Lehrstuhl für Ökophysiologie der Pflanzen

Competitiveness of young beech (*Fagus sylvatica*) and spruce (*Picea abies*) trees under ambient and elevated CO_2 and O_3 regimes

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SUMMARY

The competitiveness of young beech (*Fagus sylvatica*) and spruce plants (*Picea abies*) was analysed in a process-based, mechanistic way. Since the competitive success of the individual plant basically depends on the ability to acquire resources from external pools which are attractive also to neighbouring plants, and since such pools are intrinsically represented by spatial dimensions, competitiveness was quantified in terms of costs (resource investments) and benefits (resource gains) associated with structures (i.e. leaves, axes, roots) that are involved in a sustained above- and belowground sequestration and exploitation of space. Three kinds of cost/benefit ratios (efficiency ratios) were defined for measuring competitiveness: (1) Efficiency ratio of space sequestration (i.e. occupied aboveground space per unit of resource investment, e.g. biomass), (2) efficiency ratio of resource gain (i.e. resource acquisition per unit of resource investment or occupied above or belowground space) and, (3) efficiency ratio of “running costs” in terms of respiration and transpiration per unit of sequestered above and belowground space for sustaining the structural functionality related to competition. The major aims of this study were to quantify the resource budgets that occur along structures and within occupied space of relevance for competition, and then to examine to what extent the proposed efficiency ratios may be applicable to expressing competitiveness of plants in quantitative terms.

Competition between beech and spruce saplings was investigated in a two-year phytotron study. In 1998, two and three-year-old beech and spruce individuals of same height, respectively, were planted in mixed and monocultures in containers that were filled with forest soil. Although each container (32 in total) harboured 20 trees, measurements were concentrated on the six central plants (preventing bias by edge effects). In the year preceding the phytotron study (i.e. 1998), the containers were kept in the greenhouse at ambient and elevated CO_2 concentration (ambient + 300 ppm CO_2). In the following two growing seasons (1999 and 2000), the containers were placed into the phytotrons at ambient and elevated CO_2 concentrations in combination with ambient ($1xO_3$) and twice-ambient ($2xO_3$) ozone levels, resulting in four CO_2/O_3 gaseous regimes: Ambient $CO_2/1xO_3$ (gaseous control regime), ambient $CO_2/2xO_3$ ($+O_3$), elevated $CO_2/1xO_3$ ($+CO_2$) and elevated $CO_2/2xO_3$ ($+CO_2/+O_3$). Elevated levels of CO_2 and O_3 were used as disturbants to provoke changes in growth and resource allocation in order to more readily distinguish and derive underlying mechanisms of relevance for competitiveness from plant response. Four hypotheses were tested: (1) the

competitiveness of beech rather than spruce is affected by the elevated O_3 regime, whereas (2) spruce profits from the increase in resource availability (CO_2) in mixed culture. However, (3) tree responses to CO_2 and O_3 depend on the type of competition (i.e. intra or interspecific). (4) The competitive ability of plants can be characterized and quantified in terms of the introduced efficiency ratios of competitiveness.

In the studied plant systems, spruce was a better competitor than beech. After two growing seasons in the phytotrons, beech plants in mixed plantation showed significant reduction in above- and belowground biomass, seasonal biomass increments and crown volume as compared with beech in monoculture. In contrast, spruce tended to increase its total biomass in the presence of beech, especially under $+CO_2$. The results confirmed hypothesis 1: Beech was more sensitive to the enhanced O_3 regime than spruce. In 1999, beech plants in monoculture increased the number of leaves and foliage area under elevated O_3, while no changes were observed in mixed plantation. One year later, beech plants under $+O_3$ reduced most growth parameters (mean leaf area, shoot and root biomass, foliage area and crown volume) as compared with individuals in the gaseous control regime. The reductions were larger in mixed than in monoculture. These results suggest a higher susceptibility to ozone of beech in mixed culture, confirming hypothesis 3. Contrasting with beech, spruce did not respond to the enhanced O_3 regimes. Similar to the findings under $+O_3$, responses of beech to enhanced CO_2 varied depending on the plantation type (confirming hypothesis 3). In 1999, the number of leaves, foliage area and shoot biomass of beech were stimulated under $+CO_2$ in mono but not so in mixed culture. In 2000, elevated CO_2 led to reduced beech growth in both plantation types**,** however, reductions were larger in mixed culture. On the other hand, spruce profited from the enhanced CO_2 by increasing its total biomass in mixed plantation, which was supportive of hypothesis 2. Also the counteracting effect of elevated CO_2 relative to adverse ozone impact on beech varied between plantation types (counteraction only in monoculture). One major outcome was that competition modified tree responses to CO_2 and O_3 and that, vice-versa, these two gases influenced the competitive ability of the trees. In addition, the extent of tree responses varied or gradually substantiated during the course of the two experimental years: Effects of gaseous regimes and competition required more than one growing season to become statistically significant.

In the presence of the gaseous regimes, the main factor driving changes in resource partitioning was the plantation type (i.e. the kind of competition). The extent of biomass

production in plants of mono and mixed culture was consistent with the respective photosynthetic performance. In 2000, beech plants showed, e.g., lower instantaneous CO_2 assimilation rates in mixed as compared with monoculture, especially under $+O_3$ and $+CO_2$. The lower photosynthetic rates of beech in mixed culture were not caused by light limitation, leaf nitrogen content, ozone injury (necroses) nor by the electron transport rate of the photosynthetic light reactions, but was associated with lowered stomatal conductance. In addition to the lower stomatal conductance, reduced foliage area and crown volume, and the delay in shoot development of beech plants in mixed plantation as compared with those plants in monoculture also contributed to the lower seasonal C gain of beech under interspecific competition. Regarding stem respiration, the rates of CO_2 release strongly varied at a given temperature throughout the year. Elevated respiration rates occurred between mid-June and the end of July, in parallel with the radial stem growth. The reduced stem respiration of beech in mixed plantation as compared with plants in monoculture related to the lowered aboveground increment in biomass (in particular under $+O_3$) and perhaps to an increased CO_2 refixation in the cortex cells, as photosynthetic photon flux density (PPFD) was enhanced in the shade canopy of mixed cultures. In spruce, stem respiration rates did not differ between plantation patterns.

The efficiency ratios proved to be adequate for characterizing competitiveness (confirming hypothesis 4). Under the given experimental scenarios, the competitive disadvantage of beech in mixed culture was not related to the C gain efficiency (i.e. C gain per unit of occupied aboveground volume), as higher seasonal performance of this efficiency ratio was found in mixed rather than monoculture. The lower competitiveness of beech in the presence of spruce was rather related to a reduced efficiency in aboveground space sequestration. In 2000, beech displayed smaller crown volumes per unit of shoot biomass in mixed as compared with plants in monoculture, and the “running costs” for sustaining occupied crown volume in terms of foliage respiration and transpiration were, in general, slightly higher in mixed plantation. Spruce, in contrast, showed similar efficiencies in C gain and “running costs” in both plantation types and tended to enlarge its space sequestration in mixed plantation. Regarding belowground structures, beech plants responded to the interspecific competition by enhancing specific fine-root length (SRL). In contrast, spruce did not show significant differences in SRL between plantation types. Despite the higher SRL of beech in mixed culture, its capability to sequester and retain nutrients (calculated as whole-plant content of N and P per unit of root biomass) was in general lower in mixed than in monoculture. Apparently,

morphological changes at the leaf (SLA) and fine-root (SRL) level were not related to the competitive performance of the studied plants. In conclusion, the decisive parameter in determining competitive success is, at least in juvenile beech of this case study, related to the ability of enlarging the crown volume at low structural costs. Spruce profited from the declining space sequestration of beech, as the narrowing crown shape of the latter species tended to allow enhanced light penetration into the mixed-stand canopy. The favoured production of spruce appeared, in addition, to exacerbate the belowground competition for nutrients at the expense of beech.

The decline in the efficiency of aboveground space sequestration in beech in mixed culture was based on reduced investments into leaf formation per unit of shoot axes biomass, and into current-year axes growth per unit of older shoot axes biomass, in particular under $+O_3$. Contrasting with changes in the root/shoot and C gain/shoot biomass ratios, which merely were a function of plant size (i.e. allometry), the shift in biomass partitioning between foliage and shoot axes was size-independent. In conclusion, severe stress as imposed by exacerbating competition and O_3 impact apparently overrules the ontogenetic control in the whole-plant resource allocation of beech.

ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurde das Konkurrenzverhalten junger Buchen- (*Fagus sylvatica*) und Fichtenindividuen (*Picea abies*) funktionell und mechanistisch analysiert. Der Erfolg der einzelnen Pflanze ist grundsätzlich abhängig von der Fähigkeit limitierende Ressourcen von der Umwelt - in Konkurrenz mit den Nachbarpflanzen – aufzunehmen. Da Ressourcen in dreidimensionaler, räumlicher Verteilung vorliegen, wurde die Konkurrenzstärke eines Individuums durch Bestimmung der Kosten (Ressourceninvestition) und Nutzen (Ressourcengewinne) quantifiziert und in Verbindung gesetzt mit der Struktur, die in der ober- und unterirdischen Raumbesetzung und Raumausbeutung aktiv ist. Drei Arten dieser Kosten/Nutzen-Bilanzen (Effizienzen) wurden zur Quantifizieren der Konkurrenzstärke definiert: (1) Effizienz der Raumbesetzung (d.h. besetzter oberirdischer Raum pro Ressourceninvestition, z.B. von Biomasse), (2) Effizienz des Ressourcengewinns (d.h. Ressourcenaufnahmen pro Ressourceninvestition oder pro besetztem ober- oder unterirdischem Raum) und (3) Effizienz der laufenden Kosten im Sinne von Atmung und

Transpiration, die zum Strukturunterhalt aufgewendet werden pro besetztem ober- oder unterirdischem Raum. Die Hauptziele dieser Arbeit waren den Ressourcenumsatz entlang der Strukturen und innerhalb der besetzten Räume, welche für die Konkurrenzprozesse bedeutend sind, zu quantifizieren. Ferner sollte ergründen werden in welchem Ausmaß die vorgeschlagenen Effizienzen Anwendung finden können, um die Konkurrenzkraft der Pflanzen quantitativ auszudrücken.

Die Konkurrenz zwischen jungen Buchen und Fichten wurde während eines zweijährigen Phytotronexperiments untersucht. Im Jahr 1998 wurden zwei- bzw. dreijährige Buchen- und Fichtenindividuen gleicher Größe in Mono- und Mischkultur in Container mit Waldboden gepflanzt. In jeden Container (insgesamt 32) wurden 20 (5 Reihen à 4) Bäume eingebracht, die Messungen aber auf die sechs zentralen Pflanzen konzentriert, um Randeffekt abzumindern. Im ersten Jahr (1998) wurden die Pflanzen im Gewächshaus unter ambientem und erhöhtem CO_2 (ambient + 300 ppm CO_2) vorakklimatisiert. In den folgenden zwei Vegetationsperioden (1999 und 2000) wurden sie im Phytotron weiterhin unter den beiden CO_2-Konzentrationen, in Kombination mit ambientem ($1xO_3$) und doppelt-ambientem ($2xO_3$) Ozon behandelt. Dadurch entstanden insgesamt vier CO_2/O_3-Gasregime: ambient $CO_2/1xO_3$ (Kontrollbegasung), ambient $CO_2/2xO_3$ ($+O_3$), erhöht $CO_2/1xO_3$ ($+CO_2$) und erhöht $CO_2/2xO_3$ ($+CO_2/+O_3$). Das Wachstum und die Ressourcenallokation der Pflanzen sollten durch die eingebrachten CO_2/O_3-Regime gestört werden, um Änderungen hervorzurufen, die das Studium der zugrundeliegenden Mechanismen der Konkurrenz erleichtern. Insgesamt wurden vier Hypothesen geprüft: (1) Die Konkurrenzstärke der Buche im Gegensatz zur Fichte wird von erhöhtem O_3 beeinflusst, während (2) die Fichte von der Zunahme der Ressourcenverfügbarkeit (CO_2) in der Mischkultur profitiert. (3) Die Antworten der Bäume auf CO_2 und O_3 hängen von der Art der Konkurrenz (d.h. intra- oder interspezifisch) ab. (4) Das Konkurrenzverhalten der Pflanzen kann mit Hilfe der vorgestellten Effizienzen sinnvoll charakterisiert und quantifiziert werden.

Unter den Versuchsbedingungen zeigte sich die Fichte als der bessere Konkurrent gegenüber der Buche. Nach zwei Vegetationsperioden zeigte letztere in Mischpflanzung niedrigere ober- und unterirdische Biomasse, jährlichen Biomassenzuwachs und Kronenvolumen im Vergleich zur Monokultur. Im Gegensatz hierzu zeigte die Fichte bei interspezifischer Konkurrenz eine tendenziell erhöhte Biomasse, insbesondere unter $+CO_2$. Die Ergebnisse bestätigten Hypothese 1: Die Buche reagierte empfindlicher auf die erhöhten O_3-Regime als die Fichte.

Im Jahr 1999 hatten Buchenindividuen in Monokultur die Anzahl der Blätter und die Laubfläche unter erhöhtem O_3 vergrößert, während keine Änderung in der Mischkultur beobachtet wurde. Ein Jahr später waren die meisten Wachstumsparameter (mittlere Blattfläche, Spross- und Wurzelbiomasse, Laubfläche und Kronenvolumen) von Buchenindividuen in Mono- und Mischkultur unter $+O_3$ verringert (im Vergleich zur Kontrollbegasung). Die Reduktionen waren in Mischkultur größer als in Monokultur. Entsprechend Hypothese 3 deuten diese Ergebnisse auf eine höhere Empfindlichkeit der Buche auf O_3 in der Mischkultur hin. Im Gegensatz zur Buche reagierte die Fichte nicht auf die Erhöhung von O_3. Ähnlich zu den Befunden unter $+O_3$ war die Reaktion der Buche auf erhöhtes CO_2 abhängig vom Pflanzmuster (Bestätigung von Hypothese 3). Im Jahr 1999 wurden die Anzahl der Blätter, die Laubfläche und die Sprossbiomasse der Buchen in Mono- (aber nicht in Mischkultur) unter + CO_2 stimuliert. Im Jahr 2000 führte erhöhtes CO_2 zu verringertem Wachstum der Buche in beidem Pflanztypen, jedoch war die Reduktion in der Mischkultur größer. Entsprechend Hypothese 2 profitierte die Fichte vom erhöhten CO_2 in der Mischkultur (größere Gesamtbiomasse). Auch der O_3-Schäden kompensierende Effekt von erhöhtem CO_2 bei Buche ist durch den Pflanztypen beeinflusst (kompensierende Wirkung nur in Monokultur). Ein Hauptergebnis war, dass die Konkurrenz die Reaktion der Bäume auf CO_2 und O_3 modifizierte und, vice-versa, dass diese zwei Gase wiederum die Konkurrenzstärke der Bäume beeinflussten. Darüber hinaus zeigte sich, dass die Pflanzen im Verlauf der beiden Versuchsjahre zunehmend auf die Behandlungen reagierten: Die Wirkungen der Gasregime und der Konkurrenzeffekte brauchten mehr als eine Vegetationsperiode um statistisch signifikant zu sein.

Die primäre treibende Kraft für die Änderungen der Ressourcenallokation war der Pflanztyp (d.h. die Art der Konkurrenz). Die Photosyntheseleistung spiegelte die Biomasseproduktion der Pflanzen in Mono- und Mischkultur wider. Im Jahr 2000 zeigte die Buche z.B. niedrigere CO_2-Assimilationsraten in Misch- im Vergleich zur Monokultur, besonders unter + O_3 und $+CO_2$. Diese niedrigeren Raten der Buche in Mischkultur wurden nicht vom Lichtklima, Blattstickstoffgehalt, Nekrosen (O_3-Effekt) oder einer reduzierten Elektrontransportrate der photosynthetischen Lichtreaktion verursacht, sondern beruhten auf einer reduzierten stomatären Leitfähigkeit. Neben der niedrigen stomatären Leitfähigkeit haben eine verringerte Laubfläche, ein reduziertes Kronenvolumen und der spätere Austrieb der Buchen in der Mischpflanzung zum niedrigeren jährlichen C-Gewinn der Buche unter interspezifischer Konkurrenz beigetragen. Der temperaturkorrigierte CO_2-Verlust durch Atmung von Stamm

und Achsen änderte sich stark während des Jahresverlaufs. Die höchsten Atmungsraten wurden zwischen Mitte Juni und Ende Juli (parallel zum Stammdickenwachstum) gemessen. Die verringerte Stammatmung der Buche in Misch- im Vergleich zur Monokultur scheint auf das reduzierte oberirdische Wachstum (vor allem unter +O_3) und eventuell auch auf eine Erhöhung der CO_2-Refixierung in den Rindenzellen zurückzuführen zu sein. In der Mischkultur war die photosynthetische Photonflussdicht (PPFD) in der Schattenkrone höher als in der Monokultur. Dagegen waren die Stammatmungsraten der Fichte nicht vom Pflanztyp beeinflusst.

Die oben eingeführten Effizienzen sind zur Charakterisierung des Konkurrenzverhaltens der jungen Buchen und Fichten gut geeignet (Bestätigung von Hypothese 4). Der unter den experimentellen Szenarien aufgetretene Konkurrenznachteil der Buche in der Mischkultur konnte nicht durch eine niedrigere Effizienz des C-Gewinns (d.h. C-Gewinn pro besetztem oberirdischen Volumen) erklärt werden, da eine höhere Effizienz in der Misch- und nicht in der Monokultur gefunden wurde. Die reduzierte Konkurrenzstärke der Buche in Anwesenheit von Fichte war dagegen mit einer verringerten Effizienz in der oberirdischen Raumbesetzung verbunden. Im Jahr 2000 zeigte Buche ein kleineres Kronenvolumen pro investierte Biomasse in Misch- im Vergleich zur Monokultur, und die laufenden Kosten (Atmung und Transpiration) zum Strukturunterhalt des besetzten Kronenvolumens waren im Allgemeinen in der Mischkultur erhöht. Im Gegensatz hierzu zeigte die Fichte ähnliche Effizienzen des C-Gewinn und der laufenden Kosten in beiden Pflanztypen und zeigte tendenziell eine erhöhte Effizienz der oberirdischen Raumbesetzung in der Mischpflanzung.

Bezüglich der unterirdischen Strukturen antwortete die Buche auf die interspezifische Konkurrenz durch eine Erhöhung der spezifischen Feinwurzellänge (SRL). Im Gegensatz hierzu zeigte die Fichte keine bedeutsamen Unterschiede in SRL zwischen den Pflanztypen. Die Fähigkeit der Buche Nährstoffe aufzunehmen und zu behalten (kalkuliert als N- und P-Gehalt eines Individuums pro Wurzelbiomasse) war trotz der SRL-Erhöhung in der Mischkultur niedriger als in der Monokultur. Die morphologischen Änderungen auf Blatt (SLA) und Wurzelebene (SRL) scheinen also keinen Einfluss auf die Konkurrenzstärke der untersuchten Pflanzen zu haben. Zusammenfassend kann gesagt werden, dass der entscheidende Parameter zur Bestimmung des Konkurrenzerfolges, die Fähigkeit das Kronenvolumen bei niedrigen strukturellen Kosten zu vergrößern, zu sein scheint. Fichte profitierte von der reduzierten Raumbesetzung der Buche, da die kleineren Kronen der Buche die Lichtdurchdringung in der Mischkultur erhöhte. Zusätzlich scheint das bessere Wachstum der Fichte die unterirdische Konkurrenz um Nährstoffe auf Kosten der Buche zu entscheiden.

Die Abnahme der Effizienz der oberirdischen Raumbesetzung der Buche in der Mischkultur basierte auf einer verringerten Investition in Blattproduktion pro Achsenbiomasse und in geringerem diesjährigem Achsenwachstum pro ältere Achsenbiomasse, vor allem unter +O_3. Im Gegensatz zu den Änderungen in den Wurzel/Spross- und C-Gewinn/Sprossbiomasse-Verhältnissen, die im Wesentlichen eine Funktion der Pflanzengröße sind, war die Verschiebung der Stoffverteilung („Partitioning“) zwischen Laub und nicht-grünen Sprossteilen unabhängig von der Pflanzengröße. Unter starkem Stress (hier: Konkurrenz und O_3) scheint die Ressourceallokation der Buche also nicht mehr der ontogenetischen Steuerung zu folgen.

COMPETITIVENESS OF YOUNG BEECH (*FAGUS SYLVATICA*) AND SPRUCE (*PICEA ABIES*) TREES UNDER AMBIENT AND ELEVATED CO_2 AND O_3 REGIMES.

1 INTRODUCTION

The processes involved in the plant's life cycle continuously influence and are influenced by the physical environment and by the activity of other individuals of the same or different species (e.g. competitors, parasites, symbionts and consumers). The way an individual plant is linked into these biotic interactions, especially with other plants and parasites, is the subject of an interdisciplinary research program which is currently conducted in the Munich area (Sonderforschungsbereich (SFB) 607: "Growth and Parasite Defence – Competition for Resources in Economic Plants from Agronomy and Forestry", Matyssek *et al.*, 2002a). Any response of plants to a stimulus inherently requires an internal adjustment of the resource allocation between the different organs. The aim of the SFB program is to identify the mechanisms underlying the control of resource allocation at different organizational levels (cell, organ and plant) and in a broad range of plant life forms, ontogenetic stages and growth conditions. It is to be clarified to what extent general principles in the regulation of resource allocation exist in plants.

In this context, the present work was carried out in order to analyse plant-plant interactions, namely, the responses of plants to intra and interspecific competition. Contributing to understanding competition in a more mechanistic way (cf. Grams *et al*. 2002; Berntson & Wayne, 2000; Schwinning & Weiner, 1998), the competitive ability of plants was analysed in terms of costs and benefits of resource allocation within the plant. Beech (*Fagus sylvatica* L.) and spruce (*Picea abies* (L.) Karst.), two species of major economic interest in Central-European forestry, were chosen for this study as they represent functional groups (evergreen conifer *versus* deciduous angiosperm tree) with distinct growth dynamics and canopy morphology (Yokozawa *et al.*, 1996). Although natural mixed stands of both species are restricted to high elevation in Central Europe, mono and mixed plantations are widely spread over a large range of soil types and regional climates from northern Sweden to central Italy (Bauer, 1997, Ellenberg, 1996). During the last decades, the area of mixed beech-spruce plantations has continuously increased in Germany (Nüsslein, 1993), however, the mechanisms underlying their competitiveness are poorly understood (Grams *et al.*, 2002). The increasing need in understanding the mechanisms of competition has raised the attention towards appropriate ways of assessment. As pointed out by Freckleton & Watkinson (2000), the way of assessment may result in different interpretations of processes in competition. Thus, the choice of parameters to be analysed in order to unravel the determining mechanisms of above and belowground competition must be critically considered when deciding upon the experimental design (Connolly *et al.* 2001; Weigelt, 2001) – so that this issue needs to be raised first in the following.

According to Begon *et al.* (1996): "*competition is an interaction between individuals, brought about by a shared requirement for a resource in limited supply, and leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned*". Thus, it is clear that the success of the individual plant basically depends on the ability to acquire resources from external pools shared with neighbours. In order to ensure this, plants must invest into those plant organs which are involved in the process of resource acquisition. As plants compete primarily for light, nutrients and water, main investments should be assigned to the morphology and physiological activity of leaves and fine roots, but also supporting structures are required. The size and architecture of these latter structures determine the positioning of leaves and roots in the above and belowground space, improving the individual's ability to explore the environment for resource availability (Suzuki, 2002; Lemaire & Millard, 1999; Umeki, 1995; Küppers, 1984). On the other hand, plants also have demands other than growth and space sequestration, like running costs (transpiration, respiration), storage, reproduction and defence. Thus, the competitive success of plants depends on an efficient allocation of nutrients and assimilates to the different resource sinks (Bazzaz & Grace, 1997). Plants must balance their resources between growth and maintenance of structures that are responsible for space sequestration within the canopy and the soil. The gained space should ensure the effective acquisition of new resources (Tremmel & Bazzaz, 1995; Matyssek & Schulze, 1987; Schulze *et al.*, 1986; Küppers, 1985). Consequently, to understand competitiveness of plants, it is necessary to assess the relationships between resource investments (costs) and resource gains (benefit). On these grounds, three types of cost/benefit ratios (called "efficiency ratios") are suggested which appear to be of relevance for quantifying the competitiveness of plants (Grams *et al.*, 2002) – considering the competitive success to begin with the occupation of space and, through this, perhaps even exclusion of neighbours.

The efficiency ratios defined in this study are:

- Efficiency ratio of space sequestration: occupied above or belowground space per unit of resource investment (e.g. biomass)
- Efficiency ratio of resource gain: resource acquisition (gain) per unit of resource investment (or occupied above or belowground space)
- Efficiency ratio of "running costs": respiration and transpiration for sustaining occupied above and belowground space (i.e. per unit of sequestered volume)

One major aim of this study was to quantify the resource budgets that occur along structures and within occupied space of relevance for competition, and then to examine to what extent the proposed efficiency ratios may be applicable to express competitiveness of young beech and spruce plants in quantitative terms.

Plants were grown in mixed and monoculture under ambient and/or elevated CO_2 and O_3 regimes throughout two growing seasons. Elevated levels of these two gases were used as disturbants to provoke changes in growth and allocation in order to more readily distinguish and derive underlying mechanisms from plant response (Matyssek *et al.*, 2002a). In general, plants growing under elevated CO_2 increase their primary production (unless other resources are limiting) and shift their allocation of resources towards the roots, whereas O_3 diminishes net carbon uptake and reduces root rather than shoot growth (Matyssek & Sandermann, 2003; Andersen, 2003; Karnosky *et al.*, 2001). Moreover, elevated CO_2 and O_3 regimes may change crown architecture due to modification of branching patterns (angles, bud activity, leaf positioning, and internode lengths; Pritchard *et al.* 1999, Dickson *et al.*, 2001, Matyssek *et al.*, 1993). In birch for example, branch formation and leaf expansion were suppressed under enhanced O_3 (Maurer & Matyssek, 1997; Matyssek *et al.*, 1992). It can be expected that changes in net carbon uptake, allocation patterns and crown architecture alter the competitive ability of plants to acquire above and belowground resources (Dickson *et al.*, 2001, Pritchard et al., 1999; Tremel & Bazzaz, 1995; Matyssek *et al.*, 1993; Matyssek & Schulze, 1987; Küppers, 1985), especially in species like beech, which is believed to be rather responsive to CO_2 and O_3 (Skärby *et al.*, 1998; Epron *et al.*, 1996). However, the responses to these gases can vary strongly depending on species, organs of the plant, ontogeny and growth conditions (see reviews about different species and responses to CO_2: Poorter & Navas, 2003; Tingey *et al.*, 2000; Körner, 2000; Pritchard *et al.*, 1999; Ward & Strain, 1999; Medlyn *et al.*, 1999;

Curtis & Wang, 1998; Saxe *et al.*, 1998; reviews about responses to O_3: Matyssek & Sandermann, 2003; Andersen, 2003; Polle *et al.*, 2000; Matyssek & Innes, 1999; Paludan-Müller *et al.*, 1999; Skärby *et al.*, 1998; Langebartels *et al.*, 1997). For beech and spruce most studies in recent years concentrated on the effects of CO_2 and/or O_3 on leaf gas exchange (Wieser *et al.*, 2002a; Liozon *et al.*, 2000; Lütz *et al.*, 2000; Maurer *et al.*, 1999; Grams & Matyssek, 1999; Grams *et al*, 1999). However, increasing information has been gained about the effects of these two gases on growth and biomass partitioning (Hättenschwiler & Körner, 2000, 1998; Bruhn *et al.*, 2000; Landolt *et al.*, 2000; Paludan-Müller *et al.*, 1999; Egli *et al.*, 1998; Epron *et al.*, 1996; Lippert *et al.*, 1996a), phenology, visual injury and antioxidants (Nunn *et al.*, 2002; Wieser *et al.*, 2002a,b; Tegischer *et al.*, 2002; Bortier *et al.*, 2000; Baumgarten *et al.*, 2000; Lippert *et al.*, 1996b), carbohydrate levels (Lux *et al.*, 1997; Barnes *et al.*, 1995), root respiration (Dyckmans & Flessa, 2002), mycorrhizae (Blaschke & Weiss, 1990) as well as genetic diversity (Longauer *et al.*, 2001). However, none of these studies were carried out including interactions with neighbouring plants, while submitting plants to elevated CO_2 and O_3 regimes in parallel and for more than one growing season. It has been demonstrated that plants can show different responses to the same stimulus, depending on growth in isolation or in competition with other plant individuals (Fuhrer *et al.*, 2003; Navas *et al.*, 1999; Ward & Strain, 1999; Lemaire & Millard, 1999; Tremmel & Bazzaz, 1995; Küppers, 1985), and that responses observed in short-term experiments can differ from findings obtained from prolonged observation periods (Epron *et al.*, 1996; Heath *et al.*, 1997; Grams *et al.*, 1999). Another issue is the effect of combined CO_2 and O_3 exposure on plant functions. The main question here is whether enhanced CO_2 does counteract adverse effects of elevated O_3 (Karnosky *et al.*, 2001). The few studies on beech and spruce growing under combined CO_2 and O_3 regimes presented conflicting evidence (Lütz *et al.*, 2000; Grams *et al.*, 1999; Grams & Matyssek, 1999; Barnes *et al.*, 1995; Polle *et al.*, 1993).

In the present study, it was suggested that plants are able to adjust their resource allocation in response to competition and gaseous regimes in a way that promotes competitive success. Thus, the proposed cost/benefit ratios (efficiency ratios) were to be examined for their capacity to integrate plants responses to neighbouring individuals, CO_2 and O_3. Although internal, functional or structural ratios are commonly used to describe responses of plants to environmental stimuli (Müller *et al.*, 2000), they may sometimes lead to misinterpretation of results (Jasienski & Bazzaz, 1999). Pearsall (1927) and several other authors (Troughton, 1955; Weiner & Fishman, 1994; Gedroc *et al.*, 1996) reported that the proportional distribution of biomass across the different plant organs changes with plant size (i.e. biomass) rather than with chronological (i.e. calendarical) plant age. In some herbaceous plants, for example, the ratio of root:shoot biomass declines rapidly within the first few weeks of growth (Bazzaz *et al.*, 1989). In response to different environmental conditions (e.g. experimental treatments), plants may adjust their growth rates, which results in different sizes and developmental stages at the same chronological age (cf. Gedroc *et al.*, 1996). If plants of different treatments have achieved different sizes and/or ontogenetic stages at a given chronological age, they probably will show different patterns of biomass partitioning between plant organs. However, when comparing plants of same size in the different treatments, often no differences in allocation and biomass partitioning are found, regardless of the treatment (Müller *et al.*, 2000; Gedroc *et al.*, 1996; Weiner, 1988). In order to analyse if allocation and biomass partitioning are determined by treatments or plant size, several authors suggest to perform an allometric analysis (Osada *et al.*, 2002; Müller *et al.*, 2000; Gedroc *et al.*, 1996). In the present study, the approach by Müller *et al.* (2000) was pursued to distinguish in biomass partitioning between effects by the gaseous regimes, competition and ontogeny.

The following hypotheses were tested

- that (1) the competitiveness of beech rather than spruce is affected by the elevated O_3 regime,
- whereas (2) spruce profits from the increase of resource availability (CO_2) in the mixed culture;
- that (3) the responses of plants to CO_2 and O_3 depend on the type of competition (i.e. intra or interspecific), and
- that (4) the competitive ability of plants can be characterized and quantified by the efficiency ratios defined above.

2 MATERIALS AND METHODS

2.1 Plants and treatments

The investigation was conducted in the phytotrons of the "GSF National Research Center for Environment and Health" in Munich/Germany (Thiel *et al.*, 1996; Payer *et al.*, 1993), where young individuals of beech (*Fagus sylvatica*, seed source 810-24 Freising) and spruce (*Picea abies*, seed source 840-27 Altötting), were analysed – in a 2-year study – for mechanisms of plant competitiveness (cf. Grams *et al*. 2002).

The GSF phytotrons system allows a field-relevant, realistic simulation of environmental conditions, including diurnal and seasonal fluctuations in climate conditions and air pollution regimes. The phytotron system consists of four walk-in environmental chambers (area 9 m^2 each, Fig. 2.1), which are subdivided into four sub-chambers with temperature-controlled root compartments. Parameters as air (-20 °C to 40 °C) and soil (-15 °C to 25 °C) temperature, relative humidity (25% to 95%), soil moisture, wind and exposure to gaseous pollutants can be controlled separately in each of these sub-chambers. A combination of different lamps, glass and water filters can reproduce the light spectrum (PAR, UV-A and UV-B) with high precision (Thiel *et al.*, 1996). Photosynthetically active radiation (PAR 400-700 nm) at plant canopy can reach up to 1000 $\mu mol\ m^{-2}\ s^{-1}$.

In spring 1998, two- and three-year-old individuals of beech and spruce, respectively, were planted into the containers (0.084 m^3 each, 20 trees per container, arranged by rows of 4 x 5 individuals, Fig. 2.1, and Fig. 2.2b,c), which had been filled with natural soil from a beech stand (mixed brown-earth, Ah-B horizon, "Höglwald", Bavaria/Germany, 540 m a.s.l; see Kreutzer *et al.*, 1991). The plantations of the total of 32 containers consisted of either mixed or monocultures (16 with mixed, and 8 containers each with monocultures of spruce or beech; replacement series). Analysis carried out in the containers by Dr. Argerer (personal communication) confirmed intense mycorrhization in beech and spruce plants. In order to avoid edge effect, the investigations were concentrated on the six central individuals of each container. Plants had been kept during the summer of 1998 (i.e. one year prior to the phytotron study), in a climate-controlled greenhouse programmed to track outside climate conditions under ambient and elevated CO_2 levels (ambient +300 ppm).

