3 times greater CO_2 efflux rates than under $2xO_3$, being not significant though.

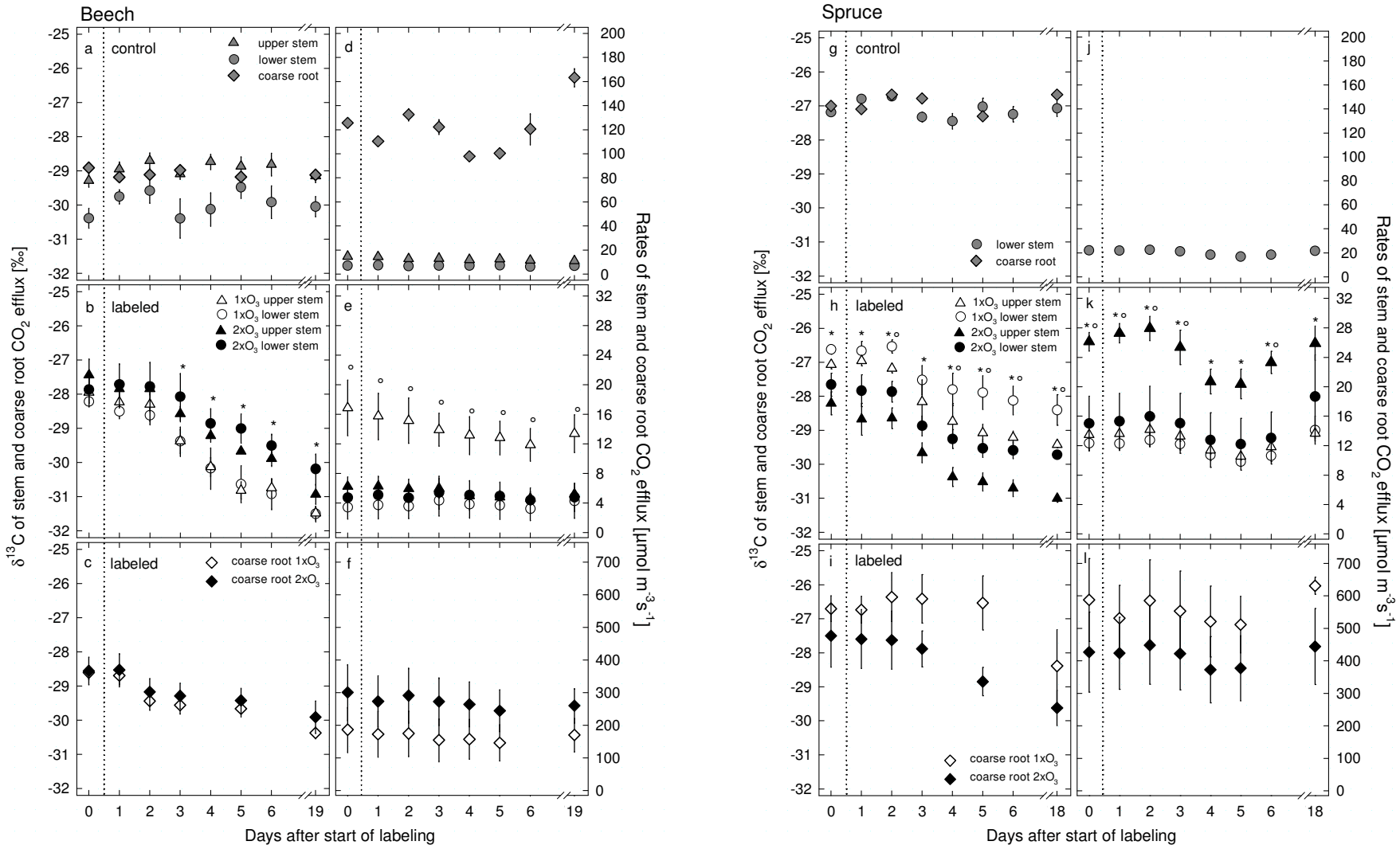

Fig. 3.4 $\delta^{13}C$ as well as corresponding rates of the CO_2 efflux of stems and coarse roots of beech and spruce. A total of six beech and six spruce trees were labeled over the course of 19 days (Aug 18 through Sep 5) and 18 days (Aug 26 through Sep 12), respectively. Half of the labeled trees were exposed to either $1xO_3$ (white) or $2xO_3$ concentrations (black). Data of the unlabeled control beech and spruce tree are represented by gray symbols (n = 1-4) (a, d, g, j). Stem CO_2 efflux (b, e, h, k) and coarse root CO_2 efflux data (c, f, i, l) of labeled beech and spruce are daily means (± SE; n = 3 trees). Triangles denote the upper, and circles the lower stem CO_2 efflux; diamonds denote the coarse root CO_2 efflux. The dotted vertical line indicates the start of the labeling. Significant differences between O_3 regimes are indicated by *, and between upper and lower stem CO_2 efflux by °, respectively ($p < 0.05$).

3.2.3 $\delta^{13}C$ of foliage and fine roots

Before start of labeling, the leaves of the labeled beech trees reflected an initial $\delta^{13}C$ close to that of the unlabeled control beech tree, being -28.2 ‰ (sun leaves) and -31.0 ‰ (shade leaves) (Tab. 3.3 A). The spruce needles were significantly enriched in ^{13}C by 1 to 2 ‰ compared with the beech leaves, irrespective of the needle age (Tab. 3.3 B). Overall, the $\delta^{13}C$ in the sun leaves exceeded that of the shade leaves significantly by *c.* 3 ‰ in the unlabeled control beech tree and the labeled beech trees, and by *c.* 2 ‰ in the labeled spruce trees.

Tab. 3.3 Initial $\delta^{13}C$ in beech (A) and spruce leaves (B) under $1xO_3$ and $2xO_3$ regime (means ± SE, n = 3 labeled trees; n = 1 unlabeled control beech tree). In spruce, data are shown for current-year (2006) and 1-year-old (2005) needles. ° denotes significant differences between sun and shade leaves ($p < 0.05$).

A: Beech	Crown	Control	Labeled $1xO_3$	$2xO_3$
	sun	-28.2	-28.3±0.1 °	-28.0±0.3 °
	shade	-31.0	-31.3±0.3	-31.6±0.3

B: Spruce	Year	Crown	Labeled $1xO_3$	$2xO_3$
	Current-year	sun	-26.4±0.5 °	-27.3±0.2 °
		shade	-28.6±0.4	-29.6±0.6
	1-year-old	sun	-27.0±0.3 °	-27.4±0.4 °
		shade	-28.9±0.2	-29.3±0.7

Before start of labeling, the $\delta^{13}C$ in the fine roots of the labeled beech trees was similar to that of the unlabeled control beech tree, being -28.2 ‰ (Tab. 3.4 A). At that time, the fine roots of the labeled spruce trees were significantly enriched in ^{13}C (by *c.* 2.0 ‰) compared to beech (Tab. 3.4 B).

Tab. 3.4 Initial $\delta^{13}C$ in beech (A) and spruce (B) fine roots under $1xO_3$ and $2xO_3$ regime (means ± SE, n = 3 labeled trees; n = 1 unlabeled control beech tree). ° denotes significant differences between beech and spruce ($p < 0.05$).

Species	Control	Labeled $1xO_3$	$2xO_3$
A: Beech	-28.2	-28.6±0.2 °	-28.4±0.2 °
B: Spruce	-	-26.4±0.3	-26.5±0.2

3.2.4 $\delta^{13}C$ of phloem sap

Before start of labeling, the phloem sap $\delta^{13}C$ of the unlabeled control tree, was -29.5 ‰ in beech and -28.3 ‰ in spruce (Fig. 3.5). The initial $\delta^{13}C$ in the phloem sap of the labeled spruce trees exceeded that of the beech trees significantly by *c.* 2‰ ($p < 0.05$).

