Wood formation in *Fagus sylvatica* L. and *Populus x canescens* (Aiton) Sm. under elevated CO_2 and with different nutrient supply

Dissertation

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by

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To whom it may concern:

Neda Lotfiomran is submitting her doctoral dissertation in English. The title of his thesis is: "Wood Formation in *Fagus sylvatica* L.and *Populus x canescens* (Aiton) Sm. under Elevated CO_2 and with Different Nutrient Supply".

I hereby certify as a native speaker and holder of Bachelor of Arts degree in both English and Journalism from the University of Connecticut that the English language used in this thesis is sufficiently correct for submission.

Joshua Brackett

Pittsburgh 12.02.2013

Summery

In view of the rising CO_2-concentration in the atmosphere accompanied by increasing global warming forests play a major role as CO_2-sinks. According to this background the impact of elevated CO_2 (770/950 ppm), in combination with different nutrient regimes on two tree species (*Fagus sylvatica* and *Populus canesence*) were studied in two growing season.

During each growing season some physiological properties of the saplings such a photosynthetic activity and transpiration rate were measured. After each growing season the trees were harvested and their phenological status such as total biomass and anatomical characteristics of them such as average vessel lumen area (AVLA) were measured. Additionally, the nitrogen and carbon content in the plants were determined. All data obtained were tested for differences.

The results revealed that the two species responded very inconsistently to the experimental conditions.

In both growing seasons the poplar saplings grown under elevated CO_2 (770/950 ppm) had a lower photosynthetic rate than in ambient air, as a consequence the total biomass was less under elevated CO_2 than in ambient air. The height growth rate of poplar was not affected by fertilization, whereas elevated CO_2 resulted in larger leaves. But fertilization led to a lower C/N ratio in the saplings. The different experimental conditions resulted in the different effect on the wood anatomical properties such as ring width and vessel characteristics.

Beech trees under elevated CO_2 had a significantly lower stomatal conductance of their leaves than saplings in ambient air. Elevated CO_2 resulted heavier and wider ring.

In beech saplings elevated CO_2 decreased the N content in the tissues and a lower C/N ratio. Lignification in the wall of fibres and vessels of beech was measured.

Total vessel lumen area (TVLA) and vessel density (VD) in beech increased by elevated CO_2 but unlike poplar no correlation was observed between ring width and vessel characteristics.

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List of Abbreviations

A_c	Photosynthesis rate
ANOVA	Analysis of variance
AVLA	Average vessel lumen area
B	Boron
C	Carbon
C/N ratio	Carbon/Nitrogen ratio
CCI	Chlorophyll concentration index
Cl	Chlorine
CO_2	Carbon dioxide
Cu	Copper
E_w	Transpiration rate
Fe	Iron
Fig.	Figure
g_S	Stomatal conductance
K_2O	Potassium oxide
kPa	kilopascal (1 kPa ≡ 1000 Pa)
LMA	Leaf mass per area
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
n.s.	Insignificant
P_2O	Phosphorus dioxide
pH	potentia hydrogenii
ppm	Parts per million
RW	Ringwidth
st.dev	Standard deviation
Tab.	Table
TVLA	Total vessel lumen area
UMSP	Cellular ultraviolet microspectrophotometry
VD	Vessel density
Zn	Zinc

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1. Introduction

In the 19th century, it was discovered that the atmospheric CO_2 is the carbon source for the photosynthetic activity of plants and in consequence for the production of the plant biomass (Fangmeier & Jäger 2001). Subsequently, this knowledge has been applied in practice to increase the yield of gardening under intentionally elevated CO_2 levels in greenhouses. But due to industrialization around the beginning of the 19th century, the CO_2 started to rise also in the atmosphere. At present, this rise is continuously increasing and since about 50 years also proven by measurements. But not only since man has interfered with the carbon cycle, CO_2 level was changing. The composition of trace gases in cores taken from the polar ice shield prove that the amount of CO_2 in the atmosphere has been severely fluctuating during the past 220,000 years resulting in cold and warm periods (Fangmeier & Jäger 2001). At present, the CO_2 concentration amounts to about 385 ppm what is higher than ever during the last 200,000 years. Only if the time axis is further extended, CO_2 concentrations of more than 3,000 ppm can be assumed.

The recent rise of the CO_2 content in the ambient air and its implications for the biosphere, including the global warming, are in the focus of politics since more than two decades and science started to study the effects of elevated CO_2 on plants. Trees as long-living organisms have permanently to respond to various short-term or long-term changes in their environment, among them to the rising CO_2 content in the ambient air. Forests as a sink for CO_2 play an important role in the discussion about global warming (e.g., Matyssek et al. 2010). On a global scale, more CO_2 is presently released than sequestered by photosynthesis. This CO_2 surplus comes from the combustion of coal and oil but also from large-scale clear cuts of forests in the tropics. This rise increases the CO_2 content in the ambient air and thus intensifies the greenhouse effect. For the next decades, an average increase of the global temperature of 1.5°C is predicted (e.g., Burschel & Weber 1988). It is at present not foreseeable how the forests will respond to this development. Presumable, the spectrum of tree species will change in favour of thermophilic trees from more southern latitudes.

However, the rise of CO_2 stimulates photosynthesis and growth of trees also appears to depend on the availability of nutrients and on the capability of trees to slowly adapt to such changes but the processes behind are only fragmentarily known (e.g., Kriebitzsch et al. 1999). Also the effects on the anatomy and technological quality of wood gown under elevated CO_2 were studied only to some extent (e.g., Burgert et al. 2000; Overdieck et al. 2007).

In view of this situation, we wanted to close some gaps of knowledge and therefore have designed a research project for two years to study various physiological and phenological as well as anatomical features of the growth of beech and poplar saplings under controlled conditions of elevated CO_2 and of different nutrient supply in a greenhouse.

2. Literature Review

[This chapter is based on review articles and on a selection of recently issued publications; literal quotations are printed in italics]

Since the pre-industrial CO_2 level in the atmosphere has reached the current level, the scientific community became increasingly aware of problems possibly connected with it. Therefore, very soon the effect of elevated CO_2 on crop plants and woody plants started to be studied (Cure & Acock, 1986; Sionit & Kramer, 1986; Strain, 1987; Bazzaz, 1992) and a large body of information of tree responses to elevated CO_2 has been produced (Ceulemans & Mousseau, 1994; Curtis, 1996; Medlyn et al., 1999; Nowak et al., 2004; Körner, 2006; Taub, 2010). CO_2 enrichment experiments have been made in growth chambers of greenhouses, open top chambers (OTC), branch bags, free air CO_2 enrichment (FACE) facilities and CO_2 springs (Ceulemans & Mousseau, 1994).
Most studies in the greenhouse are performed with seedlings or young trees for short periods of time (Ceulemans & Mousseau, 1994; Poorter & Navas, 2003) But due to the longevity of trees, also long-term studies are urgently necessary (Eamus & Jarvis, 1989) in order to allow for a tree's ability to acclimate to an elevated CO_2 level by adapting, for example, the photosynthetic rate and stomatal conductance (Donovan & Ehleringer, 1991). FACE experimental systems, originally designed for agronomic crops (Allen et al., 1992), are increasingly applied to investigate the response of adult trees to elevated CO_2 in the long-term and under natural growth conditions (Hendrey, 1992; Hendrey, Lewin et al., 1993). In the case of long-term exposures of trees to elevated CO_2, large differences in their responses should be expected depending on whether slow growing or fast growing trees species are studied.

2.1 Physiological responses of plants to elevated CO_2

2.1.1 CO_2 and photosynthesis

Photosynthesis is directly affected by a varying CO_2 content in the ambient air and therefore is one of the major physiological processes being monitored during all kinds of

experiments. Numerous short-term studies showed an increase of the photosynthesis rate caused by an increased CO_2 concentration (Cure & Acock, 1986; Strain, 1987; Bazzaz, 1990; Long & Drake, 1992; Curtis, 1996; Saxe et al., 1998; Ceulemans et al., 1999).

Evidence from FACE sites frequently supports these results and shows a stimulated photosynthesis rate under elevated CO_2, and the degree of this enhancement varies among species and under different growth conditions (Curtis & Wang, 1998; Nowak et al., 2004; Ainsworth & Long, 2005; Leakey et al., 2009). A short-term increase of photosynthesis due to elevated CO_2 has been explained by Farquhar et al. (1980) as follows:

''This model defenses the rate of net leaf photosynthesis as the minimum of two sub-processes: photosynthesis is co-limited by the rate of carboxylation and the rate of electron transport. Both of these processes are saturating functions of the CO_2 *concentration in the cells and, in the case of electron transport, of the absorbed photosynthetically active radiation (PAR). The saturated rate of carboxylation (i.e.the maximum rate of Rubisco activity, Vcmax) and the potential rate of electron transport (Jmax) govern the rate of photosynthesis. Unlike other important parameters of the Farquhar model, these two vary substantially among plant canopies, but also within canopies and among leaves of the same plants* (Ceulemans et al., 1999)''.

But the photosynthetic behavior of trees under long-term exposure to elevated CO_2 is less clear (Medlyn et al., 1999). Although it is generally agreed that short-term growth under elevated CO_2 causes a stimulation of the photosynthesis rate in trees, it has frequently been reported that this enhancement may decline or even disappear with time (Kohen et al., 1993; Woodward, 2002). Furthermore, in several studies a ''down regulation'' of the photosynthesis rate was shown in plants grown under elevated CO_2 over longer periods (weeks or months) (see reviews by Ceulemans & Mousseau, 1994; Gunderson & Wullschleger, 1994; Curtis, 1996; Saxe et al., 1998; Nowak et al., 2004). A “down regulation” of photosynthesis means that the photosynthetic activity is more intense in plants grown in ambient air than under elevated CO_2 (Medlyn et al., 1999).

So far, at least three hypotheses have been proposed to explain this phenomenon:

Ceulemans & Mousseau (1994) suggested that the availability of more C causes a nutrient limitation, which induces a reduced level in the concentration of leaf nutrients. This procedure will reduce the photosynthetic rates (Ceulemans & Mousseau, 1994).
Sage (1994) found the down-regulation as a result of re-allocation of leaf nitrogen, away from the principal CO_2-fixing enzyme, Rubisco, and towards other nitrogenous compounds.
As the third hypothesis, Stitt (1991) pointed out that down-regulation is caused by a negative feedback on photosynthesis due to an imbalance between the demand for carbon and photosynthetic productivity.
A number of studies made in a greenhouse mentioned the pot size as a factor that can limit root growth and thus is causing the phenomenon of ‘’down regulation’’ (Arp, 1991; Tomas & Strain, 1991).

2.1.2 CO_2 and transpiration

Transpiration is the evaporation of water from plants organs especially from leaves and is part of the water cycle. Transpiration depends on several factors such as a stomatal conductance (gs), net radiation receipt (R), air saturation deficit (D), temperature (T) and wind speed (u) (Jarvis & McNaughton, 1986).
Elevated CO_2 generally causes a decrease in stomatal conductance (see 2.3.1) and this in turn results in a reduced transpiration (Bazzaz, 1990; Saxe et al., 1998; Ward & Strain, 1999).

2.2 Interaction effects between elevated CO_2 and environment factors on plants

The responses of plants to elevated CO_2 strongly depend on environmental conditions (Bazzaz, 1990; Saxe et al., 1998; Poorter & Navas, 2003; De Graaff et al., 2006). Several investigators have focused on the interaction effect of elevated CO_2 with, for example, light level (Sionit et al., 1982; Kubiske & Pregitzer, 1996; Marfo & Dang 2009), temperature (Overdieck et al., 2007), soil moisture (Arp et al., 1998), mineral nutrient availability (El Kohen et al., 1992; Arp et al., 1998; De Graaff et al., 2006), other

greenhouse gases (O_3, SO_2, NO_x.) (Polle et al., 1993; Tausz et al., 1996; Lütz et al., 2000; Sallas et al., 2001).

2.3 CO_2 and leaves

Leaves as the strongest morphological driver (Poething, 1997) show the highest structural plasticity in response to different environmental conditions (Esau, 1977). They are vital for photosynthesis and water movement and therefore crucial for the whole plant function (Murthy & Dougherty, 1997). Structural adaptations of leaves clearly play a central role for a plant's adaptation to environment changes (Lewis, 1972; Ashton & Berlyn, 1994).

2.3.1 Stomata

Stomata allow communication between the internal and external environment of plants. Their main function is to allow gases such as CO_2, water vapor and oxygen to move rapidly into and out of the leaf. Stomata and their response to the environment can be altered by several factors such as temperature, soil moisture, light level and CO_2 (Bazzaz, 1990; Morison, 1998).

First studies on the stomatal response to CO_2 were made by Freudenberger (1940) and Heath (1948) and his co-workers (Heath & Russell, 1954; Heath & Meidner, 1957). The stomatal conductance and density can be affected by elevated CO_2 (Bettarini et al., 1998).

Early and current evidences of FACE and non-FACE experiments reported that the stomatal conductance decreases under elevated CO_2 (Bazzaz, 1990; Curtis & Wang, 1998; Pritchard et al., 1999; Ward & Strain, 1999; Medlyn et al. 2001; Wullschleger et al., 2002; Nowak et al., 2004; Ainsworth & Rogers, 2007).

As to the response of the stomatal density of leaves to elevated CO_2, conflicting results are being published. A number of studies reported that elevated CO_2 induces an increase (Thomas & Harvey, 1983; Gaudillere & Mousseau, 1989) or a decrease in stomatal density (Woodward & Bazzaz 1988; Field et al., 1995; Lin et al., 2001) or no significant effect (Mousseau & Enoch, 1989; Estirate et al., 1994).

2.3.2 Specific leaf area (SLA)

SLA (leaf area/total leaf dry weight) frequently exhibits a reduction under elevated CO_2 (Penuelas & Matamala, 1990; Poorter & Navas, 2003). For example, Pritchard et al., (1999) reviewed 49 observations; 78% of them showed a significant decrease in SLA, in 18% of the observations the SLA was not affected, and in 4% of them the SLA increased under elevated CO_2.

The SLA reduction is a consequence of an increased concentration of starch (Eamus & Jarvis, 1989) and of leaf total non-structural carbohydrates (TNC), which occurs when the fixation of C exceeds its utilization (Pritchard et al., 1999).

2.3.3 Leaf area per plant

The leaf area per plant generally increases by elevated CO_2 in most tree species (see reviews Ceulemans & Mousseau, 1994; Pritchard et al., 1999; Poorter & Navas, 2003; Ainsworth & Long, 2005). Nevertheless, some exceptional cases have been observed (Norby et al., 1992; Mousseau, 1993; EL Kohen et al., 1993). However, the amount of leaf area increase varies among tree species. It can result from an increased number of leaves per tree or by larger individual leaves.

Two hypotheses suggest an explanation for the increase in leaf area:

a) an increase in the number of cells (cell division) (Gaudillere & Mousseau, 1989),

b) an increase in leaf cell expansion through changes of the cell-wall properties (Taylor et al., 1994).

2.4 CO_2 and dry biomass of plants

The production of biomass is one of many paths along which carbon is metabolized (Körner, 2006). Several measurements on seedlings and young trees indicate that through an increased carbon uptake, total growth increases (see reviews by Bazzaz, 1990; Ceulemans & Mousseau, 1994; Saxe et al., 1998; Pritchard et al., 1999; Poorter & Navas, 2003; Ainsworth & Long, 2005). There is also strong evidence that plant biomass production under elevated CO_2 is mostly larger than in ambient air. But a number of

studies showed no significant increase or even a decrease of biomass production under elevated CO_2 (Reekie & Bazzaz, 1989; Bazzaz & Miao, 1993; Bazzaz & Garbutt, 1998). The magnitude of the biomass enhancement widely varies between observations. Growth stimulation depends on various factors such as the developmental stage of the plants (Norby et al., 1992; Hättenschwiler et al., 1997), genetic factors, environmental conditions and water and nutrient availability (Bazzaz, 1990; Ceulemans & Mousseau,, 1994; Roberntz & Stockfors, 1996; De Graaff et al., 2006; Leakey et al., 2009; Reddy et al., 2010). The enhancing effect of elevated CO_2 can decline or totally disappear with time (Sionit et al., 1985; Garbutt et al., 1990; EL Kohen et al., 1993; Saxe et al., 1998; Bloom, 2009).

2.5 CO_2 and N content in plant tissues

Nitrogen is part of all living cells and of all proteins, enzymes and metabolic processes involved in the synthesis and transfer of energy. Trees require a considerable amount of N for their growth, especially when growing under elevated CO_2 because then they usually grow faster (Reddy et al., 2010).

One of the most common observations is a lower N concentration in plant components grown under elevated CO_2 than in ambient air (Cotrufo et al., 1998; Poorter et al., 1997; Yin, 2002; Ainsworth & Long, 2005; Taub & Wang, 2008).

According to several reports, the concentration of N is reduced on average by 10-15% under elevated CO_2. The range of reduction varies among species. Furthermore, the N content in leaves frequently decreases more than in roots (Cotrufo et al., 1994). Several hypotheses are suggested to explain this phenomenon (Gifford et al., 2000; Taub & Wang, 2008). However, it is generally accepted that elevated CO_2 causes a decrease of N, even if some observations show an increase when the supply with N is ample (Cotrufo et al., 1994).

2.6 CO_2 and anatomy characteristics of plants

Wood anatomy is directly associated with wood quality and with various applications of wood. In comparison with the high number of studies related to physiological responses

of plants to elevated CO_2, few studies have been done about how wood quality changes under elevated CO_2. Most of them have been done with coniferous tree species (Donaldson et al., 1987; Conroy et al., 1990; Telewski et al., 1999; Yazaki et al., 2001; Ceulemans et al., 2002; Atwell et al., 2003; Kilpeläinen et al., 2003; Kostiainen et al., 2004; Ziche & Overdieck 2004; Kilpeläinen 2007; Kostiainen et al., 2009). The reports available on wood quality changes in broad-leaved tree species are rare (Atkinson & Taylor, 1996; Luo et al., 2005; Overdieck et al., 2007; Watanabe et al., 2010; Wiemann et al., 2008; Kostiainen et al., 2009; Watanabe, 2010).

2.6.1 Coniferous tree species

Under elevated CO_2, the tracheid diameter in Scot pine (Ceulemans et al., 2002) and mean tracheid lumen area in Siberian larch *(*Yazaki et al., 2001) increased and in radiate pine mean tracheid lumen area was unaffected by the CO_2 level (Atwell et al., 2003).
The tracheid wall was thinner in Siberian larch and Scot pine (Yazaki et al., 2001; Ceulemans et al., 2002) and thicker in Monterey pine under elevated CO_2 (Conroy et al., 1990). In contrast, Donaldson et al. (1987) did not find any significant CO_2 effect on tracheid characteristics in Monterey pine.
In a number of the studies mentioned, elevated CO_2 caused wider tree rings (Telewski et al., 1999; Ziche & Overdieck; 2004) or wider early wood (Ceulemans et al., 2002) or had no effect at all (Kostiainen et al., 2009).
Often, it has been reported that wood density increases in response to elevated CO_2 (Conroy et al., 1990; Tognetti et al., 1998; Telewski et al., 1999), but this cannot be generalized; for example, mean wood density of Scots pine and Norway spruce remained unaffected by elevated CO_2 (Ceulemans et al., 2002; Beismann et al., 2002).

2.6.2 Broad-leaved tree species

A review of publications shows that the total vessel lumen area increased in English oak and remained unchanged in cherry (Atkinson & Taylor, 1996) and Mongolian oak (Watanabe et al., 2008) in exposure to elevated CO_2. Response of mean vessel area to elevated CO_2 varies among species; mean vessel area increased in English oak

(Atkinson & Taylor. 1996), black poplar and a hybrid poplar (Luo et al., 2005), decreased in European beech (Overdieck et al., 2007), and was unaffected by elevated CO_2 in and Mongolian oak (Watanabe et al., 2008).

No effect of elevated CO_2 was shown in mean vessel number (vessel density) of cherry (Atkinson & Taylor. 1996) and European beech (Overdieck et al., 2007); in contrast, mean vessel number in English oak enhanced by CO_2 (Atkinson & Taylor. 1996). The results are very inconsistent between tree species and between genotypes of the same species.

3. Material and Methods

3.1 Greenhouse experiments

Test plants of two tree species, European beech (*Fagus sylvatica* L.) and gray poplar (*Populus x canescens* (Aiton) Sm.), were studied in a greenhouse for the effects of elevated CO_2 and nutrient supply on growth and wood formation during two vegetation periods (May to September 2010 and 2011).

3.1.1 Plant material and treatments

In the greenhouse of the Thünen Institute (TI) for wood science in Hamburg, located 53°30´ N and 10°12´ E at an elevation of 25 m a.s.l., two growth chambers were used. In one of them, the CO_2 concentration was the same as in ambient air (on average 385 µmol mol^{-1}) (control). In the other one, the CO_2 concentration was raised to 770 µmol mol^{-1} in the 1st study year and to 950 µmol mol^{-1} in the 2nd study year (Fig. 3.1). The one-year old saplings of beech and poplar have been planted in Ø 11x13 cm and Ø 17x19.2 cm pots, respectively (Fig. 3.1). The soil consisted of 50% of sand and 50% of a standard commercial substrate (TKS1) (Tab. 3.1).

Tab. 3.1: Soil properties used for the experiments.

Substrate TKS 1	pH ($Ca Cl_2$)	salinity (H_2O) (g/l)	N (mg/l)	P_2O (mg/l)	K_2O (mg/l)
	5.6	**0.8**	**140**	**80**	**190**

In each chamber, the saplings were divided into two groups. One of them was fertilized with a 0.2% liquid fertilizer (Tab. 3.2). The beech plants obtained 50 ml and the poplar plants 100 ml once a week during the growing seasons.
All plants were regularly irrigated with 80 ml of tap water. The whole study design is summarized in Table 3.3.

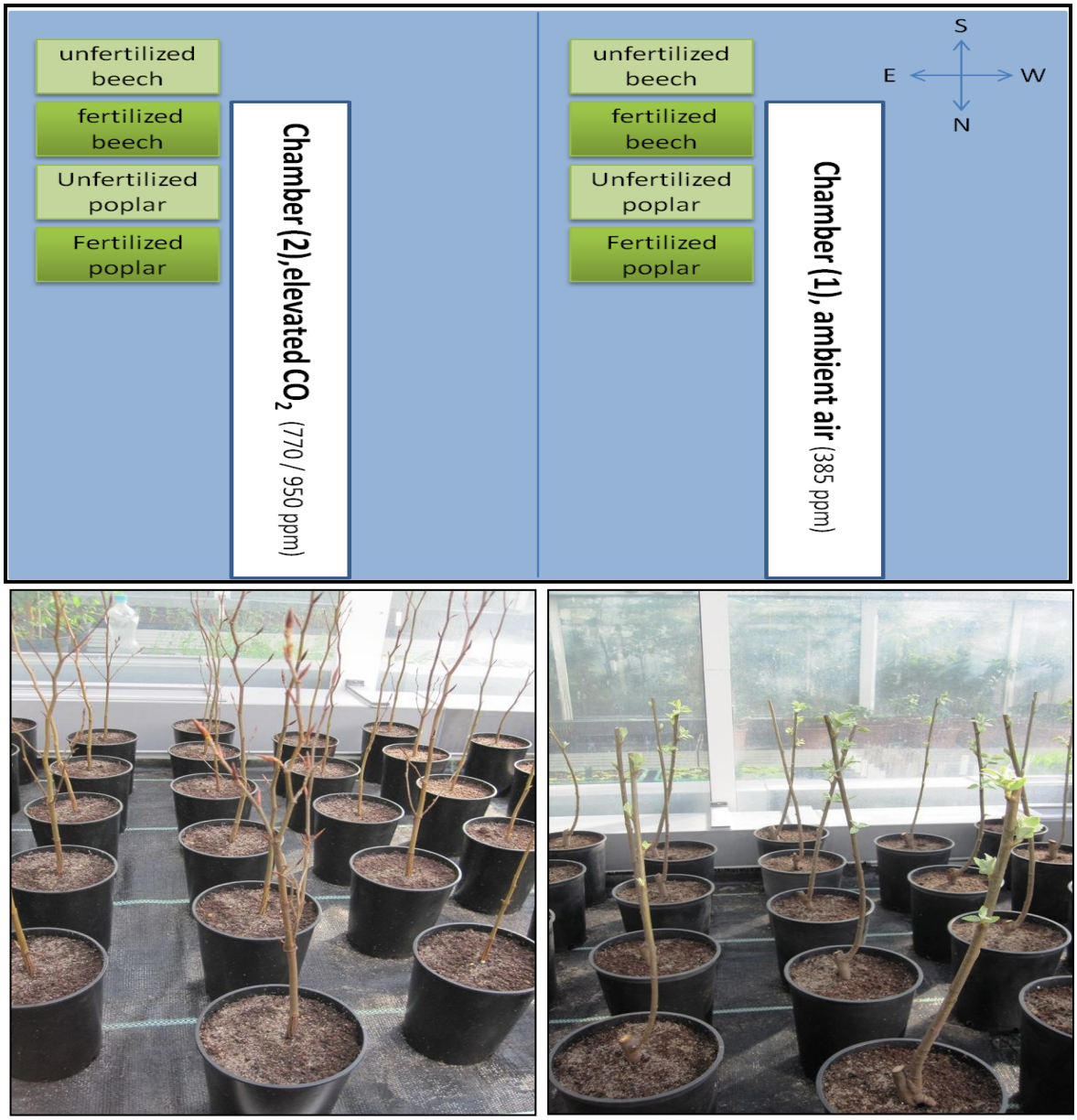

Fig. 3.1: Study design (top); European beech (left) and gray poplar (right) at the beginning of the growing seasons (bottom).