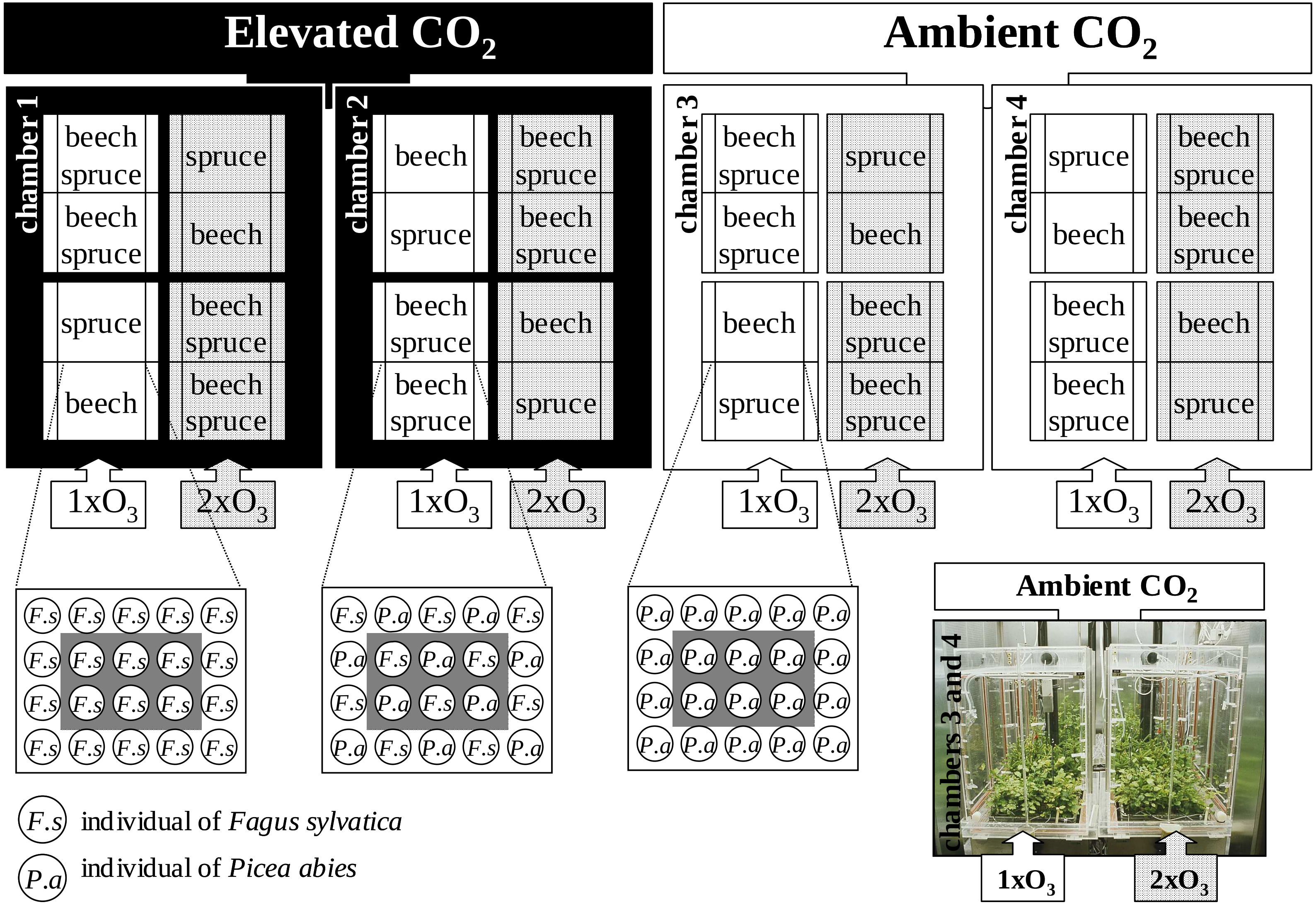


Figure 2.1. Placement of the containers with mono and mixed cultures of beech (*F.s*) and spruce (*P.a*) in the phytotron chambers 1 to 4. Each phytotron contains four sub-chambers where two plant containers were located each. The elevated CO_2 regime (ambient CO_2 + 300 ppm) was employed in the chambers 1 and 2, whereas the ambient CO_2 regime was conducted in the chambers 3 and 4. In addition to the CO_2 regime, the two sub-chambers on the left of each phytotron received the 1xO_3 regime, and the two on the right, the 2xO_3 regime. The circles represent plant individuals in the joint soil volume of containers. Investigations were conducted on the six central plants (shadowed area).

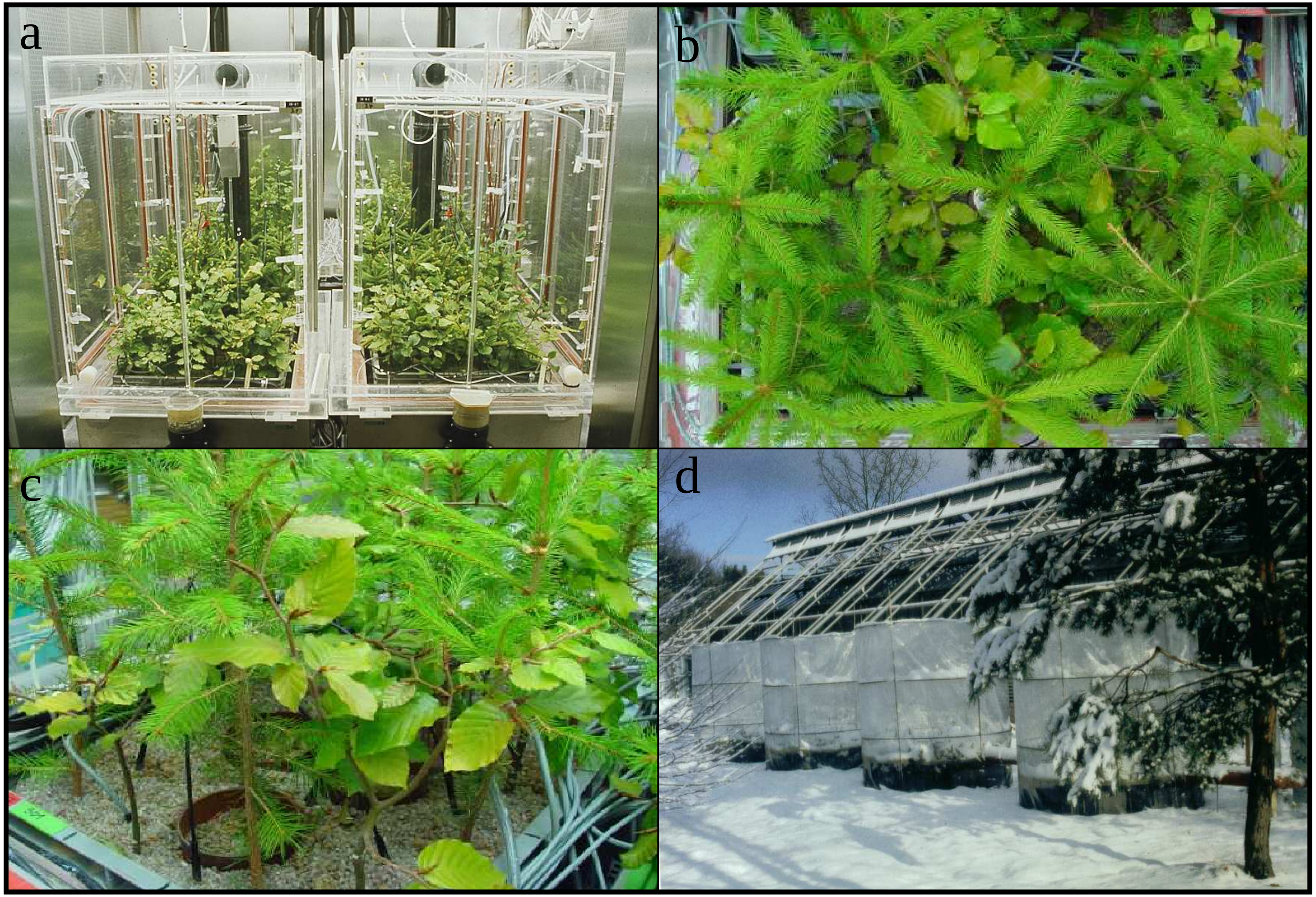


Figure 2.2. Plant containers in sub-chambers of one phytotron (a), container with mixed culture (b, c), and open-top-chambers of GSF-Research Center.

During the following two summers, the containers were transferred into the GSF phytotrons, where in addition to the CO_2 treatments, plants were exposed to either an ambient ($1xO_3$) or twice-ambient O_3 regime (i.e. $2xO_3$ levels, restricted to < 150 ppb, Figure 2.1 and Figure 2.2a). Four CO_2/O_3 regimes were established in this way (Table 2.1). During the winter months of 1998/99 and 1999/2000, plants were placed into open-top chambers in the open field while maintaining the corresponding CO_2 regimes (Figure 2.2d).

Table 2.1. Plants grew under four different gaseous regimes according to the combination of ambient and elevated CO_2 and O_3 concentrations.

CO_2	O_3	gaseous regimes
Ambient	Ambient ($1xO_3$)	Gaseous control regime
Ambient	Twice-ambient ($2xO_3$)	$+O_3$
Elevated (ambient + 300 ppm)	Ambient ($1xO_3$)	$+CO_2$
Elevated (ambient + 300 ppm)	Twice-ambient ($2xO_3$)	$+CO_2/+O_3$

During cultivation in the phytotrons, the climate conditions and O_3 regime measured during the previous year at the study site “Kranzberg Forest” near Freising (Bavaria/Germany, 490 m a.s.l.; see Pretzsch, 2002) were reproduced on an hourly basis throughout the seasonal courses (with the ambient O_3 regime of the field site providing the basis of the experimental “$2xO_3$” regime). In this way, the climate conditions of Kranzberger Forest occurring during 1998 and 1999 were simulated in the phytotrons during 1999 and 2000, respectively. Monthly means of irradiance, air temperature, relative humidity, CO_2 and O_3 concentrations during daylight and night time in the phytotrons are given in Table 2.2. In 1999, plants stayed in the climate chambers from May 15 until September 30, and in 2000 from May 12 until August 31**.** The AOT40 was calculated according to Fuhrer (1994) as the sum of 1-hourly mean O_3 concentrations over 40 nl O_3 l^{-1} air during daylight hours. AOT40 from May 15 through September 27 (in 1999) and August 31 (in 2000) for the $1xO_3$ and $2xO_3$ regimes were 10.4 and 62.8 µl l^{-1} h in 1999 and 9.2 and 60.6 µl l^{-1} h in 2000 (monthly means are given in the Table 2.2). In 1999, the highest AOT40 calculated for the $1xO_3$ and $2xO_3$ regimes were found in August. In 2000, the highest AOT40 was found in July at the $1xO_3$ regime, and in June and July at the $2xO_3$ regime. In general, plants received lower irradiance in the second growing season (2000), and about by 1 to 7 % lower CO_2 concentration than did plants in 1999. In 2000, mean RH was higher in May and August, and lower in June and July than during the same months in 1999. Soil moisture of each container was monitored continuously by tensiometers (Model T5, UMS, Munich), triggering irrigation whenever soil water tension had reached 350 hPa. Plants were irrigated with deionised water ensuring non-limiting supply throughout the entire experiment. Fertilization (Hoagland solution, Hoagland & Arnon , 1950) was regularly applied in order to maintain nutrient levels similar to that found in natural soils of Bavarian forests. This means that plants did not grow under non-limiting resource availability. Fertilization occurred four, six and eight times during the growing seasons of 1998, 1999 and 2000, respectively.

Table 2.2. Monthly mean photosynthetic active radiation (PAR), air temperature (T), relative humidity (RH), ambient and elevated CO_2 and O_3 concentrations as well as AOT40 (only during daylight hours, according to Fuhrer, 1994) as occurring in the phytotrons throughout the growing seasons of 1999 and 2000.

Month	Year Day/Night	PAR [µmol m^{-2} s^{-1}]	T [°C]	RH [%]	Amb. CO_2 [ppm]	Elevated CO_2 [ppm]	$1xO_3$ [ppb]	$2xO_3$ [ppb]	AOT40 $1xO_3$ [µl l^{-1} h]	AOT40 $2xO_3$ [µl l^{-1} h]
May	Day –1999	531.8	17.5	55.0	406	701	39.0	72.9	1.7	10.1
	2000	427.0	18.3	57.0	381	665	37.2	76.5	1.3	10.4
	Night-1999	0	12.7	72.6	417	716	24.2	40.5		
	2000	0	13.0	77.0	410	675	23.1	45.6		
Jun	Day - 1999	526.0	19.8	61.2	408	702	36.0	70.8	2.8	17.8
	2000	482.6	18.4	60.3	380	673	35.4	72.9	1.9	18.1
	Night-1999	0	14.8	82.0	430	725	15.9	33.0		
	2000	0	13.4	81.7	421	716	19.9	38.4		
Jul	Day -1999	458.0	19.7	65.6	399	690	34.2	67.6	2.0	15.2
	2000	452.2	20.7	62.2	385	683	40.3	71.5	3.9	18.0
	Night-1999	0	15.3	83.4	431	724	15.4	30.3		
	2000	0	15.5	81.4	411	707	21.2	33.8		
Aug	Day - 1999	473.3	19.7	58.7	405	703	41.0	85.1	3.9	19.8
	2000	447.2	20.1	63.2	391	688	35.0	65.0	2.1	14.1
	Night-1999	0	13.7	80.4	432	728	15.2	31.2		
	2000	0	15.1	84.4	440	736	19.4	36.2		
Sep	Day - 1999	408.1	15.4	67.1	418	714	21.3	47.0	0.1	4.8
	Night -1999	0	11.5	85.6	449	746	8.6	23.4		

2.2 Assessment of climatic conditions

Air temperature and relative humidity were measured in the phytotrons as described elsewhere (Payer *et al.*, 1993). Photosynthetic photon flux density (PPFD) was registered above and inside the canopy of the plantations, using one photodiode at a light-exposed, and two photodiodes at shaded positions in each container (Type G1118, Hamamatsu Ltd, Japan). All 96 photodiodes had been calibrated *prior* to installation with a LI-189 unit (LI-190SA quantum sensor, LI-COR Inc., Lincoln, Nebraska, USA).

2.3 Assessment of plant biomass and relative biomass increment

Biomass aboveground: Non-green aboveground biomass of the six central individuals of each container was assessed in March 1999 and 2000 volumetrically by measuring diameters and lengths of the shoot axes. Biomass to volume relation (measured on comparable plants) was used to convert measured volumetric data into shoot biomass (g DW). At the end of 2000, the biomass of five individuals (randomly chosen) of each species was calculated in this way and trees were subsequently harvested for the determination of the actual dry mass. Figure 2.3 shows the relationship between volumetrically calculated and measured biomass of these trees. The aboveground biomass assessed in March of each year is considered to represent the final biomass of the previous year. Hence, the biomass determined in March 1999 and March 2000 are regarded as the biomass at the end of growing season 1998 and 1999, respectively.

At the end of August 2000, the six central individuals were harvested in each container. During harvest, temperature and relative humidity were set to 21°C and 55 %, respectively, while PPFD was kept at about 700 µmol m^{-2} s^{-1}. The biomass (fresh weight) of leaves/needles, new and old branches and the stem was determined in each tree. Beech leaves were separated according to sun, shade and second-flush foliage, and in spruce, current-year twigs were separated from older twigs. Aliquots of each plant organ were frozen immediately and ground in liquid nitrogen for subsequent analysis of carbohydrates and amino acids (processed by Prof. Dr. Rennenberg, Freiburg). Additional aliquots were used for determining the fresh/dry weight ratio as a basis for the dry mass assessment of plant organs and for the analysis of macro and micronutrients (processed by Prof. Dr. Göttlein, Freising).

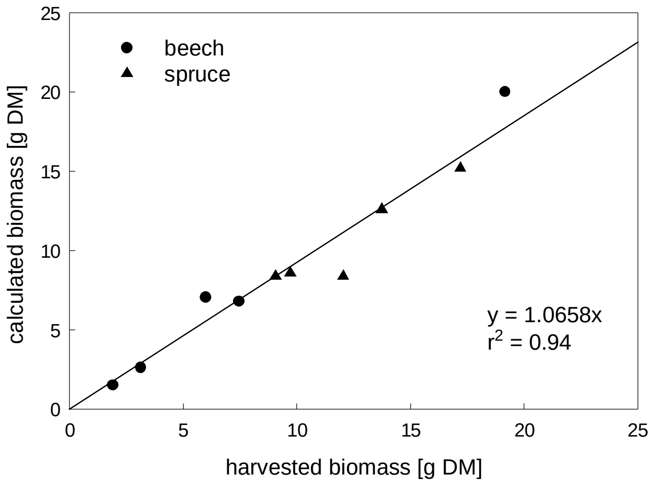


Figure 2.3. Relationship between volumetrically calculated and actually harvested non-green aboveground biomass of five beech (circles) and five spruce (triangles) individuals.

Relative aboveground biomass increment (RBI): the biomass increment aboveground during 1999 and 2000 was calculated according to:

$$RBI = \frac{(Bio_{(x)} - Bio_{(x-1)})}{Bio_{(x-1)}} *100\ \%$$

Where:

$Bio_{(x)}$: non-green aboveground biomass (g DW) at the end of one growing season

$Bio_{(x-1)}$: non-green aboveground biomass (g DW) at the end of the preceding growing season

Biomass belowground: At the harvest in August 2000, root biomass of two (randomly chosen) out of the six central plants was determined quantitatively. A square-shaped ground area of 0,0134 m^2 was assigned to each tree, and a soil volume of 0,004 m^3 underneath that area (down to the bottom of the container) was excavated with a customized metal cutter. This soil volume was regarded to contain the entire root mass of a plant, assuming amounts of roots extending from this volume to be similar to amounts intruding from neighbouring plants of the same species (Bengough *et al*, 2000). Beech and spruce roots were separated from each other. Soil particles were removed by washing (Oliveira *et al*, 2000), while separating fine from coarse roots. Root sampling for biochemical analysis and dry mass assessment was performed according to the same rationale as applied to the procedures on aboveground material.

In March 1999, the initial fine root biomass was determined in two cores (2 cm in diameter, volume 75,8 cm^3) per container. Additional cores were taken in June and October 1999 and March and August 2000, always at same positions, and the biomass of fine roots that had re-grown into the cores until each sampling date was determined. These data were also used to assess the efficiency of belowground space sequestration (see efficiency parameters of competitiveness, section 2.12).

2.4 Foliage area

At the end of July of 1999 and 2000, the total number of beech leaves (separated into sun, shade and second-flush foliage) was counted on each individual tree. The maximum width was measured of all leaves in three individual trees per treatment using a ruler. Five leaves of each leaf type per plant were excised, scanned, and the maximum width and leaf area were determined using a DT-Scan Program. High correlations were found between leaf width and leaf area (Fig. 2.4). Therefore, the foliage area of each individual was calculated by multiplying the mean leaf area by the total number of leaves. For spruce, the length of all current-year and older twigs per plant was measured in March/April of 1999 and 2000. The mean number of needles per unit of axis length, their weight and projected area were determined. Foliage area of each individual was calculated, multiplying the total axes length of a tree by the mean needle area per unit of axis length. At the end of August 2000, the total foliage area was determined directly by harvesting all current-year and older needles. Comparing the actual foliage area harvested at the end of August 2000 with the calculated area (axes length x mean needle area per unit of axis length) of five randomly chosen individuals, an overestimation of about 26 and 32 % for current-year and older twigs, respectively, was found (Figure 2.5). Thus, the calculated foliage area of spruce in March 1999 and 2000 was corrected using the equations of the linear correlations for current-year and older needles in the Fig. 2.5.

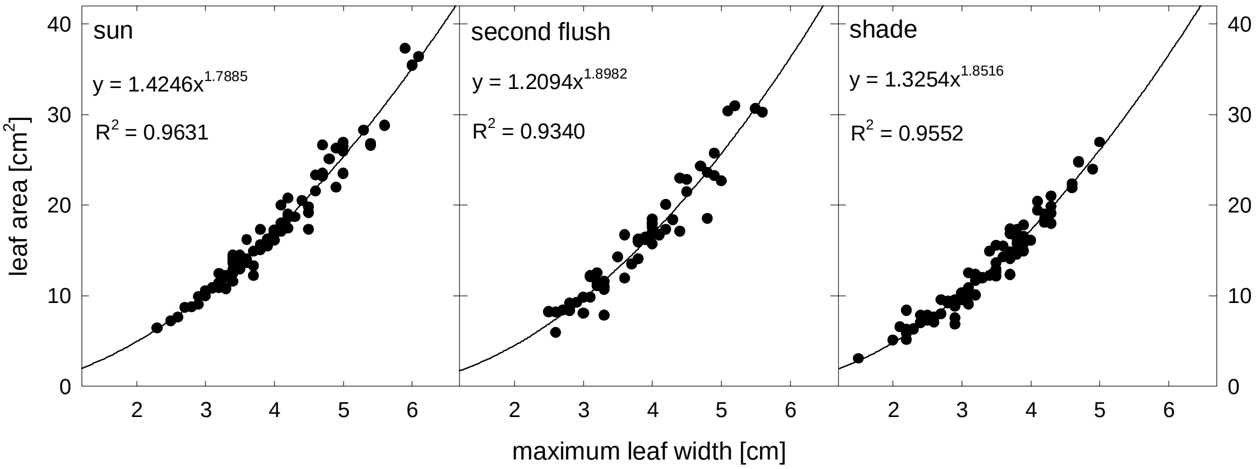


Figure 2.4. Relationship between maximum leaf width and leaf area for sun, second flush and shade leaves of beech.

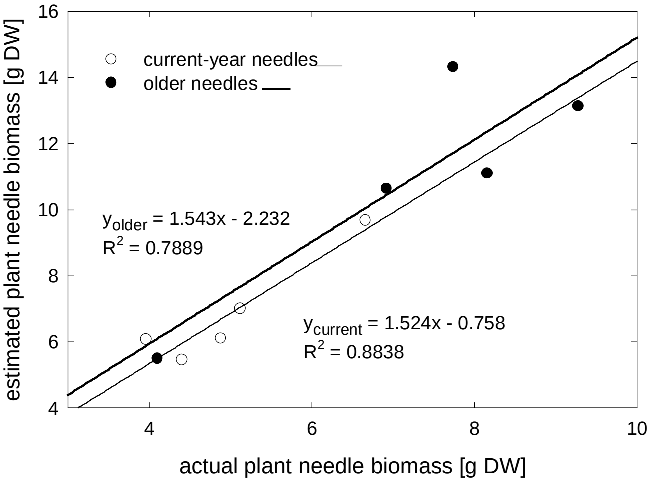
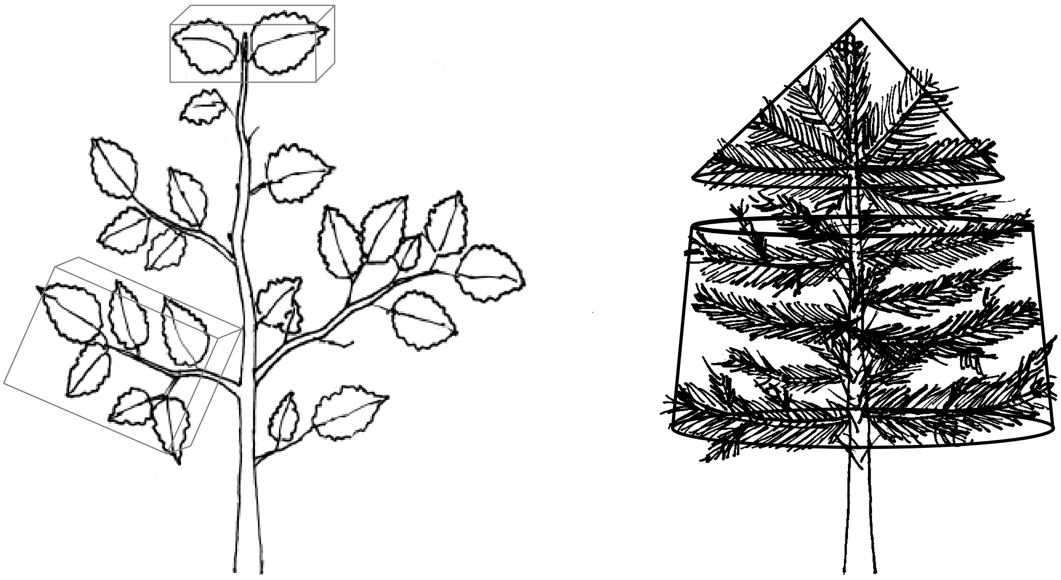


Figure 2.5. Relationship between actual and estimated current-year (open symbols) and older (closed symbols) needle dry mass per individual (n=5).

2.5 Crown volume

The crown volume of individual beech trees was determined at the end of July 1999 and 2000 (when axis growth was completed) through approximations by cuboid volumes enclosing the foliage shoots (Figure 2.6). In this way, the volume of all branches were summed up per beech individual. For spruce, a “cylinder + cone” model was applied that enclosed the entire, densely branched crown. The approaches in branch and crown volume assessment accounted for the specific growth patterns due to tree ontogeny and growth conditions in each species (Küppers, 1994). While the crown architecture of beech plants does not develop into any fixed geometrical form, spruce grows in a regular way resulting in a "cylinder + cone" crown shape. Both approaches used to measure crown volume consider the empty space between leaves of a branch (as in beech) or between twigs (as in spruce) as occupied crown volume. In this latter respect, the kind of assessment is comparable and consistent between the two species.



(a) (b)

Figure 2.6. Determination of crown volume of beech (a) through approximations by cuboid volumes enclosing the foliage shoots, and of spruce (b), applying a "cylinder + cone" model that enclosed the entire crown.

2.6 Phenology and visual O_3-induced damage

The phenology of the leaves and twigs was regularly observed to assess the seasonal development of the plants. As suggested by Häberle (1995), we classified beech leaves into six developmental stages: (0) buds closed, (1) buds swollen, (2) first leaf visible, folded (3) leaves unfolded but hanging, (4) leaves horizontally positioned, advanced length growth, axes still hanging, and (5) leaf and shoot development completed. We included a new class (6) for the appearance of second-flush leaves. For spruce we used the following classification: (0) buds closed, (1) bud break, (2) new axis shorter than 1 cm, (3) beginning of axis length growth, new axis < 3 cm, (4) advanced length growth, new axis longer than 3 cm, (5) needles of new shoots slightly spread, and (6) length growth completed. Leaf abscission was assessed only in 1999 by counting the number of attached leaves every two weeks, beginning at the end of July (when 100 % of the formed leaves were still attached to the tree). From the end of August throughout the end of September the number of attached leaves were counted by weekly intervals.

Trees were also checked regularly during the two growing seasons for the appearance of visual symptoms on leaves and needles. Injury induced by O_3 establishes on beech as bronze-green leaf discoloration, chlorotic and necrotic dots or small areas of necrosis that occur as isolated areas or spread across the whole leaf lamina. Plants were classified according to the percentage of foliage area (<1%, 1 to 5%, 6 to 10%, 11 to 20% and 21 to 40%) showing such symptoms. On spruce, injury caused by ozone occurs as chlorotic mottling and banding. In spruce the symptoms were not quantified, however plants were assigned into two classes: no symptoms (0) or appearance of chlorotic mottling (1).

2.7 Assessment of leaf gas exchange

2.7.1 Porometry: Measurements of the net CO_2 uptake rate (J_{CO2}) were conducted with a stead-state diffusion porometer (CQP130 – Walz, Effeltrich, Germany) by three to four-week intervals throughout the vegetation periods of 1999 and 2000. The measurements were performed under ambient (phytotron) light, air temperature, air humidity and CO_2 concentration on sun (n=12), shade (n=6) and second flush-leaves (sun, n=6) of beech, and current-year (sun, n=12) and older twigs (shade, n=6) of spruce. Leaves and twigs representing the mean phenological status of the plants under a treatment were chosen for repeated measurements. All leaf gas exchange and chlorophyll fluorescence measurements were done using the same leaves and twigs during each growing season. The CO_2 assimilation and transpiration rates were based on the one-sided surface area of the beech leaves and the projected area of the spruce needles. At the end of the growing seasons, the needles of assessed twigs sections in spruce were harvested, scanned for the determination of the projected needle area, and dried at 65 °C for 72 h for dry mass determination.

***2.7.2 Modelling of gas exchange (performed by Dr. B. Winkler – GSF)*:** Light and CO_2 response curves of the net CO_2 uptake rate of leaves were measured in beech and spruce by means of programmable gas exchange equipment (HCM-1000 CO_2/H_2O diffusion porometer, H. Walz, Effeltrich, Germany). Subsequently, J_{max} and V_{cmax} were calculated from the CO_2

response curves according to von Caemmerer and Farquhar (1981), while quantum yield of CO_2 gas exchange (Φ_{CO2}) was derived from the initial slope of the light response curves (at saturating CO_2 concentration). J_{max}, V_{cmax} and Φ_{CO2} were determined separately in the sun and shade crowns, and during early and late summer to account for potential seasonal effects. The parameters as derived for beech and spruce were used to parameterise – for each species separately – a leaf gas exchange model for trees (Falge *et al.*, 1996). Based on the time courses of irradiance, relative humidity and air temperature, the model was employed for calculating the area-based seasonal net carbon gain and transpiratory water loss of beech leaves and spruce needles. By relating the C assimilation and transpiration rates to the foliage area, whole-tree carbon gain and transpiratory water loss were calculated. Since the canopy closure was high in 2000, suppressing evaporation from the soil, the compensatory irrigation reflected transpiration. In 2000, amounts of water applied to the containers were used, therefore, for validating modelled water loss through transpiration (Fig. 2.7).

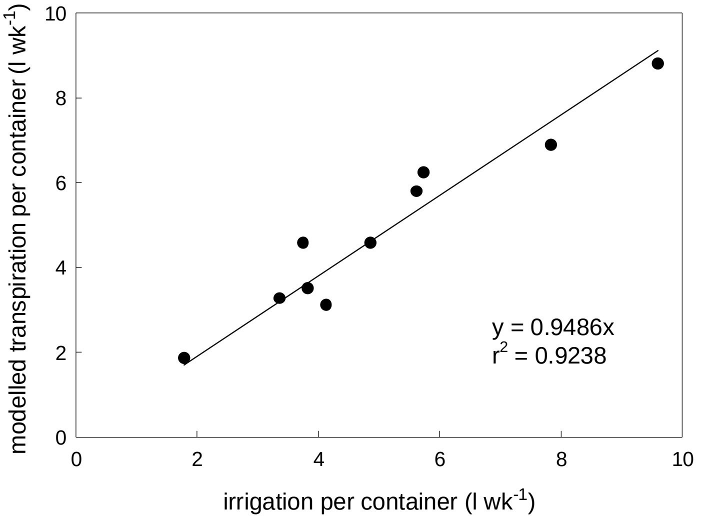


Figure 2.7. Validation of the modelled transpiration rate in a container with the amounts of irrigation water supplied per week. Transpiration rate per container (n = 5) was calculated using the PSN6-modell for one week in July (2nd to 9th) and August (15th to 22nd) 2000 (pooled data set). Each container comprised 20 individuals of *Fagus sylvatica*.

During 2000, leaf area of beech in mixed culture was very small. Just few sun-leaves were big enough to allow gas exchange measurements. Under $+O_3$, no measurement could be done. The V_{cmax} and J_{max} in monoculture varied strongly within treatment. Although the few V_{cmax}

and J_{max} data from plants in mixed plantation tended to be lower than in monoculture, they were within the range found in monoculture (data not shown, J. B. Winkler, personal communication). In addition, no differences in such parameters between plantation types were found in 1999, and under $+CO_2/+O_3$ in 2000. Thus, it was decided to use one mean value for both plantation types together. Except for $+CO_2/+O_3$, this procedure was estimated to result in over and underestimations of the seasonal C gain within 10 % relative to beech plants in mixed, and monoculture, respectively.

2.8 Chlorophyll fluorescence

Measurements of Chlorophyll *a* fluorescence of PSII were conducted using a portable pulse-amplitude modulation fluorometer (Mini-PAM, Walz, Effeltrich, Germany). The measurements were performed by three to four-week intervals in 1999 from May through September, and in 2000, from May through August on the same leaves each year which were used for porometry. Leaves were maintained in their actual position and inclination, and the fibre optics were kept at a constant angle (60°) and distance (1 cm) from the adaxial leaf surface. Care was taken to not shade the investigated leaf during measurements. One light flash of a duration of 0.8 s and photon flux density (PPFD) of more than 2000 µmol m^{-2} s^{-1} was employed to obtain the maximum fluorescence in the light-adapted state (F´m). Effective electron quantum yield of non-cyclic electron transport (Φ_{PSII}) was calculated according to Genty *et al.* (1989):

$$\Phi_{PSII} = \frac{(F'_m - F_t)}{F'_m}$$

Where,

F'_m = maximum fluorescence in the light-adapted state

F_t = steady-state fluorescence in the light

The apparent electron transport rate (ETR) was calculated as:

$$ETR = \Phi_{PSII} * PPFD * 0.5 * 0.84$$

with PPFD measured directly at the surface exposed to chlorophyll fluorescence analysis; 0.5 is a factor accounting for the partitioning of energy between PSI and PSII, and 0.84 is a standard factor corresponding to the fraction of incident light absorbed by a leaf.

2.9 Assessment of stem respiration

Stem respiration was measured in a total of 16 beech and 16 spruce individuals, i.e. two plants in a species per treatment. Clamp-on chambers made of two half-cylinders of a transparent, hard plastic (Plexiglas) were attached to the stem in May 2000, and respiration was measured during the entire growing season throughout 28th August. Chambers were 10 cm long and five cm in diameter (see Fig. 2.8b). The two halves of the chambers were pressed together using ring-shape metal clamps. To seal the horizontal walls at the upper and lower ends of the chamber to the stem, we used 1-cm-thick silicone discs with an opening similar in diameter to that of the stem. Sealing was tested by breathing to the joints and checking for leaks by means of changes in IRGA signals. Stem respiration was measured with an open gas exchange system using an IRGA (Binos 100 4P, Rosemount). A constant flow rate of 0.65 l min^{-1} through the chambers was sucked continuously by a series of pumps (Fig. 2.8a Sketch). The equipment was programmed to take samples of the 16 stem respiration chambers and of the two control chambers (chambers without stem) in series. Since each measurement required about 3.3 minutes, stem respiration was recorded every 54 min for each chamber. The respiration rate of 8 beech and 8 spruce individuals was measured under ambient or elevated CO_2 concentration by two-week intervals.