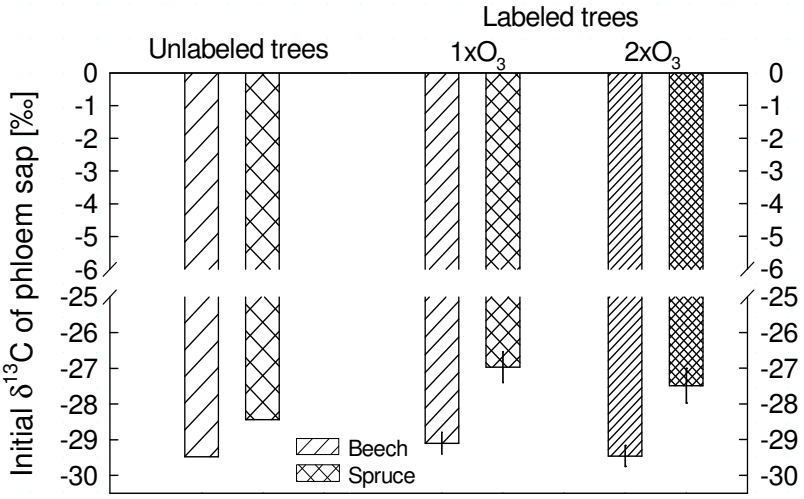

Fig. 3.5 Initial $\delta^{13}C$ of phloem sap of beech (diagonal hatching) and spruce (crossed hatching), sampled from a lower stem position. Half of the labeled trees, six beech and spruce, each, were exposed to $1xO_3$ and $2xO_3$ regime, respectively (means ± SE, n = 3 trees). Unlabeled controls: n=1 tree, each species.

3.2.5 Shift in $\delta^{13}C$

The shift in $\delta^{13}C$ (i.e. change in $\delta^{13}C$ through $^{13}CO_2/^{12}CO_2$ labeling) was compared for the canopy air as well as for the sun and shade leaves, the fine roots, the upper and lower stem CO_2 efflux, the phloem sugars (assessed from a lower stem position) and the coarse root CO_2 efflux of beech and spruce (Fig. 3.6 A, B).

The canopy air $\delta^{13}C$ of the unlabeled control tree was changed by 0.4 ‰ (beech) and 0.2 ‰ (spruce). The $\delta^{13}C$ of the unlabeled beech leaves and fine roots was changed by 0.5 ‰, in each case. In both species, the $\delta^{13}C$ shift of the phloem sugars in the stem was 0.3 ‰. The $\delta^{13}C$ in the stem CO_2 efflux was changed by 0.3 ‰ (beech) and 0.1 ‰ (spruce). The $\delta^{13}C$ shift of the coarse root CO_2 efflux was 0.2 ‰ (beech) and 0.3 ‰ (spruce).

The decrease in canopy air $\delta^{13}C$ of the labeled spruce trees (Fig. 3.6 B) was about 2 ‰ lower as compared to the air around beech (Fig. 3.6 A) (*c.* 6 ‰ vs. 8 ‰, respectively). The $\delta^{13}C$ shift in the leaves of beech exceeded that of spruce significantly by 0.5 ‰ (current-year) and 0.8 ‰ (1-year-old). The $\delta^{13}C$ shift in the stem CO_2 efflux of beech exceeded that of spruce significantly by 33 % (upper stem) and 46 % (lower stem) under $1xO_3$, and by 20 % (upper stem) and 11 % (lower stem) under $2xO_3$, respectively. Irrespective of the O_3 regime, the $\delta^{13}C$

shift in the upper stem CO_2 efflux exceeded that of the lower one by 25 % (beech) and 34 % (spruce); not significant though. The $\delta^{13}C$ shift of the phloem sugars amounted to 4.0 ± 1.4 ‰ ($1xO_3$) and 3.5 ± 0.6 ‰ ($2xO_3$) in beech, and to 3.3 ± 0.3 ‰ ($1xO_3$) and 2.5 ± 0.2 ‰ ($2xO_3$), in spruce. The $\delta^{13}C$ shift in the coarse root CO_2 efflux was 1.8 ‰ ($1xO_3$) and 1.4 ‰ ($2xO_3$) in beech, and 1.7 ‰ ($1xO_3$) and 2.1 ‰ ($2xO_3$) in spruce. The largest shift in fine root $\delta^{13}C$ was observed in spruce under $1xO_3$, being 0.9 ± 0.2 ‰.

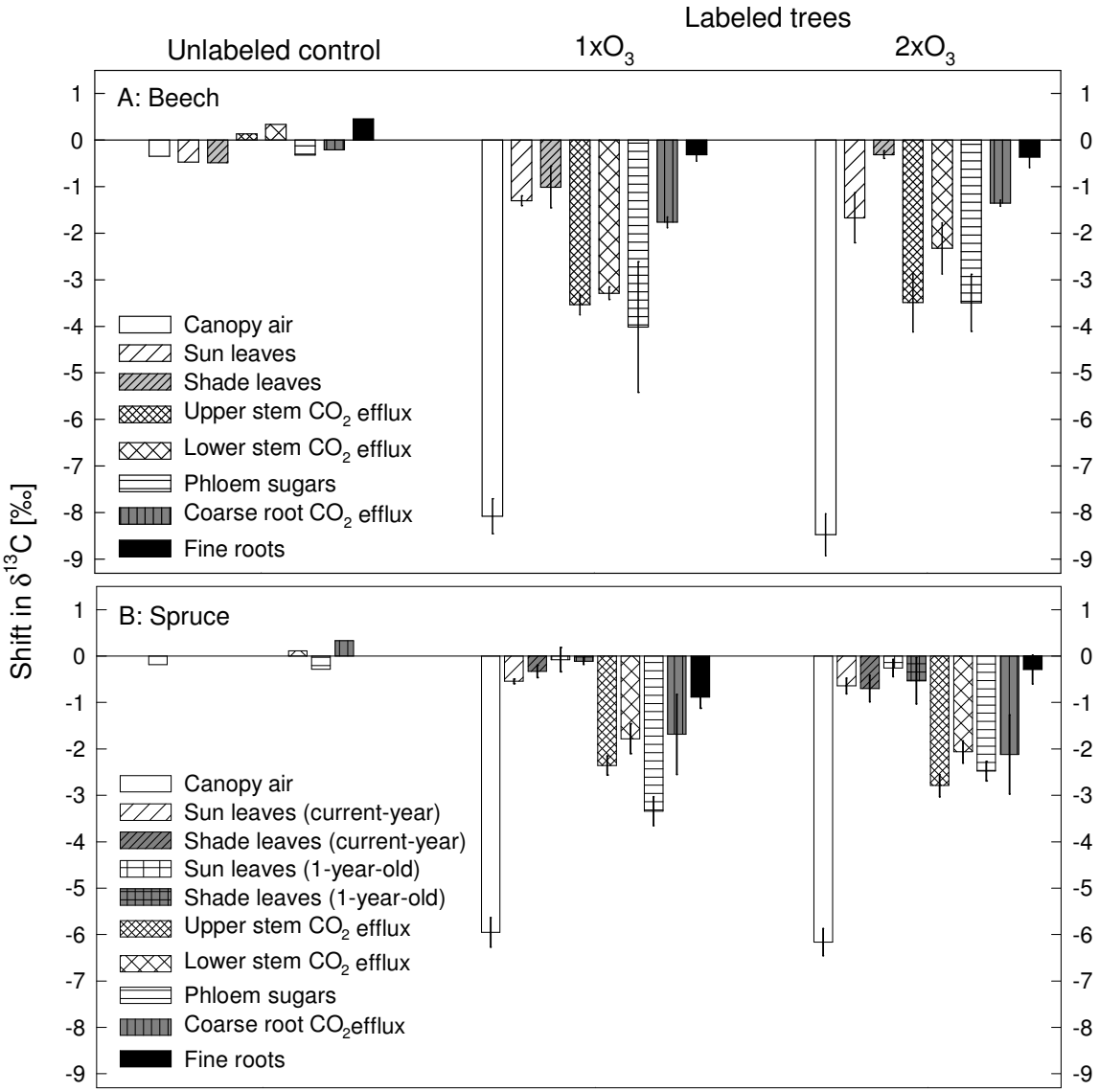

Fig. 3.6 Shift in $\delta^{13}C$ through $^{13}CO_2/^{12}CO_2$ labeling of beech (A) and spruce (B) for 19 and 18 days, respectively. Data are presented for canopy air (white), sun (white) and shade (gray) leaves (diagonal hatching, current-year; checked hatching, 1-year-old spruce needles), upper and lower stem CO_2 efflux (crossed hatching), phloem sugars (horizontal hatching), coarse root CO_2 efflux (gray, vertical hatching) and fine roots (black). Six beech and spruce trees, each, were labeled. Half of them had been subjected to $1xO_3$ or $2xO_3$ regime, respectively (means ± SE; n=3). Unlabeled controls are represented by n=1 tree, each species.