Tab. 3.2: Properties of the fertilizer (Wuxal top N).

Wuxal Top N **NPK liquid fertilizer**	**NH2-N**	**N**	**P_2O**	**K_2O**	**B**	**Cu**	**Fe**	**Mn**	**Mo**	**Zn**	**Cl**
[%]	**12**	**12**	**4**	**6**	**0.01**	**0.004**	**0.02**	**0.012**	**0.001**	**0.004**	**1.2**

Tab. 3.3: Study design.

	Treatments 2010	Number of saplings
***Fagus sylvatica* L.**	**unfertilized - ambient air (control)**	**8**
	fertilized - ambient air	**8**
	unfertilized - elevated CO_2 (770 ppm)	**8**
	fertilized - elevated CO_2 (770 ppm)	**8**
***Populus canescens* (Aiton) Sm.**	**unfertilized - ambient air (control)**	**8**
	fertilized - ambient air	**8**
	unfertilized - elevated CO_2 (770 ppm)	**8**
	fertilized - elevated CO_2 (770 ppm)	**8**
2011		
***Fagus sylvatica* L.**	**unfertilized - ambient air (control)**	**10**
	fertilized - ambient air	**10**
	unfertilized - elevated CO_2 (950 ppm)	**10**
	fertilized - elevated CO_2 (950 ppm)	**10**
***Populus canescens* (Aiton) Sm.**	**unfertilized - ambient air (control)**	**8**
	fertilized - ambient air	**8**
	unfertilized - elevated CO_2 (950 ppm)	**8**
	fertilized - elevated CO_2 (950 ppm)	**8**

3.1.2 Environmental conditions in the greenhouse

During the vegetation periods, the temperature in the greenhouse was kept constant at about 20°C, the length of photoperiod was the same as in the Hamburg area and the relative air humidity (RH) was about 70%. During the experiments, the CO_2 level, temperature, photoperiod and air humidity were monitored by the Computer Climate model CC 600 (RAM co.) every 12 minutes (Fig. 3.2). The CO_2 levels (ambient air, 770

and 950 ppm) were kept from 7:00 to 21:00 o'clock per day. Moreover, all plants were visually controlled for any conspicuous features once a week during the growing season.

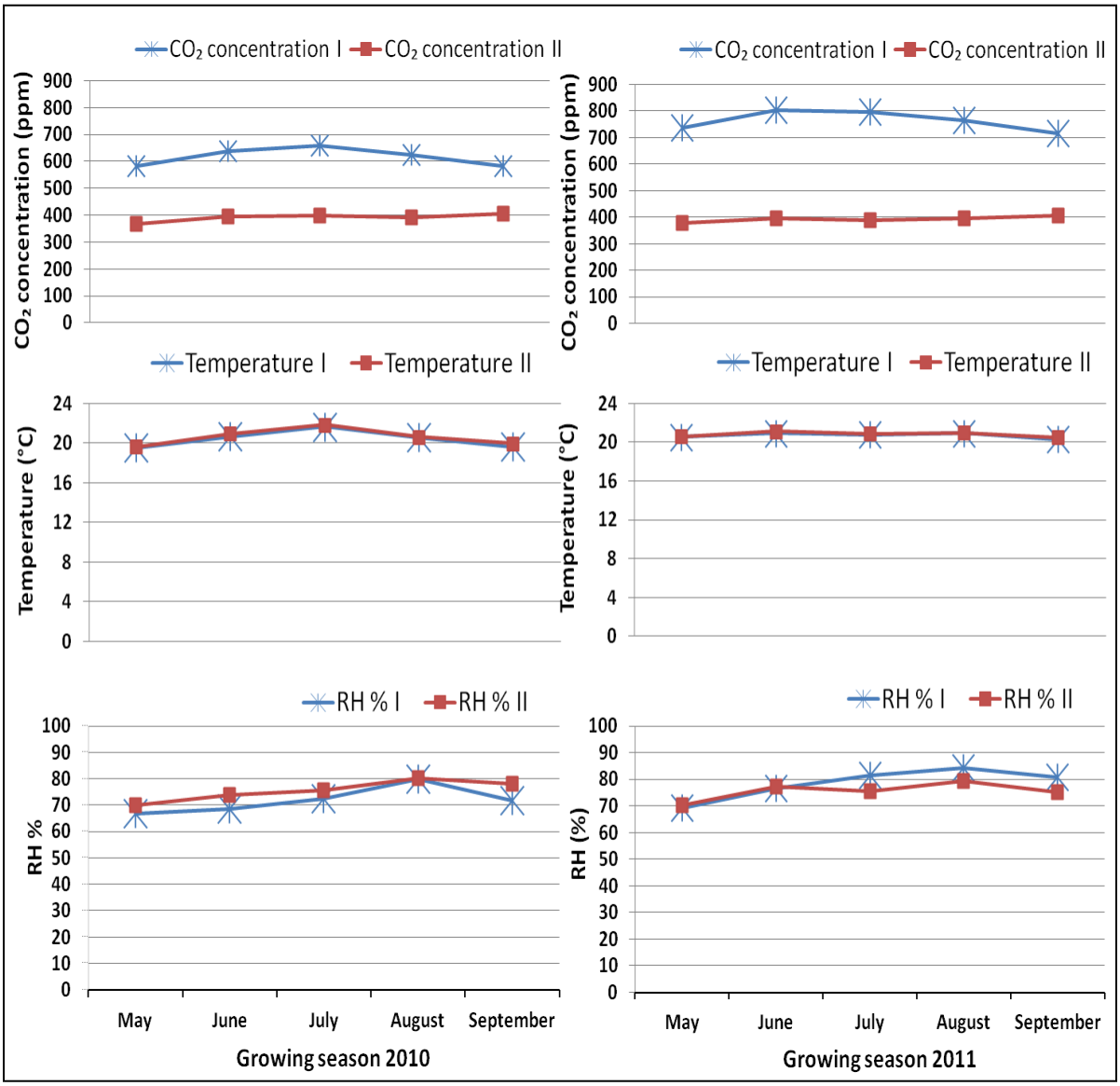

Fig. 3.2: CO_2 concentration, temperature and relative air humidity (RH) during both growing seasons; I, chamber with elevated CO_2; II, chamber with ambient air.

3.1.3 Seasonal gas exchange measurements

In the growing season 2010, the rates of CO_2 assimilation (A_C) and of transpiration (E_w) of one leaf from each plant were measured once a week by a CO_2/H_2O-promoter CMA-400 (Walz Mess- und Regeltechnik 1988) (Fig. 3.3). During the experiment, the light intensity was 800 µmol m^{-2} s^{-1} and the flow rate of air through the system was set to about 1200 µmol s^{-1}.

Fig. 3.3: Promoter CMA-400 (Walz Mess- und Regeltechnik 1988).

In the growing season 2011, the net photosynthesis rate (A_C), transpiration rate (E_W) and stomatal conductance (g_s) were measured using a portable Infra-Red Analyzer (IRGA; LI-6400, Li-Cor, Lincoln, NE, USA) on one leaf of each sapling once a week (Fig. 3.4). The rate of air flow through the system was set to 600 µmol s^{-1} and the light intensity, provided by a red-blue light source, was set to 800 µmol m^{-2} s^{-1}. The air humidity was regulated by adjusting the air flow through a desiccant tube.

Fig. 3.4: Portable Infra-Red Analyzer (IRGA; LI-6400, Li-Cor, Lincoln, NE, USA).

During both study seasons, the chlorophyll content in the leaves of all saplings was non-destructively determined by a chlorophyll meter (SPAD-502 plus Konica Minolta) once a week on 15-20 leaves per sapling (Fig. 3.5). The values measured correspond to the percentage of chlorophyll in the leaves. They were calculated from the amount of light, transmitted through a leaf of two wavelengths for which the absorbance of chlorophyll is different.

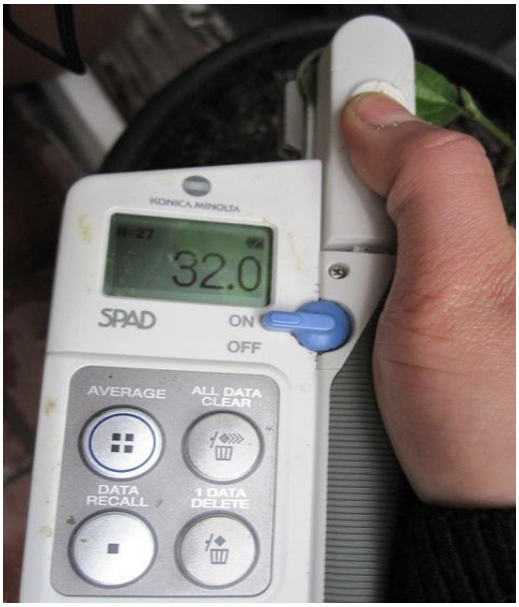

Fig. 3.5: Chlorophyll meter (Model; SPAD-502 plus).

3.2 Growth measurements

At the end of the growing season in each experimental year, the tree height was measured and then all trees were harvested and separated into leaves, stems and roots. After that, the fresh weight of these three fractions has been determined to calculate the dry weight biomass, after the samples have been dried at 70°C for one week (Fig. 3.6).
At the end of the growing season 2011, the total leaf area of all saplings was measured by a non-destructive leaf area meter (LI-3000C, Li-Cor, Lincoln, NE, USA) (Fig. 3.7). For each leaf, the LI-3000C recorded values of leaf area, leaf length, average width and maximum width. Each of these values can be shown on the display. The instrument utilizes an electronic method of rectangular approximation providing a 1 mm² resolution. The major components are the scanning head and the readout console. Area data are recorded by the readout console as the scanning head is passed over the leaf.

Fig. 3.6: Plants were harvested and separated into leaves, stems and roots.

Fig. 3.7: Leaf area meter (LI- 3000C, Li-Cor, Lincoln, NE, USA).

3.3 Sample preparation for biochemical measurements

3.3.1 Carbon and nitrogen content in leaf, stem (without bark) and root

After having determined the dry biomass weight, all three fractions of the saplings per treatment were sampled for elemental analysis. Leaves, stems without bark and roots were ground to powder using a mill (Fig. 3.8). Then, 5-10 mg of the powder were filled in tin capsules and the total carbon and nitrogen content of each sample were measured using an element analyzer instrument (Vario EL cube; Hanau, Germany) (Fig. 3.8). During analysis, the temperature in the oxidation oven was 1050°C and in the thermal conductivity detector and chromatographic column was 115°C. The carrier gas pressure was 80 kPa and the flow rate 125 ml/min, the oxygen addition was 20 ml, and the oxygen pressure was 50 kPa.

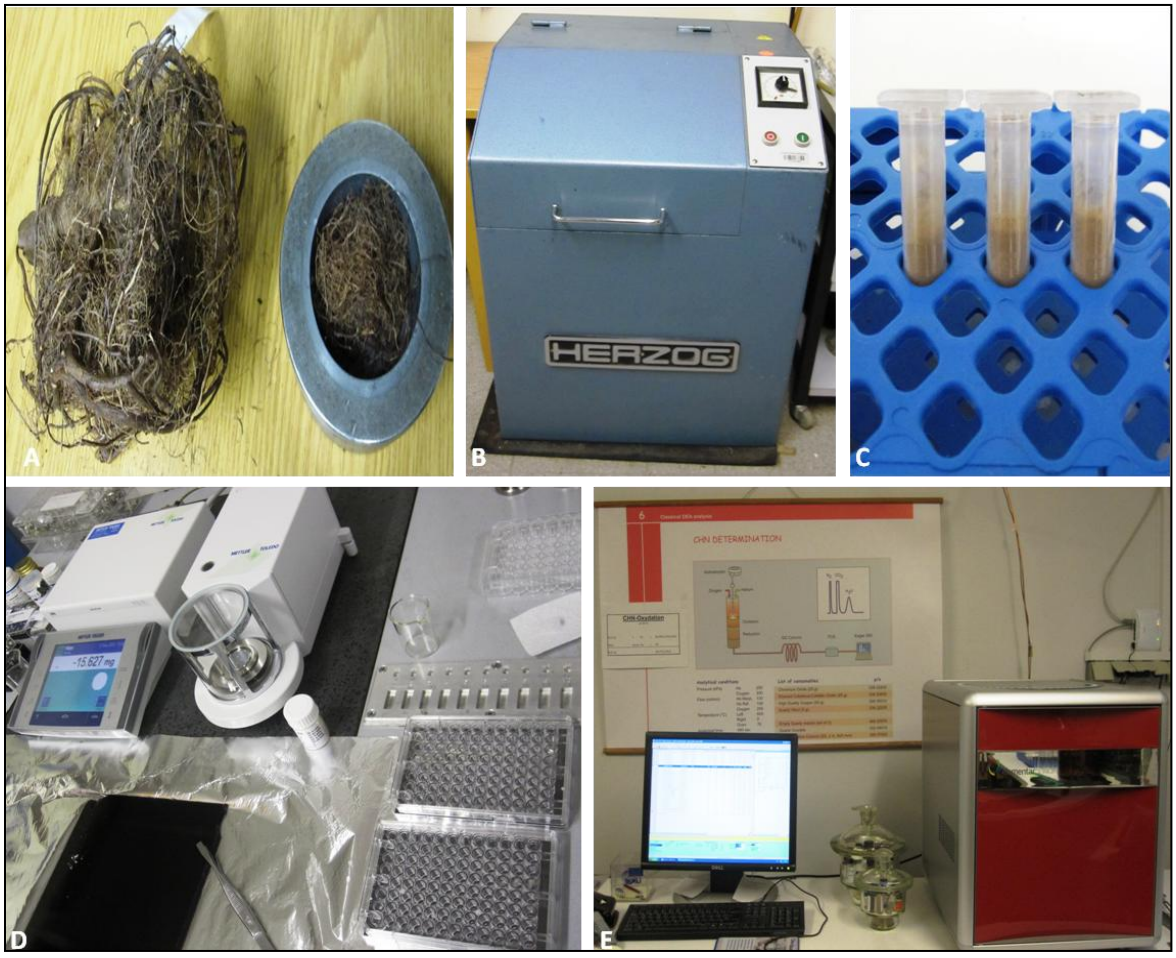

Fig. 3.8: Equipment for carbon and nitrogen analysis. (A) dried plant material; (B) mill; (C) ground material; (D) scales; (E) element analyzer instrument (Vario EL cube; Hanau, Germany).

3.3.2 Distribution of lignin in beech cell walls

Cellular ultraviolet microspectrophotometry (UMSP) was applied to localize the lignin in the walls of fibers and vessels. For the analysis, small blocks (approx. 1 x 1 x 0.5 mm^3) (l x r x t) were dissected from the wood which was formed during the experimental years. Such blocks were taken from three saplings per treatment.

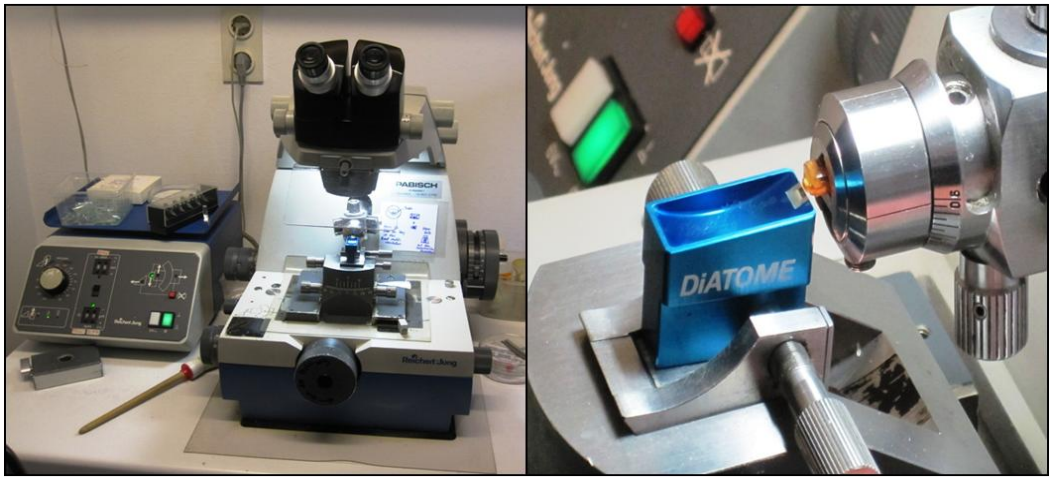

Fig. 3.9: Ultramicrotome (Ultracut E, Reichert-Jung, Wetzlar, Germany) (left), equipped with a diamond knife (right).

The specimens were dehydrated in a graded series of ethanol (80%, 90%, 95% and 100%) and 100% of acetone, and then embedded in Spurr's epoxy resin (Spurr 1969). For UMSP, the embedded blocks were trimmed to provide a face of approximately 0.5 mm². Transverse sections, 1 µm thick, were cut by an ultramicrotome (Ultracut E, Reichert-Jung, Wetzlar, Germany) equipped with a diamond knife (Fig. 3.9), transferred to quartz microscope slides, immersed in a drop of non UV-absorbing glycerine (Zeiss, Germany) and covered with a quartz cover slip (Fig. 3.10).

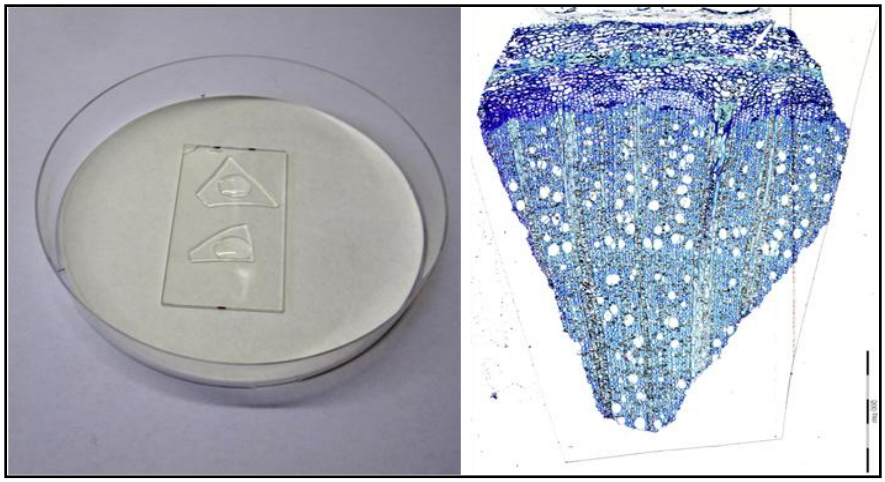

Fig. 3.10: Quartz slides (left) and light microscopic image (right).

The measurements were carried out using a ZEISS UMSP 80 (Zeiss, Germany) equipped with an immersion ultrafluar lens 1:32 (Koch & Kleist 2001) and with a scanning stage allowing the determination of image profiles at a constant wavelength with the scan programme APAMOS (Zeiss, Germany) (Fig. 3.11). Lignin was detected at 278 nm (Koch & Kleist 2001). The scan programme digitized rectangular fields with a local geometric resolution of 0.25 µm x 0.25 µm and a photometric resolution of 4096 gray scale levels, which were converted into 14 basic colors to visualize the absorbance intensities. The scans were depicted as two dimensional (2D) image profiles, including a statistical evaluation (histograms) of the UV-absorbance.

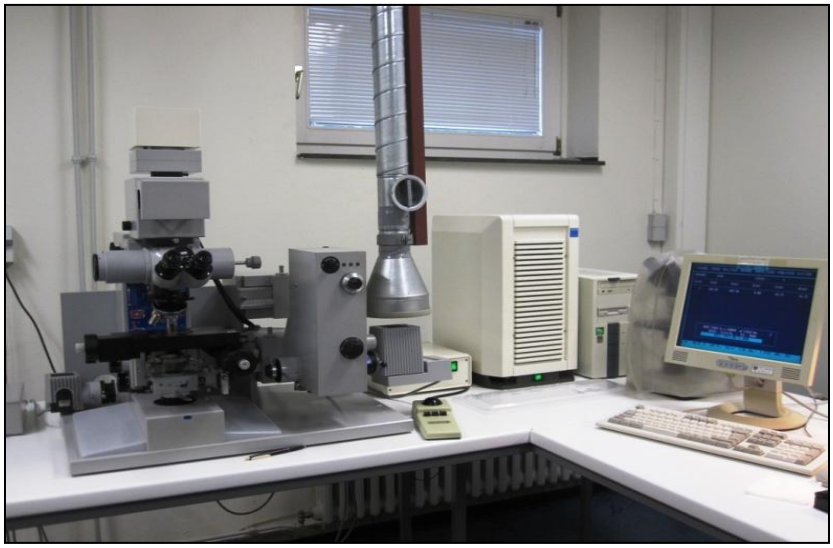

Fig. 3.11: Microspectrophotomet ZEISS UMSP 80 (Zeiss, Germany).

3.4 Sample preparation for wood anatomical measurements

At the end of each growing season, stem discs were collected from 5 cm above-ground from beech, and from 10 and 30 cm above-ground from poplar to study wood formation and some wood anatomical properties under the given experimental conditions. These samples were placed in 70% ethanol and brought to the laboratory to cut cross-sections of 18 and 30 µm thickness using a sliding microtome (Sartorius MI, 31 A 30) (Fig. 3.12) which were stained with safranin (1%) and astra-blue (5%) (Fig. 3.13).

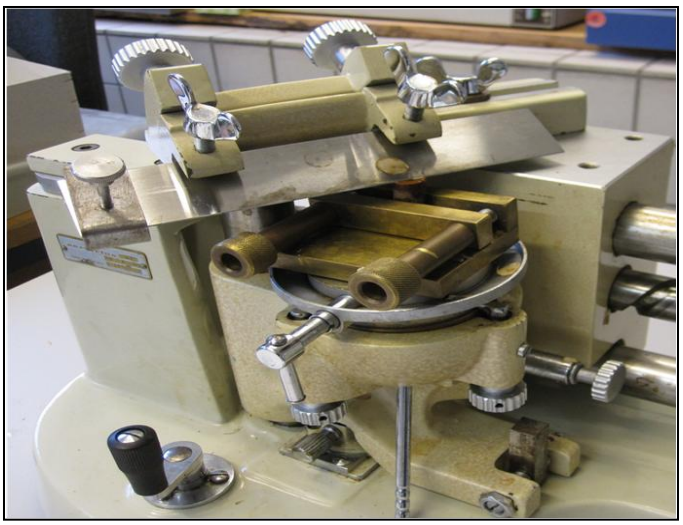

Fig. 3.12: Microtome (Sartorius MI, 31 A 30).

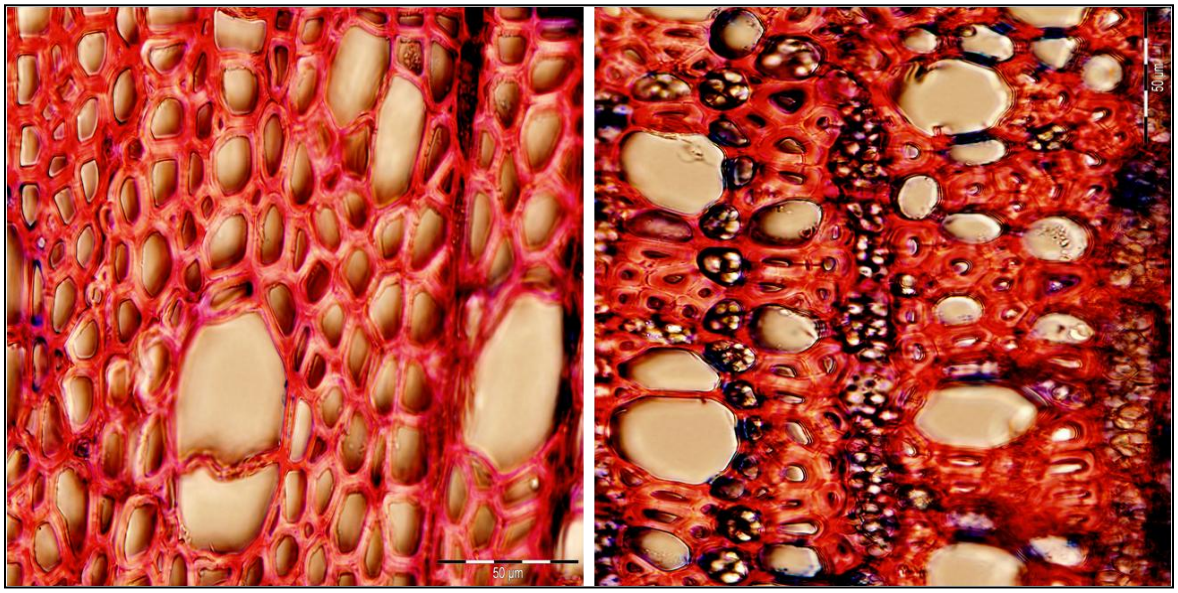

Fig. 3.13: Cross-section of gray poplar (left) and European beech (right).