The total radial area (and volume) increment of the stem enclosed in the chamber was calculated by comparing the diameters measured on May 12 and August 28. The diameter increase was considered to be linear throughout the growing season. On these grounds, stem volume enclosed in the chamber and used to calculate respiration rate (µmol CO_2 m^{-3} s^{-1}) was corrected for growth on a daily basis.

The net CO_2 release rate was calculated according to von Caemmerer and Farquhar (1981):

$$JCO_2 = \frac{\Delta CO_2 * flow * T_0 * P_1}{fCO_2 * A * T_1 * P_0} \quad [\mu mol\ m^{-3}\ s^{-1}]$$

where:

ΔCO_2: Difference in CO_2 concentration between reference and measuring gas [µmol mol]
flow: flow rate of air through the chamber [m^3 s^{-1}]
T_0: air temperature under standard conditions [273,13 K]
T_1: air temperature in the chamber [K]
P_0: pressure under standard conditions [101.325 kPa]
P_1: pressure of the flowing gas [kPa]. We used the mean local pressure (96 kPa) measured at the GSF site
fCO_2: correction factor of the BINOS dependence on CO_2 concentration of the reference gas
A: stem volume enclosed in the chamber [m^3]

Stem respiration was related to the stem temperature (which was assumed equal to the air temperature), resulting in strong linear correlations. Different temperature response curves were found during the growing season (cf. Results: Carbon balance, Figures 3.3.2 and 3.3.3). We used the apparent linear correlation between stem respiration rate and the temperature to calculate the total carbon released per unit of stem volume during the growing season of 2000. Multiplying by the total volume of the non-green aboveground structures, seasonal aboveground respiration (C_{resp}shoot) was calculated of each individual.

2.10 Nutrient analysis in the biomass

As mentioned above, aliquots of each plant organ harvested at the end of August 2000 were transferred to the analysis of macro and micronutrients (including carbon). The material was dried at 65 °C for one week, milled, digested with HNO_3 and analysed by ICP-AES (Perkin Elmer, USA). Nitrogen concentration was determined using a CHN analyser (Leco, USA). Analyses were processed by Prof. Dr. Göttlein and his team. The concentrations of N, P, Mg and Fe in each organ were used to calculate the total element content per plant as well as an parameter related to belowground resource gain (see Efficiency ratios of competitiveness, 2.12.2b).

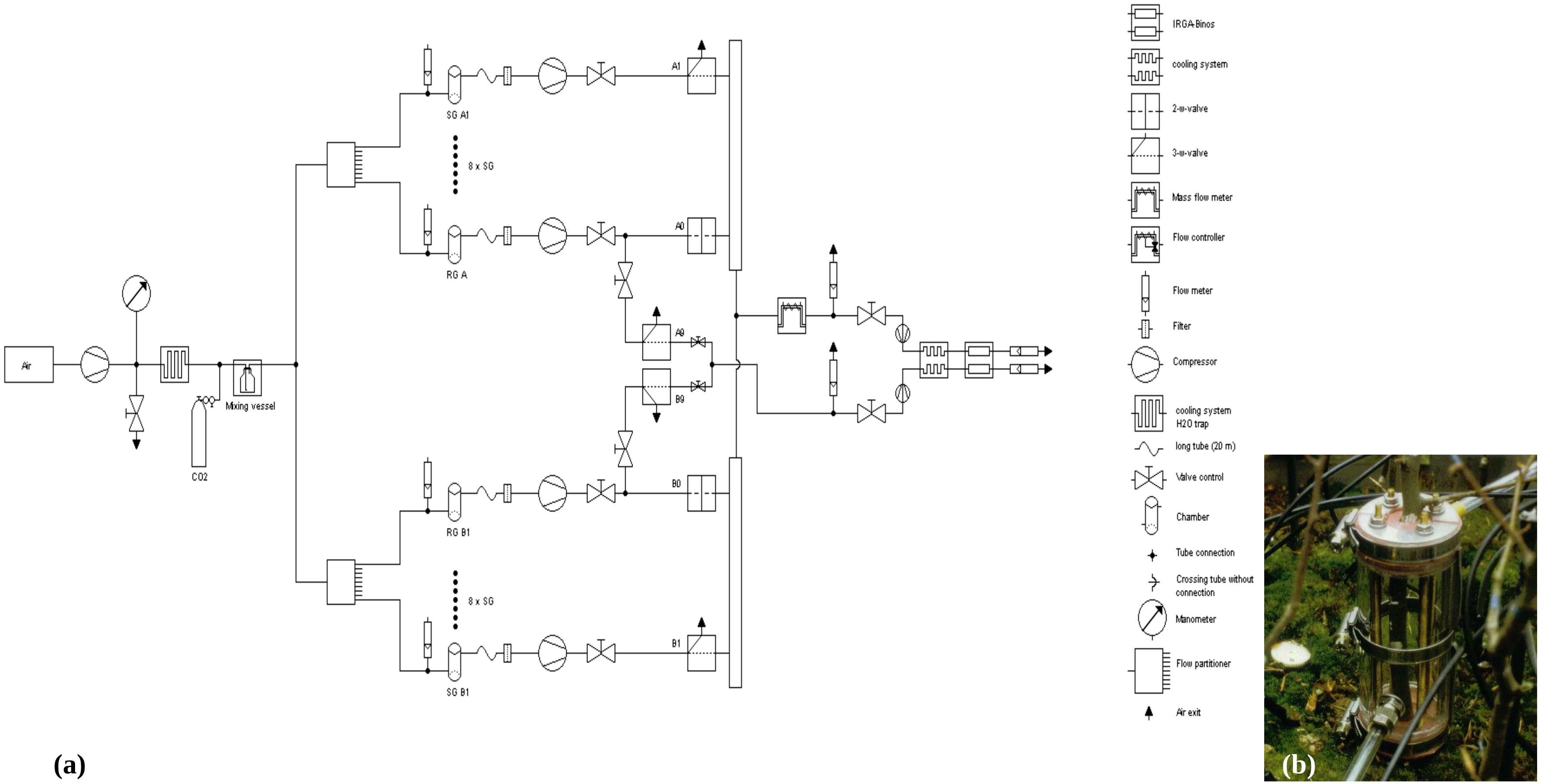


Figure 2.8a. Sketch of the open gas exchange system used to measure stem respiration. SG and RG represent sample gas and reference gas, respectively. **Figure 2,8b** shows a respiration-chamber attached to the stem of a beech individual.

2.11 Seasonal carbon balance

The seasonal carbon balance of tree individuals was calculated for the second growing season in the phytotrons (2000), as based on the net C gain, net respiration of non-green structures and C investment into biomass increment:

$$C_{gain} = \underbrace{(C_{resp}\text{shoot} + C_{bio}\text{shoot})}_{\text{C invested aboveground}} + \underbrace{(C_{resp}\text{root} + C_{bio}\text{root} + C_{exud})}_{\text{C invested belowground}}$$

Where,

C_{gain}: annual net C assimilated per individual [g C]
C_{resp}shoot: annual net C respired by stem and axes [g C]
C_{resp}root: annual C respired by roots [g C]
C_{bio}shoot: C invested in biomass increment aboveground [g C]
C_{bio}root: C invested in biomass increment belowground [g C]
C_{exud}: C release by the roots as exudates [g C]

Since root respiration rate, root biomass increment and root exudates were not assessed through measurements, the total belowground C investment was calculated as follows:

C invested belowground = C gain – C invested aboveground

2.12 Efficiency ratios of competitiveness

The following efficiency ratios were defined as based on resource gain per resource investment:

2.12.1 Efficiency ratio of space sequestration

a) Aboveground:

$$\frac{\text{occupied aboveground (crown) volume}}{\text{standing shoot biomass}} \ [\mathrm{m}^3\ \mathrm{g}^{-1}]$$

This efficiency ratio is calculated accounting for the biomass investments (costs) into stem, axes and foliage which determine the space sequestration of the crown (gain).

b) Belowground:

$$\frac{\text{fine root length}}{\text{fine root biomass}} \quad [\text{cm g}^{-1}]$$

In a consistent way, this efficiency ratio relates the fine root length (as a measure of gain in belowground space sequestration) to the biomass invested into fine roots (structural costs), resulting in a ratio typically named specific fine root length (SRL). SRL was determined in March, June and October 1999, and in March and August 2000. At each time, two in-growth cores per container (2 cm in diameter, 75.8 cm^3) were taken from the soil. The root material that had re-grown into the cores since the preceding sampling date was washed carefully, separated into beech and spruce roots, and scanned for determination of root length (using WinRhizo software, version 4.1 a, Reagent Instruments Inc., Quebec, Canada; data processed by Dr. H. Blaschke, Freising).

2.12.2 Efficiency ratio of resource gain

a) Aboveground:

$$\frac{\text{C gain}}{\text{occupied aboveground (crown) volume}} \quad [\text{g C m}^{-3}]$$

While the efficiency parameter of space sequestration (2.12.1) accounts to the costs of gain in space (measured as crown volume), the present ratio evaluates the efficiency of this sequestrated space in gaining new resources, namely carbon. Seasonal C gain modelled for the growing seasons 1999 and 2000 was related to crown volume of the plants measured at the end of July in both years.

b) Belowground:

$$\frac{\text{whole-plant nutrient incorporation}}{\text{root biomass}} \quad [\text{mg g}^{-1}]$$

For methodological reasons it was not feasible – at the current status of this ongoing SFB study – to relate the process of nutrient uptake to a discrete soil volume that surrounds the root system. Hence, the belowground efficiency in resource sequestration could not be expressed in a way conceptually consistent with the aboveground efficiency in resource sequestration. As a substitute for the time being, whole-plant nutrition (i.e. the amount of nutrients eventually incorporated into whole-plant biomass by the time of harvest) was expressed rather on the basis of the root biomass, as the latter – as a whole – represents an investment into belowground space and, at least in part, is involved in nutrient uptake. By this approach, the nutritional status of the whole plant is regarded as an outcome of resource investment into the root system. This analysis was done at the end of the experiment (August 2000).

2.12.3 Efficiency ratio of "running costs"

a) $\dfrac{\text{occupied aboveground (crown) volume}}{\text{transpiration}}$ $[\mathrm{cm}^3\ \mathrm{mol}^{-1}]$

b) $\dfrac{\text{occupied aboveground (crown) volume}}{\text{foliage respiration}}$ $[\mathrm{m}^3\ \mathrm{g\ C}^{-1}]$

The ratios represent the "running costs" in terms of transpiration and respiration as related to the sequestered space. Seasonal foliage transpiration and respiration were calculated with the PSN6 model (Falge *et al*, 1996) for the period of May 15 through August 31 in 1999 and 2000. At the present status of this ongoing SFB study, stem respiration was measured only on two individuals per species, plantation type and gaseous regime, due to logistic reasons. In addition, variation in response between the two individuals each could be high. Therefore, it was refrained at this stage from calculating the efficiency of "running costs" associated with shoot axes respiration in sustaining the crown volume. Rather, this kind of efficiency ratio was restricted to the nightly foliage respiration within the occupied crown volume. Nevertheless, stem respiration was integrated into the calculation of the whole-tree carbon balance (cf. section 2.11).

2.13 Statistical analysis

Differences between treatments and main effects

Data are presented as means ± standard error (SE). The SE gives an interval of confidence to the mean with 67% of probability. Differences between means and treatment main effects were tested with the general linear model approach (GLM) for analyses of variance (ANOVA) using the SPSS-statistic package (Bühl & Zöfel, 2000).

Allometric analysis

Allometric relationships between plant components/organs or between crown volume and shoot biomass were analysed by the general model:

$$\ln y = b_0 + b_1 \ln x$$

as derived from allometric relationship

$$y = b_0 \, x^{b1}$$

where x and y are any two components of plant structure, and the slope b_1 represents the relative change in allocation between components with treatments. To test whether plantation types and gaseous regimes directly influenced patterns of biomass partitioning between plant components or whether the differences observed reflect size-dependent shifts in allocation, an analysis of covariance (ANCOVA) was performed. Natural log-transformed estimates of one component (dependent variable) were plotted against natural log-transformed estimates of another component (covariate), and shifts in biomass partitioning were identified by significantly different slopes and/or intercepts between treatments (Müller *et al.*, 2000).

3 RESULTS

3.1 BIOMASS AND CROWN VOLUME

3.1.1 Total biomass

After two growing seasons under the different CO_2/O_3 regimes, beech appeared to have a disadvantage under interspecific competition, showing less total biomass in mixed than in monocultures, independent of the gaseous treatment. In contrast, spruce tended to enhance its biomass in the mixed plantations. Significant differences of beech between plantation types were found for shoots under all gaseous regimes, but for roots only under elevated CO_2 regimes (Fig. 3.1.1 arrows). Spruce enhanced significantly its shoot and root biomass in the mixed as compared with the monoculture only under $+CO_2$. Overall, no significant differences were found between the biomasses of beech in the monocultures. Comparing beech in mixed cultures, the enhanced ozone regime slightly reduced the above as well as belowground biomass under ambient CO_2 concentration (not significant), whereas no changes were found under elevated CO_2. Elevated CO_2 concentration increased the total biomass of spruce in the mono as well as in the mixed cultures. In general, the main factors causing changes in biomass were plantation types in beech and CO_2 regimes in spruce (ANOVA).

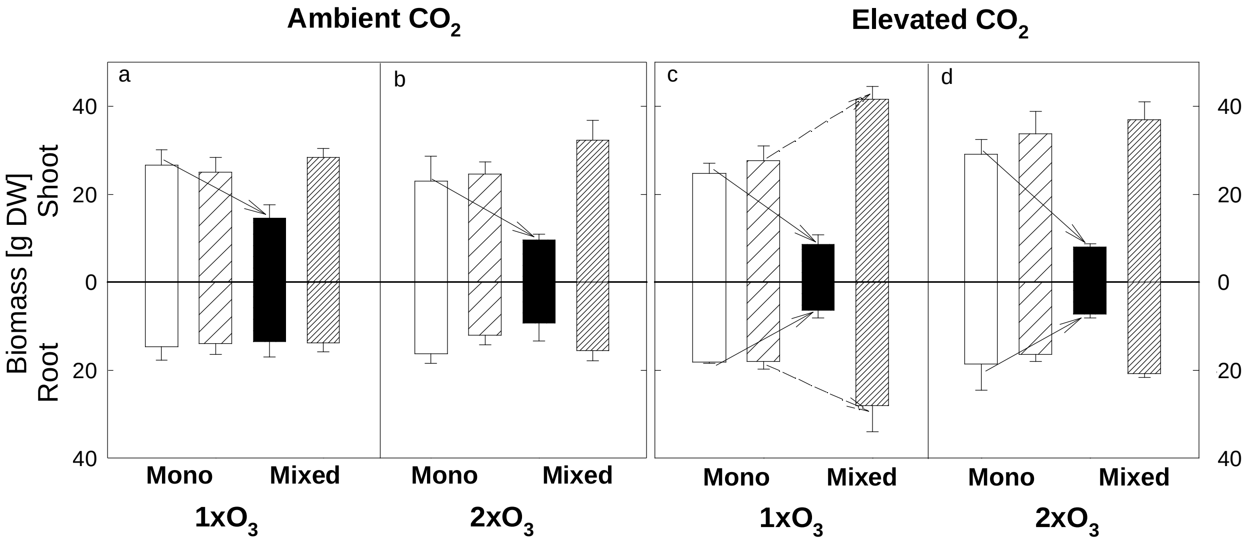


Figure 3.1.1. Shoot and root biomass of beech and spruce saplings at the end of the experiment (August 2000), growing under (a) control, (b) $+O_3$, (c) $+CO_2$ and (d) $+CO_2/+O_3$. Open and solid bars denote beech, and bars with wide and narrow hatching represent spruce in mono and mixed cultures, respectively (means ± standard error, n= 3 to 12). Arrows indicate significant differences between treatments at $p<0.05$.

3.1.2 Root/shoot biomass ratio

Overall beech showed higher root/shoot biomass ratios in the mixed as compared to the monocultures, but a significant difference was found only under the $+O_3$ and $+CO_2$ treatments (Table 3.1.1). In spruce, higher investments into roots were found in the mixed cultures under elevated CO_2, in particular under $+CO_2$.

Table 3.1.1. Root/shoot biomass ratios of beech and spruce trees at the end of August 2000 under different CO_2 and O_3 regimes.

Treatments	beech	Spruce
Control		
monoculture	0.69 ± 0.10	0.52 ± 0.10
Mixed culture	0.89 ± 0.12	0.52 ± 0.11
$+ O_3$		
monoculture	*0.54 ± 0.07	0.59 ± 0.05
Mixed culture	0.90 ± 0.06	0.57 ± 0.17
$+ CO_2$		
monoculture	*0.63 ± 0.01	*0.49 ± 0.03
Mixed culture	0.87 ± 0.03	0.68 ± 0.05
$+CO_2/+O_3$		
monoculture	0.75 ± 0.11	0.53 ± 0.10
Mixed culture	0.96 ± 0.15	0.68 ± 0.12

* indicates significant difference between mono and mixed cultures at $p<0.05$.

3.1.3 Development of the non-green aboveground biomass

At the end of the growing season 2000, beech under all treatments showed a significantly higher biomass of stem and branch axes in the mono than in the mixed plantations, in particular under elevated CO_2 (Fig. 3.1.2). The monocultures under control, $+O_3$, $+CO_2$ and $+CO_2/+O_3$ conditions had 1.73, 2.60, 2.45 and 3.65 times more biomass, respectively, than had, accordingly, the mixed cultures. Slight but significant differences in biomass between mono and mixed cultures were also found in the previous year (1999) under elevated CO_2. In 2000, the enhanced ozone regime diminished the biomass of beech in the mixed culture under amb. CO_2, however such an effect was absent under elevated CO_2. Here, enhanced ozone tended to increase the biomass of beech in the monoculture. Differences in the biomass of spruce between plantation types occurred for the first time in 2000, with the mixed cultures showing more biomass than the monocultures. However, the differences were not significant, except for plants under $+CO_2$. At the beginning of the phytotron study in 1998, spruce had four times more biomass than beech, however, by the end of 2000, beech had reached a biomass similar to that of spruce in the monocultures.

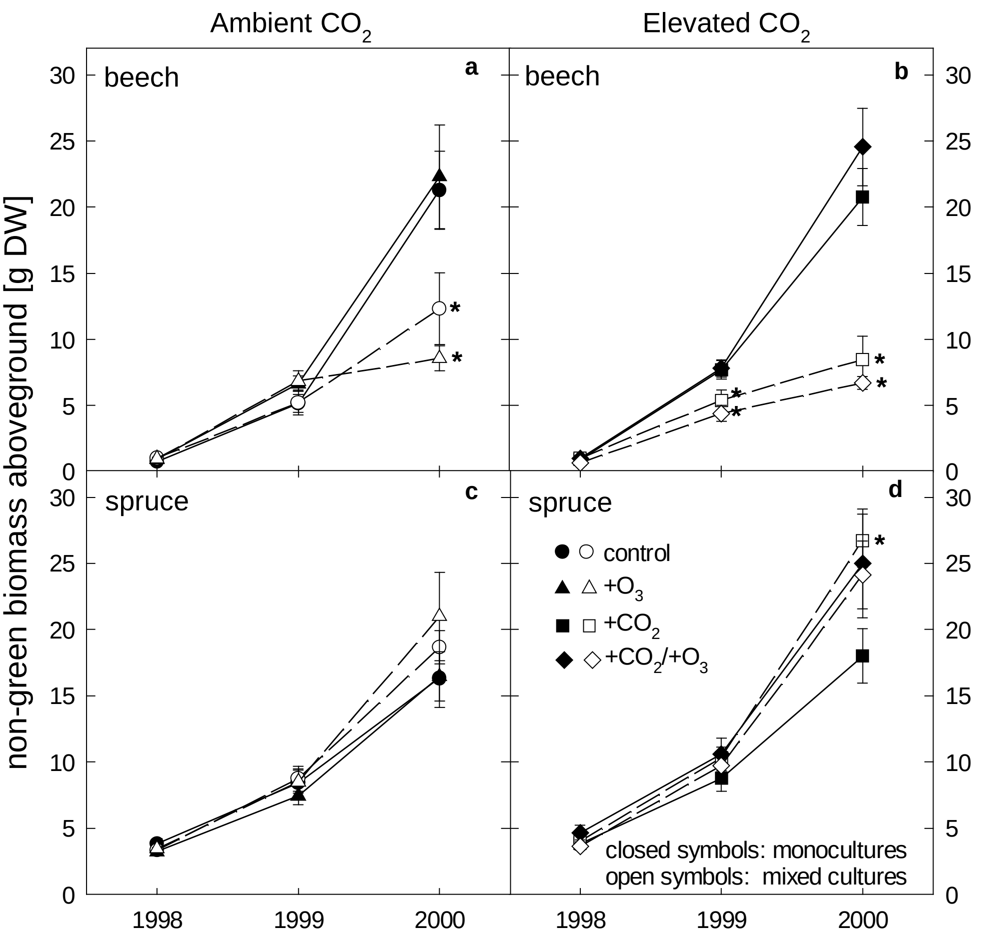


Figure 3.1.2. Biomass of stem and axes of beech (a and b) and spruce (c and d) growing under ambient (a and c) and elevated CO_2 (b and d). Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles $+O_3$; squares $+CO_2$, and rhomboid symbols are $+CO_2/+O_3$ (means ± standard error, n=5 to 12). Measurements were conducted in March of 1999 and 2000, and at the end of August 2000, representing the biomass achieved during the growing seasons 1998, 1999 and 2000, respectively.

3.1.4 Relative aboveground biomass increment

In 1999, beech plants in the monocultures (Fig. 3.1.3, solid symbols) tended to have higher relative aboveground growth increment as compared with plants in the mixed cultures (open symbols), and the opposite trend was found for spruce. However, significant differences did occur only under +CO_2 in beech, and under control and +CO_2/+O_3 conditions in spruce. In 2000, differences between mono and mixed plantations of beech and spruce became significantly larger under all treatments. Beech showed higher proportional biomass increments in the mono as compared to the mixed cultures, whereas spruce appeared to have an advantage in the latter plantation type, enhancing its relative increment at the expense of beech. This is most evident in the mixed culture under +O_3 (open triangle), where beech displayed the lowest and spruce the highest relative increment in 2000. In 1999, beech had higher and more variable biomass increments as compared with spruce. In 2000, both beech and spruce displayed reduced increments relative to 1999: The highest reductions occurred in the mixed cultures under enhanced O_3 for beech. In contrast, enhanced ozone stimulated the growth of spruce in 2000, independent of the CO_2 regime.

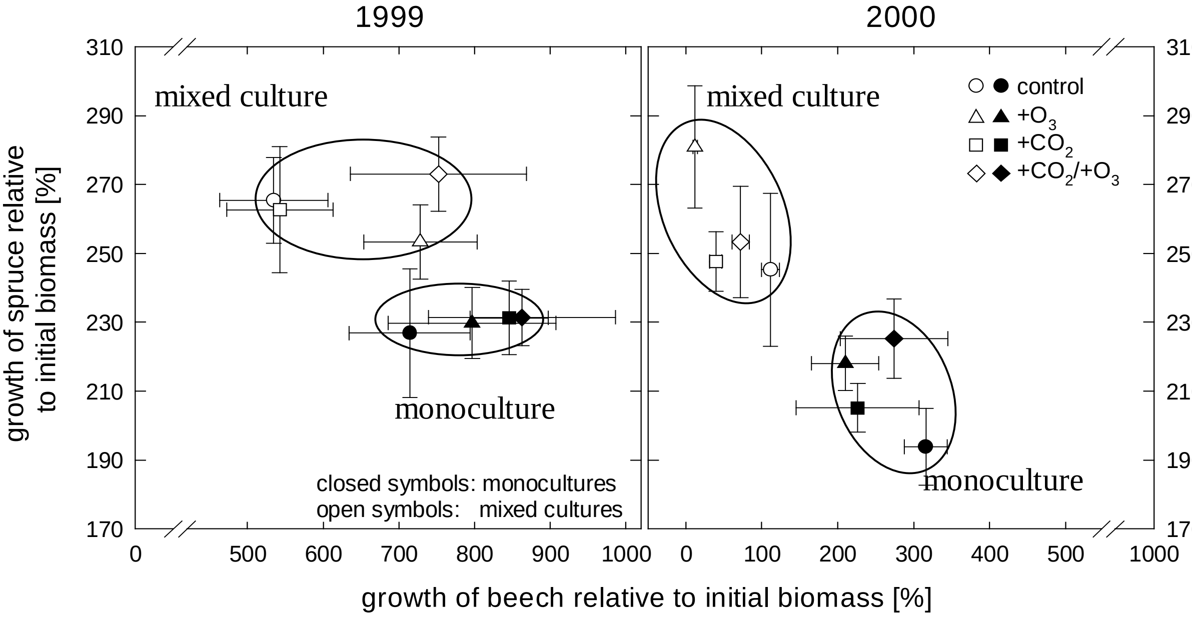


Figure 3.1.3. Growth (non-green aboveground biomass) of beech (x-axis) and spruce (y-axis) relative to the corresponding initial biomass at the beginning of each growing season under the different CO_2 and O_3 regimes. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles +O_3, squares +CO_2 and rhomboid symbols are +CO_2/+O_3 (means ± standard error, n=5 to 12).

3.1.5 Foliage area

In 1999, beech showed no differences in the foliage area between plants in mono and mixed cultures under amb. CO_2 (Fig. 3.1.4). Under elevated CO_2, beech displayed significantly lower foliage area in the mixed cultures. Differences between plantation types became larger and significant under all gaseous treatments in 2000, with the lowest foliage area being found in mixed culture under $+O_3$. Ozone appeared to have no effect on the foliage of beech under elevated CO_2. The foliage area of spruce did not differ between plantation types and gaseous regimes in 1999. In 2000, the mixed cultures showed a higher area as compared with the monocultures, but a significant difference was found only under $+CO_2$. The foliage area of spruce was hardly affected by the gaseous regimes. Overall, differences in foliage area were determined mainly by plantation types.

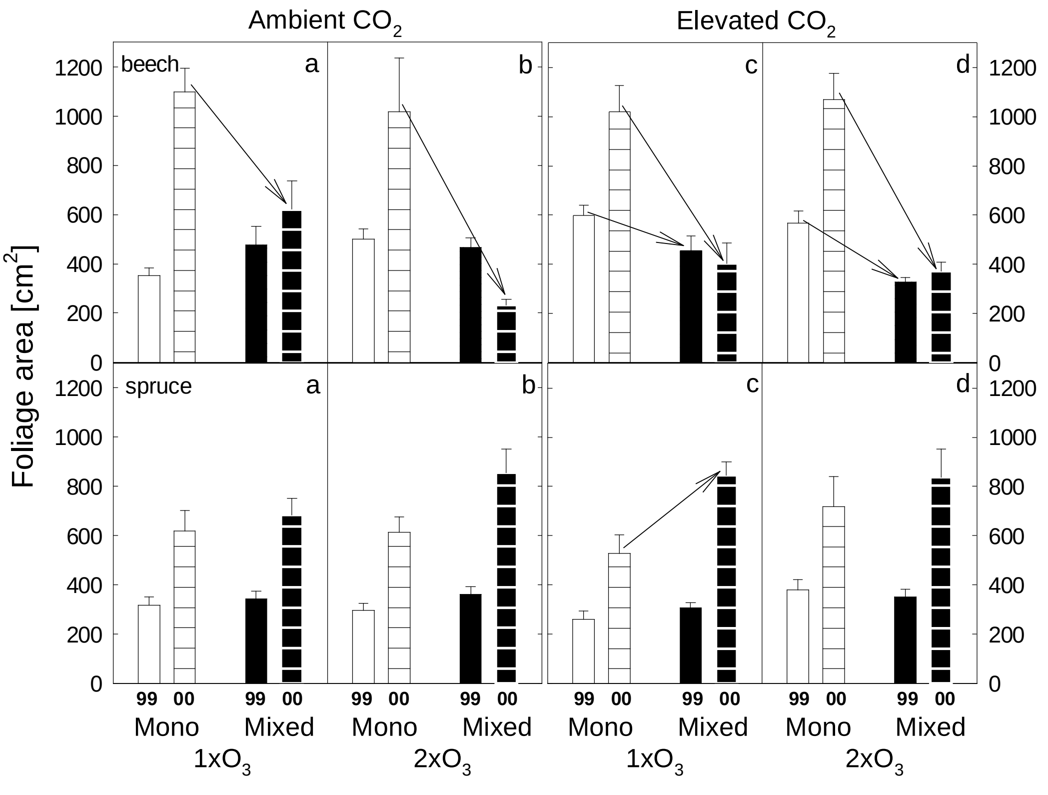


Figure 3.1.4. Foliage area of beech and spruce in 1999 and 2000 under (a) control, (b) $+O_3$, (c) $+CO_2$ and (d) $+CO_2/+O_3$. Bars without hatching represent the foliage area in 1999, and bars with horizontal hatching denote area in 2000. White and black bars represent mono and mixed cultures, respectively (means ± standard error, n= 5 to 12). Arrows indicate significant differences between treatments at $p < 0.05$.

3.1.6 Crown volume

During the first growing season in the phytotrons (1999), similar crown volumes of beech were found in all plantation types and gaseous regimes, however, except for +CO_2/+O_3, where crown volume in the mixed culture was smaller than in the monoculture (Fig. 3.1.5). In 2000, beech showed significantly lower crown volumes in the mixed than in the monocultures under all treatments. The smallest volume was found under +O_3. Plants in monocultures under elevated CO_2 concentration were not capable to enlarge the crown volume in the following growing season. In the mixed cultures, crown volumes were even smaller than during the first growing season. In general, spruce in both growing seasons showed slightly higher crown volumes in the mixed than in the monocultures. However, a significant difference was found only under +CO_2. The gaseous regimes did not influence the crown volume of the spruce plants. It were the plantation types that mainly determined changes in crown volume.

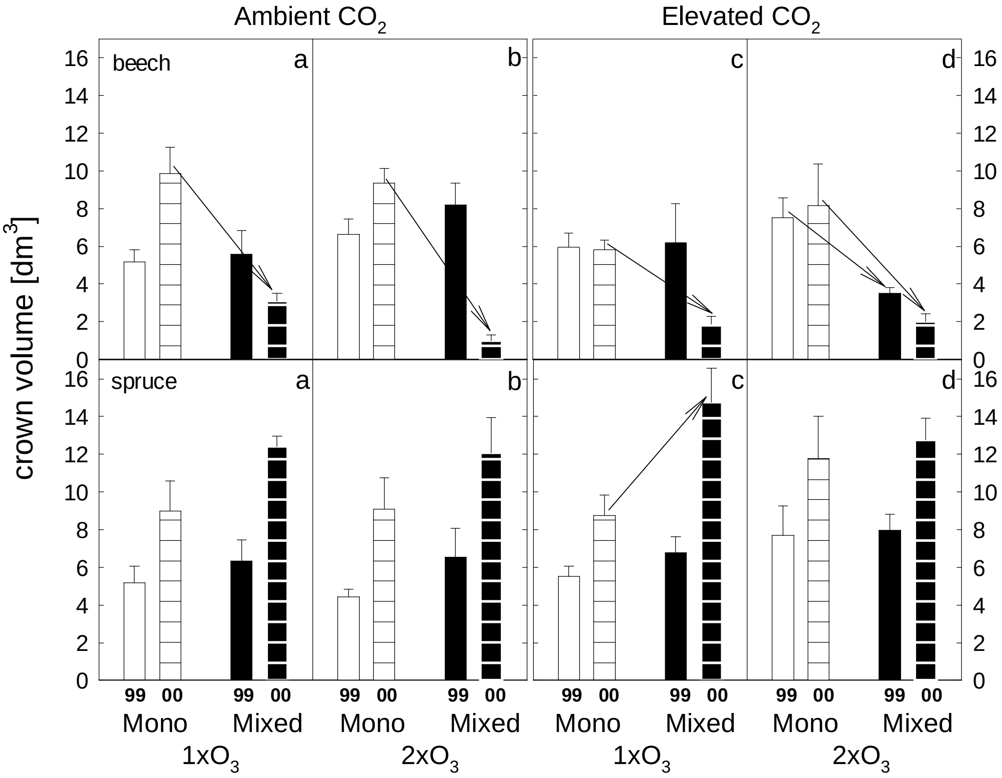


Figure 3.1.5. Crown volume (cm^3) of beech and spruce in 1999 and 2000 under (a) control, (b) +O_3, (c) +CO_2 and (d) +CO_2/+O_3. Bars without hatching represent the foliage area in 1999, and bars with horizontal hatching denote area in 2000. White and black bars represent mono and mixed cultures, respectively (means ± standard error, n= 5 to 12). Arrows indicate significant differences between treatments at $p < 0.05$.

3.1.7 Fine and coarse root biomass

At the end of August 2000, beech presented a belowground biomass investment that was 1.79 to 4.39 times higher in coarse than in fine roots (Fig. 3.1.6). On the other hand, spruce maintained a rather constant and proportional biomass distribution between fine and coarse roots, except for the mixed cultures under ambient CO_2. Here, spruce diminished the relative biomass investment into the fine roots. Beech displayed under all treatments a higher coarse/fine biomass root ratio in the mixed compared to the monocultures (not significant). The highest ratio was found under $+O_3$.