3.3 Discussion

In the following, the findings on adult beech and spruce trees are discussed. Study trees were subjected to elevated O_3 during seven consecutive growing seasons. Continuous $^{13}CO_2/^{12}CO_2$ labeling (19 and 18 days in beech and spruce, respectively) allowed to follow the labeling kinetics of CO_2 released by stem and coarse root CO_2 efflux. The isotopic shift, resulting from labeling, was evaluated in the leaf and fine root tissue, the phloem sugars in the stem as well as the stem and coarse root CO_2 efflux.

The hypothesis tested was:

II. Elevated O_3 reduces the allocation of recently fixed C to stem CO_2 efflux of adult beech and spruce.

The C label was successfully applied into the canopy atmosphere *via* the "isoFACE system" (cf. GRAMS *et al*. 2010b) and incorporated by the *c.* 25 m high beech and spruce trees. Relative to the unlabeled control, the CO_2 concentration of the canopy air was elevated by *c.* 110 µmol mol^{-1} (beech) and 75 µmol mol^{-1} (spruce), and the $\delta^{13}C$ was decreased by *c.* 8 ‰ and 6 ‰, respectively (Fig. 3.3). The increase in CO_2 concentration had no effect on the sap flow density of both species (cf. GRAMS *et al*. 2010b, pers. comm. D. KUPTZ, Ecophysiology of Plants, Technische Universität München), suggesting unchanged stomatal conductance of the leaves. Likewise, other field studies on adult beech (KEEL *et al*. 2007, DUFRÊNE *et al.* 1993) and conifers (NORBY *et al*. 1999, TESKEY *et al.* 2005) have reported low stomatal response upon atmospheric CO_2 enrichment. At the same time, the ratios of leaf internal to leaf external CO_2 concentration of juvenile and adult beech (GRAMS *et al.* 1999, GRAMS *et al.* 2007, M. LÖW, Center for Plants and Environment, University of Western Sydney, Australia, pers. comm.) were only slightly reduced (< 0.02) by an increase in canopy CO_2 concentration of 100 µmol mol^{-1}. Hence, changes in discrimination of $^{13}CO_2$ by photosynthesis are assumed to have been below 0.4‰ (FARQUHAR *et al.* 1989).

In both species, $2xO_3$ had no significant impact on the $\delta^{13}C$ in the unlabeled leaf tissue (Tab. 3.3). However, KITAO *et al*. (2009) observed a more positive $\delta^{13}C$ of leaf organic matter across sun and shade foliage of adult beech under $2xO_3$, indicating increased photosynthetic water-use efficiency (FARQUHAR *et al*. 1989). Photosynthesis of beech was mainly limited through the reduced internal CO_2 concentration upon O_3-induced stomatal closure (KITAO *et al*. 2009). Starch levels were reduced in beech leaves under $2xO_3$ (BLUMENRÖTHER *et al*. 2007), which may reflect a raised repair and detoxification demand and/or inhibited translocation (cf. MATYSSEK *et al*. 2010, LANDOLT *et al.* 1997). Also in spruce, the photosynthesis was limited by $2xO_3$, decreasing already during the first year of the needle life span under elevated O_3 exposure (NUNN *et al.* 2006).

Similar to unlabeled fine roots (Tab. 3.4), unlabeled spruce needles had $\delta^{13}C$ signatures being about 2 ‰ higher than in beech leaves ($p < 0.05$) (Tab. 3.3). Conifers tend to have lower

foliar c_i/c_a ratios, resulting in higher tissue $\delta^{13}C$ compared to deciduous species (GARTEN & TAYLOR 1992, STEINMANN *et al*. 2004). The low $\delta^{13}C$ shift in beech leaves (< 1.7 ‰) and spruce needles (< 0.7 ‰), relative to the ^{13}C-depletion in the canopy air (see above), suggested that the tracer signal in the leaves was diluted by a strong "background signal" (i.e. "old C" compounds stored in the leaf tissue; cf. STEINMANN *et al*. 2004).

In beech, the $\delta^{13}C$ of the stem CO_2 efflux was lowered by *c.* 3-4 ‰ and in spruce by *c.* 2-3 ‰ (Fig. 3.6), indicating that only about half of the sampled C originated from C fixed during labeling. At the same time, the $\delta^{13}C$ in phloem sugars was lowered by *c.* 3-4 ‰ (beech) and 2-3 ‰ (spruce) (Fig. 3.6), suggesting turnover of only 40-50 % and 40-60 % in beech and spruce, respectively. The similar $\delta^{13}C$ shift in phloem sugars and stem CO_2 efflux indicated that phloem sugars represented the main C source for the CO_2 efflux. Unlabeled C in phloem sugars may derive from "old C" atoms in C skeletons of currently synthesized sucrose as a consequence of slow turnover of precursor molecules or from remobilized C compounds (GESSLER *et al*. 2008, TCHERKEZ *et al.* 2003). Consequently, xylem-transported CO_2 deriving from soil or root respiration (cf. TESKEY *et al.* 2008) was considered to have only a minor impact on $\delta^{13}C$ of stem CO_2 efflux in beech and spruce (cf. KODAMA *et al.* 2008, UBIERNA *et al.* 2009). Throughout experimentation, $2xO_3$ had no significant impact on the carbon isotope signature in the phloem sugars of beech and spruce (Fig. 3.6). A similar observation was made by GESSLER *et al.* (2009), regarding the $\delta^{13}C$ of the phloem sap in the sun and shade crown of adult beech (*Fagus sylvatica*).

In both species, current photosynthates were used for the upper and lower stem CO_2 efflux from day 3 on (Fig. 3.4). In beech, the drop in $\delta^{13}C$ was significantly retarded under $2xO_3$, indicating that a substantial proportion of C was derived from C storage pools rather than labeled C. Hence, the allocation of recently fixed C to stem respiration of adult beech was strongly reduced under $2xO_3$ (support of hypothesis II). Conversely, spruce under $2xO_3$ showed significantly increased allocation of recently fixed C to stem CO_2 efflux, i.e. stimulation of C allocation to stem respiration (rejection of hypothesis II). Such a stimulation following O_3 exposure has been reported in several studies on herbaceous plants (GRANTZ & SHRESTHA 2006, REILING & DAVISON 1992). In addition, stem CO_2 efflux in spruce was significantly increased under $2xO_3$. Chronic exposure to elevated O_3 can raise the physiological activity of respiring stem tissues (MATYSSEK *et al*. 2002) for sustaining repair- and detoxification processes (MATYSSEK *et al*. 1995, RENNENBERG *et al*. 1996).