After that, the width of the growth ring formed during the study year was measured to the nearest 0.01 mm at eight positions around the stem, using a moving table connected to a PC and the tree-ring software CATRAS (Aniol 1983) (Fig. 3.14).

Fig. 3.14: Work station for tree-ring width measurements using CATRAS (Aniol 1983).

Then, from each microscopic cross-section images were taken from two areas across the tree ring using an Olympus BX51 microscope equipped with a digital microscope camera (Olympus DP 70) linked to a computer (Fig. 3.15).

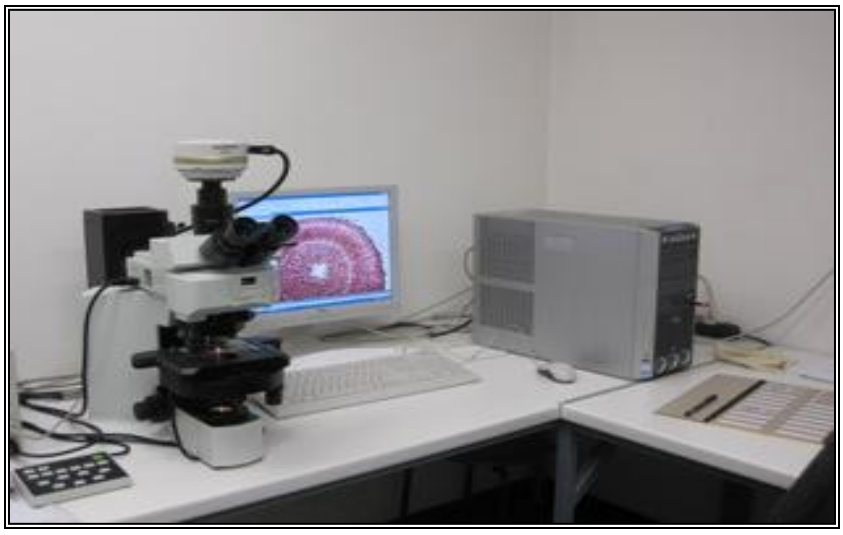

Fig. 3.15: Olympus BX51 microscope equipped with a digital microscope camera (Olympus DP 70).

The color images were taken at 4x magnification with a resolution of 4080x3072. In the case of beech, the photographic area was positioned between two multi-seriate rays (Fig. 3.16).

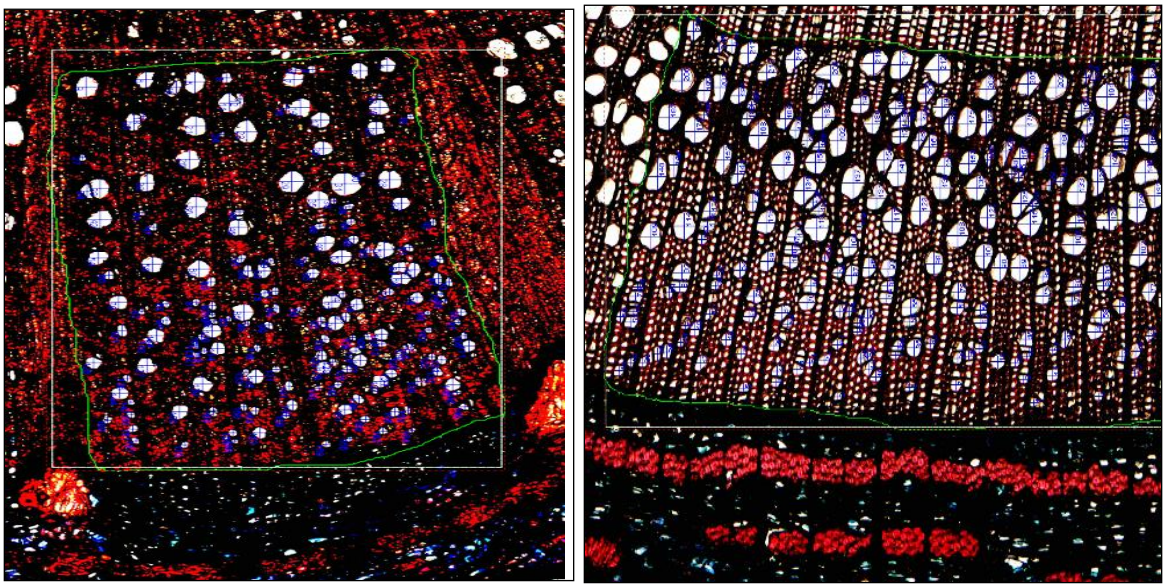

Fig. 3.16: WinCELL analysis area of beech (left) and poplar (right).

The average vessel lumen area (AVLA) (µm²), number of vessels per mm² (vessel density (VD)) and percentage of the total vessel area (vessel coverage) were measured by the image processing software WinCELL Pro (version 2010a, Régents Instruments Inc., 2001) (Fig. 3.15); the equations for calculation are given below. WinCELL is specifically designed for wood cell analysis using a different filter setting for each tree species. Only vessels larger than 120 and 200 µm² were measured from beech and poplar, respectively. The anatomical measurements can be visualized graphically using XLCell.

$$\text{TVLA} = \frac{Total\ Vessel\ Lumen\ Area}{Total\ Analyzed\ Area} \times 100$$

$$\text{AVLA} = \frac{Total\ Vessel\ Area}{Total\ Number\ of\ Vessels}$$

$$\text{VD} = \frac{Total\ Number\ of\ Vessels}{Total\ Analyzed\ Area}$$

3.5 Statistical analysis of the data

A Complete Randomized Design (CRD) was considered for the greenhouse experiments. All data were pre-tested for normality and equality by a *Kolmogorov-Smirnov* test to check whether parametric tests can be applied.

All statistical analyses were performed using SPSS 18 (SPSS Inc. Chicago, Illinois USA). The data of each experimental year were analyzed separately and independently from each other. The effects of the CO_2 levels (ambient or elevated) and fertilization were analyzed with repeated measures multivariate ANOVA. The statistical significance was set at $p \leq 0.05$ for all tests. Standard deviation (SD) was used to show the distribution of the data around the mean. The Pearson's correlation test was used to check the correlation between ring width and vessel variables and between chlorophyll concentration index and leaf N content at the end of vegetation periods.

4. Results

4.1 Seasonal measurements

4.1.1 Seasonal measurements of poplar in the greenhouse

4.1.1.1 Net photosynthesis rate

In 2010, the net photosynthesis rates of the saplings grown under elevated CO_2 (770 ppm) and in ambient air were considerably different throughout the whole growing season (Fig. 4.1.1) whereas fertilization had no effect what so ever (Tab. 4.1.1). But in 2011, the net photosynthesis rate was not significantly different between the different CO_2 level (elevated/ambient air) (Tab. 4.1.3).

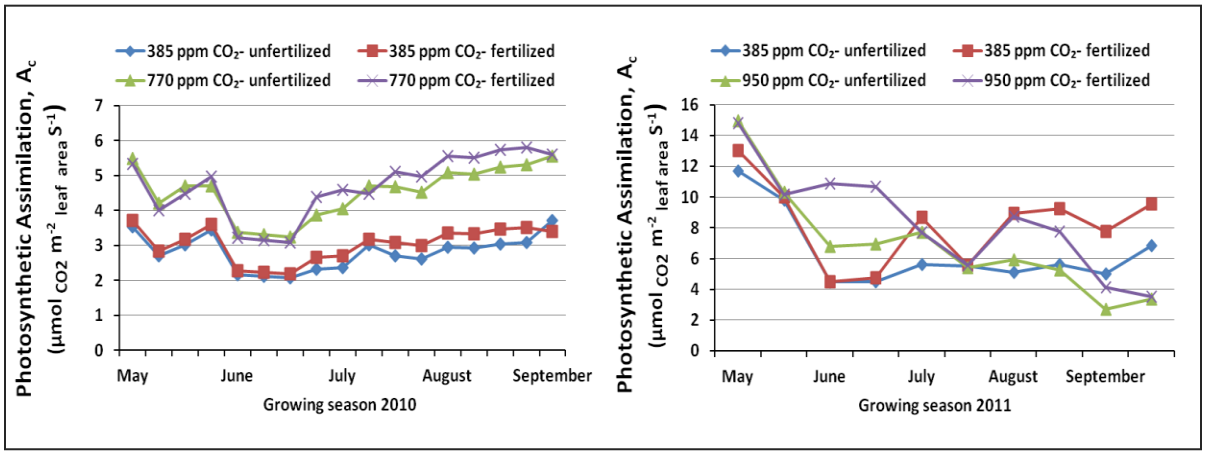

Fig 4.1.1: Net photosynthesis rate of poplar during the first (left) and the second (right) growing season.

4.1.1.2 Transpiration rate

No distinct differences of the transpiration rate were obvious between the treatments in both growing seasons (Fig. 4.1.2), saying that the transpiration rate did not respond to different CO_2 concentrations and fertilization (Tab. 4.1.3).

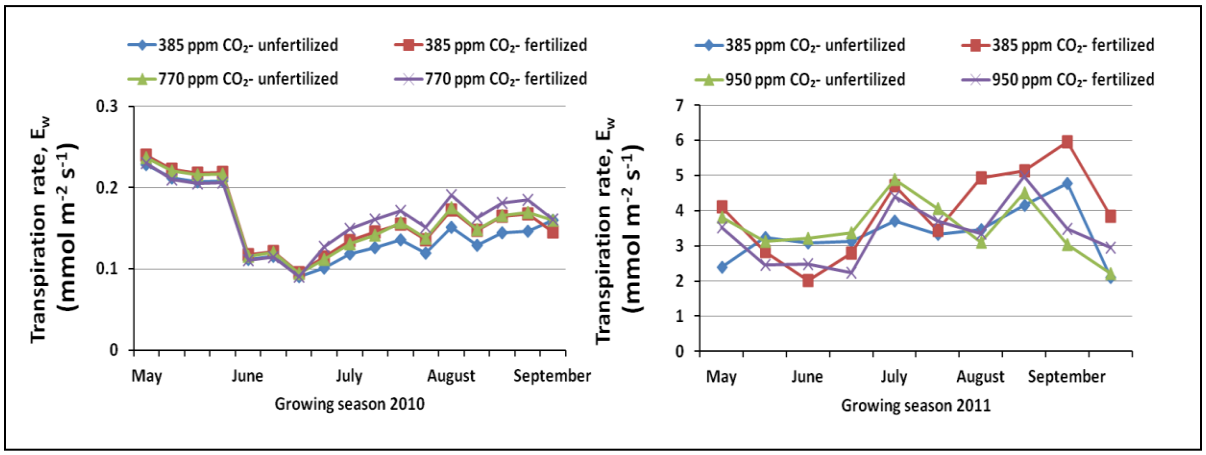

Fig 4.1.2: Transpiration rate of poplar during the first (left) and second (right) growing season.

4.1.1.3 Stomatal conductance

The stomatal conductance in the leaves was not affected by the growth conditions (Fig. 4.1.3; Tab. 4.1.3).

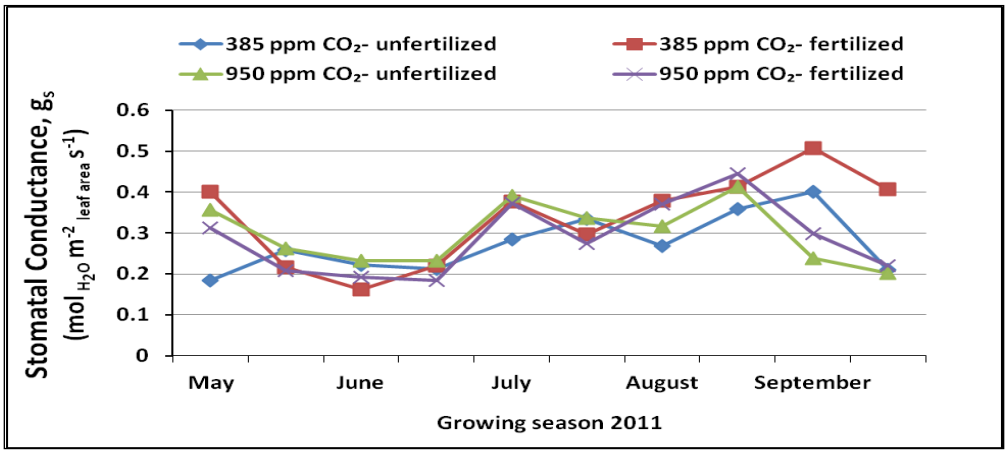

Fig. 4.1.3: Stomatal conductance of poplar during the second growing season.

Tab. 4.1.1: Mean (± st.dev.) of net photosynthesis rate and transpiration rate of poplar during the first growing season (2010).

Treatments of poplar (2010)	May	June	July	August	September
Photosynthetic rate A_C (µmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	4.61 ± 0.67	4.10 ± 2.17	3.81 ± 0.82	5.03 ± 0.31	5.67 ± 0.10
385 ppm CO_2 -fertilized	4.80 ± 0.65	4.04 ± 2.07	3.62 ± 0.42	4.75 ± 0.29	5.30 ± 0.17
Elevated CO_2 -unfertilized	3.24 ± 0.44	2.94 ± 1.60	2.45 ± 0.29	3.15 ± 0.19	3.43 ± 0.06
Elevated CO_2 -fertilized	3.08 ± 0.41	2.80 ± 1.52	2.23 ± 0.16	2.82 ± 0.17	3.19 ± 0.38
Transpiration rate E_W (mmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	0.22 ± 0.01	0.16 ± 0.10	0.11 ± 0.01	0.13 ± 0.02	0.14 ± 0.01
385 ppm CO_2 -fertilized	0.23 ± 0.01	0.17 ± 0.10	0.12 ± 0.02	0.15 ± 0.02	0.16 ± 0.01
Elevated CO_2 -unfertilized	0.22 ± 0.01	0.17 ± 0.10	0.11 ± 0.02	0.15 ± 0.02	0.16 ± 0.00
Elevated CO_2 -fertilized	0.22 ± 0.01	0.16 ± 0.10	0.12 ± 0.03	0.17 ± 0.02	0.17 ± 0.01

Tab. 4.1.2: Mean (± st.dev.) of net photosynthetic rate, transpiration rate and stomatal conductance of poplar during the second growing season (2011).

Treatments of poplar (2011)	May	June	July	August	September
Photosynthetic rate A_C (µmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	10.73 ± 1.50	4.51 ± 1.42	5.55 ± 2.24	5.37 ± 2.63	5.92 ± 0.83
385 ppm CO_2 -fertilized	11.52 ± 1.48	4.63 ± 1.17	7.12 ± 1.27	9.10 ± 1.40	8.67 ± 0.97
Elevated CO_2 -unfertilized	12.63 ± 2.42	6.90 ± 2.72	6.56 ± 2.74	5.61 ± 1.33	3.04 ± 1.04
Elevated CO_2 -fertilized	12.48 ± 2.19	10.77 ± 2.31	6.64 ± 2.52	8.24 ± 3.31	3.84 ± 1.59
Transpiration rate E_W (mmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	2.81 ± 0.47	3.11 ± 0.85	3.52 ± 0.78	3.81 ± 1.63	3.43 ± 0.64
385 ppm CO_2 -fertilized	3.47 ± 1.02	2.40 ± 0.96	4.07 ± 0.85	5.03 ± 1.05	4.90 ± 0.54
Elevated CO_2 -unfertilized	3.48 ± 0.82	3.30 ± 1.00	4.47 ± 0.60	3.80 ± 0.77	2.63 ± 0.32
Elevated CO_2 -fertilized	2.99 ± 0.97	2.35 ± 1.21	4.06 ± 0.65	4.16 ± 1.28	3.21 ± 0.83
Stomatal conductance g_S (mol_{H2O} m^{-2} leaf area s^{-1})					
385 ppm CO_2 -unfertilized	0.22 ± 0.04	0.22 ± 0.07	0.31 ± 0.05	0.29 ± 0.09	0.31 ± 0.09
385 ppm CO_2 -fertilized	0.31 ± 0.12	0.19 ± 0.09	0.34 ± 0.09	0.40 ± 0.06	0.46 ± 0.07
Elevated CO_2 -unfertilized	0.31 ± 0.10	0.23 ± 0.07	0.36 ± 0.07	0.36 ± 0.08	0.22 ± 0.04
Elevated CO_2 -fertilized	0.26 ± 0.10	0.19 ± 0.15	0.32 ± 0.07	0.41 ± 0.11	0.26 ± 0.05

Tab. 4.1.3: Statistical strength of the effects of fertilization and CO_2 concentrations on the rates of net photosynthesis and transpiration and on stomatal conductance between the experimental variants of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar		Photosynthetic rate	Transpiration rate	Stomatal-conductance
CO_2 concentration	**2010**	***	**n.s.**	--
	2011	**n.s.**	**n.s.**	**n.s.**
Fertilization	**2010**	**n.s.**	**n.s.**	--
	2011	**n.s.**	**n.s.**	**n.s.**
Interaction effect between CO_2 level and fertilization	**2010**	**n.s.**	**n.s.**	--
	2011	**n.s.**	**n.s.**	**n.s.**

4.1.1.4 Chlorophyll concentration index (CCI)

In both years, fertilization caused a significantly higher chlorophyll concentration index (CCI) as compared to unfertilized plants (Fig. 4.1.4; Tables 4.1.4 and 4.1.5); particularly at the end of the growing seasons, the difference between fertilized and unfertilized plants became most obvious. In contrast, the CO_2 content remained meaningless for the CCI.

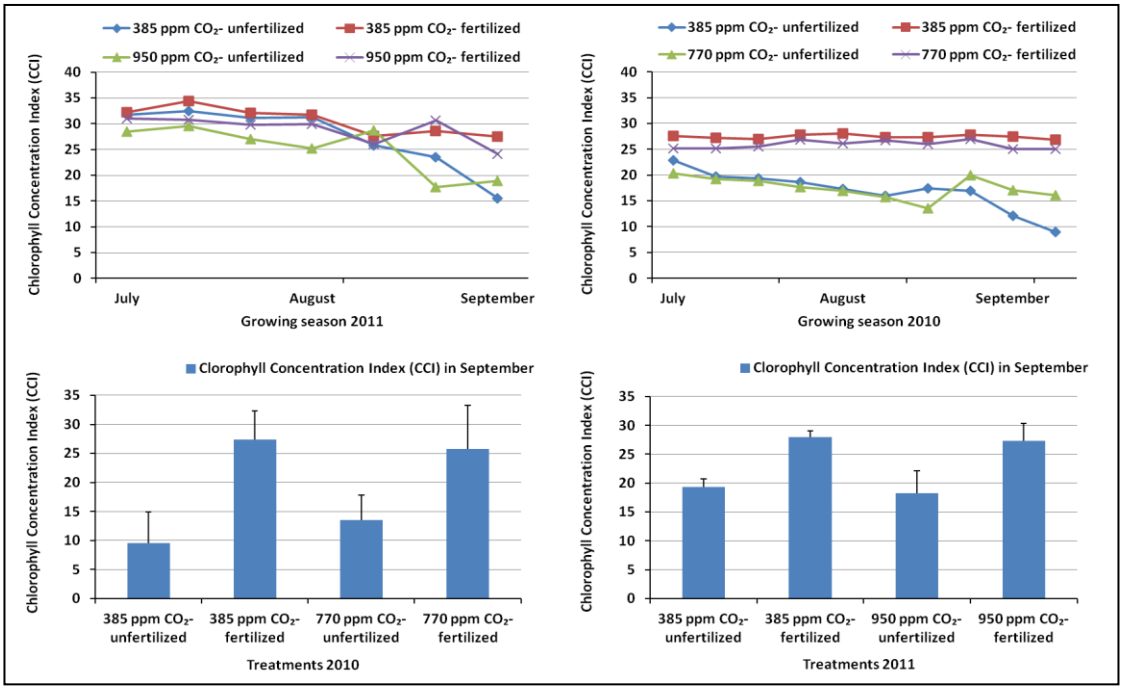

Fig. 4.1.4: Chlorophyll concentration index of poplar during (top) and at the end of both growing seasons (below).

Tab. 4.1.4: Mean (± st.dev.) of chlorophyll concentration index (CCI) of poplar during both growing seasons.

Treatments of poplar	Chlorophyll concentration index (CCI) (Mean ± st.dev.)		
	July	August	September
(2010)			
385 ppm CO_2 -unfertilized	21.31 ± 3.3	17.81 ± 4.0	9.52 ± 5.4
385 ppm CO_2 -fertilized	27.40 ± 6.0	27.60 ± 5.9	27.37 ± 4.9
Elevated CO_2 -unfertilized	19.81 ± 4.8	17.30 ± 3.3	13.47 ± 4.3
Elevated CO_2 -fertilized	25.12 ± 6.6	26.34 ± 6.7	25.72 ± 7.5
(2011)			
385 ppm CO_2 -unfertilized	32.07 ± 1.9	29.43 ± 0.1	19.36 ± 1.4
385 ppm CO_2 -fertilized	33.30 ± 2.5	30.50 ± 1.4	28.00 ± 1.0
Elevated CO_2 -unfertilized	29.02 ± 2.8	27.00 ± 3.7	18.29 ± 3.8
Elevated CO_2 -fertilized	30.81 ± 2.5	29.28 ± 3.8	27.34 ± 3.0

Tab. 4.1.5: Statistical strength of the effects of fertilization and CO_2 concentrations on the chlorophyll concentration index (CCI) between the experimental variants of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Chlorophyll concentration index (CCI)	
	2010	2011
CO_2 concentration	n.s.	n.s.
Fertilization	***	***
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.1.1.5 Correlation between the CCI and the nitrogen content in the leaf at the end of both growing seasons

The correlation between the CCI and the nitrogen content in the leaf of poplar (Tab. 4.1.6) was strongly positive (Fig. 4.1.5).

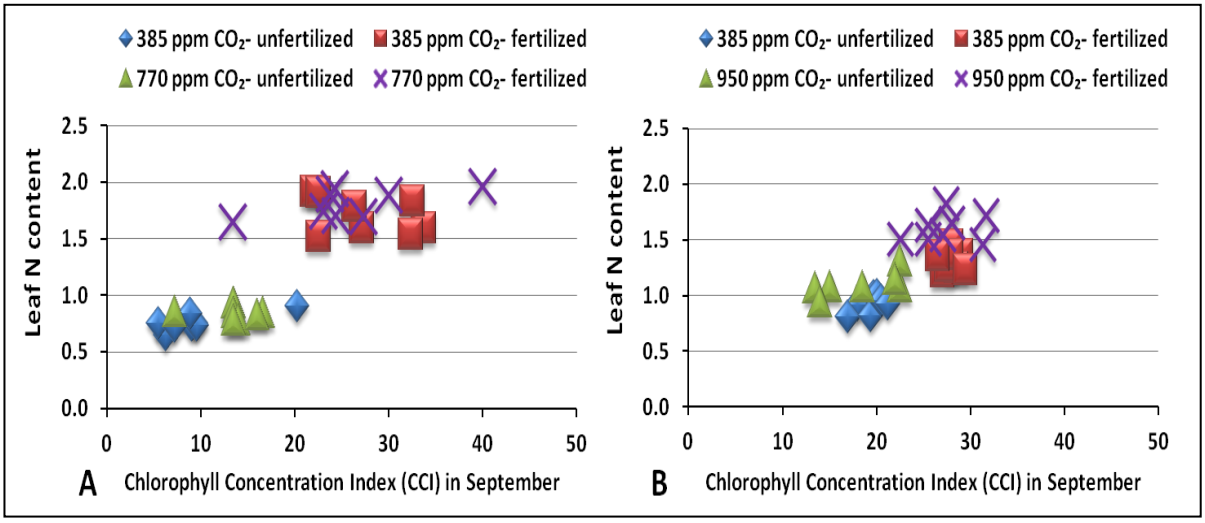

Fig.4.1.5: Correlation between the CCI and the leaf nitrogen content in the leaf of poplar saplings at the end of the first (left) and the second (right) growing season.