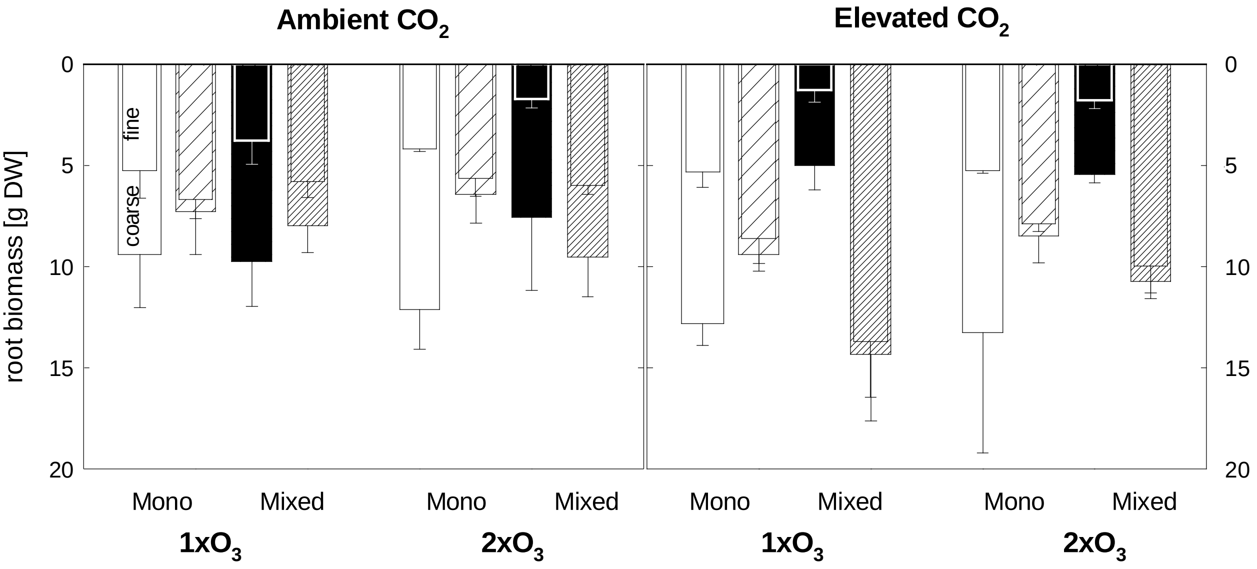


Figure 3.1.6. Fine (narrow bars) and coarse root biomass (wide bars) of beech and spruce in August 2000 under different CO_2 and O_3 regimes. Open and solid bars denote beech, and bars with wide and narrow hatching represent spruce in mono and mixed cultures, respectively (means ± standard error, n= 2).

3.1.8 Regeneration of fine root growth

The fine root biomass (Fig. 3.1.7) sampled in beech did not differ between plantation types, except under the control and $+CO_2$ conditions during 2000, where the monthly biomass regeneration was higher in the mono than in the mixed cultures (asterisks). Regeneration in beech did not differ between spring and summer of 1999. Comparing spring 1999 with the growing season of 2000, regeneration was significantly higher during 2000 in the monoculture at the gaseous control regime, and in the mixed cultures under the $+CO_2$ and $+CO_2/+O_3$ regimes. Differences between monthly regeneration in summer 1999 and 2000 were found only in the gaseous control in the monoculture, and under $+CO_2/+O_3$ regime in the mixed plantation. Winter inhibited the biomass regeneration. In summer 2000, $+O_3$ reduced the biomass regeneration of beech in the monoculture (arrow).

Comparing the mixed cultures, beech plants under +CO_2/+O_3 presented the highest fine root regeneration. In spruce, no differences between plantation types were found at each sampling date. In contrast to beech, spruce in general showed significantly higher regenerations in summer 1999 and 2000 compared to early summer in 1999. Differences in regeneration between later summer 1999 and summer 2000 were found only in the mixed cultures at the control and +CO_2/+O_3 regimes. Overall, the fine root regeneration during the winter was significantly lower as compared with all other sampling dates. Differences between gaseous regimes were found in the mixed cultures in later summer 1999, were regeneration under +CO_2 was higher than under control and +CO_2/+O_3 conditions (arrows).

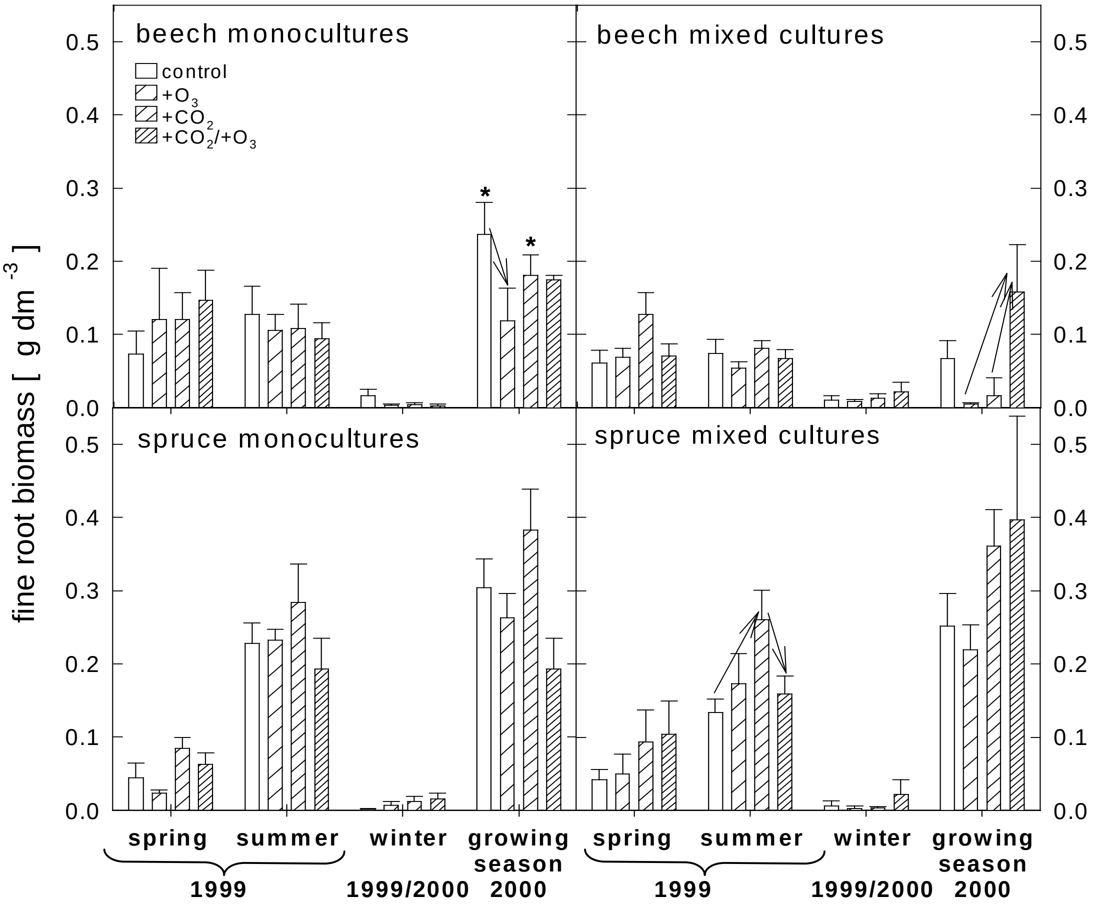


Figure 3.1.7. Monthly regeneration of fine-root biomass per unit of soil volume (g dm^{-3}) from March through June (spring) and from June through October (summer) 1999, from October 1999 through March 2000 (winter), and from March through August 2000 (growing season). Open bars, and bars with coarse, medium and fine diagonal hatching are gaseous control, +O_3, +CO_2 and +CO_2/+O_3, respectively (means ± standard error, n= 2 to 8). Arrows and asterisks indicate significant differences between gaseous treatments and between mono and mixed cultures, respectively, at $p<$ 0.05.

3.2 PHENOLOGY AND LEAF GAS EXCHANGE

3.2.1 Phenology of shoots

Figure 3.2.1 shows the phenological development of beech and spruce shoots during the growing seasons of 1999 and 2000. In 1999 the development of beech shoots was similar in all plantation types and gaseous regimes. On April 24, plants already displayed bud break, and growth was completed by mid-June (stage 5). Elevated CO_2 appeared to slightly accelerate the development of the plants. Under $+O_3$, a higher number of individuals produced the second flush in mixed culture. The same was found in the monocultures under elevated CO_2 ($+CO_2/+O_3$). In 2000, the monocultures initiated bud break one week earlier than did the mixed cultures. Under amb. CO_2 length growth was completed first by plants under $1xO_3$ in the monocultures, and was followed one and three weeks later by monocultures at $+O_3$ and mixed cultures at control conditions, respectively. Plants in mixed culture under $+O_3$ had a marked delay in development, and did not produce a second flush. Under elevated CO_2, plants in the monocultures reached each developmental stage at around one or two weeks earlier than did the mixed cultures, and more individuals produced a second-flush. Under $1xO_3$, plants in mixed culture did not grow a second-flush. In spruce, there were no developmental differences between plantation types and gaseous regimes. The enhanced ozone regime appeared to stimulate the phenological course of the plants, however, the differences were not significant. Plants reached the final stage of development (stage 6) in 2000 at about two weeks later than in 1999.

3.2.2 Senescence of beech leaves

The enhanced O_3 regime accelerated leaf abscission under ambient CO_2, in particular in the monoculture (Fig. 3.2.2). In the control, leaf loss occurred earlier in the mixed culture. Under elevated CO_2, there were no differences between plantation types and gaseous regimes, except for the monoculture under $2xO_3$, which had lost 26.8 % of the leaves by the end of September. The lowest percentage of attached leaves at this time, 66.7 %, was found in the monoculture under $+O_3$ conditions.

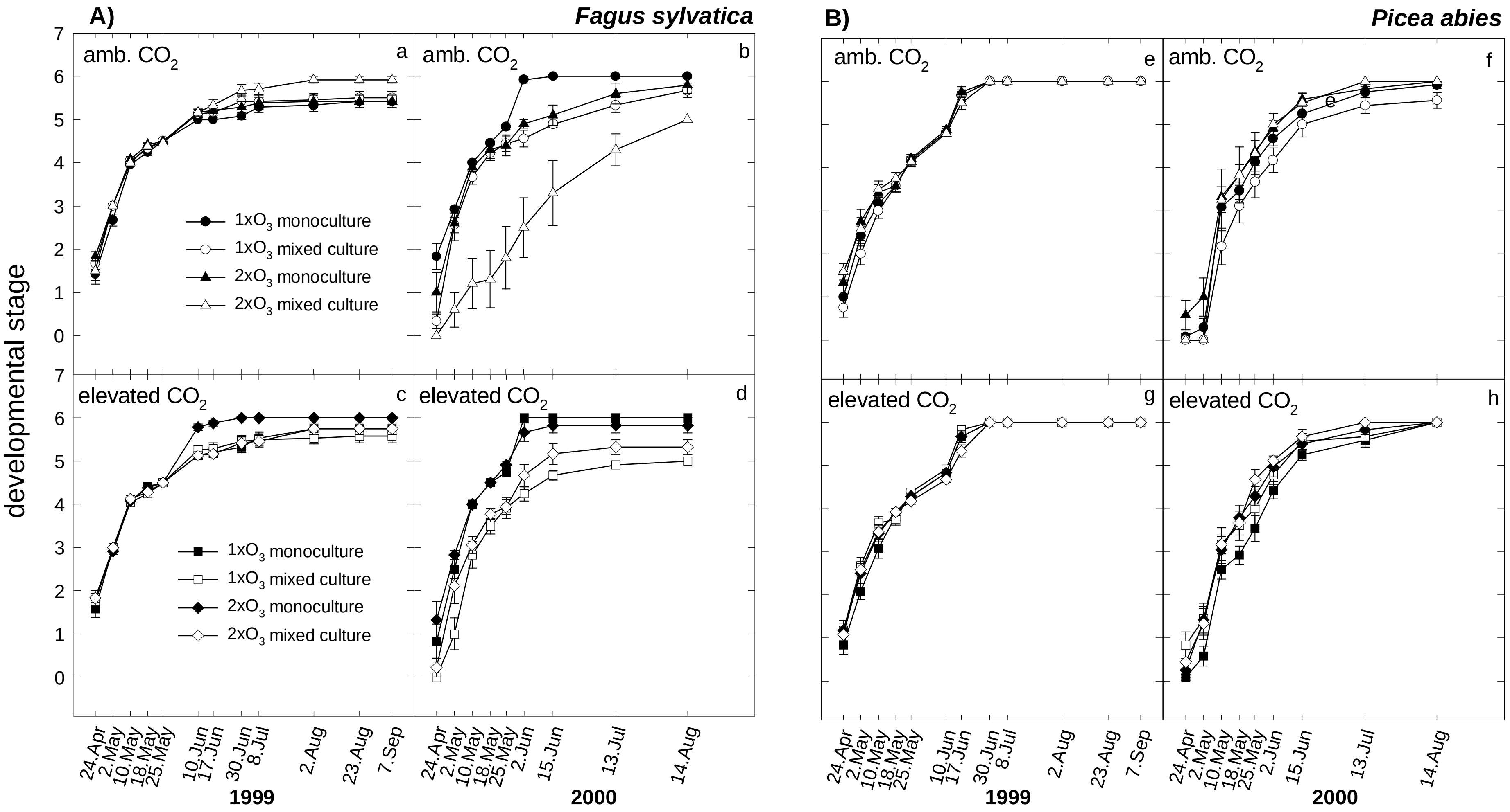


Figure 3.2.1. Phenology of the beech leaves and axes (a), and of twigs in spruce (b) throughout the growing seasons of 1999 and 2000. Beech leaves were classified by six developmental stages: (0) buds closed, (1) buds swollen, (2) first leaf visible, folded (3) leaves unfolded but hanging, (4) leaves horizontally positioned, advanced length growth, axes still hanging, and (5) leaves and shoots totally developed, (6) appearance of second-flush leaves. For spruce, the classification was: (0) buds closed, (1) bud break, (2) new axis shorter than 1 cm, (3) beginning of length growth, new axis < 3 cm, (4) advanced length growth, new axis >3 cm, (5) needles of the new shoots slightly spread, and (6) completion of length growth. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles +O$_3$, squares + CO$_2$ and rhomboid symbols +CO$_2$/+O$_3$ (means ± standard error, n=5 to 12).

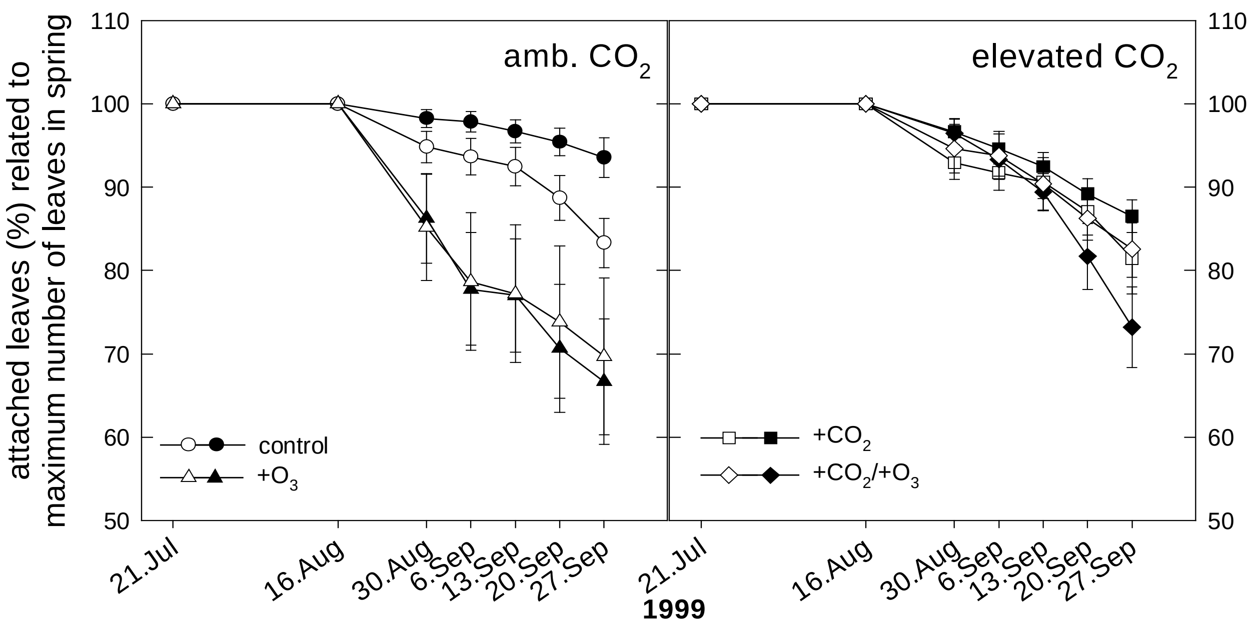


Figure 3.2.2. Percentage of remaining leaves in relation to the maximum leaf number found in spring of 1999. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles $+O_3$, squares $+CO_2$ and rhomboid symbols are $+CO_2/+O_3$. The mean maximum leaf numbers of plants was 32.8 ± 2.9 (gaseous control regime), 46.3 ± 4.0 ($+O_3$), 53.0 ± 3.9 ($+CO_2$) and 52.3 ± 4.2 ($+CO_2/+O_3$) in monoculture, and 43.9 ± 7.0 (control), 43.1 ± 3.6 ($+O_3$), 41.4 ± 5.8 ($+CO_2$) and 27.8 ± 1.5 ($+CO_2/+O_3$) in mixed plantations.

3.2.3 Ozone symptoms

Necrotic dots appeared in beech leaves at the end of June 1999 and 2000 in both plantation types (Fig. 3.2.3). Plants under the enhanced O_3 showed higher percentage of injured foliage area than did plants under $1xO_3$ regime. However, differences in injury between ozone regimes were not significant under elevated CO_2 in 1999. In both years, plants in monoculture (closed symbols) showed higher foliage injury under enhanced O_3 than did plants in mixed culture (open symbols). Under $+CO_2/+O_3$, less than 10 % of the total foliage area was injured during the first, but 20 % during the second growing season in the phytotrons.

In spruce, symptoms appeared also at the end of June 1999 and 2000. Under the enhanced O_3 regime, about 90% of all individuals in the mixed, and 67 % in the monoculture showed symptoms on older than 1-year needles at the end of the first growing season in the phytotrons. Injured current-year needles were found in 17-22 and 33-50 % of the individuals under the enhanced ozone regime in 1999 and 2000, respectively. At the beginning of the second growing season, one might have expected the same or a higher percentage of individuals showing ozone symptoms on older needles as found at the end of 1999. However, with few exceptions, injury was observed in fewer plants in 2000 than in 1999, because some plant individuals discarded their older injured needles at the end of 1999.

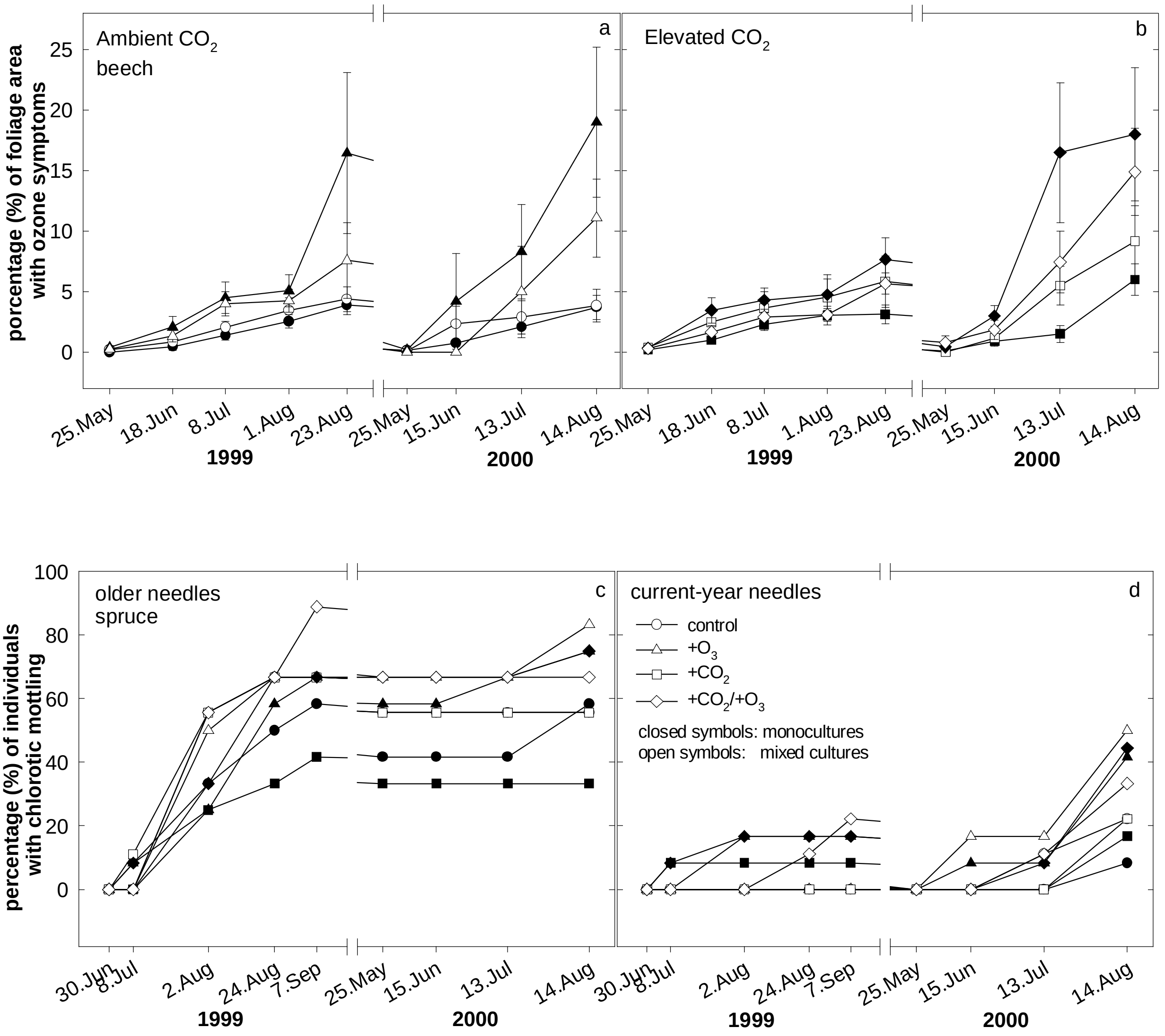


Figure 3.2.3. Percentage of beech foliage with macroscopic ozone symptoms (necrosis) (a and b) and spruce individuals with chlorotic mottling (c and d). Monocultures are given as solid and mixed cultures as open symbols. Circles denote control, triangles +O_3, squares +CO_2 and rhomboid symbols +CO_2/+O_3 (means ± standard error, n= 5 to 12).

3.2.4 Porometry

In the first growing season, the *sun leaves* of beech (c, d) showed a similar CO_2 assimilation rate in the mono and mixed cultures, and elevated CO_2 stimulated the rate at most measurement dates (Fig. 3.2.4). In 2000, beech displayed in the monocultures higher rates compared to 1999 and to mixed cultures (trend lines). The enhanced ozone regime reduced the CO_2 assimilation rate of beech under all treatments, except for elevated CO_2 in the mixed culture. This ozone effect was particularly pronounced in the mixed culture under ambient CO_2 (open triangles). In contrast, *shade leaves* (e, f) of plants in the mono and mixed cultures did not show significant differences in CO_2 assimilation, but appeared to slightly enhanced the rates in response to elevated CO_2. In August 1999, *second-flush* leaves (a, b) displayed between 60 and 124 % higher rates than did sun leaves under the same gaseous treatments, and this range increased to between 198 and 623 % by the end of September. In 2000 however, assimilation rates of second-flush leaves always were, with the exception of the monocultures under $2xO_3$, lower than those measured in sun leaves.

In spruce, the highest rates of CO_2 assimilation were found in current-year twigs (Fig. 3.2.5 a, b). No differences were found between mono and mixed plantations throughout the two growing seasons. In 1999, the highest rates were measured under the combination of elevated CO_2 and enhanced ozone. In 2000, the rates of spruce were still increased under elevated CO_2. Older than current-year twigs showed very low CO_2 assimilation rates, varying between compensation and 6 $\mu mol\ CO_2\ m^{-2}\ s^{-1}$. Rates were stimulated by elevated CO_2, and the lowest values were found under $+O_3$.

3.2.5 Chlorophyll fluorescence

The sun and second-flush leaves of beech and current-year needles of spruce presented higher ETR than did the shade leaves in beech and the older than current-year needles in spruce (Fig. 3.2.6). In 1999, second-flush leaves showed higher rates than did sun leaves, while in general, no differences between these two leaf types were found in 2000. In spruce, differences between current–year and older needles also diminished in the second growing season. The three types of beech leaves responded differently to the gaseous regimes. The enhanced ozone regime reduced ETR of sun leaves until August under the ambient CO_2 regime. Under elevated CO_2, significant reduction in ETR due to ozone occurred only in mid-September of 1999.

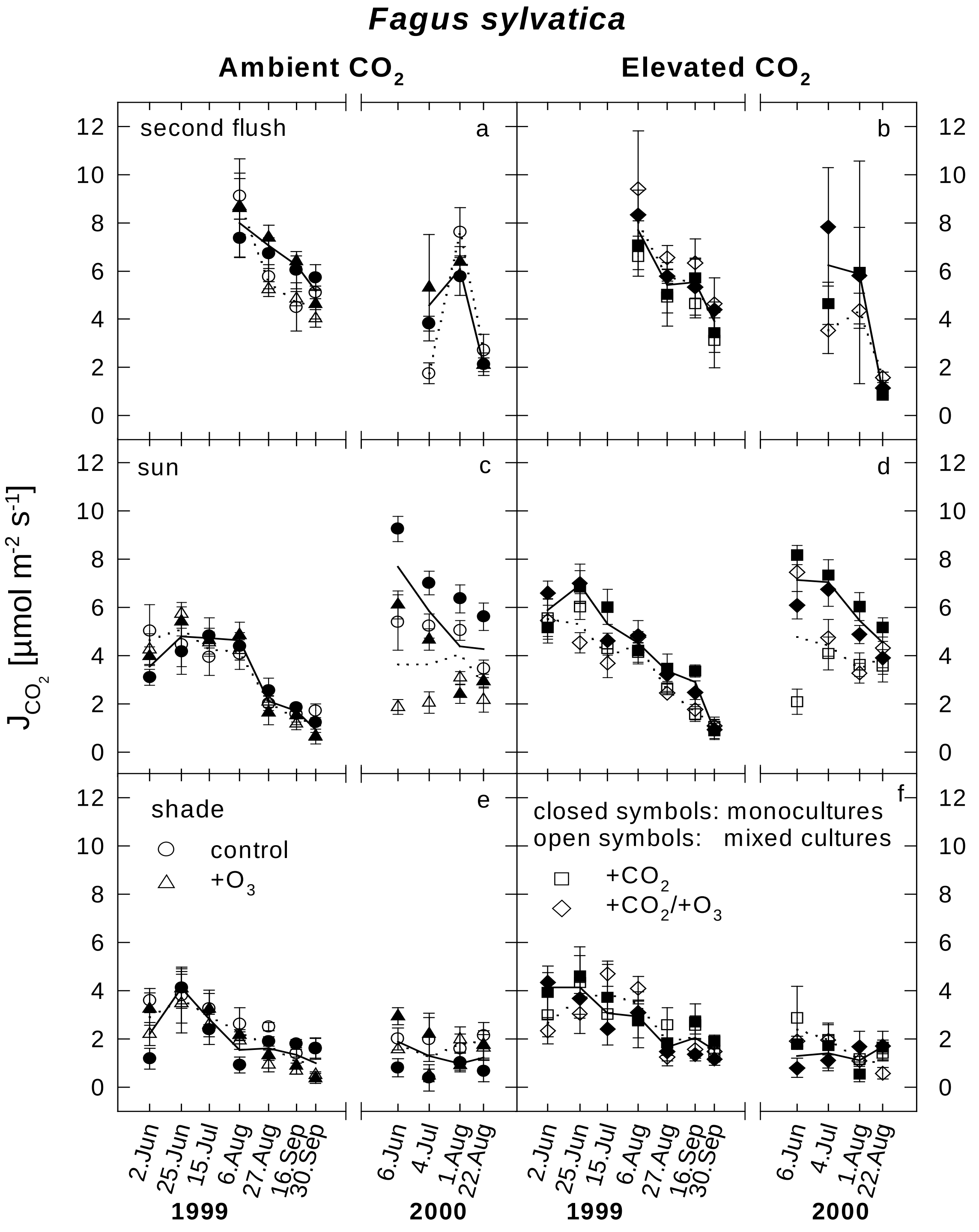


Figure 3.2.4. Net CO_2 assimilation rate (J_{CO2}) of beech, second-flush (a, b), sun (c, d) and shade (e, f) leaves under ambient light (PPFD in phytotrons), air temperature, air humidity conditions and CO_2 concentration throughout the growing seasons of 1999 and 2000. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control., triangles $+O_3$, squares $+CO_2$ and rhomboid $+CO_2/+O_3$ (means ± standard error, n=6 to 12). The solid and dotted trend lines represent the mean values of mono and mixed cultures, respectively.

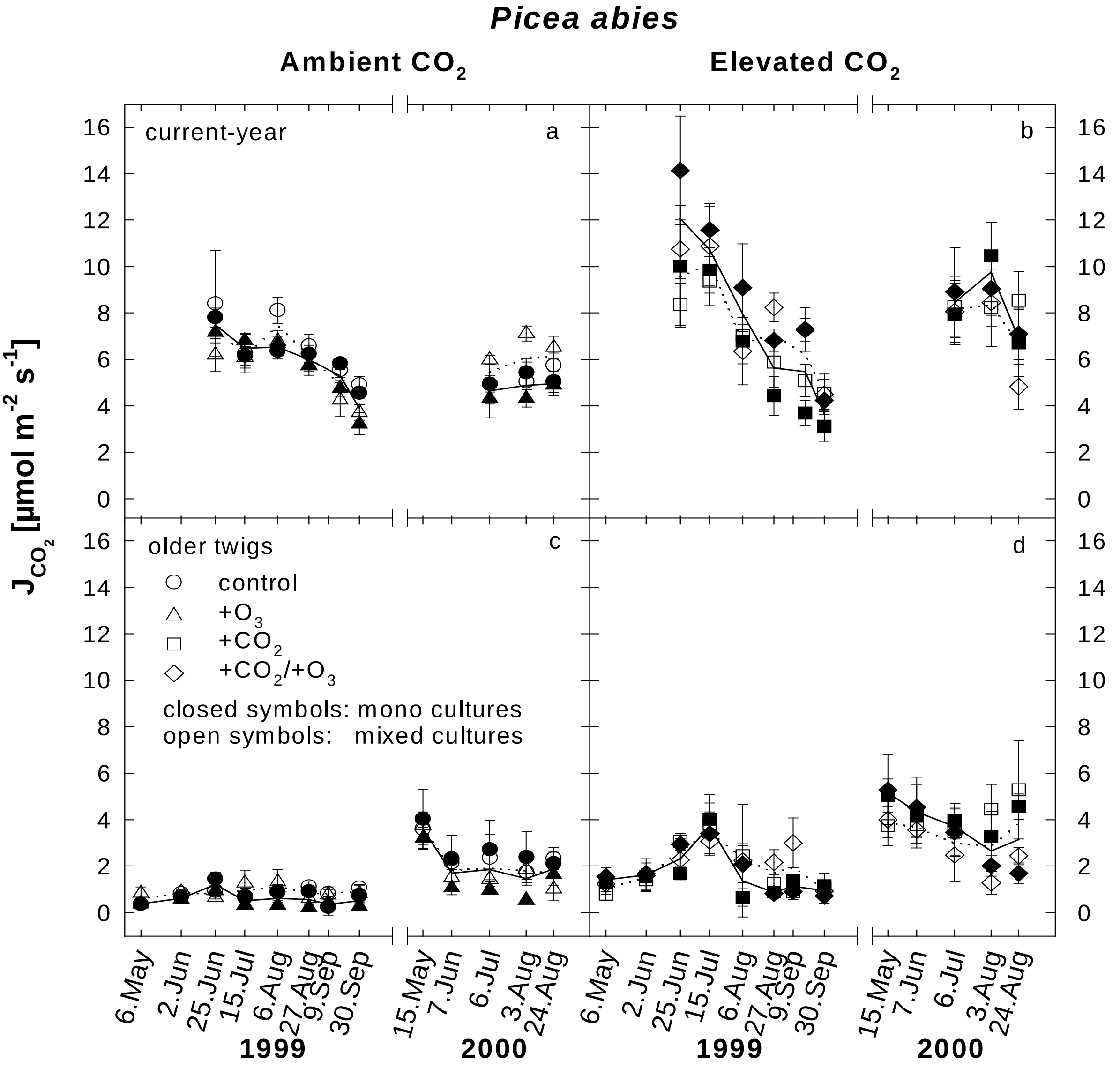


Figure 3.2.5. Net CO_2 assimilation rate (J_{CO2}) of current-year twigs (sun crown) after completion of growth (a, b), and shaded, older than current-year twigs of spruce (c, d) under ambient light (PPFD in phytotrons), air temperature, air humidity conditions and CO_2 concentration throughout the growing seasons of 1999 and 2000. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles $+O_3$, squares $+CO_2$ and rhomboid $+CO_2/+O_3$ (means ± standard error, n= 6 to 12). The solid and dotted trend lines represent the mean values of mono and mixed cultures, respectively.