Different C allocation strategies of adult beech and spruce under chronic elevated O_3 exposure may have, on the long-run, implications for stem growth (cf. MATYSSEK *et al.* 2010). BRAUN *et al.* (2007) found the shoot length growth of mature beech (*Fagus sylvatica*) significantly to be reduced by 7.4% per 10 ppm h of AOT40, whereas the shoot growth of spruce (*Picea abies*) was not affected. Throughout the free-air O_3 fumigation period (i.e. twice-ambient O_3 regime) of eight consecutive years, the stem volume growth was unaffected in spruce, however, decreased by 40 % in beech (PRETZSCH *et al*. 2009, MATYSSEK *et al.* 2010). Scaled to the stand level, the decrease was 9 m^3 ha^{-1} $year^{-1}$ in beech, in absolute terms.

Coarse root CO_2 efflux was assessed by means of the open stem gas exchange system (section 2.1.13) from roots growing near the soil surface (at *c.* 5-10 cm soil depth). Rates of root respiration may vary in dependence on the CO_2 concentration at which they are measured (DESROCHERS *et al*. 2002, BURTON *et al.* 1997). However, the applied method was considered reliable since the CO_2 concentration inside the system (*c.* 360 µmol mol^{-1}) approached that prevailing above the forest soil (390 - 400 µmol mol^{-1}; cf. GRAMS *et al.* 2010b), providing a low gradient in CO_2 concentration.

In late summer, current photosynthates in beech were transported belowground within 2 to 3 days and used in coarse and fine root respiration (ANDERSEN *et al.* 2010). CO_2 in the coarse root efflux of beech became depleted in ^{13}C one day earlier than in stem efflux (Fig. 3.4), suggesting the involvement of a faster turnover of respiratory substrate pools in the coarse roots. The drop of $\delta^{13}C$ in the coarse root CO_2 efflux was smaller by *c.* 1-2 ‰ than in stem CO_2 efflux, indicating a lower dependence of coarse root respiration on current photosynthates (BATHELLIER *et al.* 2009, WINGATE 2008).

Soil-air CO_2 around beech consisted of *c.* 25% labeled C (cf. ANDERSEN *et al.* 2010), indicating that considerable amounts of assimilates were rapidly returned back to the atmosphere. In comparison, in 20-year-old beech, the amount of label recovered in the CO_2 efflux was about 10-15 % of the assimilated $^{13}CO_2$ for soil and 5-13 % for stems (PLAIN *et al.* 2009). In contrast to beech, the C label was not detectable in the soil-air CO_2 around spruce (cf. ANDERSEN *et al.* 2010). The conifer seemed to favor allocation of labeled C to storage and/or structural pools rather than respiratory pools in the fine roots. Such an assumption was supported by a significant drop in $\delta^{13}C$ in the fine root tissue during experimentation.
The drop in $\delta^{13}C$ in the coarse root CO_2 efflux of spruce was comparable to that in the stem CO_2 efflux, reflecting short-term balance between substrate supply to and CO_2 release from respiration.
Elevated O_3 had no significant impact on the C allocation to belowground respiratory pools in either tree species (cf. ANDERSEN *et al.* 2010). Conversely, several studies have observed O_3-induced reductions in C allocation to roots and root respiration (RENNENBERG *et al*. 1996, SPENCE 1990, EDWARDS 1991) along with decreased levels of non-structural carbohydrates in roots (GRULKE *et al*. 2001, COLEMAN *et al*. 1996).

In conclusion, the fast transfer of C label from beech and spruce crowns to the stem and coarse root CO_2 efflux confirmed that stem and coarse root respiration were tightly linked to canopy photoynthesis during late summer. Elevated O_3, however, affected the substrate supply to stem respiration of beech and spruce in different ways: the conifer significantly increased the consumption of current assimilates, whereas the allocation of recently fixed C to respiration was restricted in the deciduous tree species. In consistency with model predictions (cf. SITCH *et al*. 2007), the significantly decreased C allocation to the beech stem, along with loss in annual stem production (cf. PRETZSCH *et al*. 2009) suggests that chronic O_3 stress may substantially mitigate the C sink strength of trees (cf. MATYSSEK *et al*. 2010).

4 Comparative discussion and conclusions

Examination of saplings under field-relevant conditions allowed to directly compare tree responses from phytotron observations (Chapter 2) with those from adult forest trees (Chapter 3).

The following hypothesis was examined:

III. Juvenile trees reflect higher O_3 sensitivity of the allocation of recently fixed C to stem CO_2 efflux as compared with adult trees.

The photosynthetic performance of adult beech and spruce was found to be affected under $2xO_3$, however, variations occurred amongst years and crown positions (MATYSSEK *et al.* 2007a, NUNN *et al.* 2005b, 2006, WARREN *et al.* 2007). In comparison, the photosynthesis of beech and spruce saplings examined in phytotrons was not affected by $2xO_3$, in spite of an O_3-induced stimulation ($p < 0.05$) of visible leaf injuries. Several authors have reported a greater O_3 sensitivity of young trees compared with adult forest trees in terms of visible leaf injuries, available antioxidants and photosynthesis (FELZER 2007, NUNN *et al.* 2005a, GRULKE AND MILLER 1994, WIESER *et al.* 2002, KOLB & MATYSSEK 2001). It was argued that beech and spruce saplings in phytotrons may have experienced high O_3 uptake under otherwise non-limiting experimental conditions (soil moisture, N availability), including $1xO_3$. Ample soil N supply can enhance the resistance of trees to elevated O_3, whereas high soil moisture promotes the uptake of O_3 through stomatal opening (cf. SCHAUB *et al.* 2003, NIKULA *et al.* 2009).

In adult spruce, elevated O_3 stimulated the allocation of recently fixed C to stem respiration significantly, whereas spruce saplings showed no response to ozone (rejection of hypothesis III). Both juvenile and adult beech significantly reduced the allocation of recently fixed C to stem CO_2 efflux under $2xO_3$ (rejection of hypothesis III), indicating that C supply to stem respiration was derived to a great extent from C storage pools. Ozone-induced readjustments in C allocation to stem respiration may reflect plant injury and stress compensation (cf. RANFORD & REILING 2007, PELL *et al.* 1994), being at the expense of stem growth on the long-term. The availability of mobile C to defence/repair under ozone stress is supposed to be lower in mature than juvenile trees because of proportionally higher respiratory costs for maintaining living tissues and lower photosynthetic rates of mature trees (cf. OKSANEN 2003, KOLB & MATYSSEK 2001).

Two years of $2xO_3$ treatment had no impact on both stem diameter and shoot axes biomass increment of beech and spruce saplings in phytotron chambers. In comparison, in adult beech and spruce, ozone stress shifted the resource allocation to stem height growth at the expense of diameter growth across the O_3 fumigation period of eight consecutive years (cf. PRETZSCH *et al.* 2009). Beech reduced its biomass investment in upper stem parts and favoured

allocation to the lower parts (i.e. change towards neiloidal stem form), whereas spruce altered its allometry towards a cone-shaped stem form.

In parallel to significant reductions in allocation of recently fixed C to stem respiration and stem volume growth, adult beech trees reflected reduced N uptake under $2xO_3$, along with decreased stomatal conductance and crown transpiration (pers. comm. R. WEIGT, Ecophysiology of Plants, Technische Universität München). In contrast with saplings, adult beech and spruce trees reflected reduced N acquisition under $2xO_3$ (pers. comm. R. WEIGT), suggesting an enhanced consumption of N storage pools. Such an effect might weaken on the long-run the nutrient equilibrium of the trees with implications for growth performance. As the pool sizes of C and N increase with tree age, adult trees may have a higher capacity to supply ressources to growth and respiration, which affects their ability to tolerate environmental stress. As a consequence, depletion of pool sizes under chronic elevated O_3 exposure may render adult trees more susceptible to other stressors, such as drought (GRULKE *et al*. 2001, JOHNSON & SICCAMA 1989).