Tab. 4.1.6: Correlations among the CCI and the nitrogen content in the leaf of poplar.

Treatments of poplar		leaf nitrogen content
2010	**leaf chlorophyll concentration index (CCI)**	**0.863****
2011	**leaf chlorophyll concentration index (CCI)**	**0.757****

4.1.1 Seasonal measurements of beech in the greenhouse

4.1.2.1 Net photosynthesis rate

During both growing seasons, the net photosynthesis rate of the saplings calculated from the CO_2 absorption was measured (Fig. 4.1.6; Tab. 4.1.7 and 4.1.8). In 2010, higher photosynthesis rates were observed in plants (fertilized-unfertilized) grown under elevated CO_2 (770 ppm). This positive effect was statistically significant. In contrast, any effect by fertilization or by interaction between CO_2 concentration and fertilization were insignificant (Tab. 4.1.9). In 2011, the variation of the net photosynthesis rate differed from the pattern in the first growing season. The highest rate occurred with the fertilized saplings grown under elevated CO_2 (950 ppm); nevertheless, this positive effect of elevated CO_2 was statistically not significant (Fig. 4.1.6; right). The net photosynthesis rate of fertilized plants grown under different CO_2 concentrations was significantly higher than in unfertilized plants during the whole growing season. Any interaction between CO_2 concentration and fertilization was insignificant (Tab. 4.1.9).

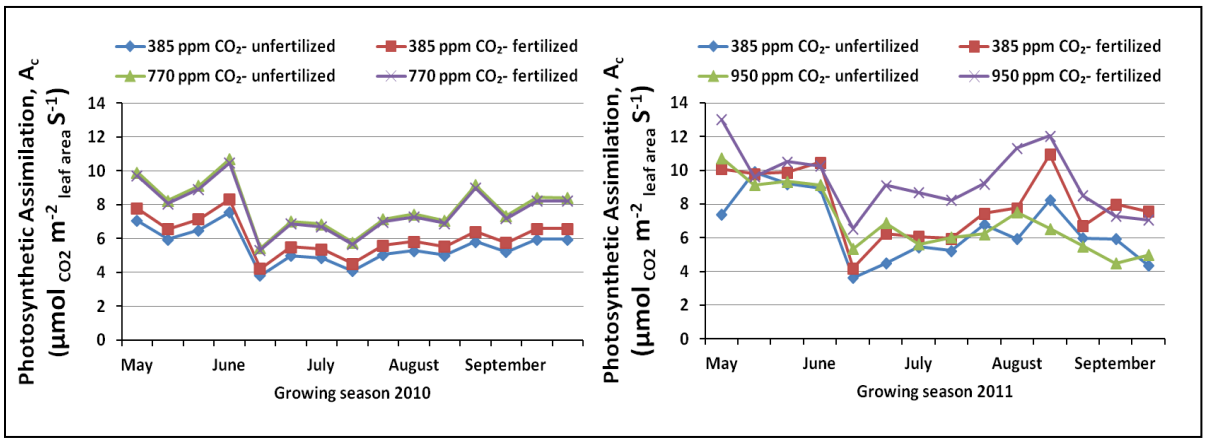

Fig. 4.1.6: Net photosynthesis rate of beech during the first (left) and the second (right) growing season.

4.1.2.2 Transpiration rate

In 2010, no significant difference of transpiration was detected between different treatments (Fig. 4.1.7). In other words, the transpiration rate did not respond to the different growth conditions given (Tab. 4.1.9). In 2011, the transpiration of saplings grown under elevated CO_2 was statistically less than of plants in ambient air. The transpiration rate of unfertilized plants grown under different CO_2 concentration was also less than of fertilized plants (Fig. 4.1.7). Elevated CO_2 significantly decreased the transpiration; in contrast, fertilization caused a significant increase of transpiration (Tab. 4.1.9).

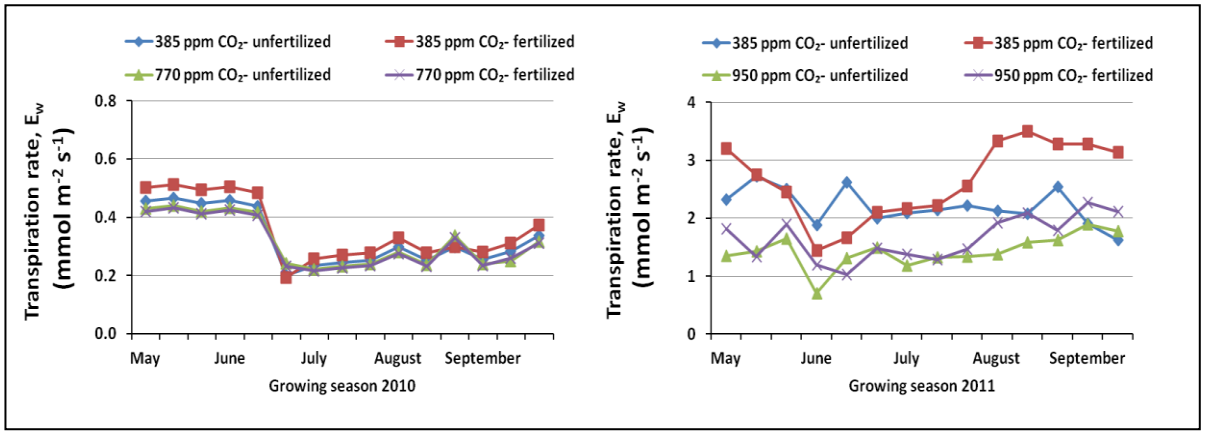

Fig. 4.1.7: Transpiration rate of beech during the first (left) and the second (right) growing season.

4.1.2.3 Stomatal conductance

The stomatal conductance of the leaves was measured only during the second growing season (Fig. 4.1.8; Tab. 4.1.8). Between the stomatal conductance and the transpiration, a similar trend was observed. That means, elevated CO_2 caused a reduction and fertilization an increase of the stomatal conductance (Tab. 4.1.9). There was no interaction between the CO_2 concentration and fertilization.

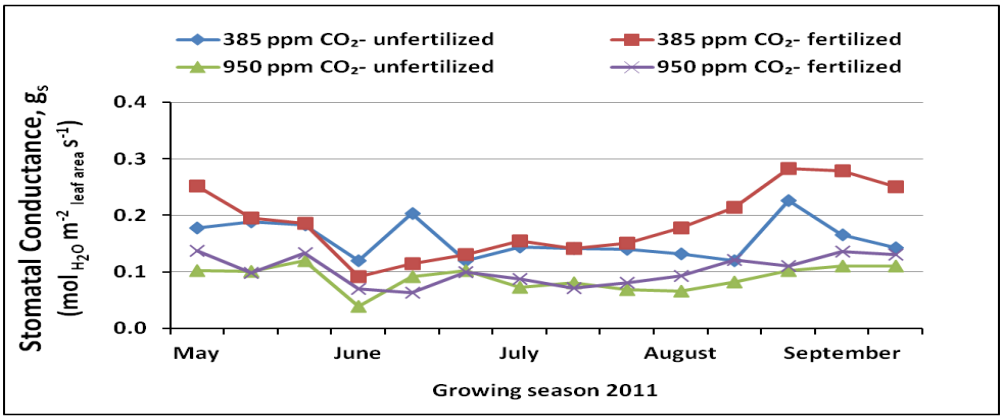

Fig. 4.1.8: Stomatal conductance of beech during the second growing season.

Tab. 4.1.7: Mean (± st.dev.) of net photosynthesis rate and transpiration rate of beech during the first growing season (2010).

Treatments of beech (2010)	May	June	July	August	September
Photosynthetic rate A_C (µmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	6.50 ± 0.55	5.46 ± 1.90	4.68 ± 0.51	5.37 ± 0.42	5.72 ± 0.44
385 ppm CO_2 -fertilized	7.17 ± 0.61	6.02 ± 2.09	5.17 ± 0.56	5.92 ± 0.45	6.31 ± 0.48
Elevated CO_2 -unfertilized	9.10 ± 0.83	7.72 ± 2.69	6.59 ± 0.71	7.89 ± 1.13	8.06 ± 0.62
Elevated CO_2 -fertilized	8.91 ± 0.81	7.56 ± 2.64	6.46 ± 0.70	7.73 ± 1.11	7.89 ± 0.60
Transpiration rate E_W (mmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	0.46 ± 0.01	0.37 ± 0.14	0.24 ± 0.01	0.28 ± 0.03	0.29 ± 0.04
385 ppm CO_2 -fertilized	0.50 ± 0.01	0.39 ± 0.17	0.27 ± 0.01	0.30 ±0.03	0.32 ± 0.05
Elevated CO_2 -unfertilized	0.43 ± 0.01	0.36 ± 0.11	0.23 ± 0.01	0.28 ± 0.05	0.27 ± 0.04
Elevated CO_2 -fertilized	0.42 ± 0.01	0.35 ± 0.11	0.22 ± 0.01	0.28 ± 0.05	0.27 ± 0.04

Tab. 4.1.8: Mean (± st.dev.) of net photosynthetic rate, transpiration rate and stomatal conductance of beech during the second growing season (2011).

Treatments of beech (2011)	May	June	July	August	September
Photosynthetic rate A_C (µmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	8.83 ± 1.31	5.70 ± 2.86	5.83 ± 0.84	5.00 ± 1.63	5.42 ± 0.92
385 ppm CO_2 -fertilized	9.92 ± 0.15	6.98 ± 3.20	6.49 ± 0.84	6.52 ± 2.25	7.42 ± 0.66
Elevated CO_2 -unfertilized	9.74 ± 0.87	7.13 ± 1.91	5.96 ± 0.31	4.79 ± 0.68	4.99 ± 0.50
Elevated CO_2 -fertilized	11.06 ± 1.75	8.64 ± 1.90	8.72 ± 0.49	7.95 ± 0.51	7.62 ± 0.78
Transpiration rate E_W (mmol $m^{-2}s^{-1}$)					
385 ppm CO_2 -unfertilized	2.51 ± 0.20	2.17 ± 0.40	2.15 ± 0.07	1.42 ± 0.04	2.02 ± 0.47
385 ppm CO_2 -fertilized	2.80 ± 0.38	1.74 ± 0.33	2.31 ± 0.21	2.35 ± 0.12	3.24 ± 0.08
Elevated CO_2 -unfertilized	1.48 ± 0.15	1.17 ± 0.42	1.28 ± 0.08	1.02 ± 0.14	1.76 ± 0.14
Elevated CO_2 -fertilized	1.69 ± 0.30	1.24 ± 0.23	1.38 ± 0.09	1.37 ± 0.12	2.06 ± 0.24
Stomatal conductance g_S (mol_{H2O} m^{-2} leaf area s^{-1})					
385 ppm CO_2 -unfertilized	0.18 ± 0.01	0.15 ± 0.05	0.14 ± 0.00	0.08 ± 0.01	0.18 ± 0.04
385 ppm CO_2 -fertilized	0.21 ± 0.03	0.11 ± 0.02	0.15 ± 0.01	0.13 ± 0.02	0.27 ± 0.02
Elevated CO_2 -unfertilized	0.11 ± 0.01	0.08 ± 0.03	0.07 ± 0.01	0.05 ± 0.01	0.11 ± 0.01
Elevated CO_2 -fertilized	0.12 ± 0.02	0.08 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.13 ± 0.01

Tab. 4.1.9: Statistical strength of the effects of fertilization and CO_2 concentrations on net photosynthetic rate, transpiration rate and stomatal conductance between variants of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		Photosynthetic rate	Transpiration rate	Stomatal-conductance
CO_2 concentration	2010	***	n.s.	--
	2011	n.s.	***	***
Fertilization	2010	n.s.	n.s.	--
	2011	**	**	*
Interaction effect between CO_2 level and fertilization	2010	n.s.	n.s.	n.s.
	2011	n.s.	n.s.	n.s.

4.1.2.4 Chlorophyll concentration index (CCI)

The leaf chlorophyll concentration index (CCI) was measured every week from July to September (Fig. 4.1.9, Tab. 4.1.10). In both years, the CCI of fertilized plants was significantly higher than of unfertilized plants and there was no interaction between fertilization and CO_2 concentration. A significant effect of elevated CO_2 was detected only in 2011 (Tab. 4.1.11) as the CCI was reduced by elevated CO_2 (Fig. 4.1.9). At the end of the vegetation period (September) in both years, the CCI in the leaves of fertilized plants grown under different CO_2 concentration was considerably higher than of unfertilized plants.

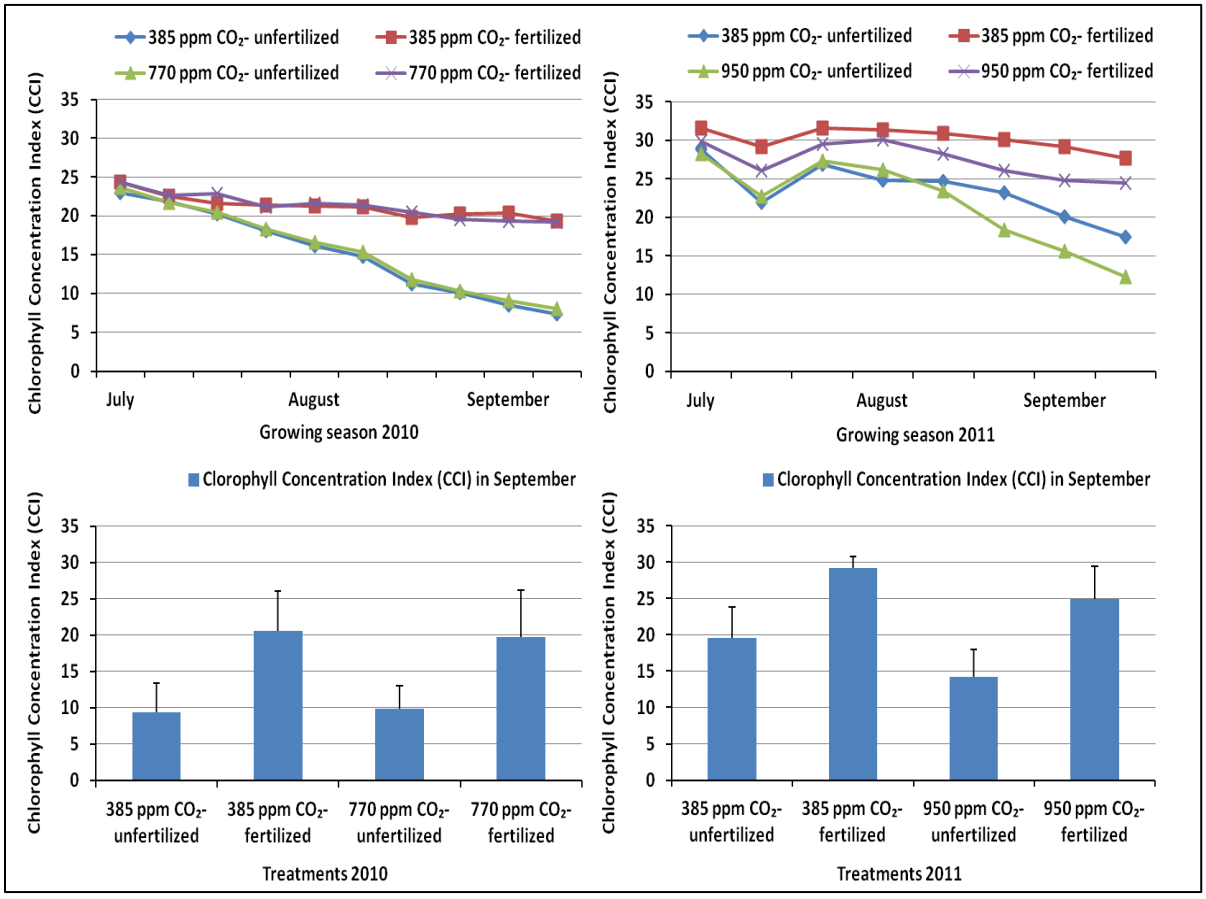

Fig. 4.1.9: Chlorophyll concentration index of beech during (top) and at the end of both growing seasons (below).

Tab. 4.1.10: Mean (± st.dev.) of Chlorophyll concentration index (CCI) of beech during both growing seasons.

Treatments of beech	Chlorophyll concentration index (CCI) (Mean ± st.dev.)		
	July	August	September
(2010)			
385 ppm CO_2 -unfertilized	**25.41 ± 3.5**	**24.91 ± 3.8**	**19.59 ± 4.3**
385 ppm CO_2 -fertilized	**30.38 ± 2.3**	**30.98 ± 1.7**	**29.23 ± 1.6**
Elevated CO_2 -unfertilized	**25.51 ± 3.8**	**23.85 ± 3.8**	**14.23 ± 3.7**
Elevated CO_2 -fertilized	**27.97 ± 3.5**	**28.47 ± 3.3**	**24.90 ± 4.5**
(2011)			
385 ppm CO_2 -unfertilized	**20.76 ± 4.9**	**15.47 ± 5.1**	**9.30 ± 4.1**
385 ppm CO_2 -fertilized	**22.43 ± 2.3**	**21.19 ± 2.5**	**20.60 ± 5.5**
Elevated CO_2 -unfertilized	**21.00 ± 2.6**	**15.97 ± 3.2**	**9.80 ± 3.3**
Elevated CO_2 -fertilized	**22.76 ± 4.2**	**21.50 ± 5.5**	**19.66 ± 6.5**

Tab. 4.1.11: Statistical strength of the effects of fertilization and CO_2 concentrations on the CCI between the experimental variants of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Chlorophyll concentration index (CCI)	
	2010	2011
CO_2 concentration	**n.s.**	*******
Fertilization	*******	*******
Interaction effect between CO_2 level and fertilization	**n.s.**	**n.s.**

4.1.2.5 Correlation between the CCI and the nitrogen content in the leaf at the end of both growing seasons

In both years, the highest values of the CCI and of the nitrogen content belonged to fertilized plants grown in ambient air (Fig. 4.1.10). Both parameters were strongly positively correlated to each other (Tab. 4.1.12).

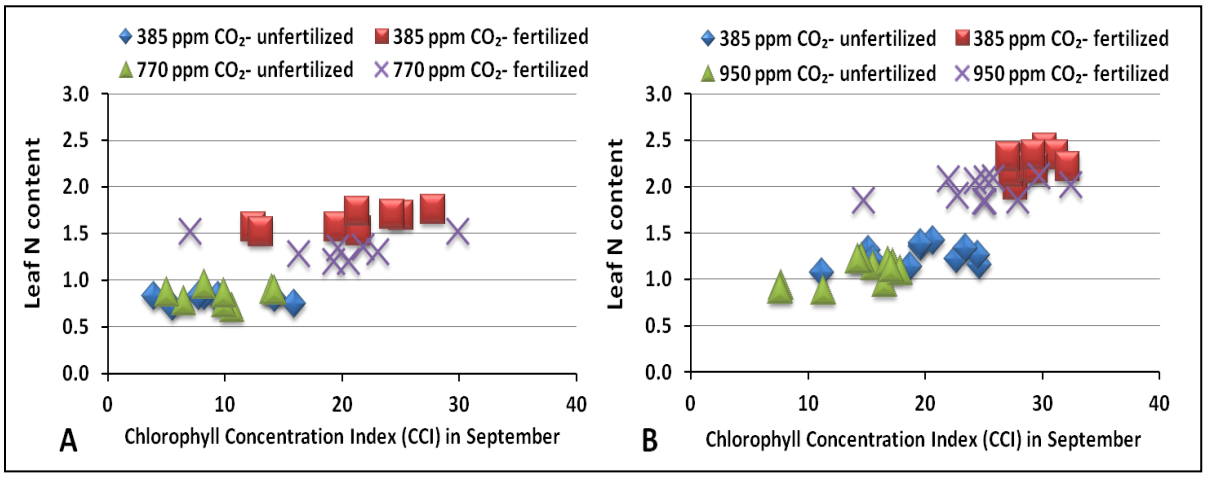

Fig. 4.1.10: Correlation between the CCI and the nitrogen content in the leaf of beech saplings at the end of the first (left) and the second (right) growing season.

Tab. 4.1.12: Correlations among the CCI and the nitrogen content in the leaf of beech.

Treatments of beech		leaf nitrogen content
2010	**leaf chlorophyll concentration index (CCI)**	**0.739****
2011	**leaf chlorophyll concentration index (CCI)**	**0.853****

4.2 Phenological and morphological analysis

4.2.1 Growth and biomass status of poplar

4.2.1.1 Tree height

At the end of each experimental year, the height of all saplings was measured and compared (Tab. 4.2.1; Fig. 4.2.1). The mean values of the four variants of treatments varied from 124.9 -165.4 cm in 2010 and from 133.3 -178 cm in 2011. The differences of the mean values between the treatments were in tendency the same in both growing seasons. In the first year, the fertilized plants, having grown under different CO_2 level, were by 21%, and in the second year by 24% taller than the unfertilized plants. These differences were highly significant (Tab. 4.2.2) whereas elevated CO_2 had no significant effect. Furthermore, there was no significant interaction effect between elevated CO_2 and fertilization.

Tab. 4.2.1: Mean (± st.dev.) height growth rate of poplar at the end of both growing season.

Treatments of poplar	Tree height (cm) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	132.3 ± 18.7	133.3 ± 13.2
385 ppm CO_2 - fertilized	165.4 ± 17.4	178.0 ± 10.3
Elevated CO_2 - unfertilized	124.9 ± 8.20	135.4 ± 21.3
Elevated CO_2 - fertilized	156.3 ± 11.0	175.6 ± 13.9

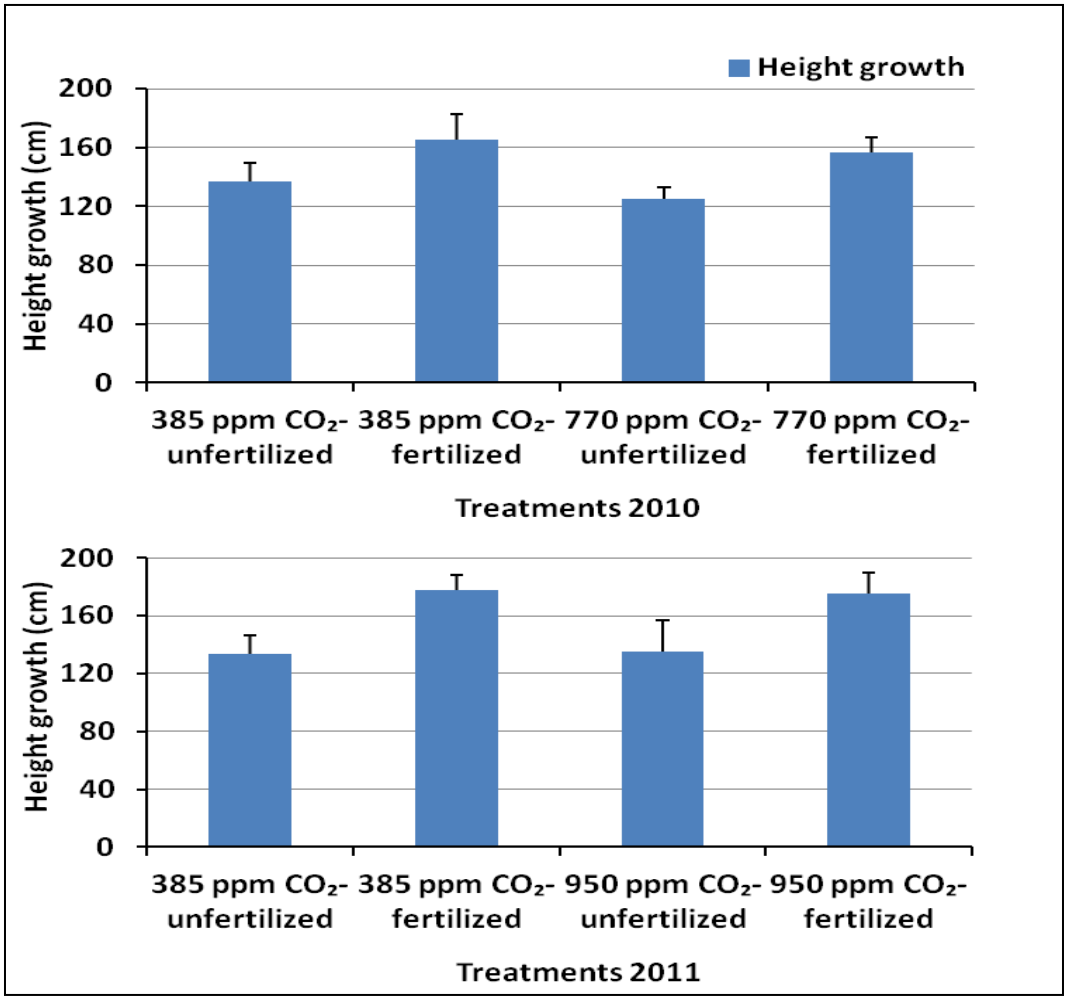

Fig. 4.2.1: Mean values of the height growth of poplar after the first (top) and the second growing season (bottom).