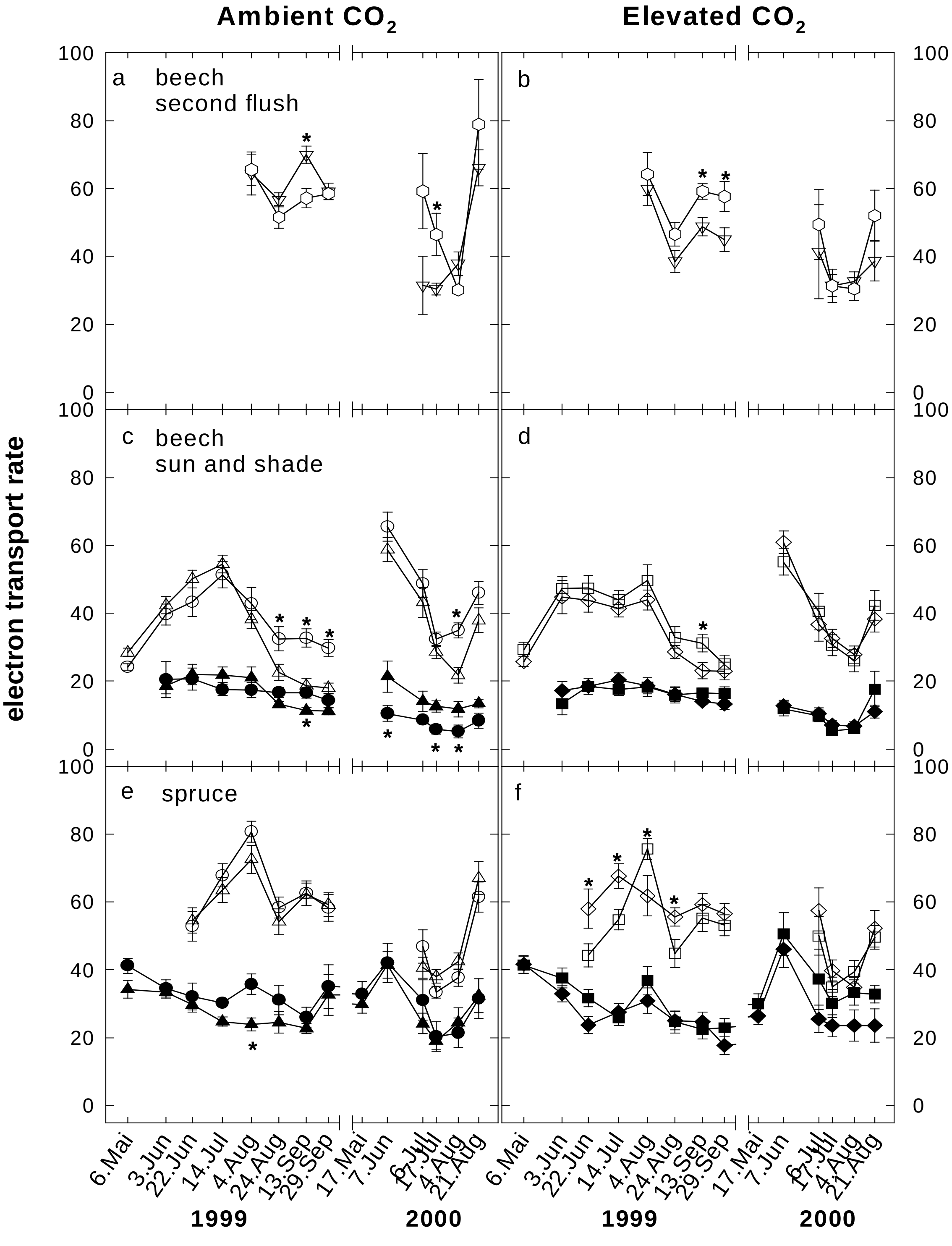


Figure 3.2.6. Apparent electron transport rate (ETR) of sun, shade and second-flush leaves of beech and of current-year and older spruce twigs throughout the growing seasons of 1999 and 2000 under ambient (a, c, e) and elevated CO_2 (b, d, f). Shade leaves and older than current-year twigs are given as solid, and sun leaves and current-year twigs as open symbols. For second-flush leaves, inverse triangle and hexagon represent $1xO_3$ and $2xO_3$. Circles denote control, triangles $+O_3$, squares $+CO_2$ and rhomboid symbols $+CO_2/+O_3$. The variance test showed no significant differences in ETR between mono and mixed cultures at $p<0.05$. Mono and mixed data were pooled to calculate the presented mean apparent electron transport rate (means ± standard error, n= 10 to 20). * indicates significant difference between $1xO_3$ and $2xO_3$ treatments.

In contrast, shade leaves hardly differed in 1999 between the ozone treatments under ambient CO_2, whereas in 2000, higher ETR was found under $2xO_3$ as compared with $1xO_3$. Under elevated CO_2, there were no differences between ozone treatments. Except for ambient CO_2 in 1999, ETR of second-flush leaves tended to be increased under $2xO_3$. In spruce, significant differences between ozone regimes were found mainly in the first growing season under elevated CO_2, with current-year needles under $2xO_3$ displaying higher ETR than needles under $1xO_3$. In older needles, the enhanced ozone regime diminished ETR under both CO_2 regimes. However, a significant difference between ozone regimes was found only in August 1999 under ambient CO_2.

3.2.6 Nitrogen content

In beech, shade and sun leaves showed the highest nitrogen concentration of all organs, followed by fine roots, current-year shoot axes, coarse roots and older shoot axes (Fig. 3.2.7). There were no differences in foliar N concentration between plantation types, except for $+CO_2$, where lower concentrations were found in sun and shade leaves of the mixed culture (arrow). Lower N concentrations in the mixed compared to the monocultures were also found for other organs like fine and coarse roots and older shoot axes at $+O_3$, and in fine roots at $+CO_2/+O_3$. On the other hand, at the control regime, plants in the mixed culture enhanced its N concentrations in fine roots and current-year and older shoot axes. The same was the case in coarse roots at $+CO_2$. Under the ambient CO_2 regime, the monocultures responded to the enhanced ozone regime by increasing the N concentration in all organs, except for sun leaves. Under elevated CO_2 and $2xO_3$ ($+CO_2/+O_3$), higher N concentrations were found in the sun and shade leaves of mixed cultures. In general elevated CO_2 significantly reduced the N concentration of the plant organs, especially of leaves and older shoot axes. The main factors that drove changes in the N partitioning were CO_2 followed by plantation type in leaves, CO_2 followed by O_3 in axes, and O_3 followed by CO_2 in roots (ANOVA-analysis).

Spruce showed a different pattern of N allocation. Current-year, older needles and fine roots had similar N levels. Under $+O_3$, plants in the mixed culture showed higher N concentrations in the current-year needles and fine roots than did plants in the monocultures. No differences between plantations patterns were found under elevated CO_2. Under ambient CO_2, the enhanced ozone regime raised the N content in current-year needles and shoot axes and in the fine roots of the mixed cultures. Elevated CO_2 reduced the N concentration in all organs (except for coarse roots) of spruce, however only in plants under $2xO_3$. CO_2 was the main factor to drive N allocation in spruce.

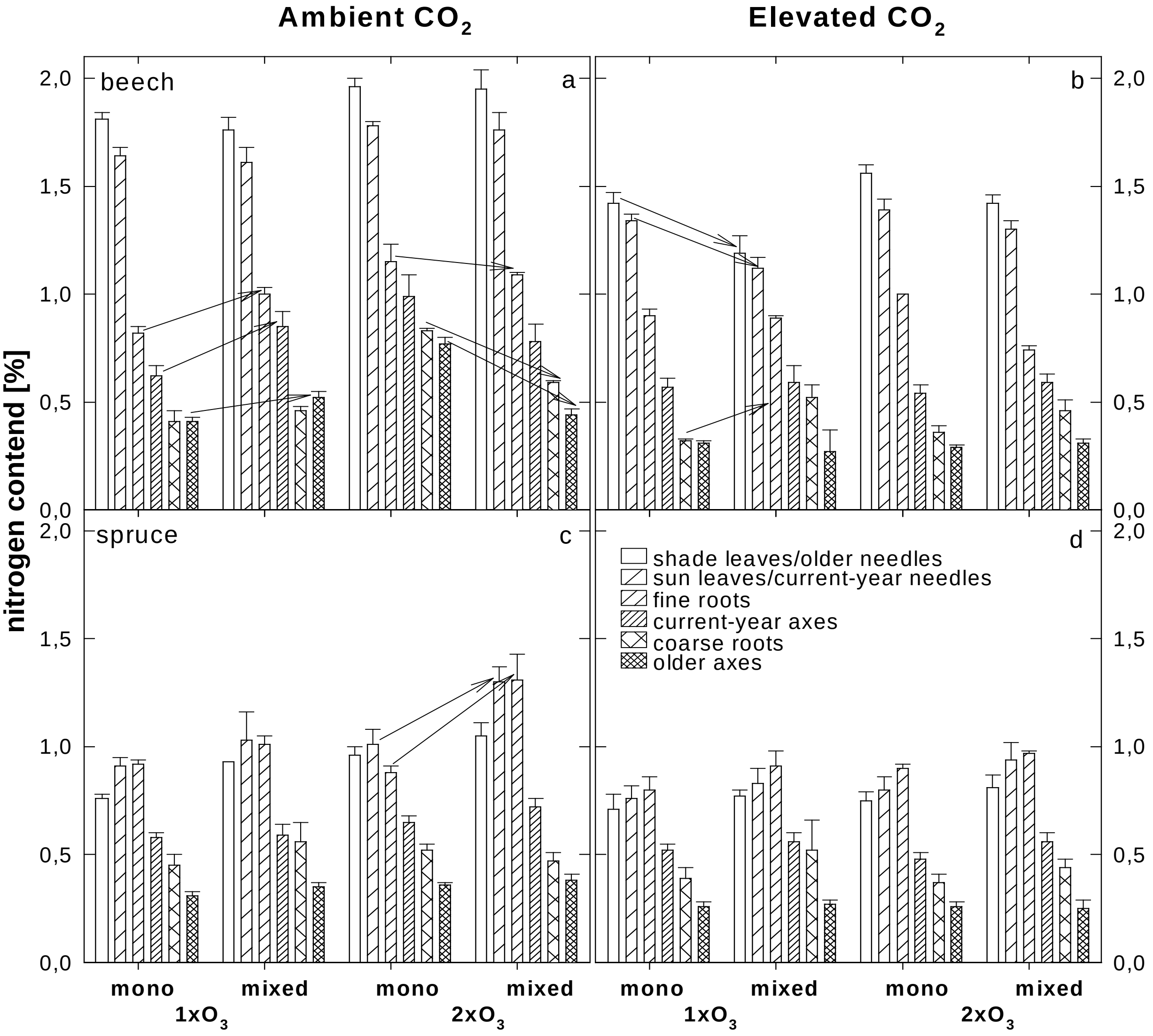


Figure 3.2.7. Nitrogen concentration in sun and shade leaves, current-year and older needles, current-year and older axes and fine and coarse roots of beech (a and b) and spruce (c and d) under the different CO_2 and O_3 treatments. Plants were harvested at the end of August 2000 (mean ± SE, n= 1 to 9). Arrows indicate significant differences between mono and mixed cultures at $p<0.05$.

3.2.7 Seasonal carbon gain, respiration and water loss

The seasonal C gain of 1999 of the sun leaves in beech did not differ between the plantation types and gaseous regimes, except for $+CO_2$ which resulted in higher assimilation on a leaf area basis

(Table 3.2.1a). In 2000, the area-based assimilation increased compared to 1999 in all other treatments. In general, sun leaves under $2xO_3$ showed lower CO_2 uptake than plants under $1xO_3$. Effects of elevated CO_2 on assimilation were not consistent across the treatments in 1999 and 2000. Respiration was similar in 1999 and lower than in 2000 under all treatments. The highest C loss in the second growing season was found at $+CO_2$. Under ambient CO_2, $2xO_3$ tended to increase respiration. In 1999, seasonal water loss of beech sun leaves was reduced by ozone, independent of the CO_2 regime. In 2000, a decrease in transpiration under $2xO_3$ occurred only under ambient CO_2. Similar to the CO_2 assimilation, effects of elevated CO_2 on transpiration were not consistent across the treatments in both years.

The current-year sun twigs of spruce showed the highest C gain in both years at $+CO_2/+O_3$ and $+O_3$, respectively. In both years, elevated CO_2 enhanced the respiration and diminished the transpiration compared to the levels under ambient CO_2. An exception was found in plants under $2xO_3$ in 2000, which did not increase respiration under elevated CO_2.

The sun leaves in beech and current-year twigs in spruce showed higher WUE at $2xO_3$ as compared to $1xO_3$, except for beech under elevated CO_2 regime. Elevated CO_2 tended to increase the WUE of both species. When compared with 1999, beech displayed higher WUE (except for $+CO_2/+O_3$) in 2000, whereas spruce maintained or slightly reduced WUE.

The shade crowns of beech and spruce in the mono and mixed plantations received different light sums (Table 3.2.1b). In general, the monocultures of beech were lighter under ambient, and darker under elevated CO_2 than were the mixed cultures. Seasonal CO_2 uptake and H_2O loss reflected the light conditions. Compared with the mixed plantations, the monocultures of beech showed higher C gains and H_2O losses under ambient CO_2, and lower under elevated CO_2. In 1999 respiration was higher in the monocultures only at $+CO_2$. In 2000, no differences in respiration between plantation types were found. When comparing the monocultures, enhanced ozone regime led to an increase in CO_2 assimilation and in transpiration in both years. $2xO_3$ increased the respiration of shade leaves under ambient CO_2 in 1999, and under elevated CO_2 in 2000. In both years, elevated CO_2 diminished seasonal assimilation and transpiration, and enhanced respiration. Shade leaves in the mixed cultures also showed higher assimilation and transpiration under elevated CO_2, but only at $1xO_3$. In general, respiration was enhanced under elevated CO_2. Enhanced ozone regime increased the assimilation and transpiration of shade leaves under ambient CO_2 in 1999 and, under all treatments, in 2000. In both years, an increase in respiration due to $2xO_3$ occurred only under elevated CO_2.

In spruce, under all treatments (with exception of the control), the shade crown in the mixed was lighter than in the monoculture. In both years, assimilation and transpiration were higher in the mixed cultures. In general, respiration was similar or higher in the mono than in the mixed plantations. In 1999, $2xO_3$ enhanced the C assimilation and transpiration of the older twigs in the monoculture under ambient CO_2. In 2000, $2xO_3$ diminished assimilation and transpiration independent of the CO_2 regime. The respiration in the monocultures was stimulated by enhanced ozone under elevated and ambient CO_2 in 1999 and 2000, respectively. In both years, elevated CO_2 diminished assimilation and transpiration under $2xO_3$ and enhanced them under $1xO_3$ in 2000. Respiration in the mono as well as in the mixed cultures was increased in 1999 under elevated CO_2, and decreased under $2xO_3$ in 2000. Similar to the monocultures, mixed plantations under elevated CO_2 also increased assimilation and transpiration under $1xO_3$, however, decreased them under $2xO_3$ in both years. In 1999, enhanced ozone concentrations tended to raise C assimilation. In 2000, $2xO_3$ reduced assimilation under elevated CO_2. In both years, $2xO_3$ increased transpiration under ambient and reduced it under elevated CO_2.

In both years, the shade leaves of beech increased their WUE under enhanced O_3 in mono as well in mixed cultures (except for plants under elevated CO_2 in 2000). Elevated CO_2 also increased WUE overall, except at $+CO_2/+O_3$ in 2000. In spruce, WUE of older needles under $2xO_3$ was higher in 1999, and lower in 2000, as compared to the $1xO_3$ regime. Elevated CO_2 enhanced the WUE of older needles in both plantation types, ozone regimes and years.

Comparing the two growing seasons, beech showed in 2000 lower seasonal C uptake under all treatments except for the monoculture at $+O_3$ and the mixed plantation at $+CO_2/+O_3$. In contrast, spruce increased its assimilation in both plantation types under $1xO_3$ and diminished it under $2xO_3$. In the second growing season, beech decreased and spruce increased the seasonal respiration under all treatments, except for $+CO_2/+O_3$, where the opposite was observed. Higher WUE were found in both species under all plantation types and gaseous regimes in the first compared to the second growing season.

Table 3.2.1a,b. Seasonal C gain (NP), respiration during night (R), and transpiration (E) of sun leaves in beech and current-year twigs (sun twigs) of spruce (a), and of shade leaves of beech and older than current-year twigs (shade twigs) of spruce (b) under the different CO_2 and ozone regimes (May 15 through August 31 of 1999 and 2000). Mean PPFD occurring in the sun crown was the same in all treatments (494.2 and 456.0 µmol m^{-2} s^{-1} in 1999 and 2000, respectively; cf. Table 2.2 in "Material and Methods"). PPFD of shade crowns see (b).

(a)	**beech sun**				**spruce sun**			
Treatment Year	R (g C m^{-2})	NP (g C m^{-2})	E(molH_2O m^{-2})	WUE (µmolCO_2/ mmolH_2O)	R (g C m^{-2})	NP (g C m^{-2})	E (molH_2O m^{-2})	WUE (µmolCO_2/ mmolH_2O)
Control								
1999	7.74	303.65	4274.0	5.9	5.02	346.97	5594.0	5.2
2000	10.11	432.39	5480.0	6.6	7.10	344.81	5768.0	5.0
+O_3								
1999	8.15	306.15	3820.0	6.7	4.78	318.57	4941.0	5.4
2000	11.78	312.62	3475.0	7.5	10.64	653.35	10405.0	5.2
+CO_2								
1999	7.80	434.11	4317.0	8.4	7.58	309.74	4245.0	6.1
2000	19.21	373.49	3704.0	8.4	11.87	364.33	4986.0	6.1
+CO_2/+O_3								
1999	7.86	299.66	3153.0	7.9	8.38	416.47	4401.0	7.9
2000	11.27	342.52	3705.0	7.7	8.07	285.34	2998.0	7.9

(b)	**beech shade**					**spruce shade**				
Treatments/Year	R (g C m^{-2})	NP (g C m^{-2})	E (molH_2O m^{-2})	WUE (µmolCO_2/mmolH_2O)	PPFD (µmol m^{2} s^{-1})	R (g Cm^{-2})	NP (g C m^{-2})	E (molH_2O m^{-2})	WUE (µmolCO_2/mmolH_2O)	PPFD (µmol m^{-2}s^{-1})
Control										
Mono 1999	8.05	114.01	2004.0	4.7	60.84	5.96	30.32	694.0	3.6	53.25
2000	4.03	67.88	1690.0	3.3	50.49	17.47	83.31	2708.0	2.6	43.18
mixed 1999	8.47	83.72	1608.0	4.3	44.54	6.32	24.14	600.0	3.4	44.54
2000	4.04	52.72	1382.0	3.2	39.04	17.54	73.43	2484.0	2.5	39.04
+O_3										
mono 1999	8.38	179.90	2665.0	5.6	102.88	4.99	62.85	1157.0	4.5	61.30
2000	1.02	210.24	3719.0	4.7	88.49	29.99	40.91	2467.0	1.4	52.90
mixed 1999	8.41	167.85	2516.0	5.6	94.21	4.84	99.42	1708.0	4.9	94.21
2000	1.02	200.82	3581.0	4.7	82.67	28.94	79.53	3544.0	1.9	82.67
+CO_2										
mono 1999	10.16	68.99	1025.0	5.6	45.19	8.92	36.81	711.0	4.3	43.45
2000	5.82	55.77	1028.0	4.5	36.18	17.99	146.56	2040.0	6.0	44.68
mixed 1999	8.40	126.27	1650.0	6.4	73.16	8.07	62.46	1062.0	4.9	73.16
2000	5.78	107.75	1734.0	5.2	70.03	17.92	221.84	2847.0	6.5	70.03
+CO_2/+O_3										
mono 1999	8.51	96.36	1339.0	6.0	53.93	9.85	36.32	595.0	5.1	41.03
2000	12.00	88.18	1777.0	4.1	50.49	7.07	20.58	393.0	4.4	36.77
mixed 1999	8.65	119.94	1530.0	6.5	73.47	9.39	73.81	956.0	6.4	73.47
2000	11.96	122.79	2276.0	4.5	70.22	6.87	44.59	633.0	5.9	70.22

3.3 CARBON BALANCE

3.3.1 Seasonal whole-plant C gain

The seasonal whole-tree C gain was assessed on the basis of the leaf area-related C gain given in Table 3.2.1a,b (cf. Chapter 3.2) and the entire foliage area of individual trees (Fig. 3.3.1). Beech displayed higher seasonal C gains in the mono as compared to the mixed cultures (highest difference of 77.9 % under +O_3).

Spruce in the mixed cultures showed similar (a and d) or higher (b and c) C gains than in the monocultures (highest difference of 42,2 % under +CO_2). The highest C gain was found under +O_3. The 2xO_3 regime increased the assimilation of spruce in both plantation types under ambient CO_2 while causing reduction in mixed culture under elevated CO_2. Elevated CO_2 also differently influenced the assimilation of spruce under 1xO_3 and 2xO_3, resulting in stimulation in the mixed culture under 1xO_3, and in reduction in both plantation types under 2xO_3.

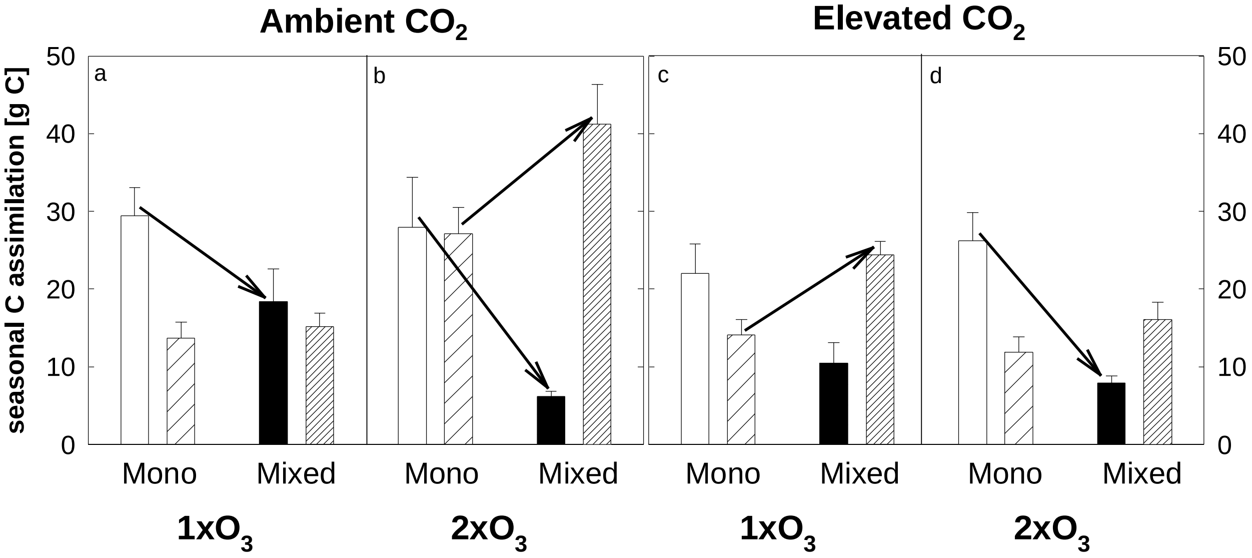


Figure 3.3.1. Seasonal net C gain (May 15 through August 31, 2000) in beech and spruce under (a) control, (b) +O_3, (c) +CO_2 and (d) elevated +CO_2/+O_3. Open and solid bars denote beech, and bars with wide and narrow hatching represent spruce in mono and mixed cultures, respectively (means ± standard error, n= 6 to 12). Arrows indicate significant differences between treatments at $p<0.05$.

3.3.2 Seasonal stem respiration

Figures 3.3.2 and 3.3.3 demonstrate the relationship between the stem respiration rate and air temperature in individuals of beech and spruce, growing in mono and mixed culture under the gaseous regimes. Respiration rates of both species were lower in mixed as compared with monocultures, except for spruce under the gaseous control regime and +CO_2/+O_3, which showed similar rates in mono and mixed plantations. The lowest stem respiration rates were found in beech in mixed culture under +O_3. The lower rates in mixed related to monoculture reflected the reduced relative aboveground biomass increment of beech, but not of spruce (cf. Fig. 3.1.3).

The relationship between stem respiration rate and air temperature varied during the growing season: In general, three different responses of stem respiration to temperature were found in both species, with low rates at the beginning of the growing season (May through beginning of June), high rates between mid-June and the end of July, and intermediate or low rates in August. In beech monoculture under the gaseous control regime, however, stem respiration showed four different responses to temperature, with the rates being high at the end of May and beginning of June, and decreasing successively from the end of June throughout August. In the mixed culture at +O_3, very low rates of respiration were found, and the variation in the response to temperature was small throughout the growing season. In the monoculture at +O_3 regime, stem respiration rates were higher at the end of August than at the beginning of June. Under +CO_2 and +CO_2/+O_3, similar rates of stem respiration were found at the end of May and mid-August. Similar to beech, in spruce the highest respiration rates in response to temperature were found between mid-June and the end of July. Except for the mixed culture under +CO_2, stem respiration rates of spruce were higher in August than in May. The variation in respiration rate throughout 2000 found in beech and spruce plants is probably related to the individual variation in stem radial growth.

Linear correlations derived from the stem respiration data of Figs. 3.3.2. und 3.3.3 were used to calculate the seasonal C release per unit of stem volume (Table 3.3.1).

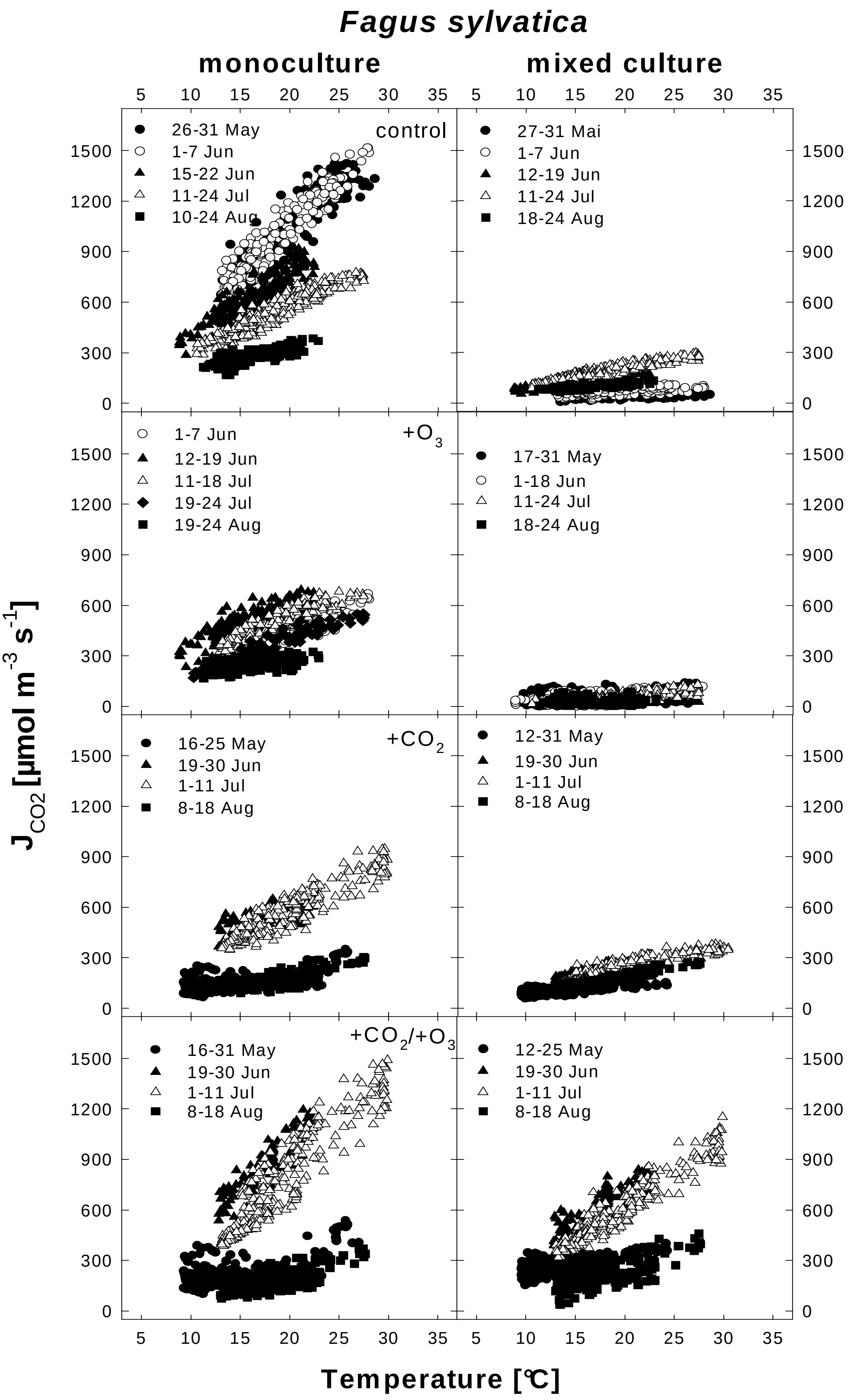


Figure 3.3.2. Relationships between stem respiration rate and air temperature, during the growing season of 2000 in individuals of beech growing in mono and mixed culture under the four gaseous regimes.

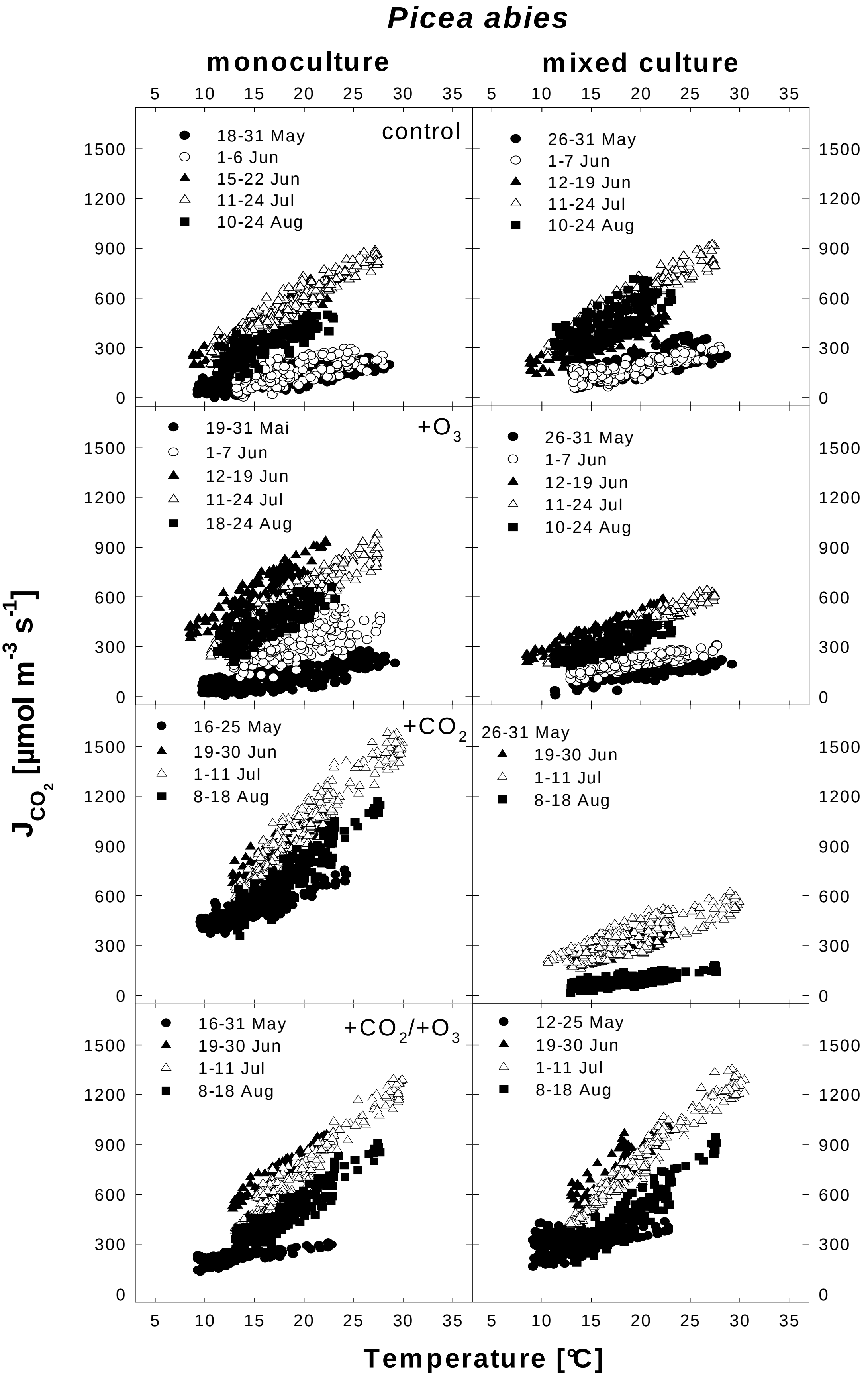


Figure 3.3.3. Relationships between stem respiration rate and air temperature, during the growing season of 2000 in individuals of spruce growing in mono and mixed culture under the four gaseous regimes.

Table 3.3.1. Seasonal respiration per unit of stem volume and per plant (non-green biomass aboveground; two individuals of beech and spruce in each treatment; May 15 through August 31, 2000).

Treatments	Individuum	Beech stem [mol C m^{-3}]	Spruce stem [mol C m^{-3}]	Beech whole-plant [g C $plant^{-1}$]	Spruce whole-plant [g C $plant^{-1}$.]
control					
monoculture	(1)	5294.94	3442.52	2.78	1.53
	(2)	5608.38	3634.97	1.02	2.84
mixed culture	(1)	1095.56	3798.91	0.57	1.71
	(2)	599.36	2072.32	0.06	1.04
+ O_3					
monoculture	(1)	2534.76	4209.17	1.19	2.16
	(2)	3457.89	2646.03	2.37	0.79
mixed culture	(1)	296.43	2860.21	0.06	2.05
	(2)	406.87	5341.55	0.04	1.98
+ CO_2					
monoculture	(1)	2896.21	4320.49	1.24	1.54
	(2)	4018.24	8181.55	1.37	4.87
mixed culture	(1)	9121.95	2099.47	3.10	1.62
	(2)	1808.26	4243.92	0.21	2.73
+CO_2/+O_3					
monoculture	(1)	4859.22	3983.93	2.16	3.05
	(2)	1898.60	5526.82	0.77	5.37
mixed culture	(1)	4102.74	5541.17	0.64	3.15
	(2)	764.80	3067.51	0.10	1.63

Seasonal rates of stem respiration per unit of stem volume and seasonal amounts of C respired per aboveground non-green organs were higher in beech in mono rather than in mixed culture under ambient CO_2. Under elevated CO_2, no clear trend was found. The highest C loss was measured in the mixed culture at +CO_2, and the lowest in the mixed culture at +O_3. Spruce did not show any differences between plantation types, and the rates did not vary to the extent found in beech: Differences in respired C per unit of stem volume between individuals under same treatments varied by a factor of 1.1 to 5.4 in beech, and 1.1 to 2.0 in spruce. When viewing the total C release by all non-green aboveground organs during the growing season, maximal differences between individuals under same gaseous regimes amounted to a factor of 14.8 (mixed culture at +CO_2) in beech, and 3.2 (monoculture at +CO_2) in spruce.