In the present work, ozone displayed no impact on the whole-tree partitioning of newly acquired N in beech and spruce saplings. In comparison, in adult trees, the N allocation of both species was influenced in opposite ways under $2xO_3$: spruce tended to increase the allocation of „new N" into the shoot (particularly the needles; pers. comm. R. WEIGT), indicating enhanced aboveground N demand. Shifts in N allocation towards defence/repair at the leaf level (ALEXOU *et al.* 2007) might occur at the expense of N investments into roots and mycorrhizae (cf. ANDERSEN 2003). Conversely, beech at $2xO_3$ tended to foster „new N" investment into belowground organs (pers. comm. R. WEIGT).
In parallel, O_3-fumigated beech displayed a stimulated fine-root and ectomycorrhizal biomass development, the latter being apparently mediated through hormonal perturbation (cf. MATYSSEK *et al*. 2007a, NIKOLOVA *et al*. 2010). In consistency with adult beech, beech saplings significantly stimulated their fine root growth in response to elevated O_3. Such O_3-induced stimulatory effects on growth processes are interpreted as a compensatory strategy (cf. OKSANEN *et al.* 2001, KARNOSKY *et al*. 1996, NIKULA *et al*. 2009).

In conclusion, adult beech resembled in O_3 sensitivity to beech saplings in terms of a reduced allocation of recently fixed C to stem CO_2 efflux. Conversely, in spruce, O_3 sensitivity was observed only in adult trees (i.e. increased allocation of recently fixed C to stem CO_2 efflux). As beech and spruce showed different reactions to elevated O_3 in terms of C and N allocation, inter-species competition may change in mixed stands. Since tropospheric O_3 levels show an increasing trend in Central Europe (cf. PRATHER *et al.* 2001, FOWLER *et al.* 1999, 2008), chronic elevated ozone impact may lower the regional C sink strength (cf. SITCH *et al.* 2007), leading to substantial losses in economical/ecological terms and increased radiative forcing in the atmosphere.

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Appendix

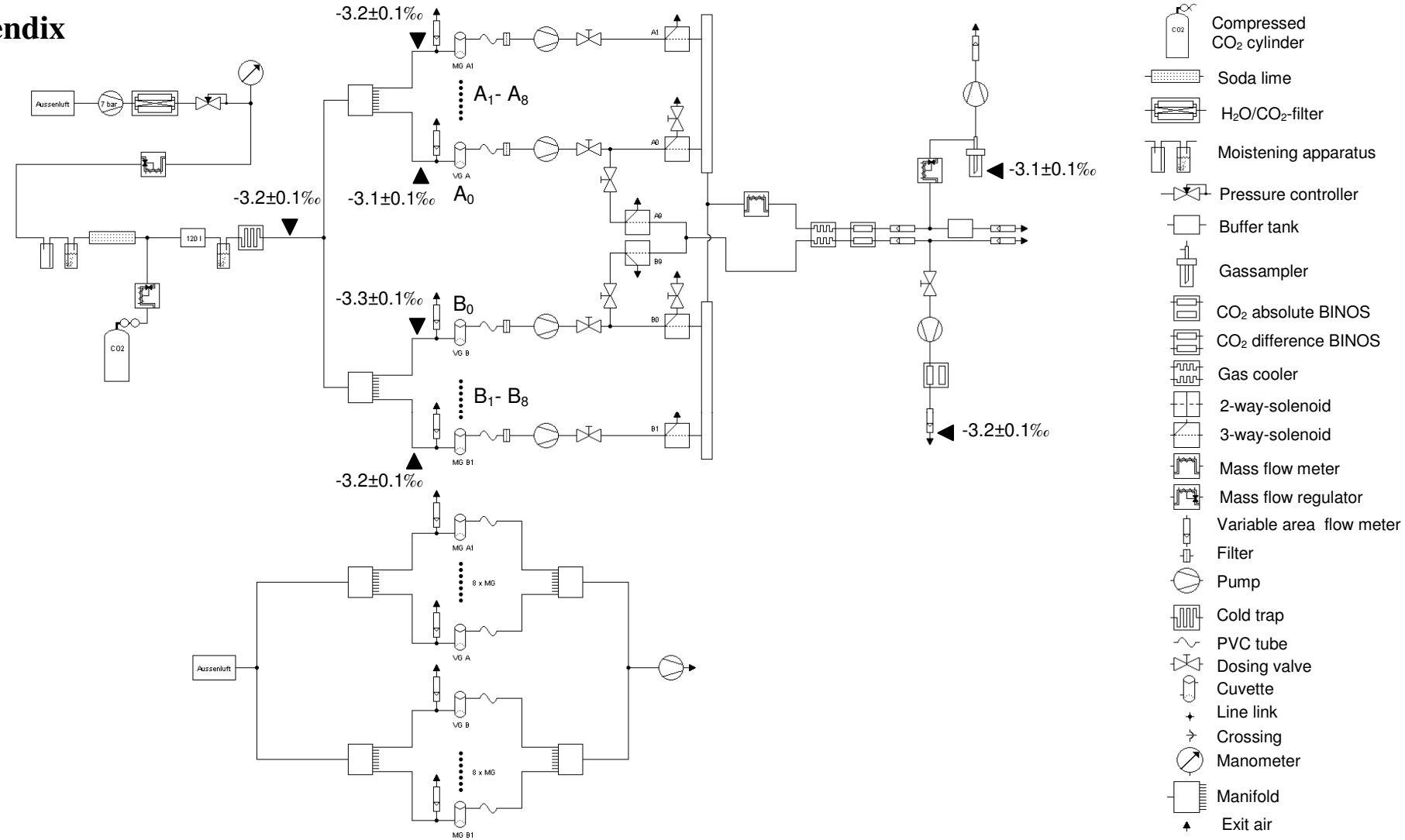


Fig. A-1 Set-up of the open gas exchange system for assessment of stem CO_2 efflux from juvenile trees (Design: Dipl.-Ing. T. FEUERBACH, Ecophysiology of Plants, TU München). The various components of the system have been checked for $\delta^{13}C$ stability by manually taking gas samples (100 mL syringe) at 7 positions along the gas way of the system as marked by black arrowheads. Inserted numbers represent $\delta^{13}C$ mean values (± SD; n=3 gas samples, each).

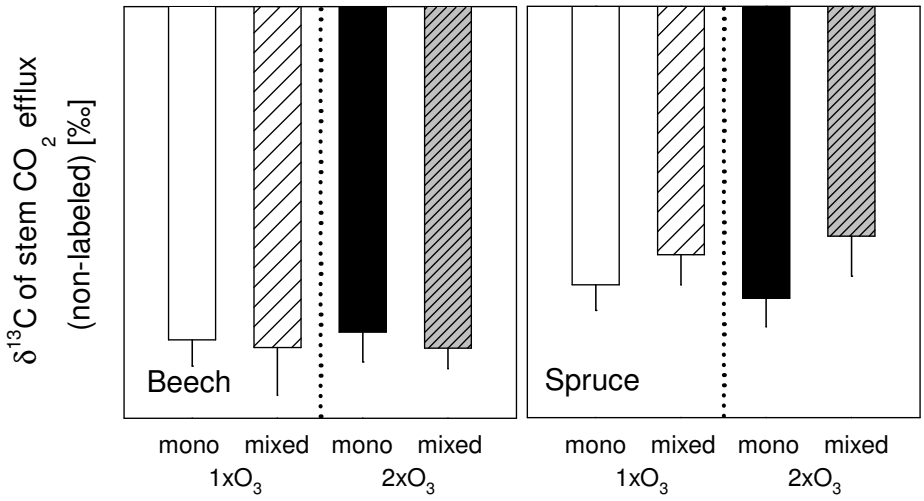


Fig. A-2 Initial $\delta^{13}C$ of stem CO_2 efflux of beech and spruce exposed to $1xO_3$ or $2xO_3$ regime prior start of $^{13}CO_2/^{13}CO_2$ labeling (September 2005). Open and solid bars denote monocultures, and hatched bars the mixed cultures (means ± SE; n = 2 to 4 containers). No significant main effect by O_3 or type of competition was observed.