Tab. 4.2.2: Statistical strength of the effects of fertilization and CO_2 concentration on the height of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Tree height	
	2010	2011
CO_2 concentration	n.s.	n.s.
Fertilization	***	***
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.2.1.2 Leaf dry weight

The dry weight of leaf varied significantly between the variants of treatments from 1 - 11.7 g in 2010 and from 22.4 - 38.9 g in 2011 (Tab. 4.2.3; Fig. 4.2.2; Tab. 4.2.4). However, at the end of the first growing season, considerably less leaves have remained on the saplings of all treatments than at the end of the second growing season. Nevertheless, in both years, the dry weight of the leaves responded in tendency similarly to the various growth conditions (Fig. 4.2.2). The leaves of the unfertilized saplings grown under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) and of the fertilized saplings grown under ambient CO_2 (385 µmol CO_2 mol^{-1}) had the lowest and the highest weight, respectively, in both years (Tab. 4.2.3; Fig. 4.2.2). Fertilization caused a significant increase in the weight of leaves but this positive influence was reduced by elevated CO_2. This interaction between fertilization and CO_2 in both years was moderately to highly significant (Tab. 4.2.4).

Tab. 4.2.3: Mean (± st.dev.) leaf dry weight of poplar at the end of both growing seasons.

Treatments of poplar	Leaf dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	1.00 ± 0.2	25.3 ± 4.5
385 ppm CO_2 - fertilized	11.7 ± 2.4	38.9 ± 2.9
Elevated CO_2 - unfertilized	1.00 ± 0.3	22.4 ± 2.7
Elevated CO_2 - fertilized	7.90 ± 0.5	31.7 ± 4.0

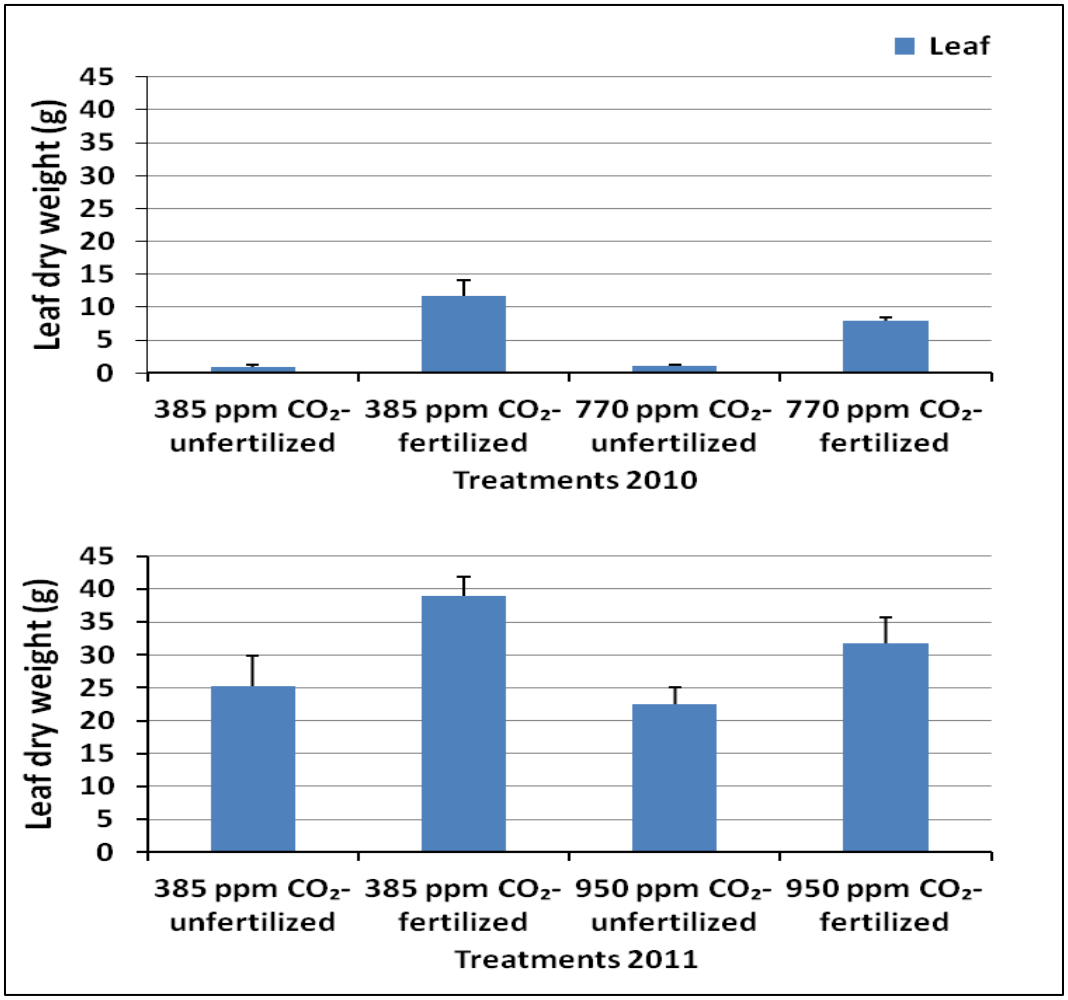

Fig. 4.2.2 Mean values of the leaf dry weight of poplar after the first (top) and the second growing season (bottom).

Tab. 4.2.4: Statistical strength of the effects of fertilization and elevated CO_2 on the leaf dry weight of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Leaf dry weight	
	2010	2011
CO_2 concentration	***	**
Fertilization	***	***
Interaction effect between CO_2 level and fertilization	***	*

4.2.1.3 Number of leaves, leaf area and leaf mass per area (LMA)

At the end of growing season 2011, the number and area of the leaves were measured and compared. The average leaf number ranged from 104.9 - 129.1 (Tab. 4.2.5). No distinct difference between treatments was obvious (Fig. 4.2.3), that is to say, the number of leaves was unaffected by the CO_2 concentration and fertilization (Tab. 4.2.6). The average leaf area varied significantly between treatments from 3554 - 5011 cm^2 (Tab. 4.2.5); both treatments, CO_2 and fertilization, caused an increase. Unfertilized plants under ambient air (control) had the smallest leaves; in contrast, the fertilized saplings under elevated CO_2 had the largest leaves (Tab. 4.2.5; Fig. 4.2.3). There were no interaction effects between fertilization and CO_2 on the leaf area. The highest and the lowest value of leaf mass per area (LMA) belonged to fertilized plants grown under ambient air and unfertilized plants under elevated CO_2, respectively. Elevated CO_2 significantly reduced the LMA; plants grown under elevated CO_2 had by 32% less LMA than plants grown under ambient air. In contrast, fertilization caused an increase in LMA (Fig. 4.2.4; Tab. 4.2.6); fertilized plants under different CO_2 concentration had about 15% more LMA.

Tab. 4.2.5: Mean (± st.dev.) number of leaves, leaf area and LMA of poplar per tree.

Treatments of poplar 2011	**Number of leaves** (Mean ± st.dev.)	**Leaf area (cm^2)** (Mean ± st.dev.)	**LMA (g/cm^2)** (Mean ± st.dev.)
385 ppm CO_2 -unfertilized	**104.9 ± 20.1**	**3554 ± 457.7**	**0.0075 ± 0.0009**
385 ppm CO_2 -fertilized	**129.1 ± 13.5**	**4564 ± 273.5**	**0.0089 ± 0.0003**
Elevated CO_2 -unfertilized	**123.3 ± 28.6**	**4253 ± 305.6**	**0.0053 ± 0.0004**
Elevated CO_2 -fertilized	**124.7 ± 17.0**	**5011 ± 199.3**	**0.0059 ± 0.0006**

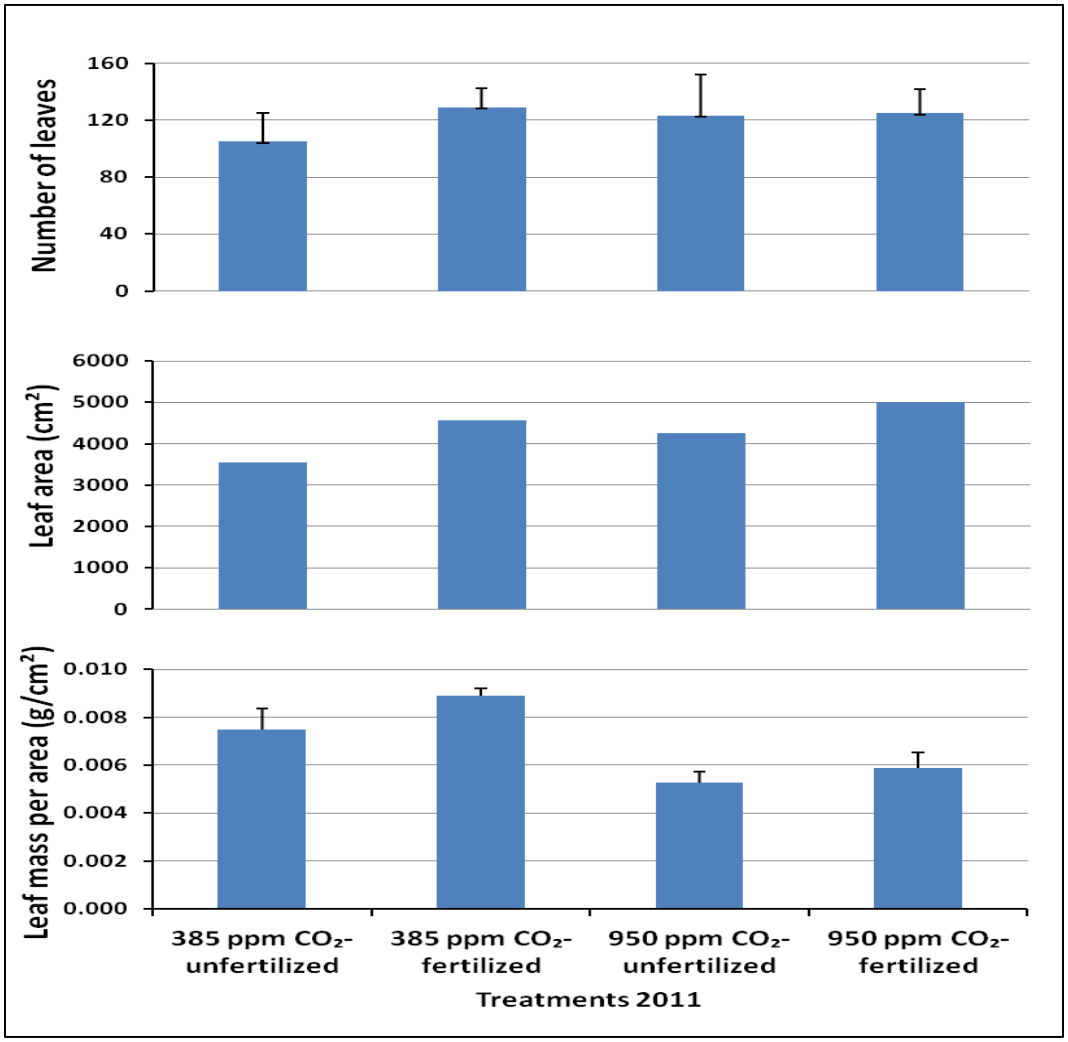

Fig. 4.2.3: Mean values of number of leaves, leaf area and LMA of poplar in 2011.

Tab. 4.2.6: Statistical strength of the effects of fertilization and elevated CO_2 on the number of leaves, leaf area and LMA of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar 2011	Number of leaves	leaf area	LMA
CO_2 concentration	n.s.	**	***
Fertilization	n.s.	***	**
Interaction effect between CO_2 level and fertilization	n.s.	n.s.	n.s.

4.2.1.4 Stem dry weight

The stem dry weight of poplar ranged from 23.9 - 44.9 g in 2010 and from 37.4 - 55.8 g in 2011 (Tab. 4.2.7). The differences of the mean values between the treatments were in tendency the same in both growing seasons. In both years, the highest and lowest values of stem weight belonged to fertilized plants grown under ambient CO_2, respectively to unfertilized plants grown under elevated CO_2 (Fig. 4.2.4). As in the case of the dry weight of leaves, elevated CO_2 significantly reduced the dry weight of the stems, whereas fertilized plants had significantly heavier stems than unfertilized plants in both growing seasons. There was no significant interaction effect between fertilization and elevated CO_2 on the stem dry weight in both of experimental seasons (Tab. 4.2.8).

4.2.7: Mean (± st.dev.) Stem dry weight of poplar at the end of both growing seasons.

Treatments of poplar	Stem dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	36.6 ± 6.0	45.7 ± 6.8
385 ppm CO_2 - fertilized	44.9 ± 9.8	55.8 ± 7.2
Elevated CO_2 - unfertilized	23.9 ± 5.0	37.4 ± 5.7
Elevated CO_2 - fertilized	38.8 ± 3.2	49.2 ± 8.8

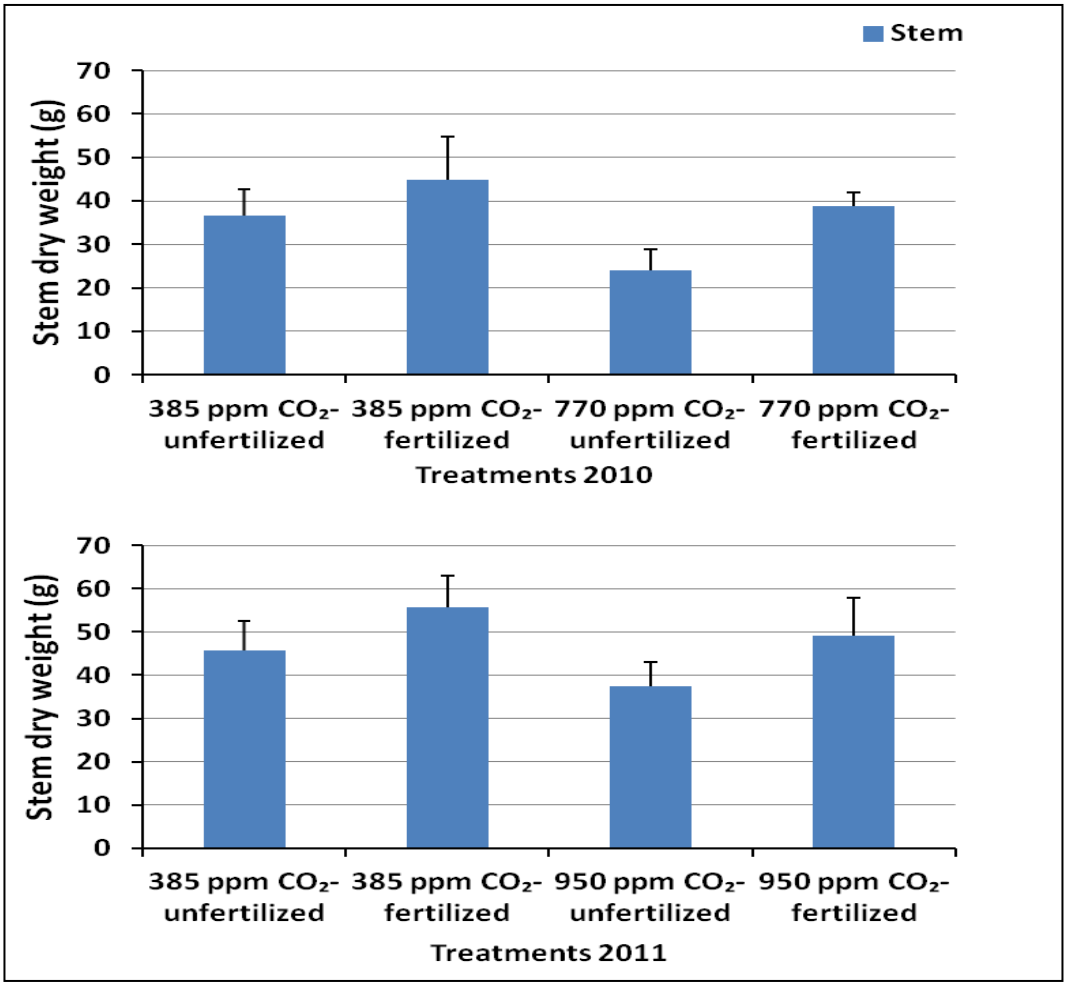

Fig. 4.2.4: Mean values of the stem dry weight of poplar after the first (top) and the second growing season (bottom).

Tab. 4.2.8: Statistical strength of the effects of fertilization and elevated CO_2 on the stem dry weight of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Stem dry weight	
	2010	2011
CO_2 concentration	*	*
Fertilization	**	*
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.2.1.5 Root dry weight

The poplar trees grown in ambient air, both unfertilized and fertilized, had heavier roots than under elevated CO_2 in both years (Tab. 4.2.9; Fig. 4.2.5). The average increase in 2010 and in 2011 was 20 and 26%, respectively. But the effect of fertilization was statistically insignificant in both years (Tab. 4.2.10). In contrast, elevated CO_2 had a significantly negative effect on root dry weight in both years.

Tab. 4.2.9: Mean (± st.dev.) root dry weight of poplar at the end of both growing seasons.

Treatments of poplar	Root dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	36.6 ± 9.80	51.1 ± 5.6
385 ppm CO_2 - fertilized	41.2 ± 10.8	56.5 ± 5.2
Elevated CO_2 - unfertilized	31.1 ± 5.40	39.5 ± 8.3
Elevated CO_2 - fertilized	30.7 ± 3.60	40.1 ± 7.6

Tab. 4.2.10: Statistical strength of the effects of fertilization and elevated CO_2 on the root weight of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Root dry weight	
	2010	2011
CO_2 concentration	**	***
Fertilization	n.s	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

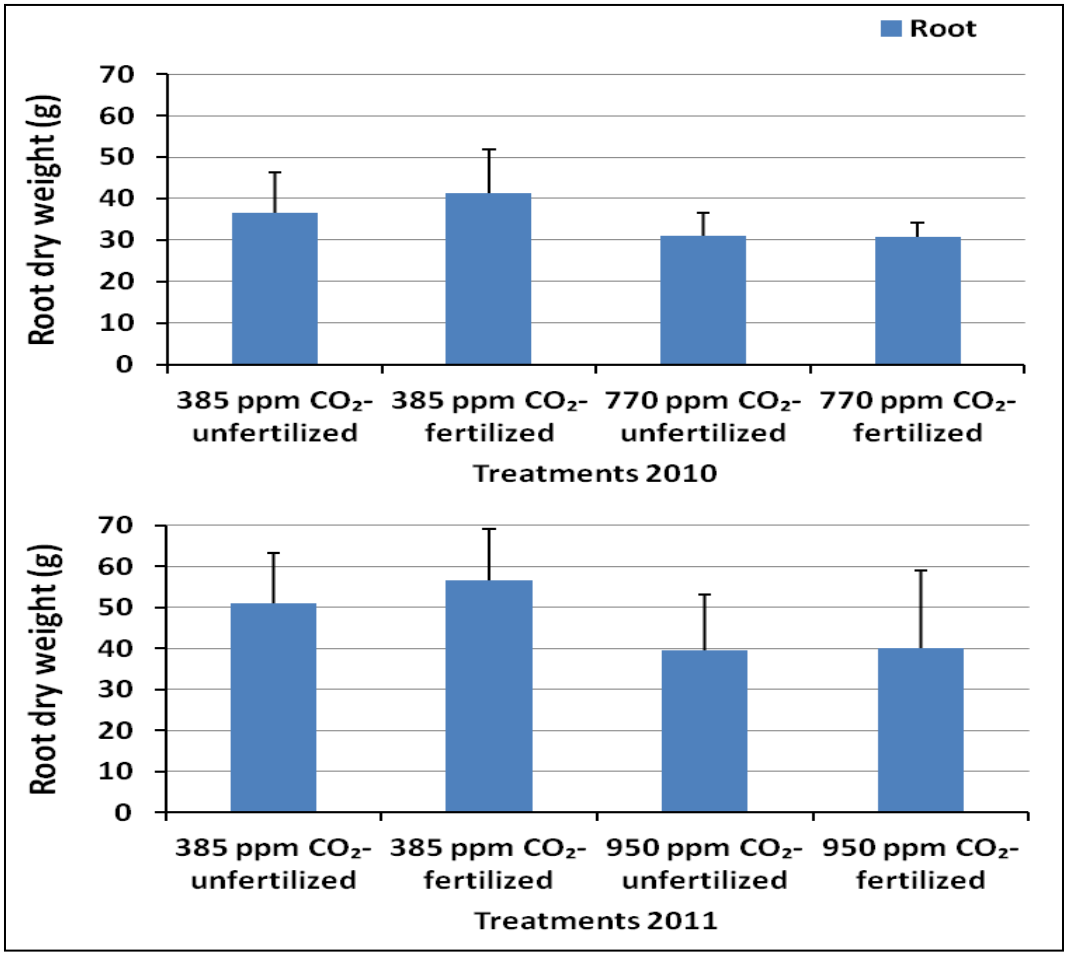

Fig. 4.2.5: Mean values of the root dry weight of poplar after the first (top) and the second growing season (bottom).

4.2.1.6 Biomass dry weight

Here, leaves, stems and roots of a sapling are understood as biomass (Fig. 4.2.6). In both experimental years, the fertilized saplings in ambient air had produced the highest amount of biomass dry weight whereas the lowest values were obtained from the unfertilized saplings grown under elevated CO_2. All three fractions of biomass dry weight were positively correlated among each other (Tab. 4.2.13).

In 2010 and 2011, plants under elevated CO_2 produced 23 and 20%, respectively, less biomass than plants grown in ambient air (Tab. 4.2.11)

Fertilization increased significantly the biomass dry weight (Tab. 4.2.12). In both years, fertilized plants grown under different CO_2 concentrations (ambient air and elevated)

produced by 26 and 19%, respectively, more biomass dry weight than unfertilized plants. There were no interaction effects between fertilization and CO_2 concentration on the dry weight of biomass (Tab. 4.2.12).

Tab. 4.2.11: Mean (± st.dev.) biomass dry weight of poplar at the end of both growing seasons.

Treatments of poplar	Biomass dry weight (gr) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	74.1 ± 14.6	122.1 ± 12.1
385 ppm CO_2 - fertilized	97.8 ± 21.7	151.2 ± 12.7
Elevated CO_2 - unfertilized	56.1 ± 8.70	99.00 ± 13.5
Elevated CO_2 - fertilized	77.4 ± 6.50	120.9 ± 18.8

Tab. 4.2.12: Statistical strength of the effects of fertilization and elevated CO_2 on the biomass dry weight of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar	Biomass dry weight	
	2010	2011
CO_2 concentration	**	***
Fertilization	***	***
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

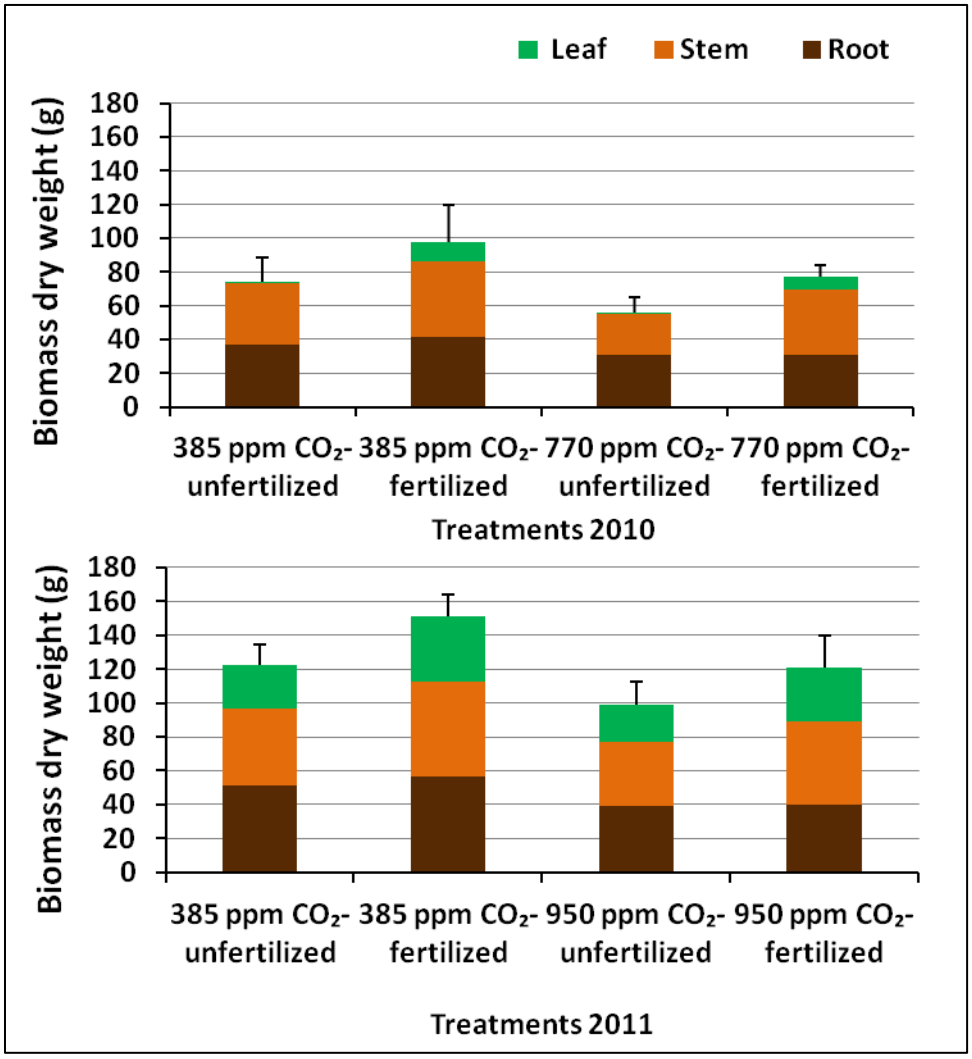

Fig. 4.2.6: Mean values of the biomass dry weight of poplar after the first (top) and the second growing season (bottom).