3.3.3 Seasonal C balance in 2000

Beech allocated the highest portion of the C gain to belowground (up to 56 %, except for the monoculture at $+CO_2/+O_3$), followed by the investment into new aboveground biomass increment. C released due to aboveground respiration consumed 3 to 13 % only of the C gain (Fig. 3.3.4). Plantation type changed the pattern of allocation. Beech plants in mixed cultures showed lower aboveground respiration and lower investment into aboveground biomass increment, and thus, higher a allocation to belowground than did plants in monocultures. In both plantation types, elevated CO_2 increased the percentage of assimilated C that was released by respiration and invested into new aboveground biomass. Enhanced ozone also increased, in proportion, the allocation to the aboveground organs.

Spruce showed a pattern of allocation that differed from beech. Except for $+O_3$, spruce plants allocated more C to the aboveground biomass increment (up to 43 %) rather than belowground. Respiration accounted for 7 to 22 % of the C gain. Similar to beech, elevated CO_2 tended to enhance C allocation aboveground (respiration and new biomass) in the monocultures, but only under $2xO_3$ ($+CO_2/+O_3$) in the mixed plantations. In both plantation types, the enhanced ozone regime had contrasting effects on the percentage of C gain invested into new aboveground biomass of plants growing under the two CO_2 regimes: Enhanced ozone diminished allocation to aboveground biomass increment under ambient CO_2, but resulted in increase under elevated CO_2.

In beech, higher net C gain was found under ambient as compared with elevated CO_2, with the exception of the plants in mixed culture under $2xO_3$ (see circle radius). In mixed culture, beech at $+O_3$ showed the lowest and spruce the highest seasonal C assimilation.

The amount of carbon allocated to belowground (i.e. the sum of biomass increment, respiration and other releases like e.g. exudates) highly correlated with the seasonal net C gain (see r^2 values in Fig. 3.3.5). In general, beech invested higher amounts into belowground per unit of C gain than did spruce. In addition, beech and spruce did not respond to an increase in C gain in a similar way: Doubling of the net C gain within the range of the largest difference between the two regression lines increased the C allocation to belowground by about a factor of 2.4 in beech, and about a factor of 3.8 in spruce.

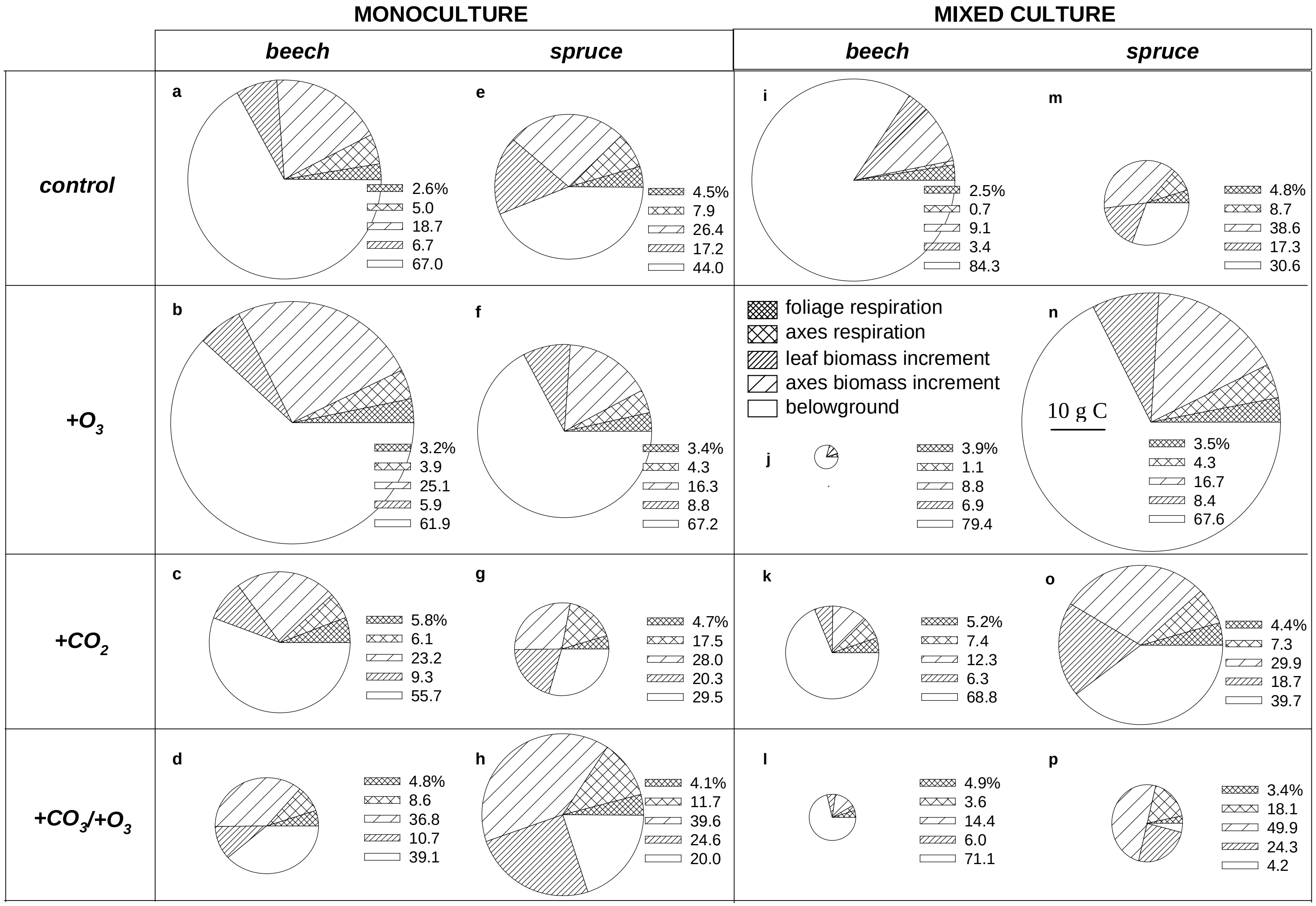


Figure 3.3.4. Seasonal C balance: Seasonal C released by respiration of foliage (narrow cross hatching), and non-green above-ground structures (coarse cross hatching), C invested in foliage and axes biomass increment (narrow and coarse diagonal hatching, respectively), and C partitioned to belowground (white area) given as percentage of the total seasonal net C gain. Circle size (radius) reflects the seasonal amount of C gain (bar represents 10 g C). Plants growing under the gaseous control regime are given in a, e, i and m; under $+O_3$ in b, f, j and n, $+CO_2$ in c, f, k and o, and $+CO_2/+O_3$ in d, h, l and p. Pie charts represent means of two plants each.

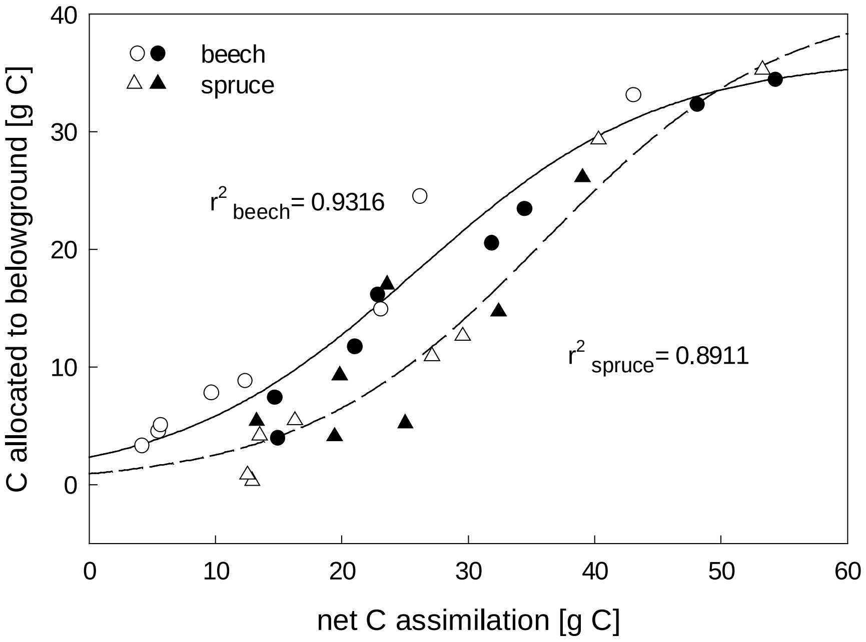


Figure 3.3.5. Relationship between the seasonal net C gain and the amount of C allocated to belowground (time period May 15 through August 31, 2000). The solid and dashed lines represent regressions for beech (circles) and spruce (triangles), respectively. Solid symbols represent monocultures, and open symbols give mixed cultures.

3.4 PARAMETERS OF COMPETITIVENESS

3.4.1 Efficiency ratio of aboveground space sequestration

During the first growing season in the phytotrons (1999), efficiencies in aboveground space sequestration of beech were lower in the mixed than in the monocultures under the ambient CO_2 regime, however, differences were not significant (Fig. 3.4.1; expressed as differences relative to the gaseous control regime). The lowest efficiencies were observed in both plantation types of beech under elevated CO_2. In contrast, spruce increased its space sequestration efficiency in the mixed as compared to the monocultures under all gaseous treatments (significant differences only under ambient CO_2). During the second growing season in the phytotrons of 2000, spruce maintained its higher performance in the mixed as compared to the monocultures (except for +O_3), whereas the opposite trend was found in beech. Beech drastically reduced its efficiencies relative to the control in 2000 and enlarged the differences between plantation types, with the mixed plantations showing efficiencies that were lower by 40 (control), 61 (+O_3), 22 (+CO_2) and 14 % (+CO_2/+O_3) relative to the monocultures. The absolute efficiency levels of the gaseous control regime declined towards the second growing season of 2000 (see legend of Fig. 3.4.1), in beech by about 46.9 %, and in spruce by about 17.7 % as compared with 1999.

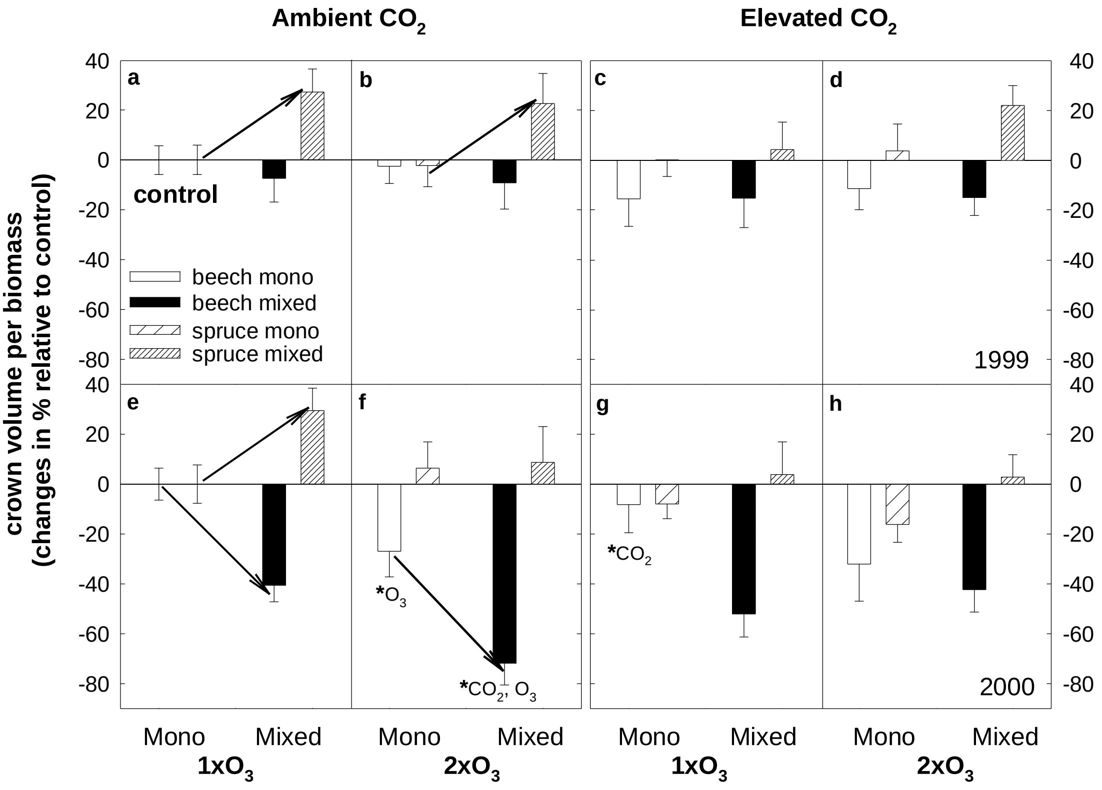


Figure 3.4.1. Efficiency of aboveground space sequestration (crown volume per unit of standing biomass, i.e. dry mass) of beech and spruce in 1999 and 2000, growing in mono and mixed cultures under (a, e) control, (b, f) +O_3, (c, g) +CO_2 and (d, h) +CO_2/+O_3. Open and solid bars are beech, and bars with wide and narrow hatching represent spruce in mono and mixed culture, respectively (means ± standard error, n=5 to 12). Data are given as percentages relative to the monocultures under the gaseous control regime). The absolute efficiency levels in beech and spruce in monoculture under the control regime were 769.4 ± 44.1 and 425.4 ± 24.3 cm^3 g DW^{-1} in 1999, respectively, and 410.2 ± 25.2 and 350.1 ± 16.8 in 2000, respectively. Arrows indicate significant differences between plantation types, and * between CO_2 or O_3 regimes at $p<0.05$.

3.4.2 Efficiency ratio of belowground space sequestration

At the end of the winter period (March of 1999 and 2000), no significant differences in the efficiency ratio of belowground space sequestration (i.e. SRL) were found between the plantation types of beech under all gaseous regimes (Fig. 3.4.2). Significant differences were present only in October 1999 and August 2000 under +O_3, where higher efficiencies were found in the mixed as compared to the monocultures. Except for March 2000**,** beech consistently showed significantly higher efficiencies in belowground space sequestration relative to spruce in the mixed culture under all gaseous regimes.

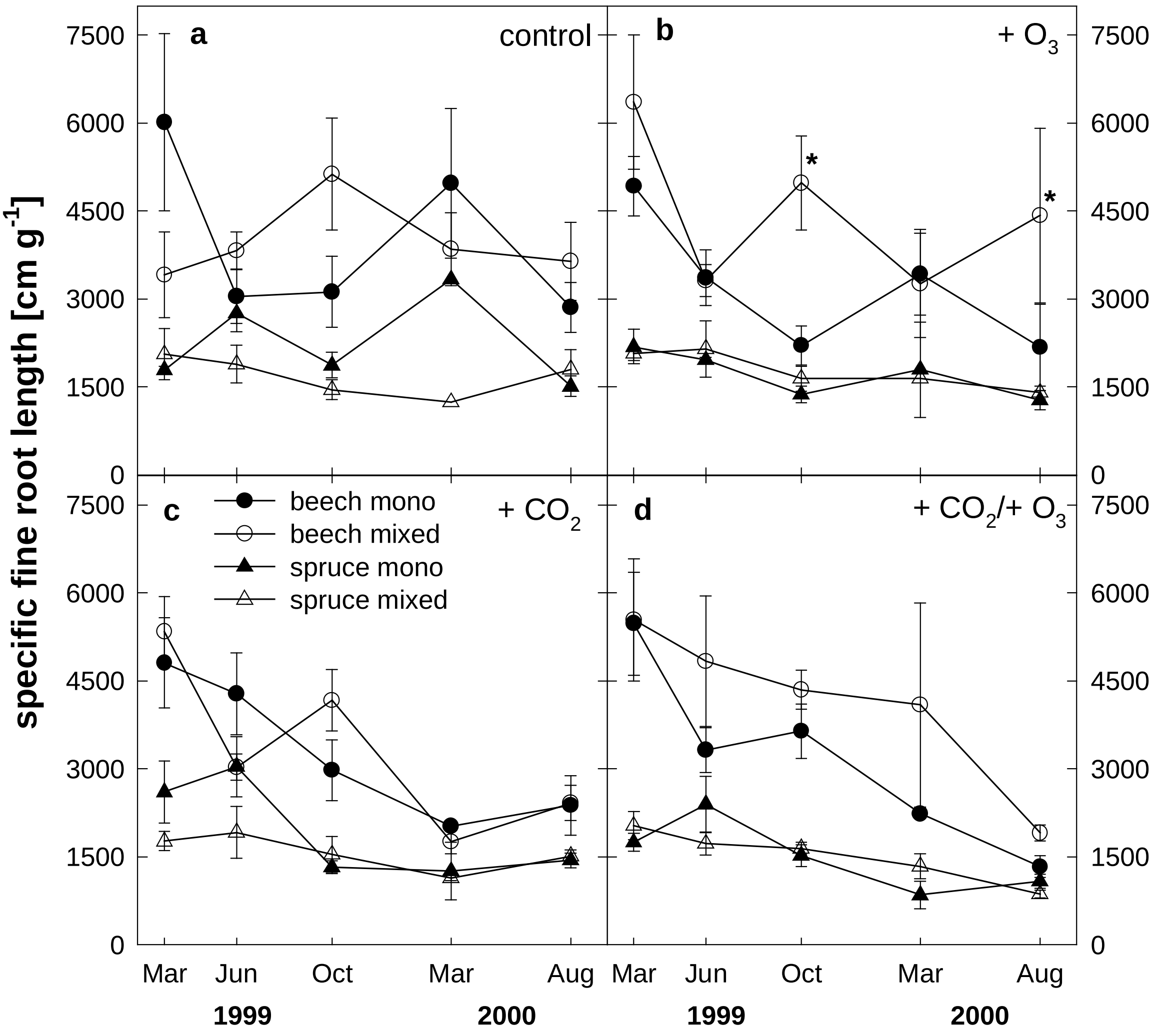


Figure 3.4.2. Specific fine-root length (length per unit of dry mass) of beech (circles) and spruce (triangles) under (a) control, (b) +O_3, (c) +CO_2 and (d) +CO_2/+O_3. Monocultures are given as closed, and mixed cultures as open symbols (means ± standard error, n= 4). * indicates significant differences between mono and mixed plantations at $p<0.05$.

Under elevated CO_2 (+CO_2 and +CO_2/+O_3), beech tended to reduce its efficiency throughout the growing seasons in the phytotrons**.** Irrespective of the gaseous regimes, spruce displayed similar SRL in both plantation types throughout 1999 and 2000, and showed lower variability (see error bars) and morphological fine root plasticity as compared with beech.

3.4.3 Efficiency ratio of aboveground resource gain

In 1999, beech plants showed a slightly higher (but not significant) C gain per unit of crown volume in the mixed as compared with the monocultures in all gaseous regimes (Fig. 3.4.3). The highest efficiencies were found under +CO_2.

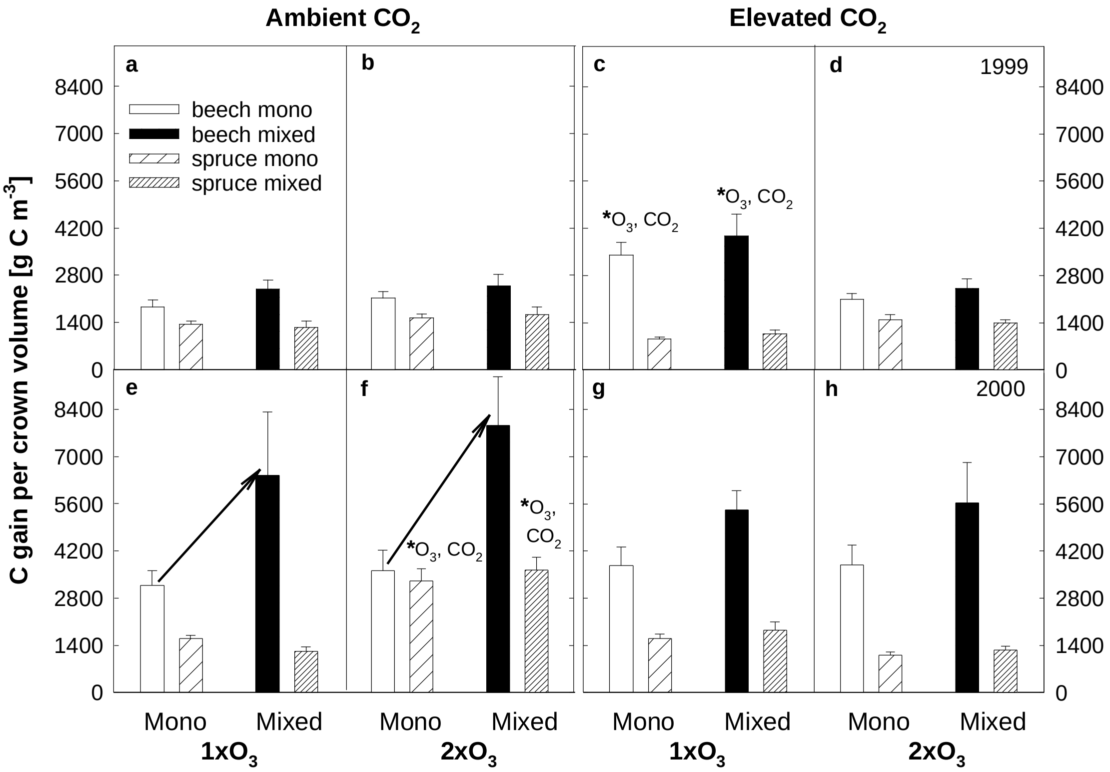


Figure 3.4.3. Efficiency in aboveground resource sequestration, calculated as seasonal (May 15 through August 31, 1999 and 2000) C gain per unit of crown volume, in beech and spruce as growing in mono or mixed culture under (a, e) the gaseous control regime, (b, f) +O_3, (c, g) +CO_2 and (d, h) +CO_2/+O_3. Open and solid bars represent beech, and bars with wide and narrow hatching give spruce in mono and mixed cultures, respectively (means ± standard error, n= 5 to 12). Arrows indicate significant differences between plantation types, and * between CO_2 (*CO_2) or ozone (*O_3) regimes at *p*<0.05.

In spruce, no differences were found between plantation types and gaseous regimes. However, during the second growing season in the phytotrons (2000), enhanced ozone increased the efficiency of spruce under the ambient CO_2 regime. In 2000, beech showed significant differences between plantation types under ambient CO_2: Mixed cultures were by about 103.3, 119.6, 44.0 and 48.1 % more efficient under the gaseous control regime, $+O_3$, $+CO_2$ and $+CO_2/+O_3$, respectively, than the monocultures. As compared with 1999, beech increased its C gain efficiencies during the succeeding growing season. Spruce showed similar C gain per unit of crown volume in both years, except for $+O_{3.}$ Here, spruce increased its C gain efficiency in the second growing season of 2000 by about 120 % relative to 1999.

3.4.4 Whole-plant nutrition as related to root biomass

For methodological reasons it was not feasible – at the current status of this ongoing SFB study – to relate the process of nutrient uptake to a discrete soil volume that surrounds the root system. Hence, the belowground efficiency in resource sequestration could not be expressed in a way conceptually consistent with the aboveground efficiency in resource sequestration. As a substitute for the time being, whole-plant nutrition (i.e. the amount of nutrients eventually incorporated into whole-plant biomass by the time of harvest) was expressed rather on the basis of the root biomass, as the latter – as a whole – represents an investment into belowground space and, at least in part, is involved in nutrient uptake. By this approach, the nutritional status of the whole plant is regarded as an outcome of resource investment into the root system.

Table 3.4.1 shows this kind of nutritional status, calculated as the whole-plant content of N, Mg, P and Fe as based on the root mass. Beech showed, under all gaseous regimes and for all elements, lower root mass-based nutrition in the mixed as compared with the monocultures. However, significant differences between plantation types were found only under $+O_3$ for N and P. At $+O_3$, the monoculture of beech significantly enhanced the incorporation of N and P through the root as compared with the other mono and mixed cultures and gaseous regimes. In contrast, spruce displayed a higher root mass-based incorporation under $+O_3$ in the mixed plantations, although differences were not significant. Overall, no significant differences between plantation types were observed in spruce. Elevated CO_2 reduced the root-related incorporation of N in both species.

Table 3.4.1. Whole-plant content of nitrogen, magnesium, iron and phosphorous as related to root biomass (mg g DM^{-1}) of beech and spruce at the end of August 2000 in all treatments (means ± standard error, n= 3 to 12).

	beech				spruce			
Treatments	N	Mg	Fe	P	N	Mg	Fe	P
Control								
monoculture	16,2 ± 2,3	2,7 ± 0,3	1,0 ± 0,4	1,9 ± 0,2	17,1 ± 1,2	2,6 ± 0,2	2,8 ± 0,7	2,6 ± 0,2
mixed culture	15,1 ± 0,9	2,3 ± 0,3	0,6 ± 0,2	1,8 ± 0,4	19,5 ± 3,8	2,9 ± 0,7	1,4 ± 0,5	3,0 ± 0,8
+ O_3	*			*				
monoculture	28,2 ± 2,5	3,0 ± 0,1	0,6 ± 0,1	3,0 ± 0,4	17,1 ± 0,8	2,4 ± 0,1	1,2 ± 0,2	2,4 ± 0,2
mixed culture	14,0 ± 0,4	2,3 ± 0,1	0,4 ± 0,1	1,5 ± 0,1	23,8 ± 3,1	2,4 ± 0,4	0,9 ± 0,2	3,2 ± 0,2
+ CO_2								
monoculture	11,7 ± 0,4	2,2 ± 0,2	0,7 ± 0,1	1,5 ± 0,1	14,2 ± 1,0	2,3 ± 0,1	1,6 ± 0,7	2,3 ± 0,2
mixed culture	11,1 ± 1,2	1,9 ± 0,4	0,7 ± 0,2	1,3 ± 0,1	13,4 ± 1,1	2,1 ± 0,2	1,3 ± 0,3	2,3 ± 0,1
+CO_2/+O_3								
monoculture	12,6 ± 0,1	3,3 ± 0,4	1,6 ± 0,3	1,7 ± 0,1	16,5 ± 2,0	2,8 ± 0,3	2,5 ± 0,4	2,6 ± 0,3
mixed culture	10,5 ± 1,2	2,5 ± 0,2	1,1 ± 0,2	1,6 ± 0,1	14,0 ± 0,6	2,2 ± 0,1	2,2 ± 0,3	2,4 ± 0,3

* differences between plantation types being statistically significant at $p < 0.05$.

3.4.5 Efficiency ratio of running costs

A) Transpiration

During the two years in the phytotrons, both species did not display any significant differences in the efficiency of "running costs", here given in terms of transpiration for sustaining the occupied crown volume, between the plantation types (Fig. 3.4.4). In spruce, elevated CO_2 enhanced the crown volume per molar unit of transpired water irrespective of the ozone regime in 1999 and 2000 in both plantation types, except for the mixed culture under +CO_2. In 2000, enhanced O_3 lowered the efficiency of spruce in mono and in mixed plantations under ambient CO_2, but enhanced the efficiency under elevated CO_2**.** Efficiencies of beech were not influenced by the gaseous regimes. In comparison with 1999, both species reduced their efficiencies in 2000, except for spruce under +CO_2/+O_3.

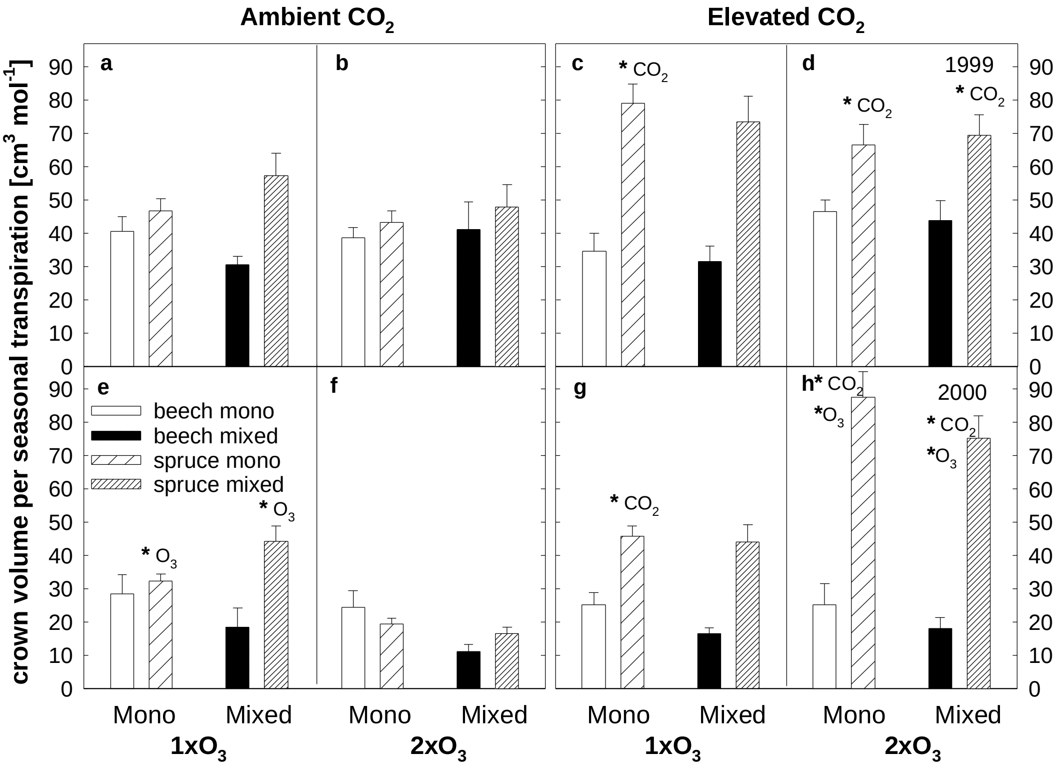


Figure 3.4.4. Efficiency of "running costs" with respect to transpiration, calculated as crown volume per seasonal transpiration (May 15 through August 31, 1999 and 2000) of beech and spruce growing in mono or mixed cultures under (a, e) control, (b, f) +O_3, (c, g) +CO_2 and (d, h) +CO_2/+O_3. Open and solid bars represent beech, and bars with wide and narrow hatching represent spruce in mono and mixed cultures, respectively (means ± standard error, n= 5 to 12). Arrows indicate significant differences between plantation types, and * between CO_2 (*CO_2) or ozone (*O_3) regimes at p<0.05.

B) Respiration of leaves

In 1999, efficiencies in “running costs” by leaf respiration were, in general, higher in spruce in the mixed rather than monocultures, however, differences between plantation types were not significant (Fig. 3.4.5). Under elevated CO_2, spruce showed lower efficiencies (i.e. lower crown volume per unit of respired C) than under the ambient CO_2 regime, except for the monoculture at $+CO_2$. The gaseous regimes did not affect the efficiencies of beech. In the second growing season in the phytotrons (2000), beech presented a higher efficiency in the mono as compared to the mixed cultures, although a significant difference between plantation types was only found in the gaseous control regime. Here, spruce displayed a higher efficiency in the mixed than in the monoculture.

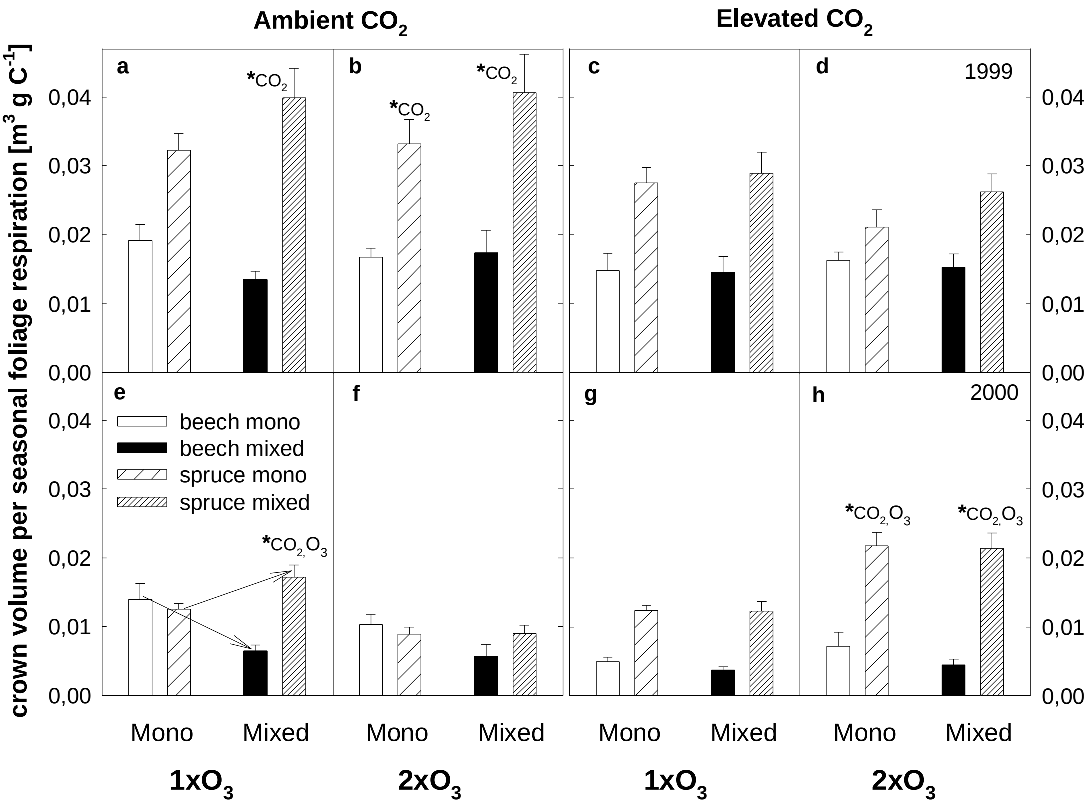


Figure 3.4.5. Efficiency of “running costs”, calculated as crown volume per seasonal C release by foliage respiration at night during 1999 and 2000 in beech and spruce as growing in mono or mixed cultures under (a, e) control, (b, f) $+O_3$, (c, g) $+CO_2$ and (d, h) $+CO_2/+O_3$. Open and solid bars represent beech, and bars with wide and narrow hatching give spruce in mono and mixed cultures, respectively (means ± standard error, n= 5 to 12). Arrows indicate significant differences between plantation types, and * between CO_2 (*CO_2) or ozone (*O_3) regimes at $p<0.05$.