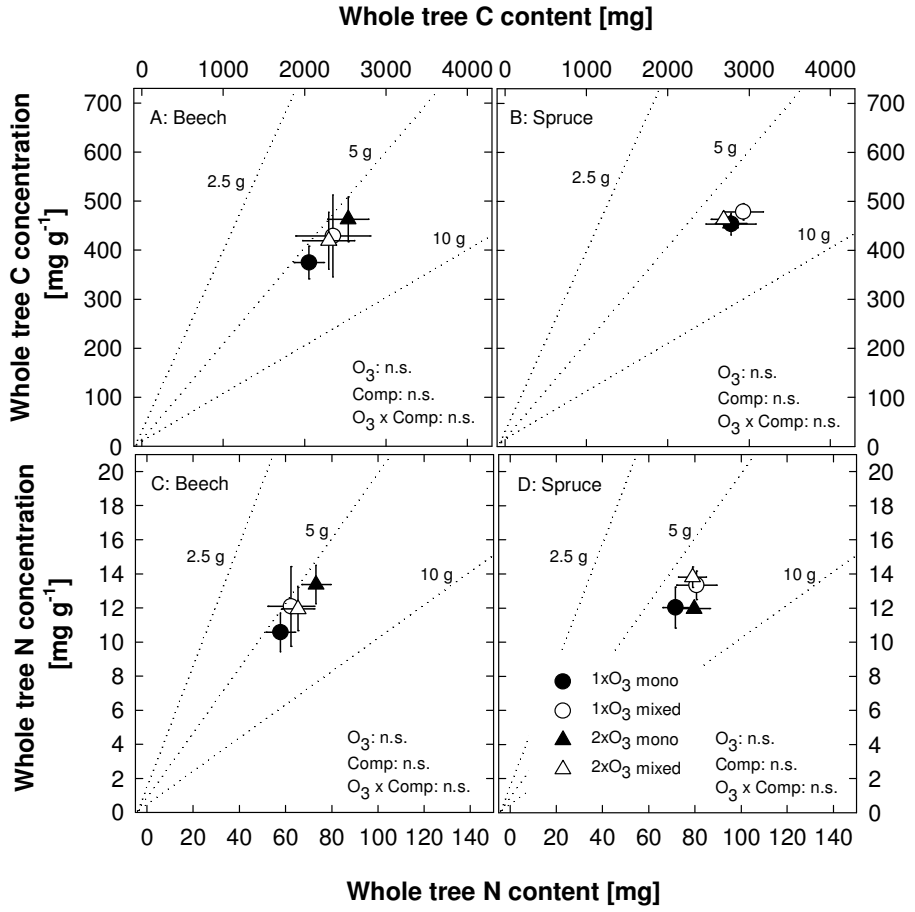


Fig. A-3 C concentration *vs*. C content (A, B) and N concentration *vs*. N content (C, D) in beech and spruce trees at the end of the study. Monocultures are given as solid, mixed cultures as open symbols. Circles denote $1xO_3$ and triangles $2xO_3$ regime (means ± SE, n = 4 containers). No significant main effect by O_3 or type of competition was observed.

Tab. A-1 Number of beech leaves in September (means ± SE, n = 4 containers). Elevated O_3 increased the number of leaves significantly ($p < 0.05$).

No. of leaves	Beech
1xO_3 monoculture	20.7 ± 0.9
1xO_3 mixed culture	23.1 ± 5.3
2xO_3 monoculture	28.4 ± 1.2
2xO_3 mixed culture	24.9 ± 5.7

Tab. A-2 Data of $\delta^{13}C$ (A) C concentrations (B) and N concentrations (C) of organs in beech (means ± SE, n = 4 containers). * denotes significant effects of O_3 and competition as well as their interaction (O_3 x comp.) ($p < 0.05$); n.s. states "not significant".

(A) $\delta^{13}C$ [‰]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	-18.9±0.8	-17.6±1.1	-18.6±1.2	-16.8±0.3	n.s.	n.s.	n.s.
Leaves	-20.8±0.6	-22.8±0.7	-22.9±0.7	-21.9±0.3	n.s.	*	*
Current axes	-25.2±0.3	-26.3±0.3	-25.2±0.2	-25.6±0.3	n.s.	*	n.s.
Old axes	-27.3±0.1	-25.3±0.4	-24.7±0.5	-24.9±0.2	*	n.s.	*
Stem	-26.2±0.3	-26.3±0.4	-26.7±0.2	-25.8±0.1	n.s.	n.s.	n.s.
Coarse roots	-24.4±0.8	-24.6±0.4	-25.4±0.3	-25.5±0.4	*	n.s.	n.s.
Fine roots	-26.9±0.2	-27.2±0.3	-26.8±0.2	-26.0±0.4	*	n.s.	*

(B) C [%]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	46.2±0.6	46.2±0.1	46.2±0.3	45.4±0.5	n.s.	n.s.	n.s.
Leaves	42.8±0.6	41.7±0.5	44.6±0.3	44.5±0.3	*	n.s.	n.s.
Current axes	48.2±0.4	49.3±0.5	48.2±0.1	47.9±0.7	n.s.	n.s.	*
Old axes	50.7±0.1	49.5±0.5	48.2±0.1	48.1±0.5	*	n.s.	*
Stem	49.0±0.3	48.3±0.2	49.0±0.6	49.7±0.4	n.s.	n.s.	n.s.
Coarse roots	44.8±0.4	44.6±0.2	44.6±0.3	44.3±0.4	n.s.	n.s.	n.s.
Fine roots	45.3±0.8	45.0±0.5	45.9±0.3	46.0±0.5	n.s.	n.s.	n.s.

(C) N [%]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	1.22±0.02	1.16±0.02	1.25±0.04	1.17±0.03	n.s.	*	n.s.
Leaves	2.01±0.05	1.97±0.10	1.91±0.06	2.05±0.16	n.s.	n.s.	n.s.
Current axes	1.06±0.08	0.94±0.03	1.06±0.07	1.02±0.05	n.s.	n.s.	n.s.
Old axes	0.85±0.01	0.87±0.02	1.06±0.21	0.91±0.06	n.s.	n.s.	n.s.
Stem	0.87±0.01	0.86±0.04	0.90±0.06	0.87±0.04	n.s.	n.s.	n.s.
Coarse roots	1.38±0.06	1.42±0.01	1.32±0.09	1.42±0.08	n.s.	*	n.s.
Fine roots	1.39±0.03	1.37±0.02	1.45±0.01	1.41±0.04	*	n.s.	n.s.

Tab. A-3 Data of $\delta^{13}C$ (A) C concentrations (B) and N concentrations (C) of organs in spruce (means ± SE, n = 4 containers). * denotes significant effects of O_3 and competition as well as their interaction (O_3 x comp.) ($p < 0.05$); n.s. states "not significant".

(A) $\delta^{13}C$ [‰]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	-25.0±0.7	-24.6±0.7	25.4±0.5	24.2±0.5	n.s.	n.s.	n.s.
Current leaves	-23.5±0.6	-22.1±0.4	-23.0±0.5	-21.4±0.4	n.s.	*.	n.s.
Old leaves	-29.9±0.2	-28.1±1.4	-29.9±0.4	-27.9±1.2	n.s.	*	n.s.
Current axes	-22.8±0.9	-22.5±0.7	-24.0±0.6	-21.6±0.7	n.s.	*	n.s.
Old axes	-24.6±0.5	-23.4±1.1	-24.7±0.5	-23.6±0.2	n.s.	n.s.	n.s.
Stem	-23.8±0.4	-21.7±0.7	-22.6±0.4	-21.0±1.0	*	*	n.s.
Coarse roots	-24.2±0.7	-22.4±0.2	-23.1±0.6	-21.8±1.1	n.s.	*	n.s.
Fine roots	-25.6±0.6	-22.8±0.9	-24.9±0.6	-23.1±0.4	n.s.	*	n.s.