Tab. 4.2.13: Correlation between the dry weights of the three plant fractions.

Treatments of poplar		Stem	Leaf
2010	Root	0.66**	0.33*
	Stem		0.71**
2011	Root	0.62**	0.40*
	Stem		0.62**

4.2.2 Growth and biomass status of beech

4.2.2.1 Tree height

The height growth of beech saplings ranged from 47.3 - 54.5 cm between treatments in 2010 and from 41.6 - 44.3 cm in 2011 (Tab. 4.2.14). No distinct differences between treatments were obvious (Fig. 4.2.7); this was supported by statistical analysis (Tab. 4.15). In conclusion, height growth of beech did not respond to the different growth conditions.

Tab. 4.2.14: Mean (± st.dev.) height growth rate of beech at the end of both growing seasons

Treatments of beech	Tree height (cm) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	47.8 ± 9.0	42.8 ± 5.4
385 ppm CO_2 - fertilized	47.3 ± 5.1	43.2 ± 4.6
Elevated CO_2 - unfertilized	48.8 ± 8.8	44.3 ± 5.5
Elevated CO_2 - fertilized	54.5 ± 2.0	41.6 ± 6.8

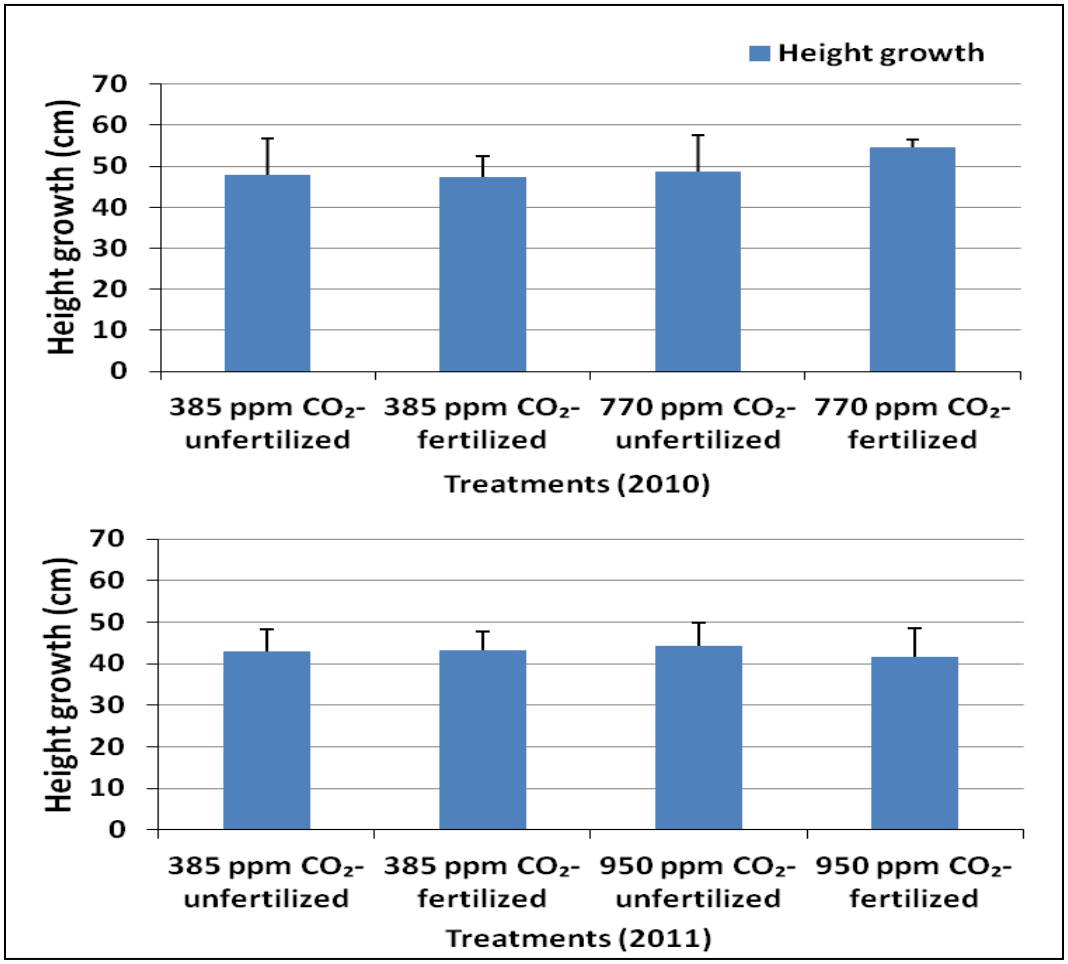

Fig. 4.2.7: Mean values of the height growth of beech after the first (top) and the second growing season (bottom).

Tab. 4.2.15: Statistical strength of the effects of fertilization and CO_2 concentration on the height of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Tree height	
	2010	2011
CO_2 concentration	n.s.	n.s.
Fertilization	n.s.	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.2.2.2 Leaf dry weight

No distinct treatment-related differences between the mean values of leaf dry weight were observed in 2010 (1.6 - 1.9 g) (Fig. 4.2.8; Tab. 4.2.16); even the slightly higher weight the in the case of the fertilized plants under elevated CO_2 is not significant (Tab. 4.2.17). But in 2011, the dry weight of the leaves varied significantly among treatments (Tab. 4.2.17). Unfertilized plants under ambient air (control) had the highest value; in contrast, the unfertilized saplings under elevated CO_2 had the lowest value. In 2011, leaf dry weight was significantly smaller under elevated CO_2 than under ambient air. Although no significant influence of fertilization on dry weight of leaves was detected, the interaction between CO_2 and fertilization was significant. This means that fertilization alone had no effect on dry weight but under elevated CO_2 the difference between the dry weight of leaves of unfertilized and fertilized plants was significant.

Tab. 4.2.16: Mean (± st.dev.) leaf dry weight of beech at the end of both growing seasons.

Treatments of beech	Leaf dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	1.6 ± 0.3	3.6 ± 0.7
385 ppm CO_2 - fertilized	1.6 ± 0.2	2.9 ± 0.3
Elevated CO_2 - unfertilized	1.7 ± 0.1	2.7 ± 0.4
Elevated CO_2 - fertilized	1.9 ± 0.4	3.1 ± 0.2

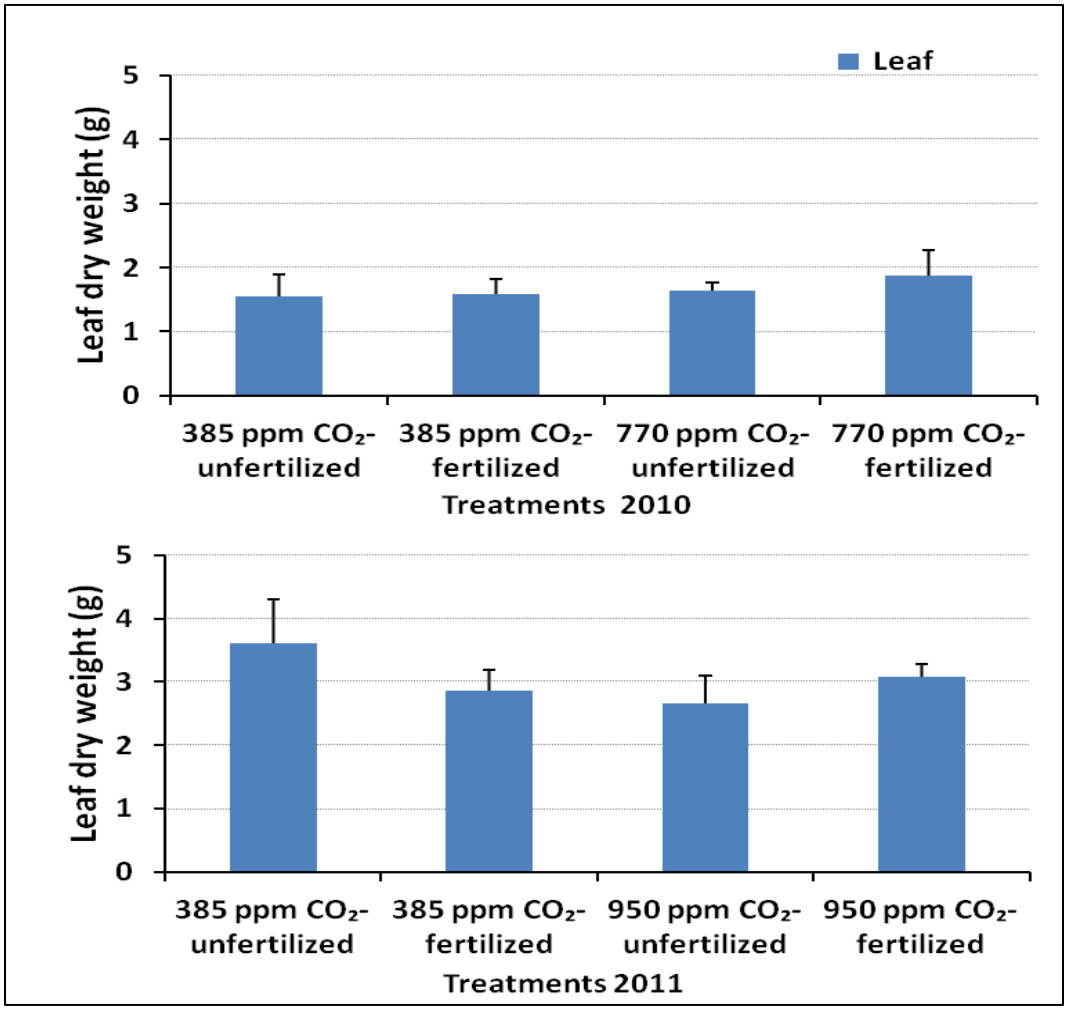

Fig. 4.2.8: Mean values of the leaf dry weight of beech after the first (top) and the second growing season (bottom).

Tab. 4.2.17: Statistical strength of the effects of fertilization and CO_2 concentration on the leaf dry weight of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Leaf dry weight	
	2010	2011
CO_2 concentration	n.s.	*
Fertilization	n.s.	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	***

4.2.2.3 Number of leaves, leaf area and leaf mass per area (LMA)

The number of leaves and the leaf area of the saplings were measured only at the end of the growing season 2011. For the number of leaves, no significant variation between treatments could be discovered (Tab. 4.2.19; Fig. 4.2.9). The leaf area ranged from 300 – 346.5 cm² (Tab. 4.2.18; Fig. 4.2.9), but these differences were insignificant. LMA varied significantly between different treatments. Plants grown under elevated CO_2 had about 20% less LMA in comparison with plants grown under ambient air. But LMA was unaffected by fertilization (Tab. 4.2.19), also no interaction was detected between CO_2 and fertilization.

Tab. 4.2.18: Mean (± st.dev.) number of leaves, leaf area and LMA of beech per tree.

Treatments of beech 2011	**Number of leaves** (Mean ± st.dev.)	**Leaf area (cm²)** (Mean ± st.dev.)	**LMA (g/cm²)** (Mean ± st.dev.)
385 ppm CO_2 -unfertilized	**20.9 ± 6.57**	**300.1 ± 65.23**	**0.0124 ± 0.0029**
385 ppm CO_2 -fertilized	**22.3 ± 7.20**	**300.0 ± 88.81**	**0.0102 ± 0.0020**
Elevated CO_2 -unfertilized	**19.9 ± 4.38**	**314.3 ± 60.23**	**0.0090 ± 0.0013**
Elevated CO_2 -fertilized	**24.8 ± 7.39**	**346.5 ± 79.54**	**0.0088 ± 0.0016**

Tab. 4.2.19: Statistical strength of the effects of fertilization and elevated CO_2 on the number of leaves, leaf area and LMA of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech 2011	**Number of leaves**	**Leaf area**	**LMA**
CO_2 concentration	**n.s.**	**n.s.**	******
Fertilization	**n.s.**	**n.s.**	**n.s.**
Interaction effect between CO_2 level and fertilization	**n.s.**	**n.s.**	**n.s.**

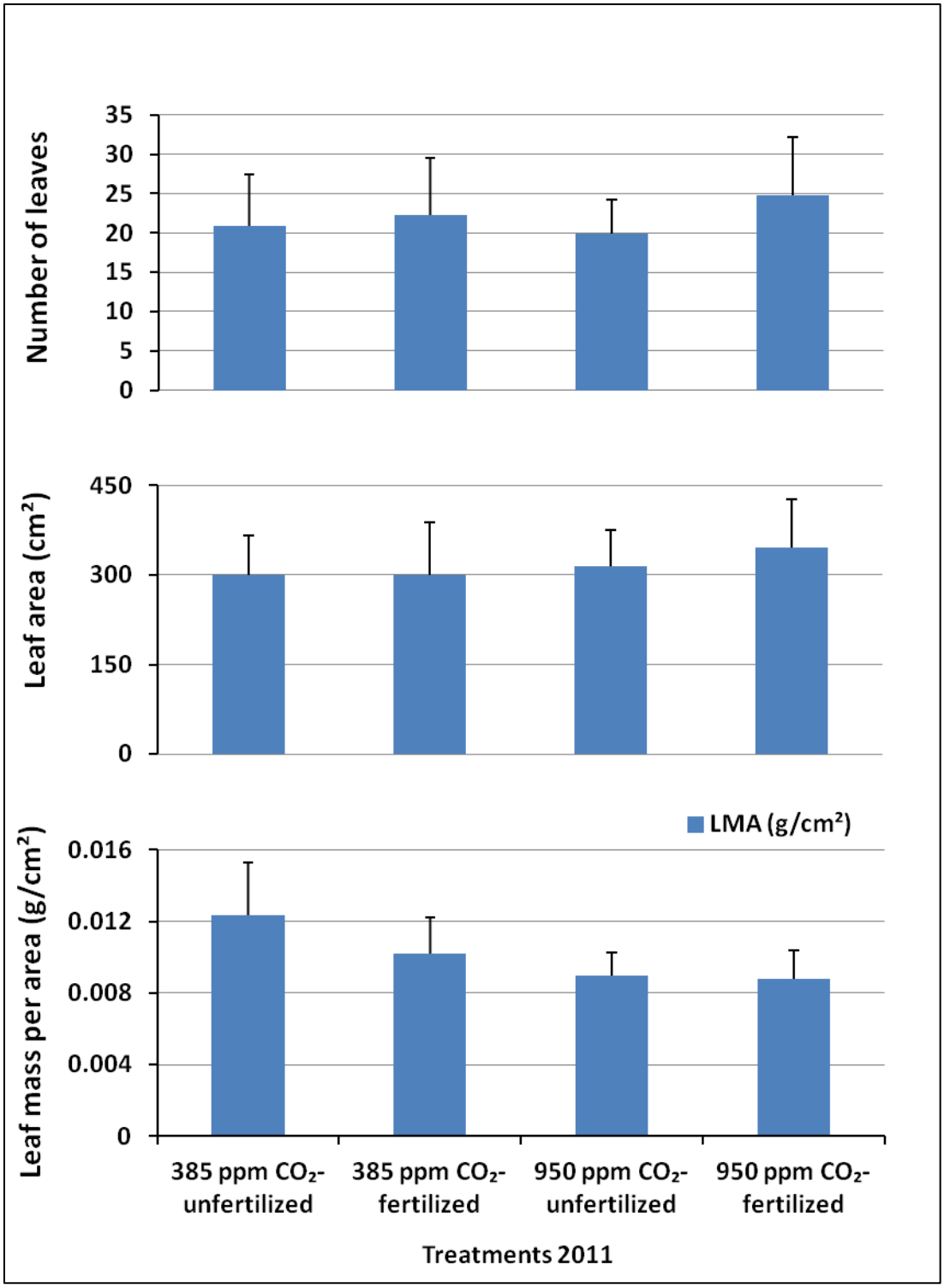

Fig. 4.2.9: Mean values of the number of leaves, leaf area and LMA of beech in 2011.

4.2.2.4 Stem dry weight

In 2010, the mean stem dry weight varied from 5.4 - 7.5 g whereas in 2011 the variation ranged from only 4.1 - 4.7 g (Tab. 4.2.20). The variation of stem dry weight between treatments showed a similar pattern in both growing seasons (Fig. 4.2.10), that is to say, plants under elevated CO_2 produced heavier stems (Tab. 4.2.20). The differences between fertilized and unfertilized plants grown under different CO_2 levels (elevated-ambient) were not significant (Tab. 4.2.21). In other words, fertilization did not affect stem dry weight in both experimental years. Interactions between CO_2 concentration and fertilization did not occur.

Tab. 4.2.20: Mean (± st.dev.) stem dry weight of beech at the end of both growing seasons.

Treatments of beech	Stem dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	5.9 ± 1.3	4.1 ± 1.0
385 ppm CO_2 - fertilized	5.4 ± 1.4	4.2 ± 1.0
Elevated CO_2 - unfertilized	6.6 ± 1.3	4.7 ± 0.7
Elevated CO_2 - fertilized	7.5 ± 2.5	4.7 ± 0.7

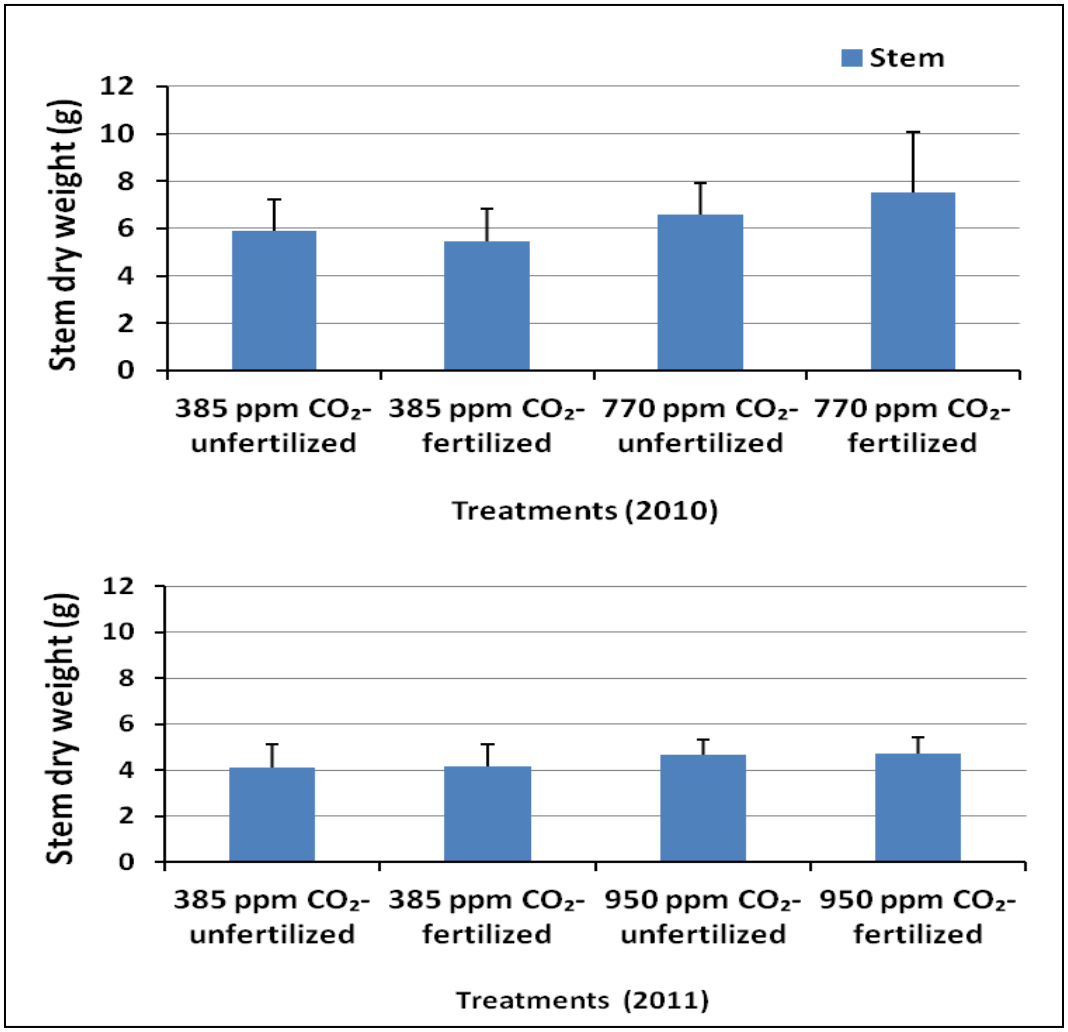

Fig. 4.2.10: Mean values of the stem dry weight of beech after the first (top) and the second growing season (bottom).

Tab. 4.2.21: Statistical strength of the effects of fertilization and CO_2 concentration on the stem dry weight of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Stem dry weight	
	2010	2011
CO_2 concentration	*	*
Fertilization	n.s.	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.2.2.5 Root dry weight

In both growing seasons, there were no significant differences between the mean values of root dry weight grown under different treatments (Tab. 4.2.22; 4.2.23; Fig. 4. 2.11). That is to say, root dry weight of beech was unaffected by CO_2 concentration and fertilization.

Tab. 4.2.22: Mean (± st.dev.) root dry weight of beech at the end of both growing seasons

Treatments of beech	Root dry weight (g) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	10.7 ± 0.8	9.6 ± 1.7
385 ppm CO_2 - fertilized	11.7 ± 2.7	9.5 ± 2.5
Elevated CO_2 - unfertilized	11.0 ± 1.6	10.1 ± 1.5
Elevated CO_2 - fertilized	11.6 ± 1.8	10.3 ± 1.2

Tab. 4.2.23: Statistical strength of the effects of fertilization and CO_2 concentration on the root dry weight of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Root dry weight	
	2010	2011
CO_2 concentration	n.s.	n.s.
Fertilization	n.s.	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

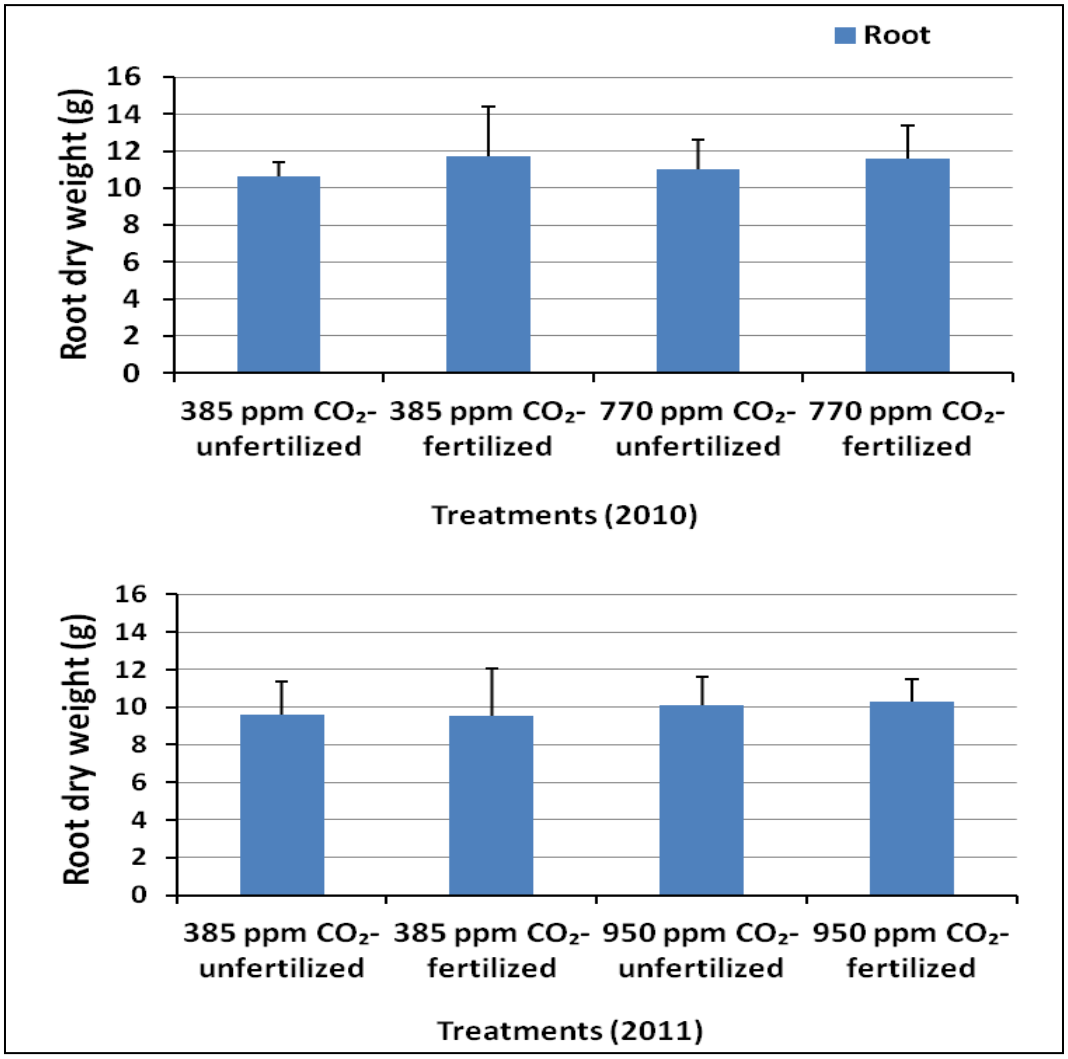

Fig. 4.2.11: Mean values of the root dry weight of beech after the first (top) and the second growing season (bottom).