In 2000, elevated CO_2 increased the efficiency of spruce only at $+CO_2/+O_3$. In comparison with spruce plants under ambient O_3 regime, plants under enhanced ozone displayed lower efficiencies in the mixed culture under ambient CO_2, and in contrast, higher efficiency in both plantation types under elevated CO_2. The highest efficiencies of spruce were found under $+CO_2/+O_3$. In comparison with 1999, both beech and spruce reduced its efficiencies in 2000.

3.5 ALLOMETRIC ANALYSIS

3.5.1 Root/shoot biomass ratio

The plantation type influenced plant size (Fig. 3.5.1): In general, beech individuals were smaller in mixed than in monoculture. However, biomass partitioning between root and shoot was not the result of re-adjustments in resource allocation: Both slopes and intercepts of the two linear regressions were not significantly different from each other, indicating that biomass partitioning was size-dependent.

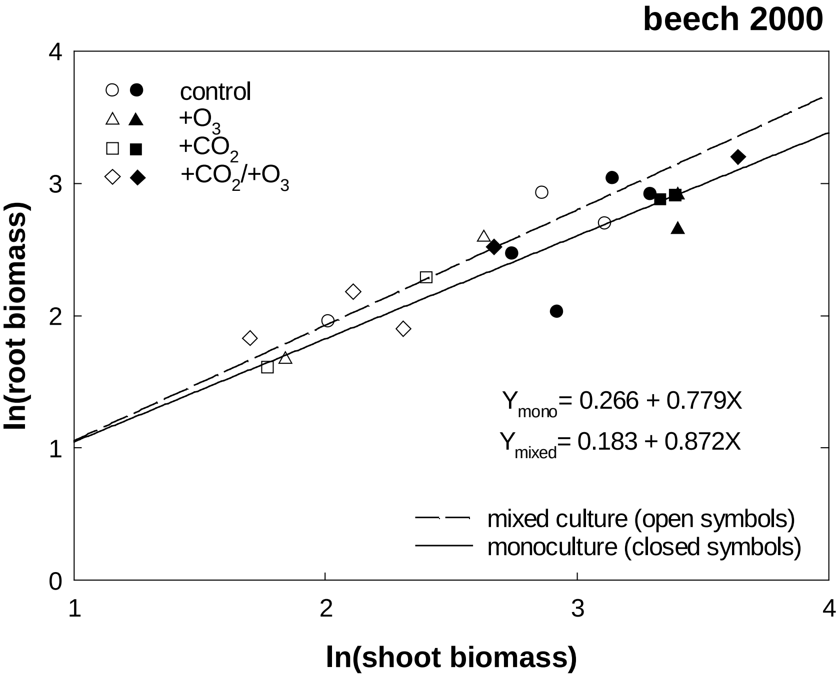


Figure 3.5.1. Relationship between log-transformed root and shoot biomass of beech saplings in mono (closed symbols) and in mixed plantations (open symbols) under the four gaseous regimes. Slopes and intercepts of the two regressions did not significantly differ.

3.5.2 C gain as related to shoot axes biomass

A high correlation existed between the seasonal C gain and shoot biomass in 1999 and 2000. Plantation types as well as gaseous regimes did not change the seasonal C gain of beech plants per unit of shoot biomass (Fig. 3.5.2).

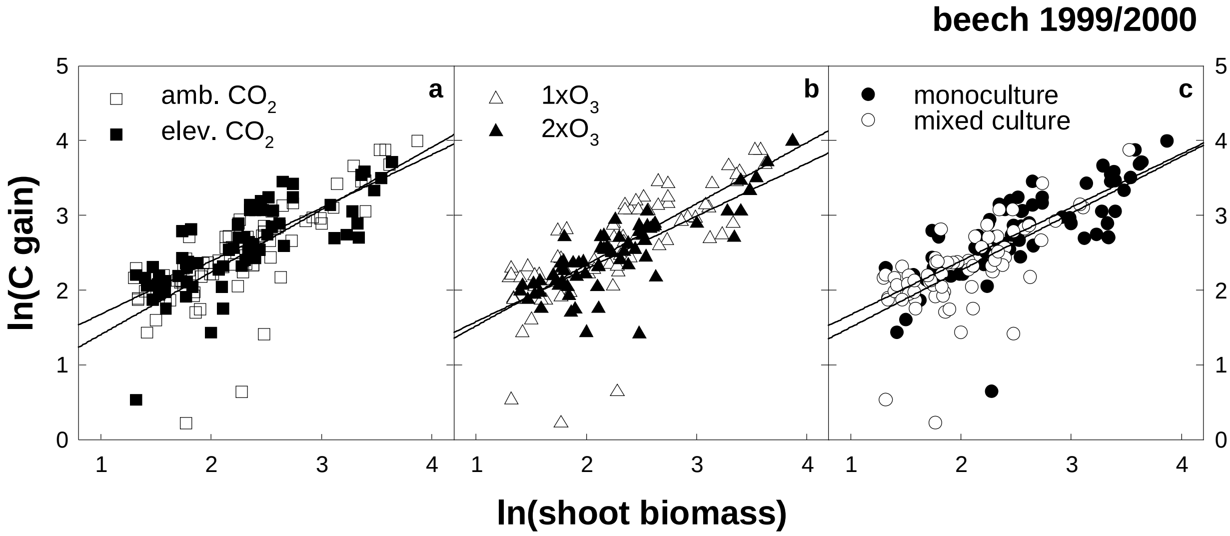
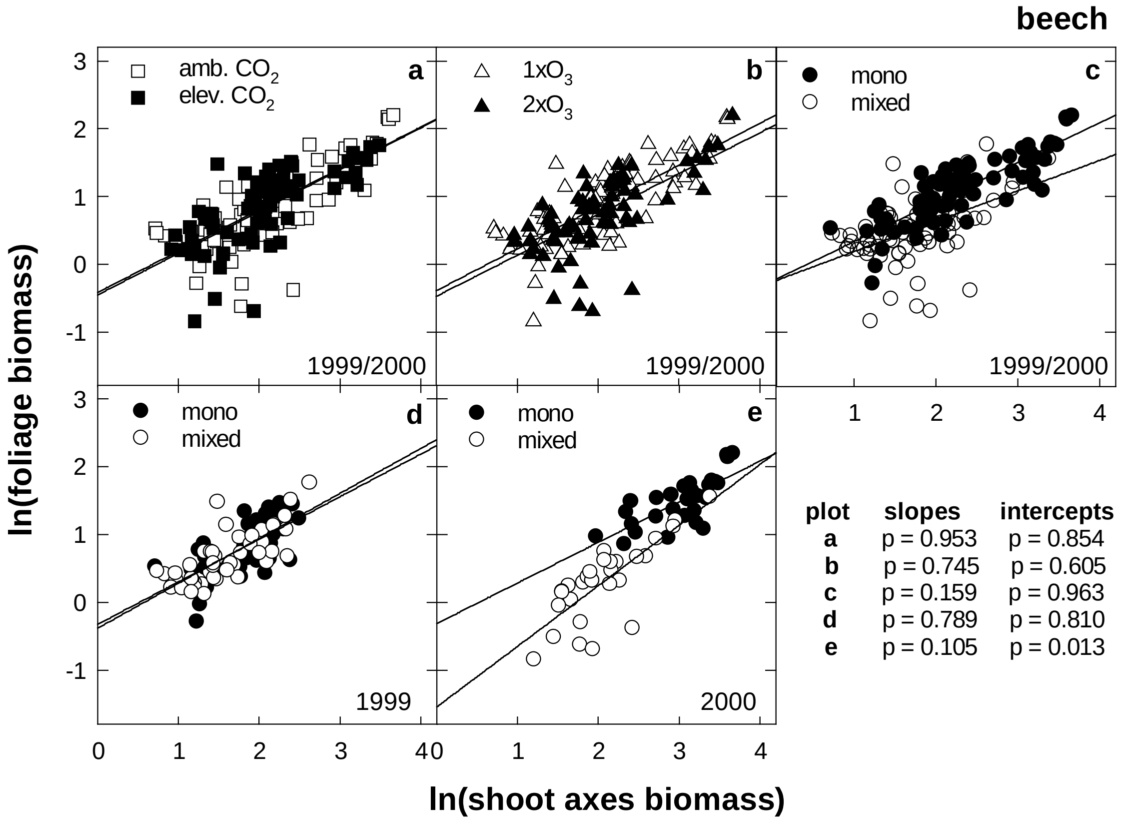


Figure 3.5.2. Relationship between log-transformed shoot biomass and seasonal C gain of beech saplings in 1999 and 2000. Linear regressions were fitted to data split into treatments by CO_2 (a), ozone (b) and plantation type (c). Slopes and intercepts of the two regressions in a, b and c did not significantly differ.

3.5.3 Biomass ratio of foliage *versus* shoot axes

The partitioning of biomass between foliage and shoot axes, which was highly related to crown volume, was not influenced by the gaseous regimes (Fig. 3.5.3a,b). As there were no differences between slopes and intercepts in both years, the yearly datasets were pooled. As well, no differences were found between mono and mixed culture (c). A separate analysis of the data from 1999 and 2000 (d,e) each showed, however, that differences in allocation occurred in 2000, and were size-independent (significant difference between intercepts): In mixed plantation, beech plants were smaller and allocated less substrate to leaves at a given shoot axes biomass than did plants in monoculture. This effect was found, in particular, under the $+O_3$ regime.



plot	slopes	intercepts
a	$p = 0.953$	$p = 0.854$
b	$p = 0.745$	$p = 0.605$
c	$p = 0.159$	$p = 0.963$
d	$p = 0.789$	$p = 0.810$
e	$p = 0.105$	$p = 0.013$

Figure 3.5.3. Relationship between log-transformed shoot axes and foliage biomass of beech saplings; data sets of 1999 and 2000 pooled (a, b, and c), or separate analysis in 1999 (d) and 2000 (e) each. Linear regressions were fitted to data split into treatments by CO_2 (a), ozone (b) and plantation type (c, d and e). Slopes of the two regressions in graphs a-e did not differ. Significant difference between intercepts was found in plantation types only in 2000 (e).

4 DISCUSSION

In the following section (4.1), the influence of CO_2 and O_3 on growth parameters (above- and belowground biomass and crown volume) of beech and spruce saplings will be discussed as based on the background information of the more recent literature. Attention is given to the different responses of plants to the gaseous regimes (including the question of whether CO_2 compensates for O_3 effects) when growing under intra or interspecific competition. Sections 4.2 and 4.3 address differences between plantation types in leaf gas exchange and whole plant C balance, respectively. Section 4.4 focuses plant competitiveness in terms of the costs and benefits of resource allocation (efficiency ratios). The size dependence of changes in biomass partitioning and in C gain per unit of shoot biomass between plantation types and gaseous regimes is analysed in section 4.5. The results discussed in the preceding sections are integrated into a synopsis (conclusions) of the present study and summarised through a conceptual model (see schematic diagram in section 4.6).

4.1 Comparison of plant responses to competition and gaseous regimes in mixed and monoculture

4.1.1 Beech

Within the scope of the experiment, the results overall indicate a competitive disadvantage of young beech when growing in mixture with spruce. After three growing seasons (including the first year of pre-acclimation to the CO_2 regimes), beech plants in mixed plantation showed, with few exceptions, significant reduction in above and belowground biomass, seasonal biomass increments and crown volume as compared with beech plants in monoculture (Table 4.1).

The negative effect of interspecific competition on beech was observed first in 1999 (second growing season under competition and different CO_2 regimes, and first season under ozone regimes), in particular under elevated CO_2 (Table 4.1). The differences in the growth parameters of beech between plantation types were a consequence of the growth stimulation in monoculture under elevated CO_2, whereas plants in mixed plantations did not respond to the high CO_2 supply.

Table 4.1. Changes in the investigated parameters observed in the mixed as compared to the monoculture of beech and spruce under the four gaseous regimes in 1999 and 2000. Arrows indicate increase (↑) or decrease (↓) of each parameter in response to interspecific competition. Significance levels: ° $p \leq 0.1$; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. n.m. denotes that the parameter was not measured.

		beech				spruce			
Year	Parameter	control	$+O_3$	$+CO_2$	$+CO_2/+O_3$	control	$+O_3$	$+CO_2$	$+CO_2/+O_3$
1999	Shoot axes biomass			↓*	↓***		↑	↑	↓
	Seasonal biomass increment	↓	↓	↓**	↓	↑°	↑	↑	↑**
	Foliage area	↑	↓	↓°	↓***	↑	↑	↑	↓
	Crown volume		↓	↓	↓***	↑	↑*	↑	
	Number of leaves	↑	↓	↓	↓***	n.m.	n.m.	n.m.	n.m.
	Leaf area		↓	↓	↓	n.m.	n.m.	n.m.	n.m.
	Leaves/shoot axes	↑	↓	↑		n.m.	n.m.	n.m.	n.m.
	Current axes/old shoot axes		↓	↓	↓	n.m.	n.m.	n.m.	n.m.
2000	Shoot axes biomass	↓*	↓*	↓**	↓***	↑	↑	↑**	
	Seasonal biomass increment	↓***	↓**	↓***	↓**	↑*	↑**	↑***	↑
	Foliage area	↓**	↓*	↓**	↓***	↑	↑*	↑**	↑
	Crown volume	↓***	↓*	↓***	↓**	↑°	↑	↑**	
	Number of leaves	↓	↓°	↓*	↓**	n.m.	n.m.	n.m.	n.m.
	Leaf area	↓**	↓***	↓***	↓**	n.m.	n.m.	n.m.	n.m.
	Leaves/shoot axes	↓**	↓°	↓		n.m.	n.m.	n.m.	n.m.
	Current axes/old shoot axes	↓***	↓*	↓	↑	n.m.	n.m.	n.m.	n.m.
	Fine root biomass	↓	↓*	↓°	↓**	↓	↑	↑	↑
	Coarse root biomass		↓	↓°	↓		↑	↑	↑
	Total root biomass	↓	↓	↓*	↓°		↑	↑	↑°
	Coarse/fine	↑	↑	↑	↑	↑	↑	↓	↑
	Root/shoot	↑	↑*	↑**	↑			↑*	↑
	Axes length/stem height	n.m.	n.m.	n.m.	n.m.	↑	↑*	↑°	

A positive CO_2 effect on the biomass of beech was found in several studies (Dyckmans & Flessa, 2002, Hättenschwiler & Körner, 2000; Heath & Kerstiens, 1997, Overdieck & Forstreuter, 1995). The increase in the number of leaves per beech individuum in monocultures by about 62% and foliage area by 66% under elevated CO_2 is in accordance with findings of Epron *et al*. (1996). In their work, foliage area increased under enhanced CO_2 by about 53%, mainly due to an increase in the number of leaves per plant. In the present study, plants under elevated CO_2 produced 1.3 (sun), 1.7 (shade) and 4.5 (second-flush) times more leaves as compared with plants exposed to the ambient CO_2 regime. On the other hand, the observed increase in shoot dry mass by 90% reported by Epron *et al*. (1996) was higher than found here (49%) so that the present finding is in accordance with the mean value (49%) observed for several deciduous species (Saxe *et al*, 1998).

During 2000, significant reductions in almost all investigated aboveground and some of the belowground parameters of beech were found in mixed relative to monoculture under all gaseous regimes. Although plants were hardly affected by ozone during 1999, the largest differences in aboveground growth parameters between plantation types were found under $+O_3$ one year later. Similarly, Bortier *et al*. (2000, 2001) did not find changes in relative growth and shoot biomass in beech seedlings during the first growing season under ozone fumigation. In a study on beech seedlings from 12 provenances, ozone did not influence shoot biomass even after two seasons of exposure (Paludan-Müller *et al*., 1999). However, as pointed out by Andersen (2003), ozone may distinctly affect belowground processes before symptoms become visible aboveground. Under enhanced O_3, beech plants in monoculture tended to increase shoot axes biomass and number of leaves as compared to those under the ambient O_3 regime. In contrast, plants in mixed culture showed significant reductions in all investigated growth parameters. These results suggest a higher susceptibility to ozone impact of beech plants in mixed culture, and confirm the hypothesis 3 which predicted that responses of plants to the gaseous regimes depend on the type of competition.

An increase in the number of leaves or foliage area in response to ozone stress has been observed in beech and other broadleaf species (Kolb & Matyssek, 2001). As O_3 inhibits assimilate translocation out of the leaves (Rennenberg *et al*. 1996; Gunthardt-Goerg *et al*., 1993; Matyssek *et al*., 1992), growth of new leaves during the growing season may be a strategy to enhance resistance to ozone by creating new C sinks (Matyssek & Sandermann, 2003). Such sinks may be fed by resource retranslocation and/or mobilization of reserve storage. However, the increase of foliage area depends on the nutrient availability (Kolb and Matyssek, 2001; Polle *et al.*, 2000). In the present study, plants under +O_3 produced, in 1999, 85% and 92% more second-flush leaves in the mono and mixed cultures, respectively, than did plants under the ambient O_3 regime. However, during the growing season of 2000, leaf area diminished in both plantation types, in particular in the mixed culture. In 2000, plants in mixed culture under +O_3 and +CO_2 did not produce a second flush. Reduction in leaf area has been associated with limiting nitrogen availability, in particular to NO_3^- in the soil (Forde, 2002), or to ozone impact on leaf differentiation (Matyssek *et al.*, 1995).

Under elevated CO_2 (+CO_2 and +CO_2/+O_3), the differences between plantation types observed in 1999 increased throughout 2000. Contrasting with 1999, elevated CO_2 did not stimulate beech aboveground growth in monoculture during 2000. Compared with beech plants under ambient CO_2, elevated CO_2, led to reduced growth both in mono and in mixed culture, however reductions were larger in the latter plantation type. On the other hand, belowground biomass tended to be enhanced by both elevated CO_2 and O_3 regimes in monoculture, and diminished in mixed cultures. These results underline that responses of beech to CO_2 and O_3 were influenced by plantation types, which confirmed hypothesis 3 (Grams *et al.*, 2002; Fuhrer *et al.*, 2003; Navas *et al.*, 1999). Moreover, as found in other studies on CO_2 and O_3 (Isebrands *et al.*, 2001; Paludan-Müller *et al.*, 1999; Egli *et al.*, 1998), the present results show that it can take more than one growing season before the responses of plants to gaseous pollutants become significant.

4.1.2 Spruce

Contrasting with beech, spruce tended to profit from the interspecific competition. However, responses of spruce to competition and gaseous regimes were much less distinct than those of beech (Table 4.1). In 1999, spruce showed significant higher seasonal aboveground biomass increment in mixed relative to monoculture only under +CO_2/+O_3. In 2000, growth was stimulated in the mixed plantation under all gaseous regimes, in particular under +CO_2 (Table 4.1). This positive response of spruce to the enhanced CO_2 is in accordance with the hypothesis 2. There was no significant effect of the "+O_3" regime on all investigated parameters, regardless of plantation type. This finding confirms hypothesis 1 which claims that beech rather than spruce is affected by the ozone regimes. Spruce, like other coniferous species, has been considered less sensitive to ozone than deciduous trees (Landolt *et al.*, 2000;

Skärby *et al.*, 1998), being aware, however, that the variation of genotypes within a species may strongly determine responsiveness (Vanderheyden *et al.*, 2001). Under elevated CO_2, the increase in the aboveground (shoot axes) biomass of spruce by 43% was much lower than the mean value of 130% found for conifers, as reported by Saxe *et al.* (1998). However, in that review, the majority of the studies presume optimal growth conditions. Hättenschwiler & Körner (1998) did not find significant effects of enhanced CO_2 on above- and belowground biomass in spruce that grew in natural nutrient-poor soil. Increasing nitrogen deposition significantly stimulated biomass production, confirming that low N levels limit plant responses to CO_2. Absent or moderate biomass increments in response to enhanced CO_2 have been observed in plants that grew under ecological meaningful conditions (limited nutrient supply and/or intra- and interspecific competition: Hättenschwiler *et al.*, 1997; Körner & Arnone, 1992; Reekie & Bazzaz, 1989).

4.1.3 Compensation of adverse O_3 effects through elevated CO_2 depends on plantation type (intra or interspecific competition)

As discussed above, plants in mono and in mixed plantations can respond very differently to CO_2 and O_3 regimes (confirming hypothesis 3), including the combination of elevated CO_2 and enhanced O_3. In recent years, it has been discussed as to whether elevation of CO_2 does counteract negative O_3 effects (Karnosky *et al.*, 2001, Wustman *et al.*, 2001, Isebrands *et al.*, 2001, King *et al.*, 2001, Dickson *et al.*, 2001, McKee *et al.*, 2000, Grams & Matyssek, 1999). Basically, high levels of CO_2 might mitigate negative effects of ozone in two ways: inducing reduction in stomatal conductance and thus diminishing ozone flux into the leaf (Allen, 1990), and increasing amounts of C skeletons for detoxification and repair (Carlson & Bazzaz, 1982). Matyssek & Sandermann (2003) and Karnosky *et al.* (2001) summarise that evidences about an interaction of O_3 and CO_2 in plant are inconsistent. In the present study, the investigated parameters, which were constrained under $+O_3$ relative to the control, were compared with the outcome under $+CO_2/+O_3$. Beech plants were able to profit from the elevation of CO_2 as adverse ozone impact on seasonal biomass increment and mean leaf area was counteracted in 2000, and growth responses (in number of leaves, foliage area, shoot axes biomass, crown volume in 1999) were even increased relative to the control plants in monoculture. In contrast, compensation was rather weak in mixed cultures (in foliage area, shoot biomass and crown volume in 2000), inexistent (in seasonal aboveground biomass increment and mean leaf area in 2000), or CO_2 even promoted the impact of ozone (in number of leaves and foliage area in 1999). Thus, the results indicated moderate amelioration by elevated CO_2 at least in the foliage area and crown volume of beech in mixed culture. Moderate counteraction of CO_2 against O_3 impact was also observed by Wustman *et al.* (2001). Volin *et al.* (1998) studying two C_3 trees (*Populus tremuloides* and *Quercus rubra*) and C_3 and C_4 grasses found that O_3-induced reduction in relative growth rate vanished in all species under the both elevated CO_2 and O_3 regime. Wustman *et al.* (2001) also found negative effects of O_3 on mean leaf size in aspen clones, but in contrast with the findings in beech monoculture of the present study, elevated CO_2 did not protect against ozone influence. Lack of amelioration in growth responses by elevated CO_2 has been found in Scots pine (Kellomäki & Wang, 1998) and in Norway spruce (Lippert *et al.*, 1997, Barnes *et al.*, 1995). In the present study, there was no effect of O_3 on spruce (see above).

4.1.4 Gas regime influenced competition, but plantation type modified responses to gaseous regimes

Research on interacting effects of CO_2 and O_3 in plants has shown contradictory results even within the same species. Varying responses of plants probably result from different experimental conditions, nutrient, water and light availability, presence or absence of competition, genetic variability and ontogenetic stage (Karnosky *et al.*, 2001). In the present study, the results overall indicate that interspecific competition impedes beech plants to respond to CO_2 as they would do in monoculture. Navas *et al.* (1999) came to similar conclusions when working on grasses and legumes. Also in the case of ozone, interspecific competition may modify effects relative to plant growth in monoculture or in isolation. Fuhrer *et al.* (2003) summarize results of studies on semi-natural vegetation, which show that the impact of ozone stress can be enhanced by interspecific competition. In a study on five perennial species (Bender *et al.*, 2003), the biomass of monocultures was not influenced by ozone regimes. When growing with the competitor species *Veronica chamaedrys* in the absence of ozone, the biomass of *Poa pratensis* was reduced relative to that achieved in monoculture. However, under conditions of AOT40 = 3.6 ppm.h (after five weeks), the competitive advantage of *V. chamaedrys* disappeared. In another study on *Trifolium pratense* and *P. pratense*, the decline in biomass of *T. pratense* due to ozone stress became more evident in the presence of *P. pratense*. In contrast, the biomass of the grass species was unaffected by ozone, both in mono or mixed culture (cf. Fuhrer *et al.*, 2003). The results from the literature, in accordance with the findings presented in this study, underline that CO_2 and

O_3 can influence the competitive ability of plants. And *vice-versa*, competition can modify responses of plants to gaseous regimes. It is important to be aware of such interactions when attempting to transfer experimental knowledge acquired from plants growing in isolation or in monoculture to natural plant communities in the field.

4.2 Beech versus spruce: Leaf gas exchange

Beech plants showed e.g. lower instantaneous C assimilation rates in mixed as compared with monoculture, being consistent with the responses in biomass discussed before. The following section addresses the question as to whether differences in leaf gas exchange observed between mono and mixed plantation, and under the four gaseous regimes, were associated with changes in light availability, leaf nitrogen content, stomatal conductance, shoot architecture and phenology.

In 1999, the fact that beech growth under elevated CO_2 (but not so under the ambient CO_2 regime) was reduced in mixed as compared to monoculture (Table 4.1) may be related to an exacerbation in light competition in the presence of spruce: Under enhanced CO_2, growth of spruce rather than beech would be stimulated, and by this, the C assimilation in beech would be limited by shading. Indeed, under $+CO_2$, spruce in the mixed culture showed significant higher shoot biomass (but not crown volume) as compared to that under the control regime. Moreover, under elevated CO_2, the sun leaves of beech in mixed culture showed slightly lower instantaneous net CO_2 assimilation rates as compared with those in monoculture (Fig. 3.2.4). However, under elevated CO_2, the mean irradiation measured in the shade crown tended to be higher in mixed than in monoculture (Table 3.2.1b), although light interception in the lower canopy was rather patchy due to the heterogeneous, “clumpy” distribution of foliage. Probably, an additional factor limited the aboveground growth of beech in response to elevated CO_2 in the mixed plantation.

Several studies reported that plant responses to elevated CO_2 might be constrained by mineral nutrient supply (Oren *et al.*, 2001, Zak *et al.*, 2000, Maurer *et al.*, 1999; Egli *et al.*, 1998, Diaz *et al.*, 1993). In a study on beech and spruce in mixed culture growing in two soil types, Egli *et al.* (1998) found growth stimulation in beech on the calcareous and limitation on the acidic soil in response to enhanced CO_2. Except for radial stem growth, which was stimulated by enhanced N deposition on acidic soil, increased N had no effect on beech aboveground biomass. In contrast, spruce profited from both enhanced CO_2 and N deposition, independent of soil type. The authors suggest that the aboveground growth of beech was predominantly limited by factors other than CO_2 and N on acidic soils. As they did not have beech and spruce monocultures as a reference, it is not clear if the general negative response of beech to enhanced CO_2 in acidic soil was a mere consequence of the soil type or of the interaction between soil type and interspecific competition. In the present study, spruce and beech plants were not only grown in similar conditions (natural acidic soil and mixed plantations), but also in monocultures each, and it was found that the aboveground biomass of beech plants was increased in monoculture in response to enhanced CO_2 (in 1999). In 2000, beech plants in monoculture showed higher biomass than those in mixed plantation under all gaseous regimes. Thus, acidic soil *per se* cannot explain the lowered growth performance of beech in mixed culture. Mixed and monocultures of beech are widely spread in central Europe, being found on almost all soil types, from rather acidic to calcareous ones. Beech is considered a strong competitor and is outcompeted only on wet or very dry soils, or at altitudes, where winters are too long and too cold (Ellenberg, 1996). In this study, competition with spruce was a more determining factor for beech growth than were the gaseous regimes. As suggested by Wang *el al*. (2001), the presence of spruce in the mixed culture may decrease the amount of nutrients available for beech. Analysing the rhizosphere of beech and spruce, they found more pronounced reductions in pH, K^+, Ca^{2+}, Mg^{2+} and NO_3^- concentrations caused by spruce rather than by beech. The authors suggest that in mixed plantations young spruce trees have a competitive advantage belowground over beech. Other studies in forests have shown that spruce changes the availability of ammonium and nitrate in its rhizosphere (Brierley *et al*., 2001; Dieffenbach & Matzner, 2000). Such modifications induced by spruce may have impact on the belowground processes of beech (Bauer, 1997; Bauer *et al*, 1997).

In 2000, beech growth in mixed plantation was reduced as compared with that in monoculture not only under elevated CO_2 (as in 1999), but under all gaseous regimes (Table 4.1). In both species, the seasonal aboveground biomass increment was, in general, lower in 2000 as compared with 1999 (Fig. 3.1.3). Throughout 2000, lower rates of net C assimilation in instantaneous measurements were found in sun leaves of beech in mixed as compared with monoculture under all gaseous regimes, especially under $+O_3$ and $+CO_2$. Similar to 1999, the lower assimilation rates were apparently not associated with light limitation in mixed culture. Nor were the assimilation rates related to the N concentration of the leaves. Under $+O_3$, beech leaves had the highest N levels, however, at the same time, the lowest C assimilation rates (perhaps, because of a reduced, O_3-induced C relative to N flux through the plant: cf. Matyssek & Sandermann 2003). Compared with plants in the control gaseous regime, the sun and shade leaves of beech under $+O_3$ showed significant decrease in ETR, but no difference was found between plantation types. Plants under enhanced O_3 also showed higher percentage of macroscopic foliage injury as compared with those under the ambient O_3 regime. However, visual injury was found in plants in mono rather than in mixed plantations (Fig. 3.2.3). The reduced C assimilation in mixed culture, and probably the lower development of visual injury, perhaps was related to lowered stomatal conductance (Fig.A1, Appendix). In beech saplings, partial stomatal closure under enhanced O_3 was also observed by Bortier *et al.* (2001) and Lippert *et al.* (1996b), but not so by Paludan-Müller *et al.* (1999).

Also responses of stomata to enhanced CO_2 may vary. In beech saplings exposed to elevated CO_2, stomatal conductance was slightly lowered during the second growing season (Grams *et al.*, 1999). However, Saxe *et al.* (1998) and Curtis & Wang (1998) report about lacking or only minor reductions in gs in long-term studies under enhanced CO_2. On the other hand, as pointed out by Eamus & Ceulemans (2001), endogenous and exogenous factors like reduction in sink strength, increased foliar carbohydrate accumulation, nutrient and water limitation may initiate photosynthetic down-regulation. In the present study, and contrasting with others (cf. Matyssek & Sandermann, 2003, Noormets *et al.*, 2001; Bortier *et al.*, 2001), stomatal closure in mixed culture was accompanied by reduced ci under $+CO_2$ and $+O_3$, at least during part of the year (Figure A1, Appendix). The possibility of lowered ci induced by elevated CO_2 and O_3 has been indicated by Farquhar *et al.* (1989), Matyssek *et al.* (1995), and Saxe *et al.* (1998). However, in the present study, reduction in ci under these gaseous regimes were only observed in mixed plantation. This would indicate that, under competition with spruce, beech plants may have experienced some restriction in water relations, although soil moisture was continuously controlled in order to prevent water limitation – and moderate soil drought is typically reflected in constant ci (Schulze & Hall 1982). $\delta^{13}C$ and $\delta^{18}O$ levels of beech plants, however, appear to indicate water limitation to some minor extent (T. E. E. Grams, personal communication).

The differences observed in seasonal C gain between plantation types (Fig. 3.3.1) were mainly due to amounts of foliage area (Fig. 3.1.4) and light availability in shade crowns (Table 3.2.1b). In addition to smaller foliage area, the reduced crown volume in mixed culture might have mitigated the capacity of light interception, leading to a decrease in total C fixation. With reduction of C fixation, less C is available for growth of new structures like shoot axes and roots, and therefore, the acquisition of above- and belowground resources becomes more difficult. In the present study, the largest difference in crown volume between plantation types was found under +O_3. This was a consequence of a lowered foliage area (by 59 %) and current-year axes biomass (by 57 %) in trees in the mixed culture under enhanced O_3 regime as compared to plants in the gaseous control treatment. Reduction in branching due to ozone was also observed in aspen (Dickson *et al*., 2001), birch (Maurer & Matyssek, 1997; Matyssek *et al*., 1992) and poplar (Matyssek *et al*., 1993). As enhanced O_3 normally decreases carbon assimilation (due to lower photosynthetic rates, lower foliage area, and premature senescence) and, in addition, impairs the assimilate translocation out of the leaves, less C is available for branch growth. Under enhanced O_3, current terminal stem lengths of aspen decreased by about 32%, and the branch weight/branch length ratio by about 54% and 59% in the two investigated aspen clones. Moreover, ozone also altered branch angles (Dickson *et al*., 2001), which also contributed to change in crown shape.

Another factor that might have influenced seasonal C gain of beech plants was the shoot phenology. In 2000, plants in monoculture initiated bud break and finished the development of new branches about three (under +O_3) and one or two weeks (under +CO_2) earlier than in mixed plantations. In 1999, senescence was also accelerated by enhanced O_3, but no differences were found between plantation types. It is possible, that the seasonal course of V_{cmax} and J_{max} of plants differed under the different treatments (B. Winkler, personal communication). Alteration in phenology of beech due to CO_2 and O_3 was also found by Nunn *et al*., (2002), Bortier *et al*., (2000), Baumgarten *et al*., (2000), but not so by Epron *et al*. (1996). Changes in V_{cmax} and J_{max} in beech were found by Liozon *et al*. (2000) during the growing season, and in response to CO_2 and nutrients. Nutrient stress decreased V_{cmax} in plants exposed to enhanced CO_2. Inspite of the different shoot phenology, modelled transpiration in beech under monoculture was highly correlated under all gaseous regimes with amounts of supplied irrigation (cf. Fig. 6 in Material and Methods).