(B) C [%]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	48.3±0.4	47.2±0.1	49.0±0.1	48.1±0.2	*	*	n.s.
Current leaves	48.0±0.3	48.0±0.5	47.0±0.2	47.7±0.4	*	*	n.s.
Old leaves	48.7±0.4	48.8±0.7	48.2±0.2	48.3±0.3	n.s.	n.s.	n.s.
Current axes	49.1±0.3	49.9±0.3	49.8±0.2	47.8±0.1	*	*	*
Old axes	50.5±0.5	52.5±0.4	52.9±0.3	49.3±0.6	n.s.	n.s.	*
Stem	49.8±0.2	48.9±0.5	49.3±0.2	50.0±0.2	n.s.	n.s.	*
Coarse roots	46.9±0.5	45.1±0.5	45.1±0.6	45.3±0.5	n.s.	n.s.	*
Fine roots	43.1±0.2	43.4±0.5	42.3±0.3	44.6±0.3	n.s.	*	*

(C) N [%]	1xO_3 mono	1xO_3 mixed	2xO_3 mono	2xO_3 mixed	O_3	Comp.	O_3 x Comp.
Buds	1.45±0.15	1.42±0.10	1.29±0.05	1.60±0.12	n.s.	n.s.	n.s.
Current leaves	2.04±0.2	1.99±0.09	1.98±0.03	2.19±0.07	n.s.	n.s.	n.s.
Old leaves	1.61±0.04	1.50±0.10	1.60±0.12	1.83±0.10	n.s.	n.s.	n.s.
Current axes	1.32±0.14	1.30±0.06	1.32±0.08	1.31±0.03	n.s.	n.s.	n.s.
Old axes	0.91±0.05	0.85±0.06	0.97±0.08	0.98±0.08	n.s.	n.s.	n.s.
Stem	0.94±0.08	1.07±0.09	0.85±0.04	0.99±0.10	n.s.	*	n.s.
Coarse roots	1.00±0.02	0.97±0.07	0.98±0.02	0.97±0.07	n.s.	n.s.	n.s.
Fine roots	1.97±0.03	1.93±0.04	1.94±0.05	1.99±0.05	n.s.	n.s.	n.s.

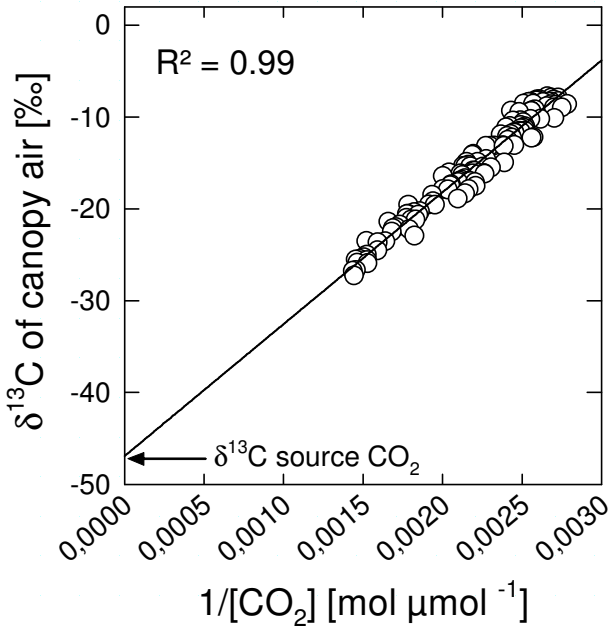


Fig. B-1 $\delta^{13}C$ of CO_2 in canopy air is plotted against the inverse of CO_2 concentration ($1/[CO_2]$ mol µmol^{-1}) ("Keeling plot"). $\delta^{13}C$ of source CO_2 utilized for $^{13}CO_2/^{12}CO_2$ labeling (-46.9 ± 1.1‰ SE) is estimated by the y-intercept of the linear regression ($r^2 = 0.99$). From Aug 18 through Sept 05 a total of 130 air samples were taken at 48-hour intervals from 14 channels of a multiplex sampling system (section 3.1.2.1). Using a 60 mL syringe (Omnifix, Braun, Melsungen AG) samples were flushed into N_2-flushed glass vials (12 mL, Exetainer, Labco Limited, Exetainer, UK) and analyzed within 48 hours on an isotope-ratio mass spectrometer (GVI-Isoprime, Elementar, Hanau, Germany).

Tab. B-1 $\delta^{13}C$ mean values of gas samples (± SD; n=3 to 5) taken along the gas way of the system as shown in Fig. B-2.

Sampling position	July 17	August 14	September 02
A	-2.9 ± 0.1	-2.2 ± 0.1	-3.2 ± 0.1
B	-2.8 ± 0.0	-2.2 ± 0.1	-3.0 ± 0.1
C	-2.7 ± 0.2	-2.2 ± 0.1	-3.1 ± 0.1
D	-2.7 ± 0.1	-2.3 ± 0.1	-3.0 ± 0.1
E	-2.7 ± 0.1	-2.5 ± 0.1	-3.0 ± 0.0
F	-2.7 ± 0.0	-2.3 ± 0.1	-3.0 ± 0.1
G	-2.7 ± 0.1	-2.5 ± 0.1	-3.0 ± 0.1
H	-2.8 ± 0.1	-2.4 ± 0.1	-3.1 ± 0.1
I	-3.0 ± 0.1	-2.4 ± 0.1	-3.0 ± 0.1
J	-2.8 ± 0.1	-2.3 ± 0.1	-3.1 ± 0.1
K	-2.9 ± 0.1	-2.4 ± 0.1	-3.0 ± 0.0