4.2.2.6 Biomass dry weight

In 2010 and 2011, the total biomass weight ranged from 18.1 – 21 g and from 16.6 - 18.1 g, respectively (Tab. 4.2.24). On average, the biomass dry weight was by about 50% determined by the roots (Fig 4.2.12). The biomass dry weight was not affected by the CO_2 level and fertilization in both growing seasons. However, some differences between treatments were visible but they were insignificant (Tab. 4.2.25).

Tab. 4.2.24: Mean (± st.dev.) biomass dry weight of beech at the end of both growing seasons.

Treatments of beech	Biomass dry weight (gr) (Mean ± st.dev.)	
	2010	2011
385 ppm CO_2 - unfertilized	18.1 ± 2.0	17.3 ± 1.7
385 ppm CO_2 - fertilized	18.7 ± 3.9	16.6 ± 2.5
Elevated CO_2 - unfertilized	19.2 ± 2.1	17.4 ± 1.5
Elevated CO_2 - fertilized	21.0 ± 4.2	18.1 ± 1.2

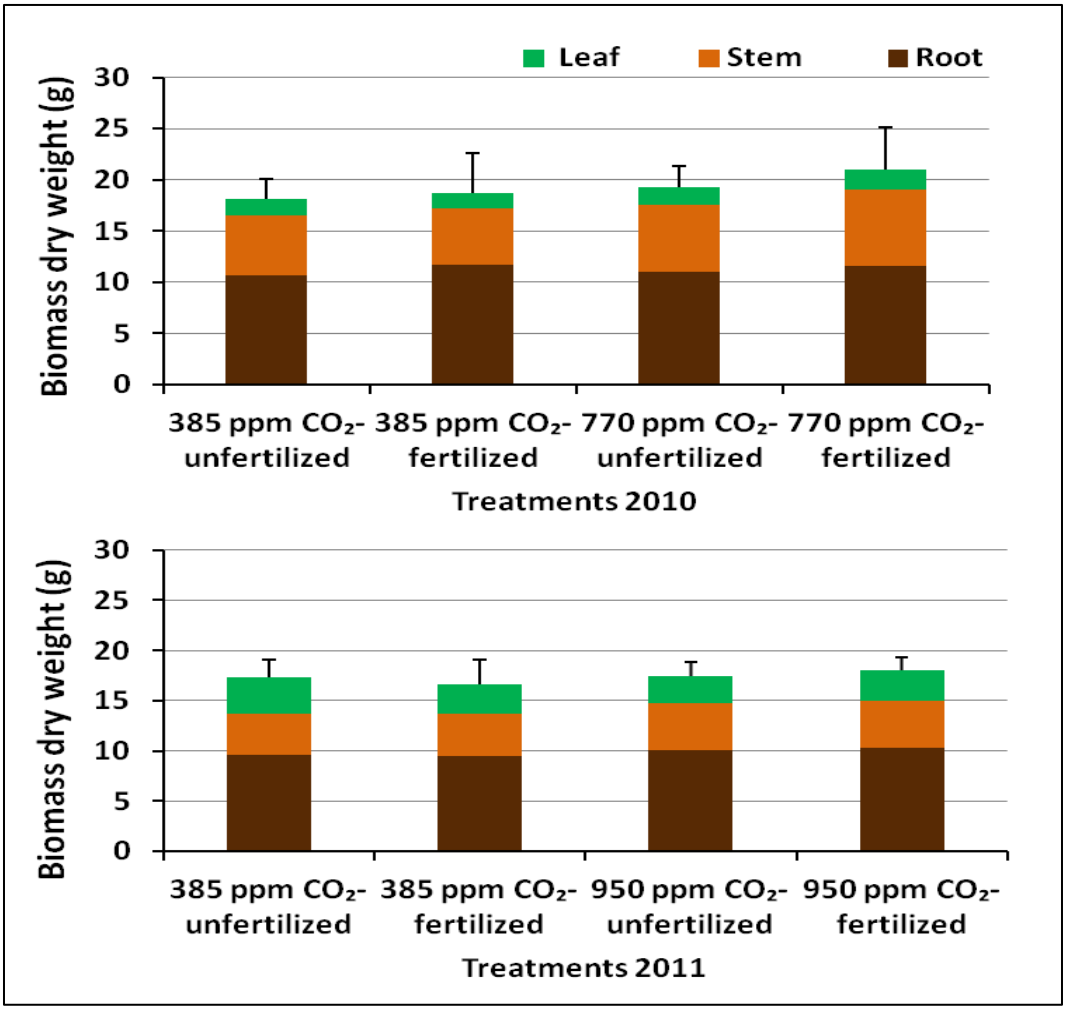

Fig. 4.2.12: Mean values of the biomass dry weight of beech after the first (top) and the second growing season (bottom).

Tab. 4.2.25: Statistical strength of the effects of fertilization and CO_2 concentration on the biomass dry weight of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech	Biomass dry weight	
	2010	2011
CO_2 concentration	n.s.	n.s.
Fertilization	n.s.	n.s.
Interaction effect between CO_2 level and fertilization	n.s.	n.s.

4.3 Biochemical analysis

4.3.1 Biochemical analysis of poplar

4.3.1.1 Nitrogen content of leaf

Leaf N content of poplar varied significantly between the treatments from 0.76 to 1.85 % in 2010 and from 0.92 to 1.65 % in 2011 (Tab. 4.3.1). In both growing seasons, the highest value of leaf N content belongs to fertilized plants grown under elevated CO_2 (Fig. 4.3.1). Fertilization and elevated CO_2 caused a significant increase in leaf N content (Tab. 4.3.2).

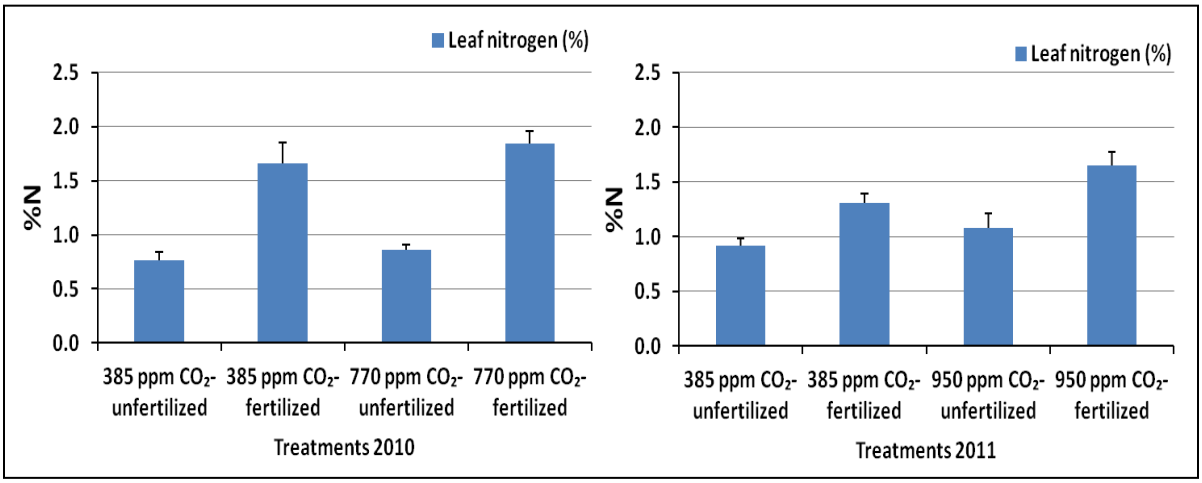

Fig. 4.3.1: Mean values of the leaf N content of poplar after the first (left) and the second growing season (right).

Tab. 4.3.1: Mean (± st.dev.) of the N content in leaf, stem and root of poplar at the end of both growing seasons.

Treatments of poplar	N content (%) (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	0.76 ± 0.08	0.17 ± 0.02	0.41 ± 0.03
385 ppm CO_2 -fertilized	1.66 ± 0.19	0.16 ± 0.02	0.66 ± 0.08
Elevated CO_2 -unfertilized	0.86 ± 0.05	0.15 ± 0.02	0.40 ± 0.01
Elevated CO_2 -fertilized	1.85 ± 0.11	0.19 ± 0.02	0.70 ± 0.07
(2011)			
385 ppm CO_2 -unfertilized	0.92 ± 0.06	0.24 ± 0.02	0.52 ± 0.02
385 ppm CO_2 -fertilized	1.30 ± 0.09	0.24 ± 0.02	0.61 ± 0.03
Elevated CO_2 -unfertilized	1.08 ± 0.13	0.20 ± 0.02	0.55 ± 0.06
Elevated CO_2 -fertilized	1.65 ± 0.13	0.23 ± 0.02	0.66 ± 0.07

Tab. 4.3.2: Statistical strength of the effects of fertilization and CO_2 concentrations on the N content of leaf, stem and root between variants of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar		N content (%)		
		Leaf	Stem (without bark)	Root
CO_2 concentration	2010	***	n.s.	n.s.
	2011	***	***	***
Fertilization	2010	***	*	***
	2011	***	*	***
Interaction effect between CO_2 level and fertilization	2010	n.s.	***	n.s.
	2011	**	n.s.	n.s.

4.3.1.2 Nitrogen content of stem

The stem (without bark) nitrogen content of poplar was very low and ranged from 0.15 - 0.19 % between treatments in 2010 and from 0.20 - 0.24 % in 2011 (Tab. 4.3.1).
The differences of the mean values between the treatments were in tendency the same in both growing seasons (Fig. 4.3.2), that means, fertilization caused only a significant increase of N in stem under elevated CO_2 (770/950 µmol CO_2 mol^{-1}); low values belong to unfertilized plants under elevated CO_2, but a negative effect of elevated CO_2 (770/950 µmol CO_2 mol^{-1}) on stem N content was significant only in 2011 (Tab. 4.3.2). Statistical analysis also shows a significant interaction effect between fertilization and CO_2 concentration in both years.

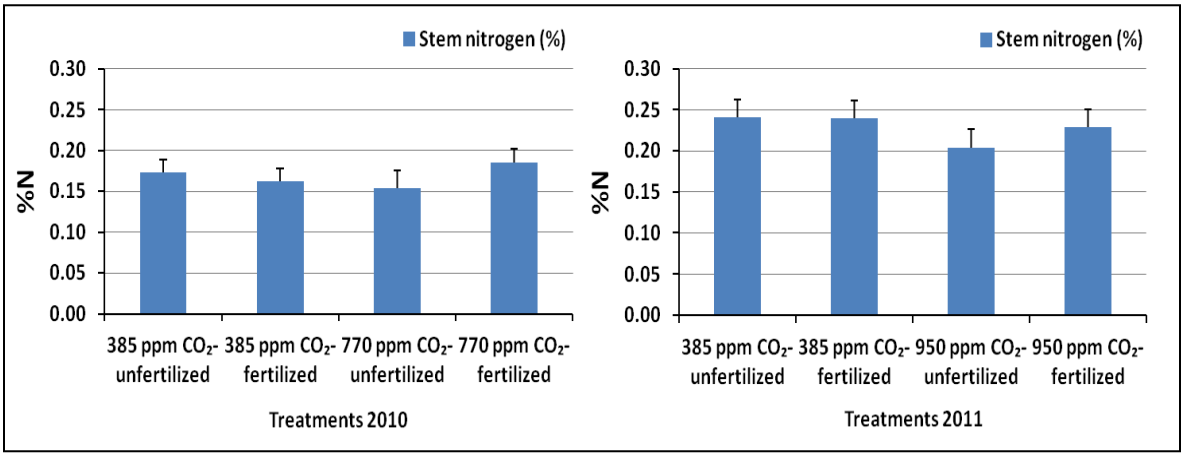

Fig. 4.3.2: Mean values of the stem N content of poplar after the first (left) and the second growing season (right).

4.3.1.3 Nitrogen content of root

In both growing seasons, N content increased significantly by fertilization (Tab. 4.3.2); fertilized plants grown under different CO_2 concentration had higher N content in their roots (Fig. 4.3.3). CO_2 concentration effect on root N content was significant only in the second growing season. No interaction effect between CO_2 concentration and fertilization was detected in both years (Tab.4.3.2).

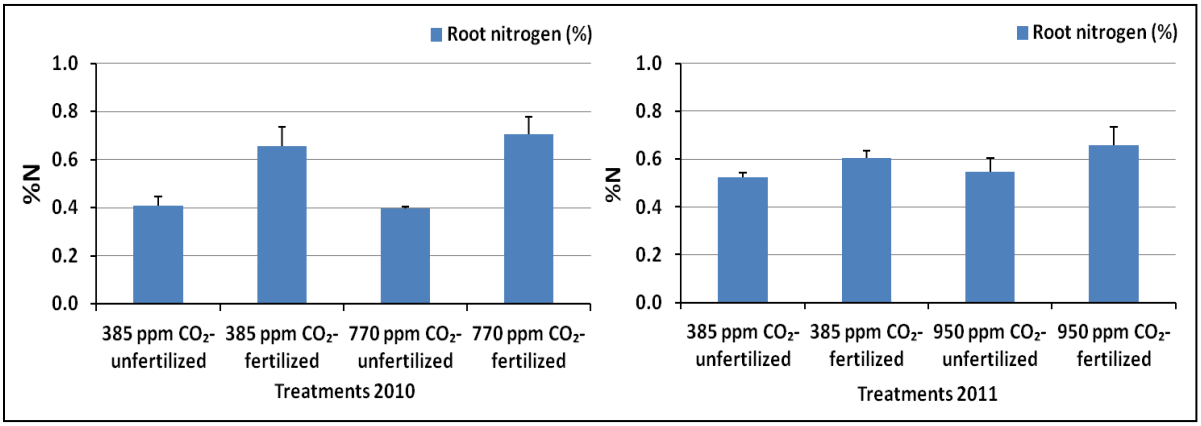

Fig. 4.3.3: Mean values of the root N content of poplar after the first (left) and the second growing season (right).

4.3.1.4 Carbon content of leaf

The carbon content in leaf varied between different treatments from 44.75 - 45.19% in 2010 and from 45.29 - 46.30% in 2011 (Tab. 4.3.3).

The carbon content in response to the different growing conditions showed different patterns in the two growing seasons (Fig. 4.3.4). In 2010, fertilized plants grown under different CO_2 had significantly less carbon in their leaves and CO_2 concentration had no significant influence on the carbon content (Tab. 4.3.4). In 2011, the only significant difference was detected between the carbon content in fertilized plants grown under elevated CO_2 (950 µmol CO_2 mol^{-1}) and other treatments.

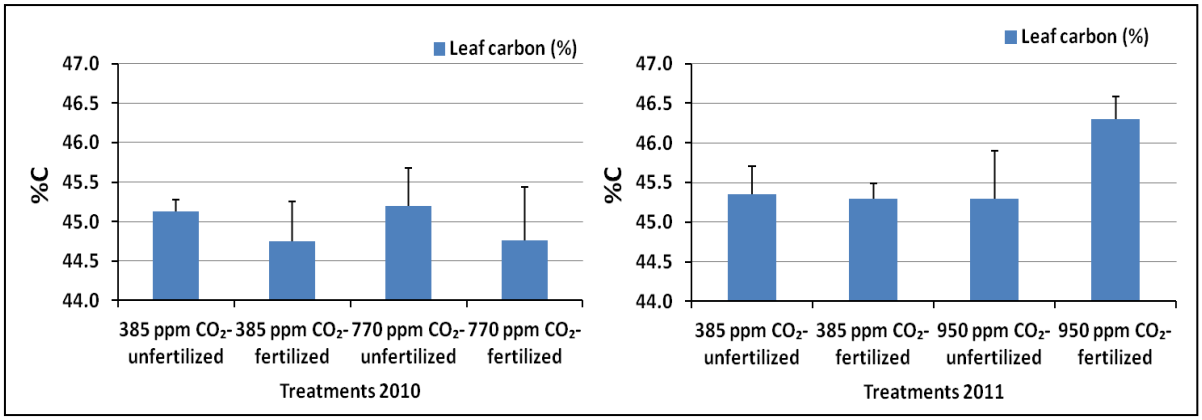

Fig. 4.3.4: Mean values of the leaf C content of poplar after the first (left) and the second growing season (right).

4.3.1.5 Carbon content of stem

The difference between the mean values of stem carbon content in trees under different growth conditions were in tendency the same in both years (Fig. 4.3.5; Tab. 4.3.3 and 4.3.4). Unfertilized plants grown under different CO_2 concentrations had higher values of carbon in their stems, although a significant effect of CO_2 on stem carbon content was detected only in 2010 (Tab. 4.3.4).

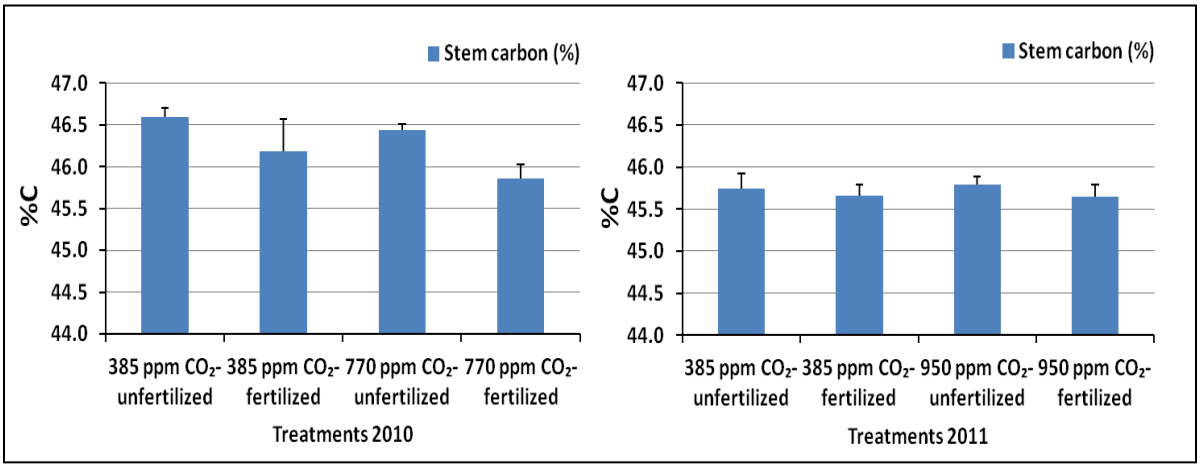

Fig. 4.3.5: Mean values of the stem C content of poplar after the first (left) and the second growing season (right).

4.3.1.6 Carbon content of root

The C content in roots had responded differently under different growth conditions in the first and second growing season (Fig. 4.3.6). Only in 2011, elevated CO_2 caused a significant increase. In both years, fertilized plants grown under different CO_2 concentration (elevated/ ambient air) had significantly less C in their roots (Fig. 4.3.6; Tab. 4.3.4).

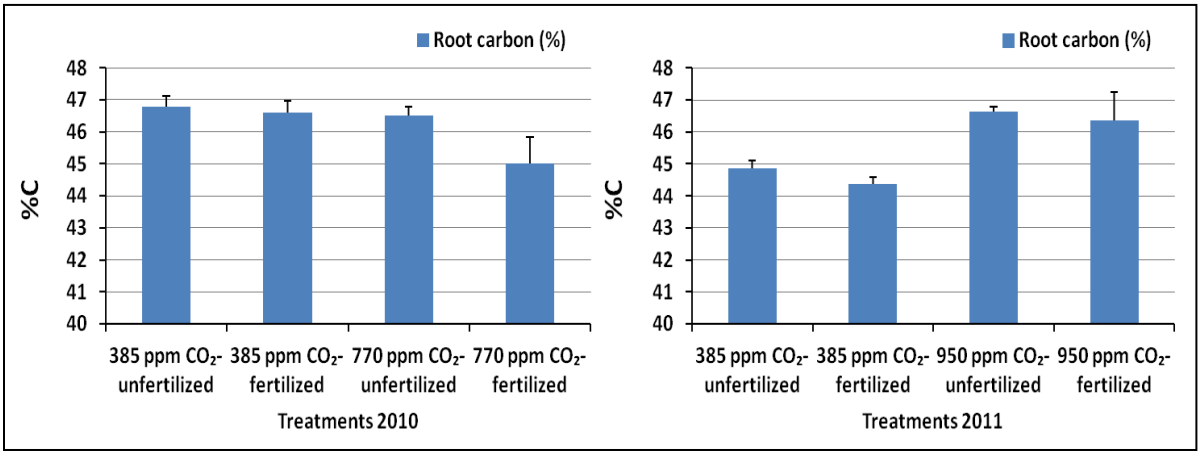

Fig. 4.3.6: Mean values of the root C content of poplar after the first (left) and the second growing season (right).

Tab. 4.3.3: Mean (± st.dev.) of the C content in leaf, stem and root of poplar at the end of both growing seasons.

Treatments of poplar	C content (%) (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	45.13 ± 0.15	46.59 ± 0.11	46.80 ± 0.32
385 ppm CO_2 -fertilized	44.75 ± 0.51	46.18 ± 0.39	46.60 ± 0.37
Elevated CO_2 -unfertilized	45.19 ± 0.49	46.43 ± 0.07	46.51 ± 0.26
Elevated CO_2 -fertilized	44.76 ± 0.68	45.86 ± 0.17	45.01 ± 0.82
(2011)			
385 ppm CO_2 -unfertilized	45.35 ± 0.35	45.75 ± 0.17	44.85 ± 0.25
385 ppm CO_2 -fertilized	45.29 ± 0.20	45.66 ± 0.14	44.39 ± 0.19
Elevated CO_2 -unfertilized	45.29 ± 0.60	45.79 ± 0.11	46.63 ± 0.17
Elevated CO_2 -fertilized	46.30 ± 0.28	45.65 ± 0.15	46.35 ± 0.88

Tab. 4.3.4: Statistical strength of the effects of fertilization and CO_2 concentrations on the C content of leaf, stem and root between variants of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar		C content (%)		
		Leaf	Stem (without bark)	Root
CO_2 concentration	2010	n.s.	***	n.s.
	2011	***	n.s.	***
Fertilization	2010	**	***	***
	2011	***	**	**
Interaction effect between CO_2 level and fertilization	2010	n.s.	n.s.	***
	2011	n.s.	*	n.s.

4.3.1.7 Leaf C/N ratio

In 2010, the mean C/N ratio in leaf varied from 24.32 - 59.80 and in 2011 from 28.29 - 49.64 (Tab. 4.3.5). The C/N ratios between treatments showed a similar pattern in both growing seasons (Fig. 4.3.7); the highest and lowest values belong to unfertilized plants under ambient air (control) and fertilized plants under elevated CO_2 (770/950 µmol CO_2 mol^{-1}), respectively (Fig. 4.3.7). Both variants (elevated CO_2 and fertilization) caused a decrease of the C/N ratio (Tab. 4.3.6).