In summary, the lower photosynthetic rates of beech plants in mixed culture were apparently not caused by light limitation, leaf nitrogen content, ozone injury (necroses) or reduced ETR. The reduced area-based C assimilation appeared to be associated with stomatal conductance, which was about 32% lower in mixed as compared with monoculture, in particular under +O_3

and +CO_2 (around 50%). Although both gases may cause stomata closure, the isotopic analysis tends to suggest that reduced gs of beech might have been induced to some degree by water limitation in the presence of spruce. The lower foliage area, crown volume, and the delay in shoot development of beech plants in mixed plantation as compared with those in monoculture also contributed to the reduced seasonal C gain of beech under interspecific competition.

4.3 Beech versus spruce: Aspects of the whole-plant C balance

The following section discusses if information gained from assessments of the standing biomass (like fine:coarse root and root:shoot ratio) and from seasonal C allocation between plant organs can be useful to understand competitiveness of plants.

Beech tended to reduce its fine root biomass in mixed compared to monoculture under all gaseous regimes (significantly under enhanced O_3 in both CO_2 regimes). However, no statistical differences between plantation types were found, regarding the proportion of fine to coarse root biomass. Contrasting results were found by Leuschner *et al.* (2001) studying root competition between beech and oak, and by Schmid (2002) and Schmid & Kazda (2001) with respect to beech-spruce mixed forests. They found higher fine and coarse root biomass, and a higher radial root growth rate of beech in beech-spruce mixed stands as compared to beech in monoculture. The authors suggest, contrasting with conclusions of Wang *et al* (2000) about beech and spruce saplings, that adult beech has a higher belowground competitive ability compared with spruce. In these latter two studies (Schmid, 2002 and Wang *et al,* 2000), conclusions about the belowground competitiveness of beech and spruce were based on different parameters: root biomass and radial growth, and nutrient uptake capability, respectively. Moreover, differences in ontogenetic stage (adult versus saplings) and experimental conditions (field versus laboratory) must be considered. As reported by Aerts (1999), in many cases increase in biomass partitioning to the root rather than to the shoot *per se* did not ensure improved competitiveness. In addition, increase in fine root density does not necessarily imply enhanced nutrient uptake and may lead to exacerbation of intraspecific root competition compared to competition among roots of other individuals/species (Rubio *et al.*, 2001, Casper & Jackson, 1997). Many studies have shown that morphological plasticity in biomass partitioning rather than increased biomass may be a more decisive factor in determining competitive success (cf. Aerts, 1999, Grime *et al.*, 1997). Plants can enhance their competitive ability by enlarging root length per unit of dry mass (Aerts, 1999; Campbell & Grime, 1992), as reflected in beech in the present study.

Beech in the present study also showed a higher root:shoot biomass ratio in mixed as compared with monoculture (significant under +O_3 and +CO_2), although the absolute root biomass was reduced – indicating favoured allocation to roots under interspecific competition. Contrasting with these findings, Curtis & Wang (1998) found no changes in the root:shoot ratio in response to enhanced CO_2 across 500 reported studies. However, as pointed out by Kubiske & Godbold (2001), the standing root biomass it is a poor indicator of total root production due to the continuous turnover of fine roots. In addition, root exudates can account for up to 40% of the whole-plant C gain (cf., Lemaire & Millard, 1999; Merbach *et al.*, 1999). In 10-year-old beech trees, Gansert (1995) found that respiration of fine roots consumed up to 16 % of the daily whole-plant net C assimilation rate in summer. Root respiration was highly correlated with soil temperature, but at the same temperature, plants growing in the understorey showed higher root respiration rates than those in clearings. Root respiration was also influenced by soil water content and mycorrhizal biomass. Thus, although the root:shoot ratio may not change in response to environmental factors, the C allocation to belowground processes like exudation, respiration and symbiosis may strongly vary (cf. Kubiske & Godbold, 2001). In mixed culture, beech plants invested a higher proportion of the seasonal C gain into the belowground organs that did plants in monoculture (Fig. 2.3). Spruce behaved in a similar way under +CO_2. Regarding stem respiration, the rates were highly correlated with the air temperature (Fig. 2.2a,b). However, at a given temperature, respiration rates strongly varied throughout the year. Higher rates were measured between mid-June and the end of July, in parallel to the radial stem growth. Seasonal variation in stem respiration of beech was also observed by Damesin *et al.* (2002) and was associated with changes in growth respiration. Stem respiration of beech was mostly lower in plants in mixed as compared with those in monoculture. In spruce, respiration rates did not differ between plantation types, except for +CO_2, which was also lower in mixed plantation. Perhaps this was a result of an increased C assimilation in the cortex cells (Matyssek *et al.*, 2002b; Pfanz & Aschan, 2001) of beech plants in mixed culture. As pointed out above, higher PPFD was indicated in the shade canopy of the stands in mixed as compared with monoculture. Hoops (2002) demonstrated that axis respiration of beech diminished with increasing light availability, and this was caused by the stimulation of axis photosynthesis (i.e. C refixation). The latter author found that in thin axes (4 mm diameter), up to 20% of the axis volume might be constituted by photosynthetic active chlorenchyma. However, also during the night hours the stem respiration was lower in plants in mixed as compared with those in monoculture. Thus, it seems that in addition to the possibly higher stem photosynthesis in beech in mixed culture, another factor may reduce the respiration rate in this plantation type. Stem respiration rate may depend on nutrient availability. Matyssek *et al*. (2002b) found a marked decrease of the CO_2 release in *Betula pendula* plants growing at low as compared to plants under high nutrient supply, especially when the O_3 regime was high. The reduction in respiration was associated with a lower stem volume increment, xylem and phloem widths, and consequently, growth and maintenance respiration. In mixed plantation, beech trees showed lower aboveground biomass increment, in particular under $+O_3$. Here, beech decreased its phloem and xylem area as compared with plants in the control gas regime (H. Rennenberg, pers. communication), and showed a significantly lower nitrogen concentration in the older than current-year shoot axes in mixed culture. All these factors can influence respiration rates.

The biomass ratios presented in this section (root:shoot and coarse:fine roots) did not offer unambiguous information about the competitive ability of the studied plants. The rates of stem respiration measured continuously during the growing season of 2000 reflected the lower aboveground growth of beech plants in mixed culture, especially under $+O_3$, and showed to be a helpful parameter to the estimation of C allocation between above- and belowground organs.

4.4 Beech versus spruce: Why is spruce the profiteer?

Spruce tended to increase its total biomass in mixed as compared with monoculture under all gaseous regimes (significantly under $+CO_2$, cf. Table 4.1). Contrasting with beech, spruce plants were able to profit from the elevated CO_2 in both plantation types, in particular in mixed culture (hypothesis 2). These results indicate that growth conditions became more favourable to spruce in the presence of beech. According to Kubiske & Godbold (2001), there is some evidence that N uptake per root dry mass may be stimulated under elevated CO_2 in conifers but not so in woody angiosperms. In mixed culture, beech displayed a reduced crown volume that increased the light availability to spruce as compared to the conditions in monoculture (except for the gaseous control regime). In monoculture, spruce showed higher stems as compared with plants in mixed plantations (except for plants in the gaseous control regime, cf. Table A3, Appendix). In addition, spruce showed, under +CO_2, longer branches per unit of stem length as compared with plants in monoculture. The preferential investment into stem height, as observed in monoculture, rather than in lateral branching (e.g. as in mixed culture) is a common response of plants to light competition (Aphalo *et al.*, 1999; Tremmel & Bazzaz, 1995; Küppers, 1985).

As addressed above, the C gain and biomass partitioning in beech differed at the end of the experiment in response to the presence of spruce and, to a lesser extent, to the gaseous regimes. Spruce, on the other hand, showed much less responsiveness to the plantation type and gaseous regimes, but e.g. profited from the interspecific competition. To which extent are the physiological and morphological responses of beech and spruce in terms of C gain and biomass partitioning related to their competitiveness? As pointed out by Aerts (1999), different standing biomasses may not be associated with changes in the efficiency in resource acquisition and hence, with competitive ability. More important appear to be changes in the morphology of structures related to resource sequestration, like leaves and fine roots.

Analysing competition among *Molinia caerulea* and *Erica tetralix*, Aerts (1999) found that the lower allocation of biomass to leaves in *M. caerulea* as compared with *E. tetralix* plants was compensated for by their higher specific leaf area (SLA). On the other hand, a lower allocation to the roots of the *E. tetralix* plants was compensated for by its higher specific root length (SRL). However, as shown above, the overall architecture of above and belowground structures in addition to SLA and SRL plays an important role in the process of plant competition. Beech and spruce plants changed crown volume in response to interspecific neighbourhood. Whenever crown and root architecture are modified, plant competitiveness may be affected, because the efficiency of space sequestration and resource acquisition is influenced (Matyssek & Sandermann, 2003; Grams *et al.*, 2002; Suzuki, 2002, Lemaire & Millard, 1999; Küppers, 1985). The next two sections will discuss the importance of the two conventional, morphological parameters SLA and SRL and of the efficiency ratios introduced in the present study for elucidating mechanisms that underlie plant competitive success. The efficiency ratios should give information about the fluxes of resources along plant structures involved in competition, by this integrating resource allocation and allometry in a quantitative way. In the present study, the analyses were concentrated on aboveground structures (section

4.4.1), although some information about belowground competitiveness will be presented in section 4.4.2.

4.4.1 Aboveground efficiency ratios of competitiveness

In the present study, the competitive disadvantage of beech in mixed culture was not associated with lower SLA (Table A2, Appendix) or C gain efficiency (seasonal C gain per unit of crown volume, Fig. 3.4.3). On the other hand, beech showed higher C gain per unit of crown volume in mixed as compared with monoculture under all gaseous regimes, although significant differences were only found under ambient CO_2 in 2000. The “running costs” (seasonal respiration and transpiration) for sustaining the crown volume of beech were in general slightly (but not significantly) higher in mixed plantations. Spruce, in contrast, showed similar C gain and “running cost” efficiencies in both plantation types. The lower competitiveness of beech in the presence of spruce was rather related to a reduction in the efficiency of aboveground space sequestration (Fig. 3.4.1). Beech displayed smaller crown volumes per unit of shoot biomass in mixed as compared with monoculture under all gaseous regimes in 2000 (significant differences under ambient CO_2), whereas spruce tended to enhance its space sequestration in mixed culture (significantly only in the gaseous control regime). The decline in the efficiency of aboveground space sequestration in beech in mixed culture was a result of lower investments into leaves per unit of shoot axes biomass and into current-year axes per unit of older shoot axes biomass as compared with monoculture. Along with the exacerbation of competition during 2000, beech drastically reduced its occupied crown volume per unit of shoot biomass as compared with 1999. These results confirm the findings of Küppers (1984, 1985) and Schulze *et al*. (1986). In their studies on plants of a secondary forest succession, the higher competitive ability of climax species like beech could not be explained by photosynthetic characteristics at the leaf level. Pioneers showed the highest photosynthetic capacity and annual C gain, but inspite of this, they were replaced by climax species. The competitive ability of the latter species was rather related to their high capacity of sequestering aboveground space and producing shade towards neighbouring plants, while keeping biomass investments into structures low.

The ability of plants to change crown architecture in response to the neighbourhood has received high attention in recent years, and has been considered a crucial mechanism of plant competition that determines population and community ecology (Suzuki, 2002; Umeki, 1997;

Yokozama *et al.*, 1996; Tremmel & Bazzaz, 1995; Küppers, 1987). Plants are able to detect the presence of neighbours by perceiving changes in light quality, and they may alter crown shape even before the light intensity becomes reduced (Ballaré *et al.*, 1988, 1987). Many studies have shown that some plants respond to decline in the red:far red ratio and associated changes in blue light by promoting stem internode elongation and lowering root:shoot as well as leaf:stem biomass ratios (Aphalo *et al.*, 1999). Such effects were claimed to reflect a strategy of shade avoidance. Other species, however, may adopt responses of shade tolerance by increasing leaf area ratio and SLA (Gilbert *et al.*, 2001; cf. Lemaire & Millard, 1999).

Different responses to neighbourhood in terms of crown architecture may play an important role in species composition in a given community. Yokozama *et al.* (1996) pointed out that the difference in crown shapes of conifers (conic crown) and angiosperm broadleaved trees (spheroidal crowns) may ensure the coexistence of these two tree types in boreal and sub-boreal zones. Kubota & Hara (1996) found for *Picea jezoensis* and *Abies sachalinensis* that intense competition between saplings that leads to habitat segregation was more important for the subsequent species composition of the forest than was the competition between the canopies of the adult trees. Although the red:far red ratio was not measured in the canopies of the present study, it is plausible to assume that light quality was modified in mixed as compared with monocultures, being one pre-requisite of the changes in the space sequestration efficiency of beech. As discussed above, in the presence of spruce, beech reduced its crown volume, increasing the light availability in mixed culture. Spruce appeared to take advantage from this situation by increasing its seasonal C gain. The higher C availability allows spruce to increase total biomass and probably to invest more C into the compensation of adverse effects caused by ozone. To sustain its increased growth capacity, spruce may have an enhanced demand for nutrients and water uptake. Beech may respond in mixed culture to this constraint in nutrient and water supply induced by spruce through decreasing leaf area and stomatal conductance. The latter changes can lead to a lowered C gain, which mitigates the C availability to growth and repair. Despite of the reduced C gain at non-limiting light supply, the exacerbating competition for nutrients and water may drive the resource allocation into the below rather than aboveground growth. Thus, in parallel to the reduced leaf area, lower investments in current-year axes eventually results in the reduced crown volume of beech trees in mixed culture. A small crown volume reduces the light interception of beech and in parallel increases the light availability to spruce.

4.4.2 Belowground efficiency ratios of competitiveness

Regarding belowground structures, beech plants changed their root morphology in response to the presence of spruce, tending to enhance specific fine-root length (SRL) in mixed compared to monoculture. On the other hand, spruce did not show significant differences in SRL between plantation types. Despite of the higher SRL of beech in mixed culture, in general, its capability to sequester and retain nutrients (calculated as content of N, P, Mg, Fe in the whole plant as related to root biomass, Table 3.4.1) was lower in mixed than in monoculture. Significant lower ratios were found for N and P under $+O_3$, while spruce increased (not significantly) its ratios of N and P content per root biomass. Kubiske & Godbold (2001) reviewed the N uptake efficiency of root systems of many species under ambient and elevated CO_2 regimes and found decline under enhanced CO_2. The results of the present study are in accordance with the findings of Kubiske & Godbold (2001). Reynolds & D`Antonio (1996) reported that in addition to root morphology, changes in root physiology may be a key response to the nutrient availability in the soil. The relative importance of root morphology and physiology depends on the ion mobility in soils. The authors also stress that under interspecific competition for nitrogen the best competitor was not necessarily the species with the highest plasticity in the root weight ratio (root per whole-plant biomass). Some studies found that the best competitor was the least plastic species. Hence, not necessarily a higher SRL leads to increased nutrient uptake. Moreover, the acquisition of elements like phosphorus generally depends on other factors like mycorrhization.

Overall, the results showed that morphological changes at the level of leaves (SLA) and fine roots (SRL) were not related to the competitive ability of the studied plants. Also the response of C and water fluxes through the leaves could not explain the better performance of spruce in mixed plantation. The competitiveness of plants was rather decided by the efficiency in aboveground space sequestration.

4.5 Allometric versus allocative control of competitiveness?

The discussion above was based on the analysis of plant responses to competition and/or gaseous regimes and on presented ratios of biomass partitioning between plant organs and crown volume-related measures of biomass and C gain. However, plant-internal ratios of resource partitioning must be interpreted within a larger functional context. According to Jasienski & Bazzaz (1999), "*focusing on average ratios, rather than allometric relationships between variables, is likely to obscure important biological phenomena*". An elucidative example was demonstrated by Müller *et al.* (2000). The authors analysed the biomass partitioning of 27 herbaceous species growing under low and high nutrient levels on the basis of biomass ratios and also allometrical relationships. Although they found statistically significant differences in the ratios between the nutrient regimes, the allometric analysis proved that plants do not re-adjust their internal resource allocation in response to the nutrient supply. The differences observed were merely a consequence of plant size, and hence, ontogeny. Plants under low-nutrient supply were e.g. smaller, and due to this, showed a higher root:foliage biomass ratio as compared with plants of the high-nutrient treatment. Most of the results of the present study are in accordance with the above findings. Under elevated CO_2 and O_3, beech plants were smaller and had, in proportion, less foliage area in mixed than larger plants had in monoculture. Hence, as C gain mainly depended on foliage area, a high correlation was found between seasonal C gain and shoot biomass. There were no significant differences between data from ambient and elevated CO_2 as well as $1xO_3$ and $2xO_3$ regimes, nor between plantation types (Fig. 3.5.2). Also root:shoot was a result of plant size (Fig. 3.5.1). The treatments influenced plant size, but did not change allocation patterns. The partitioning of biomass between foliage and shoot axes, which is highly related to crown volume, neither was directly influenced by the gaseous regimes (Fig. 3.5.3a,b). However, in mixed plantation, beech plants did allocate less substrate to leaves at a given shoot axes biomass than did plants in monoculture (see different intercepts). This result indicates a size-independent effect of competition with spruce on the biomass partitioning in beech, and this was found, in particular, under the $+O_3$ regime. Plants under enhanced O_3 showed a diminished proportion of conductive phloem area and lowered transport rate of sugars, and hence reduced sugar concentrations in shoots and fine roots in both plantation types, although effects were more distinct in mixed culture (H. Rennenberg, personal communication). Despite the reduction in sugar translocation out of the leaves under enhanced ozone, biomass partitioning between leaves and shoot axes did not differ from that of plants under the control regime in monoculture. This suggests that in monoculture the lower amount of C exported from the leaves was distributed between plant organs in a way that reflected ontogenetic control of the resource allocation. In contrast, beech plants in mixed culture re-adjusted allocation under $+O_3$. It appears that severe stress as by exacerbating competition for resources and O_3 impact may overrule the ontogenetic control in the allocation of beech.

Although the allometric analysis provides important information about the causality of changes in allocation (treatment effects versus size dependence), nevertheless this kind of analysis may face limitations (Poorter & Nagel, 2000). Applying a modified approach, the latter authors found indications that plants may indeed have the capacity to overrule ontogeny in resource allocation, namely in response to light, nutrient and water availability, but not so to CO_2 regimes; however, the outcome of such analyses may strongly be governed by the specificities of experimental scenarios. Similar to the results of the present study, Zak *et al.* (2000) found that CO_2 treatments did not re-adjust biomass partitioning in *Populus tremuloides.*

Irrespective of the influence of ontogeny on the resource allocation, plants need to adapt the growth rates of the different organs (root, stem, leaves) in order to balance the internal resource demand with the external resource availability. During the last decade, evidence has grown that carbohydrate levels in source and sink tissues regulate gene expression, providing a mechanism to adjust allocation and growth of the different plant parts in response to changes in the external resource availability (cf. Andersen, 2003; Stitt & Schulze, 1994). For example, a decrease in the carbohydrates of leaves up-regulates genes responsible for photosynthesis (activation and synthesis of the rubisco enzyme), sugar mobilization and export, whereas increased carbohydrate levels up-regulates genes for C storage and use (cf. Andersen 2003; Farrar & Jones, 2000). In situations where soil N is limiting, lowered inorganic N in leaves results in a decreased synthesis of amino acids, and hence in an increase of carbohydrates available to other functions. As expected, high sugar levels in leaves inhibit photosynthesis and C accumulation, and stimulate translocation to non-green tissues like roots. Thus, nitrogen and sugar seem to act as signals between plant organs, controlling resource allocation. If the transport of such signal molecules between organs is impaired (for example due to closure of sieve elements by callose under $+O_3$ as in beech, H. Rennenberg, pers. communication), the capability of plants to respond to changes in environmental resource availability may be lost.

4.6 Conclusions

In the studied system, spruce was a better competitor than beech. The efficiency ratio of aboveground space sequestration (crown volume per unit of shoot biomass) turned out to be sensitive to the gaseous regimes, and proved to be an adequate parameter to reflect competitiveness (confirming hypothesis 4, which predicts that the efficiency ratios can be used to characterize and quantify competitiveness of beech and spruce plants). To a lesser extent, the efficiency ratios of “running costs” (crown volume-related respiration and transpiration) also reflected the restricted competitive ability of beech. In contrast, the C gain efficiency (C gain per unit of crown volume) was not related to the competitiveness of beech in mixed culture. Besides the higher C gain efficiency ratio, beech also showed higher belowground morphological plasticity than spruce, in particular, increasing SRL in response to interspecific competition. Therefore, the results indicate that at least in juvenile beech, the decisive parameter in determining competitive success is related to the ability to enlarge the crown volume at low structural costs. Individuals which depend on high amounts of resources per unit of occupied crown volume may have less resources available for the demands of other plant functions (like root growth or repair processes of O_3 injury, for example). Consequently, a restricted aboveground space sequestration efficiency may predispose to a lowered nutrient uptake capacity and/or raised O_3 susceptibility, and this may limit, in turn, the growth and development of the aboveground structures.

A conceptual model of the competitive advantage of spruce over beech is presented in figure 4.6.1. Due to the crown shape of beech, more light tends to be available for spruce in the mixed as compared with the monoculture. Given a potentially higher capacity for nutrient uptake from the soil (Wang *et al*, 2001) along with enhanced light availability, spruce increases its total biomass in mixed culture, perhaps at the expense of nutrient and water availability to beech. This may lead to reduced C gain of beech plants in mixed culture, in particular under the $+O_3$ regime, and eventually results in decreased whole-plant growth (with the aboveground parts and crown volume being most affected).

In the present study the analysis of competitiveness was concentrated on aboveground structures. A more detailed analysis of root morphology, fine root turnover and resource uptake capacity, interaction with mycorrhizal fungi and the mycorrhizosphere, as well as analyses of belowground resource availability throughout the growing season are necessary to clarify questions about competition for nutrients and water. Belowground processes in a similar beech-spruce system are currently being studied with emphasis during the presently conducted second phase of the SFB research program.

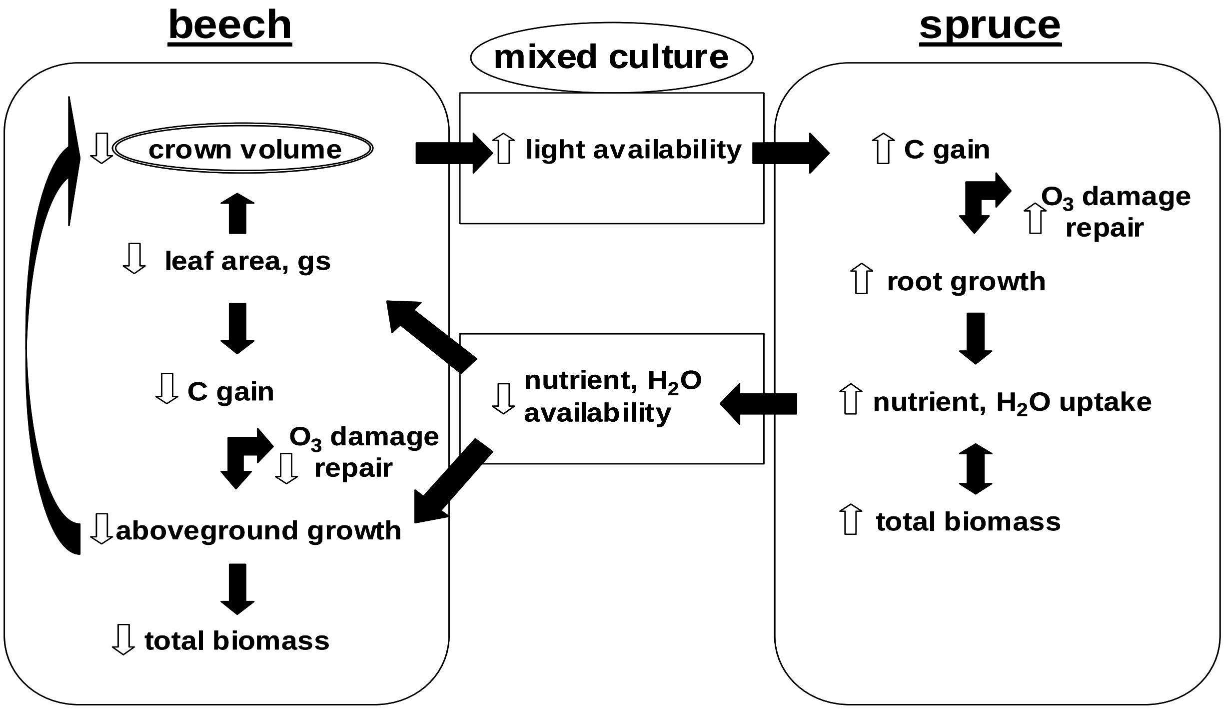


Figure 4.6.1. Conceptual model on the competitive advantage of spruce over beech in mixed plantation. Spruce profits from the restricted space sequestration of beech, as the narrow crown shape of the latter species tends to allow enhanced light penetration into the mixed-stand canopy. The favoured production of spruce (given a potentially higher capacity for nutrient uptake from the soil along with enhanced light availability) appears to exacerbate the belowground competition for water and nutrients at the expense of beech. The lowered C gain of beech, mediated in part through belowground competition, results in reduced whole-plant growth (in particular aboveground), and consequently, further promotes the constriction in crown volume (see text; black arrows represent vectors that give the direction of action, white arrows represent stimulation of tree parameters when upward, or limitation when downward oriented).

Although beech was more responsive to O_3, (confirming hypothesis 1), the main factor driving changes in biomass allocation was the type of competition (i.e. intra *versus* interspecific). Only spruce was able to profit from the elevation of CO_2 (confirming hypothesis 2). Apparently, responses to CO_2 and O_3 strongly depended on growth conditions (confirming hypothesis 3), i.e. the presence of competition in mono and mixed plantations. One should be aware of such evidence when attempts are made to extrapolate results as gained from research on isolated plants or monocultures to mixed stands in the field. In general, changes in resource allocation were size-dependent. However, under multiple stress as imposed by the presence of spruce and ozone impact, beech appeared to lose the ontogenetic control in biomass partitioning. In this situation, it is possible that fluxes of carbohydrates and nitrogen between sources and sinks are impaired, impeding plants to internally signalise – and to respond to – changes in the environmental resource availability.

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APPENDIX

Number and area of beech leaves

Table A1. Number of leaves and leaf area of beech plants at the end of July 1999 and 2000 in mono and mixed cultures under different CO_2 and O_3 regimes (means ± standard error).

Treatments	1999 number of leaves	leaf area [cm^2]	2000 number of leaves	leaf area [cm^2]
Control				
monoculture	32.8 ± 2.9	11.4 ± 0.1	117.8 ± 10.6	*9.4 ± 0.4
mixed culture	43.9 ± 7.0	11.4 ± 0.2	88.7 ± 17.6	7.2 ± 0.5
+ O_3				
monoculture	46.3 ± 4.0	11.2 ± 0.1	*134.8 ± 24.3	*7.3 ± 0.5
Mixed culture	43.1 ± 3.6	11.2 ± 0.0	75.2 ± 14.4	3.6 ± 0.4
+ CO_2				
monoculture	53.0 ± 3.9	11.7 ± 0.1	*140.7 ± 17.1	*7.3 ± 0.2
mixed culture	41.4 ± 5.8	11.6 ± 0.1	77.8 ± 16.2	5.2 ± 0.4
+CO_2/+O_3				
monoculture	*52.3 ± 4.2	*10.5 ± 0.3	*118.5 ± 17.0	*8.2 ± 0.6
mixed culture	27.8 ± 1.5	11.1 ± 0.3	62.4 ± 7.2	6.0 ± 0.2

* indicates significant difference between mono and mixed cultures with $p<0.05$.

Specific leaf area

Table A2. Specific leaf area of sun, shade and second flush leaves of beech in mono and in mixed plantations in 1999 and 2000 under different CO_2 and O_3 regimes (means ± SD).

Treatments/Year		SLA [m^2 kg^{-1}] ***sun***		***Shade***		***second flush***	
		mono	mixed	mono	Mixed	mono	mixed
Control	1999	21.4 ± 2.0	21.5 ± 2.8	27.8 ± 5.1	29.1 ± 6.1	15.1 ± 2.5	16.4 ± 2.9
	2000	21.8 ± 12.3	28.0 ± 9.0	26.2 ± 8.4	32.7 ± 13.7	18.2 ± 6.1	19.3 ± 5.2
+ O_3	1999	22.3 ± 2.8	22.5 ± 2.8	28.8 ± 3.6	29.4 ± 3.7	16.4 ± 1.8	16.0 ± 2.9
	2000	26.6 ± 8.3	25.9 ± 2.1	28.7 ± 12.5	37.8 ± 8.0	18.3 ± 7.9	n.m.
+ CO_2	1999	19.5 ± 2.2	21.3 ± 2.3	26.3 ± 3.0	27.8 ± 2.9	15.1 ± 2.2	16.3 ± 1.6
	2000	22.2 ± 3.8	27.1 ± 8.9	32.2 ± 9.8	29.9 ± 17.0	23.9 ± 14.7	n.m.
+CO_2/+O_3	1999	19.4 ± 1.7	21.5 ± 3.0	25.8 ± 3.3	28.5 ± 4.3	13.9 ± 1.9	14.8 ± 1.7
	2000	21.3 ± 6.5	27.5 ± 13.7	38.1 ± 25.4	35.6 ± 6.7	12.9 ± 5.0	18.8 ± 4.1

Number and length of spruce twigs

Table A3. Number of axes and total axes length per stem height of spruce at the begin of 2000 in mono and mixed cultures under different CO_2 and O_3 regimes (means ± standard error).

Treatments	stem height [cm]	Number of axes/stem height [n° m^{-1}]	axes length/stem height [m m^{-1}]
Control			
monoculture	36.6 ± 14.1	70.4 ± 4.0	9.4 ± 0.8
mixed culture	37.3 ± 15.3	67.3 ± 3.4	10.2 ± 1.0
+ O_3			
monoculture	37.4 ± 11.6	70.5 ± 4.4	*8.1 ± 0.5
mixed culture	35.5 ± 15.8	68.2 ± 2.9	10.2 ± 0.7
+ CO_2			
monoculture	40.3 ± 13.1	67.1 ± 4.0	*8.7 ± 1.1
mixed culture	37.1 ± 12.9	71.9 ± 3.4	11.3 ± 0.7
+CO_2/+O_3			
monoculture	41.2 ± 18.2	*77.7 ± 4.0	10.4 ± 0.5
mixed culture	38.3 ± 17.1	65.4 ± 4.1	10.7 ± 1.0

* indicates significant difference between mono and mixed cultures at $p<0.05$.

Stomatal conductance and internal CO$_2$ partial pressure

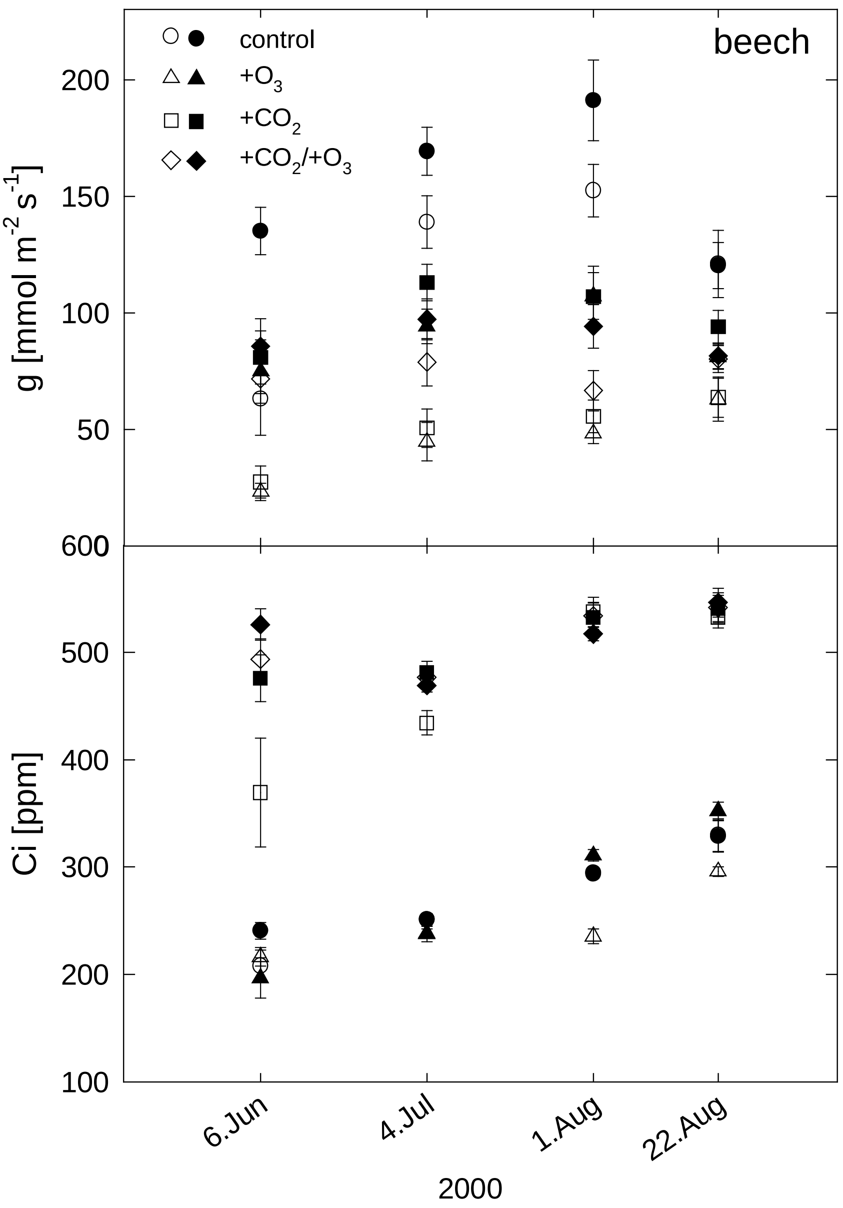

Figure A1. Stomatal conductance (a) and ci (b) in sun-leaves of beech measured under all gaseous regimes during 2000. Monocultures are given as solid, and mixed cultures as open symbols. Circles denote control, triangles +O$_3$, squares +CO$_2$ and rhomboid symbols are +CO$_2$/+O$_3$.

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