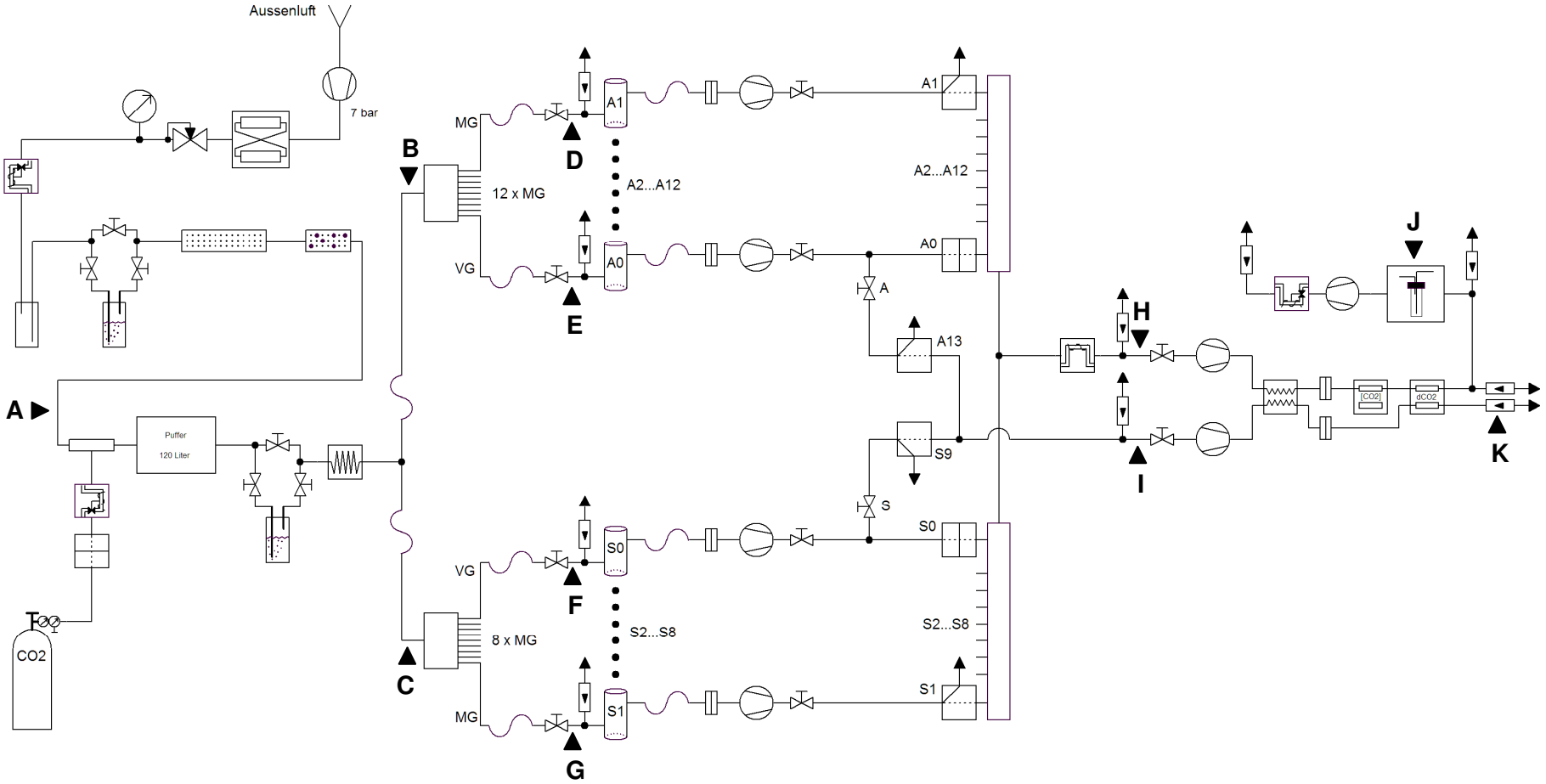
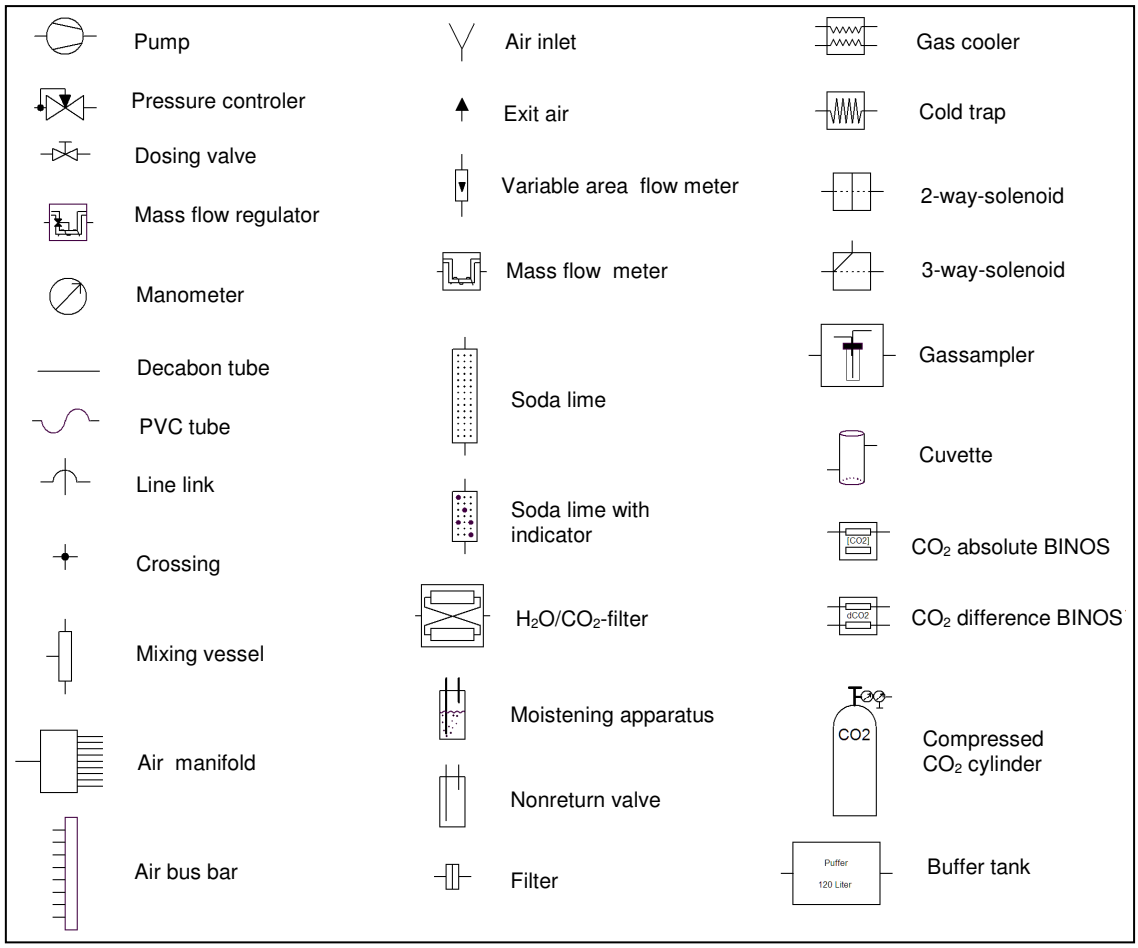


Fig. B-2 Set-up of the open gas exchange system for assessment of stem CO_2 efflux from adult trees (notice legend on the following page) (Design: Dipl.-Ing. T. FEUERBACH, Ecophysiology of Plants, TU München). Various components of the system have been checked for $\delta^{13}C$ stability by manually taking gas samples (100 mL syringe) at 11 positions along the gas way of the system (marked by letters "A" to "K", see Tab. B-1).



Legend of Fig. B-2

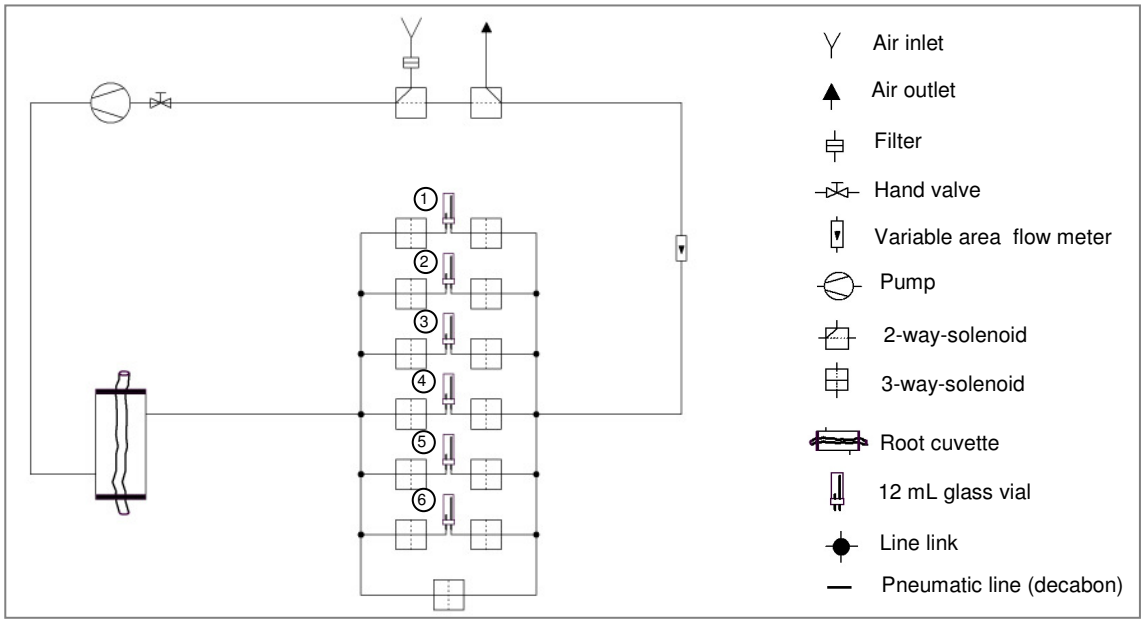


Fig. B-3 Scheme of a custom build closed sampling device for collection of coarse root CO_2 efflux (Design: Dipl.-Ing. T. FEUERBACH, Ecophysiology of Plants, TU München).