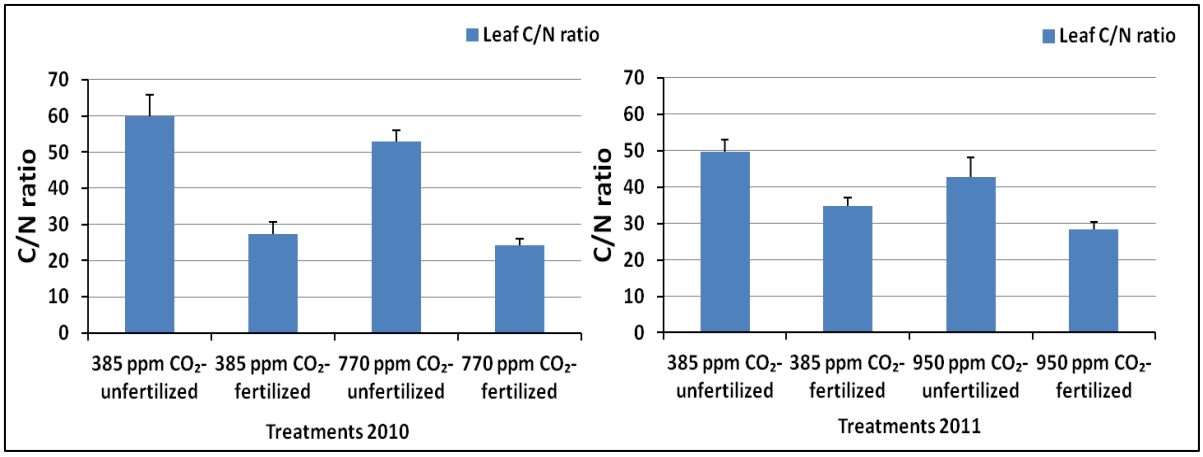

Fig. 4.3.7: Mean values of the leaf C/N ratio of poplar after the first (left) and the second growing season (right).

4.3.1.8 Stem C/N ratio

The differences of the C/N ratio in the stems under different growth conditions were in tendency the same in both years (Fig. 4.3.8; Tab. 4.3.5).

Saplings grown under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) had a higher C/N ratio in their stems; nevertheless, a significant effect of CO_2 was detected only in 2011.

In both years, the interaction effect of CO_2 concentration and fertilization was significant and significant differences were observed between the C/N ratios in fertilized and unfertilized plants grown under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) (Tab. 4.3.6).

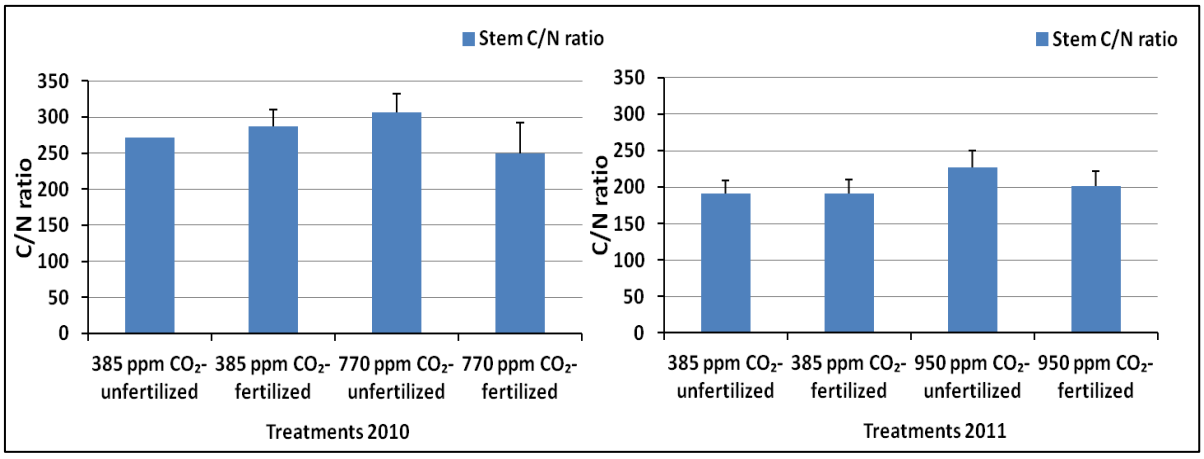

Fig. 4.3.8: Mean values of the stem C/N ratio of poplar after the first (left) and the second growing season (right).

4.3.1.9 Root C/N ratio

In both growing seasons the differences of the mean values between treatments were in tendency the same (Fig. 4.3.9). Unfertilized plants grown under different CO_2 concentrations (ambient air/elevated) had a higher C/N ratio. But the effect of the CO_2 concentration was statistically insignificant (Tab. 4.3.6). The interaction between CO_2 level and fertilization was also insignificant in both years (Tab. 4.3.6).

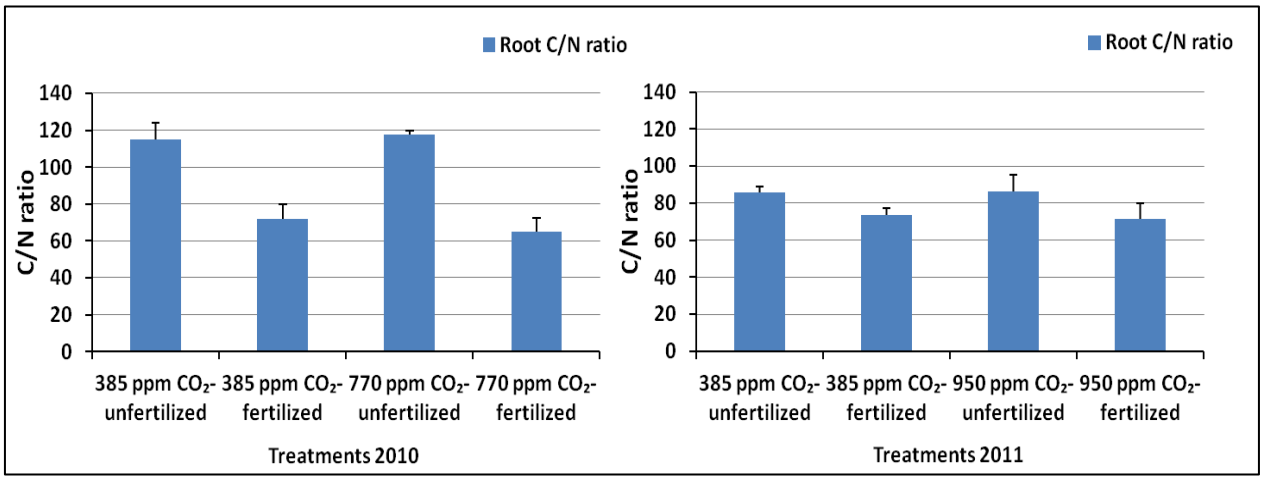

Fig. 4.3.9: Mean values of the root C/N ratio of poplar after the first (left) and the second growing season (right).

Tab. 4.3.5: Mean (± st.dev.) of the C/N ratio in leaf, stem and root of poplar at the end of both growing seasons.

Treatments of poplar	C/N ratio (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	59.80 ± 6.09	271.5 ± 23.38	115.04 ± 8.92
385 ppm CO_2 -fertilized	27.30 ± 3.35	287.2 ± 26.05	71.80 ± 8.15
Elevated CO_2 -unfertilized	52.89 ± 3.04	306.5 ± 43.06	117.77 ± 2.11
Elevated CO_2 -fertilized	24.32 ± 1.65	249.8 ± 23.19	64.74 ± 7.74
(2011)			
385 ppm CO_2 -unfertilized	49.64 ± 3.44	191.5 ± 17.94	85.55 ± 3.20
385 ppm CO_2 -fertilized	34.89 ± 2.32	191.8 ± 18.65	73.48 ± 3.67
Elevated CO_2 -unfertilized	42.66 ± 5.41	226.5 ± 23.83	86.14 ± 9.03
Elevated CO_2 -fertilized	28.29 ± 2.14	201.5 ± 20.64	71.19 ± 8.66

Tab. 4.3.6: Statistical strength of the effects of fertilization and CO_2 concentrations on the C/N ratio in leaf, stem and root between the experimental variants of poplar; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar		C/N ratio		
		Leaf	Stem (without bark)	Root
CO_2 concentration	2010	***	n.s.	n.s.
	2011	***	***	n.s.
Fertilization	2010	***	**	***
	2011	***	*	***
Interaction effect between	2010	n.s.	***	n.s.
CO_2 level and fertilization	2011	n.s.	*	n.s.

4.3.2 Biochemical analysis of beech

4.3.2.1 Nitrogen content of leaf

The N content in the leaves varied significantly between the four variants from 0.79 - 1.66% in 2010 and from 1.06 - 2.22% in 2011 (Tab. 4.3.7). The differences between the treatments were in tendency the same in both growing seasons. As expected, fertilized plants had more N in their leaves; in contrast, elevated CO_2 resulted in a lower amount of N in the leaf. Between the CO_2 concentration and the fertilization, a significant interaction was detected, but only in 2010 (Tab. 4.3.9).

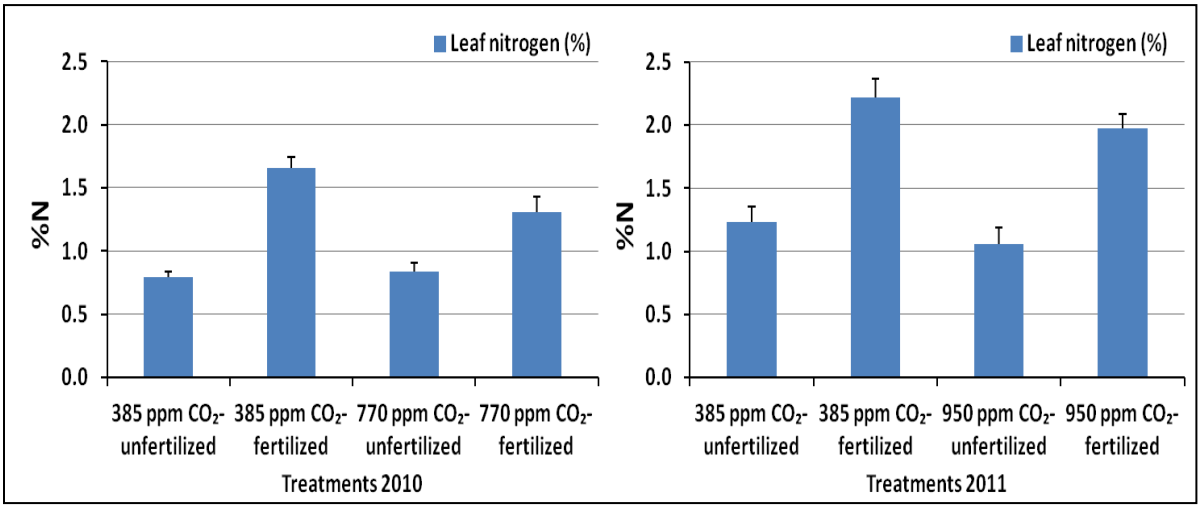

Fig. 4.3.10: Mean values of the leaf N content of beech after the first (left) and the second growing season (right).

Tab. 4.3.7: Mean (± st.dev.) of the N content in leaf, stem and root of beech at the end of both growing seasons.

Treatments of beech	N content (%) (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	0.79 ± 0.05	0.19 ± 0.02	0.60 ± 0.05
385 ppm CO_2 -fertilized	1.66 ± 0.09	0.86 ± 0.07	1.11 ± 0.15
Elevated CO_2 -unfertilized	0.84 ± 0.07	0.20 ± 0.02	0.58 ± 0.06
Elevated CO_2 -fertilized	1.31 ± 0.12	0.62 ± 0.07	0.94 ± 0.15
(2011)			
385 ppm CO_2 -unfertilized	1.24 ± 0.12	0.24 ± 0.03	0.56 ± 0.05
385 ppm CO_2 -fertilized	2.22 ± 0.14	1.17 ± 0.08	1.63 ± 0.13
Elevated CO_2 -unfertilized	1.06 ± 0.13	0.23 ± 0.03	0.60 ± 0.04
Elevated CO_2 -fertilized	1.98 ± 0.11	1.16 ± 0.13	1.43 ± 0.08

Tab. 4.3.8: Statistical strength of the effects of fertilization and CO_2 concentrations on the N content of leaf, stem and root between variants of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		N content (%)		
		Leaf	Stem (without bark)	Root
CO_2 concentration	2010	***	***	**
	2011	***	n.s.	***
Fertilization	2010	***	***	***
	2011	***	***	***
Interaction effect between CO_2 level and fertilization	2010	***	***	*
	2011	n.s.	n.s.	***

4.3.2.2 Nitrogen content of stem

Considerable differences of the amount of N were observed between fertilized and unfertilized plants (Tab. 4.3.7; Fig. 4.3.11). As with the leaves, fertilization caused an increase of N in the stem in both years. However, there was no difference of the N content between treatments under different CO_2 concentrations (ambient air/ elevated CO_2) in 2011. A significant interaction effect between CO_2 concentration and fertilization was detected in 2010 in so far as elevated CO_2 caused a significant decrease in N of fertilized saplings grown under elevated CO_2. In 2011, the effect of elevated CO_2 on the amount of N was insignificant (Tab. 4.3.8).

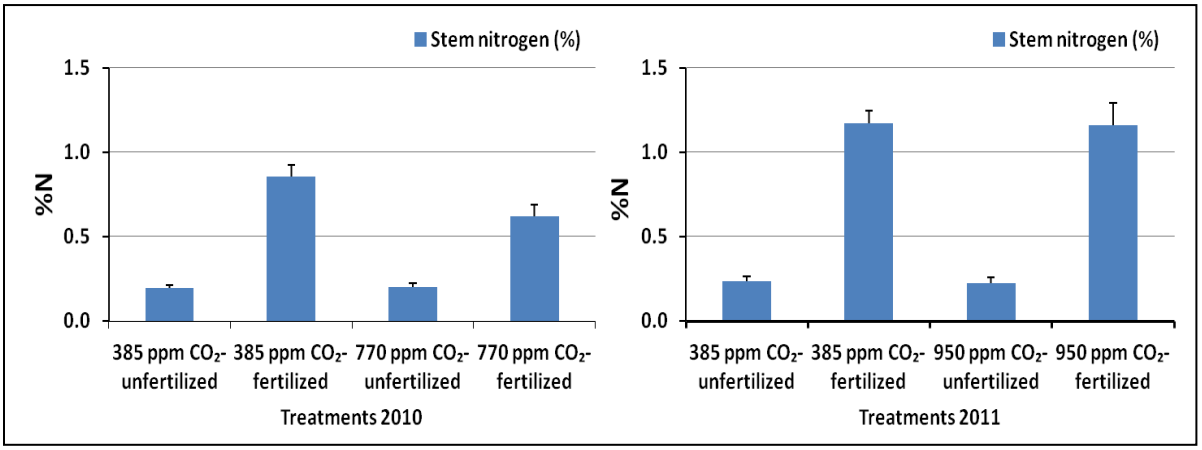

Fig. 4.3.11: Mean values of the stem N content of beech after the first (left) and the second growing season (right).

4.3.2.3 Nitrogen content of root

The differences of the amount of N in the root between the saplings under different growth conditions were in tendency the same in both years (Fig. 4.3.12; Tab. 4.3.7); highest values belonged to fertilized plants grown in ambient air. As in the leaf and stem, fertilization caused a significant increase of N in the root. The interaction effect between CO_2 concentration and fertilization was significant in both years (Tab. 4.3.8). A significant influence of elevated CO_2 was detected only in fertilized plants grown under different CO_2 concentration (elevated/ambient air). Between the roots of unfertilized plants grown under different CO_2 level, no difference in N content was observed.

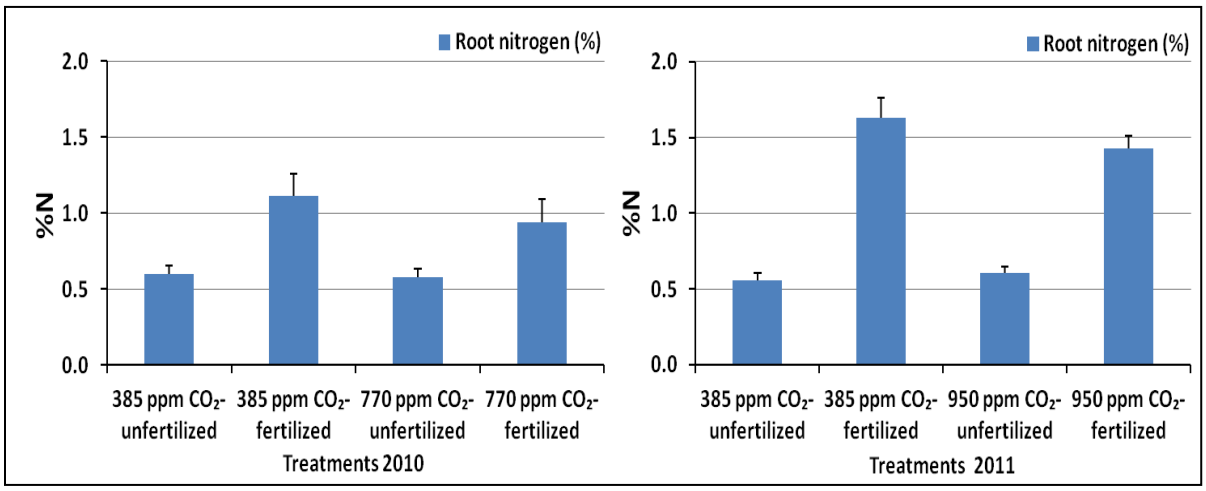

Fig. 4.3.12: Mean values of the root N content of beech after the first (left) and the second growing season (right).

4.3.2.4 Carbon content of leaf

The amount of C in the leaf ranged from 43.8 - 44.8% in 2010 and from 46.5 – 47.3% in 2011 (Tab. 4.3.9). In both years, there was no difference between the C content of fertilized and unfertilized plants grown under different CO_2 concentrations (Fig. 4.3.13). Whereas the C content significantly increased in response to elevated CO_2, no interaction between CO_2 concentration and fertilization was detected in both experimental years (Tab. 4.3.10).

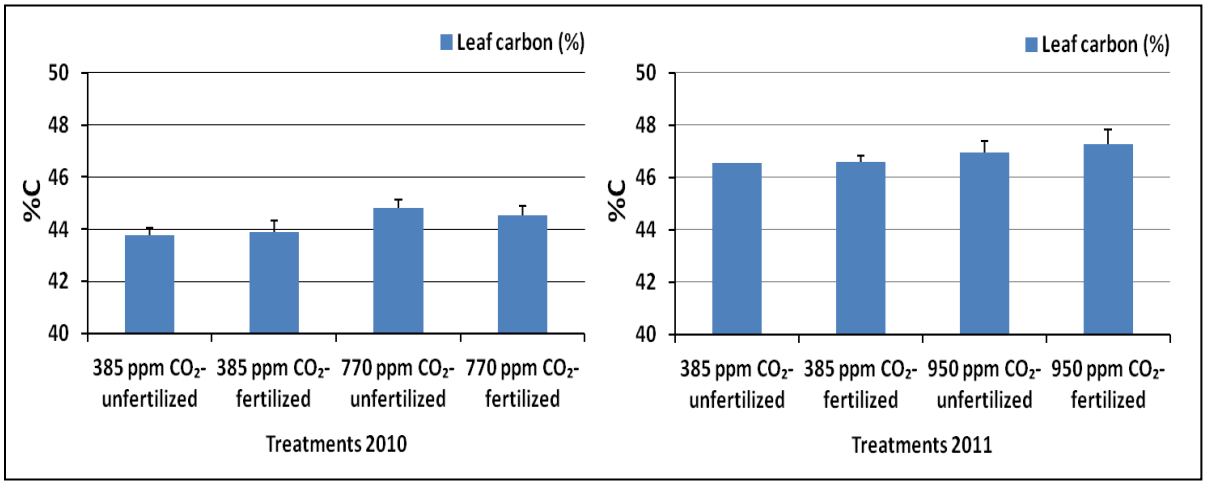

Fig. 4.3.13: Mean values of the leaf C content of beech after the first (left) and the second growing season (right).

Tab. 4.3.9: Mean (± st.dev.) of the C content in leaf, stem and root of beech at the end of both growing seasons.

Treatments of beech	C content (%) (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	43.78 ± 0.27	44.46 ± 0.14	47.81 ± 0.63
385 ppm CO_2 -fertilized	43.90 ± 0.43	44.33 ± 0.10	47.77 ± 0.41
Elevated CO_2 -unfertilized	44.80 ± 0.34	44.49 ± 0.29	48.01 ± 0.41
Elevated CO_2 -fertilized	44.51 ± 0.39	44.61 ± 0.27	48.08 ± 0.56
(2011)			
385 ppm CO_2 -unfertilized	46.54 ± 0.25	44.04 ± 0.12	46.20 ± 0.49
385 ppm CO_2 -fertilized	46.58 ± 0.43	44.19 ± 0.19	45.70 ± 0.38
Elevated CO_2 -unfertilized	46.94 ± 0.55	44.59 ± 0.08	46.25 ± 0.44
Elevated CO_2 -fertilized	47.29 ± 0.79	44.39 ± 0.30	46.16 ± 0.56

Tab. 4.3.10: Statistical strength of the effects of fertilization and CO_2 concentrations on the C content in leaf, stem and root between variants of beech; * p≤0.05; **; p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		C content (%)		
		Leaf	Stem (without bark)	Root
CO_2 concentration	2010	***	**	n.s.
	2011	***	***	n.s.
Fertilization	2010	n.s.	n.s.	n.s.
	2011	n.s.	n.s.	*
Interaction effect between	2010	n.s.	*	n.s.
CO_2 level and fertilization	2011	n.s.	*	n.s.

4.3.2.5 Carbon content of stem

In both years, the differences of stem carbon content between treatments grown under different growth conditions were small (Fig. 4.3.14; Tab. 4.3.9): differently treated (fertilized/unfertilized) plants grown under elevated CO_2 had about 0.4% in 2010 and 0.8% in 2011 more C in their stems than saplings grown in ambient air; nevertheless these small differences were significant (Tab. 4.3.10). In contrast, fertilization caused no significant difference of stem C content between treatments in both growing seasons.

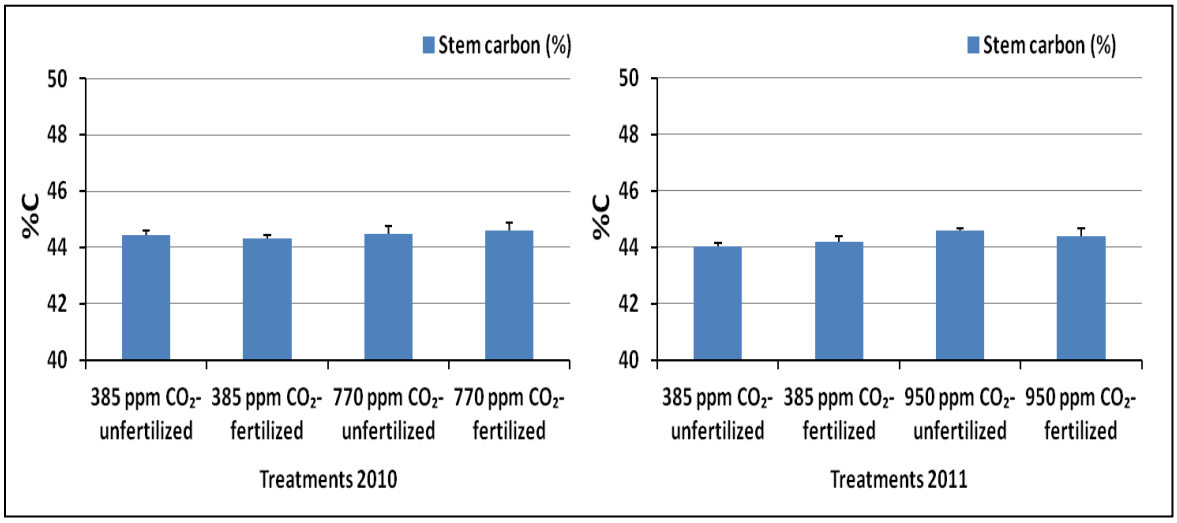

Fig. 4.3.14: Mean values of the stem C content of beech after the first (left) and the second growing season (right).

4.3.2.6 Carbon content of root

In 2010 and 2011, the C content in the roots ranged from 47.8 - 48.1% and from 45.7 - 46.25%, respectively (Tab. 4.3.9). However, in both growing seasons the value of root C content under elevated CO_2 was higher than in ambient air. Nevertheless, no significant effect of CO_2 on root C content was detected. The C content was not so much affected by fertilization; only in 2011, a significant effect of fertilization was detected between fertilized and unfertilized plants grown in ambient air (Fig. 4.3.15; Tab. 4.3.10).

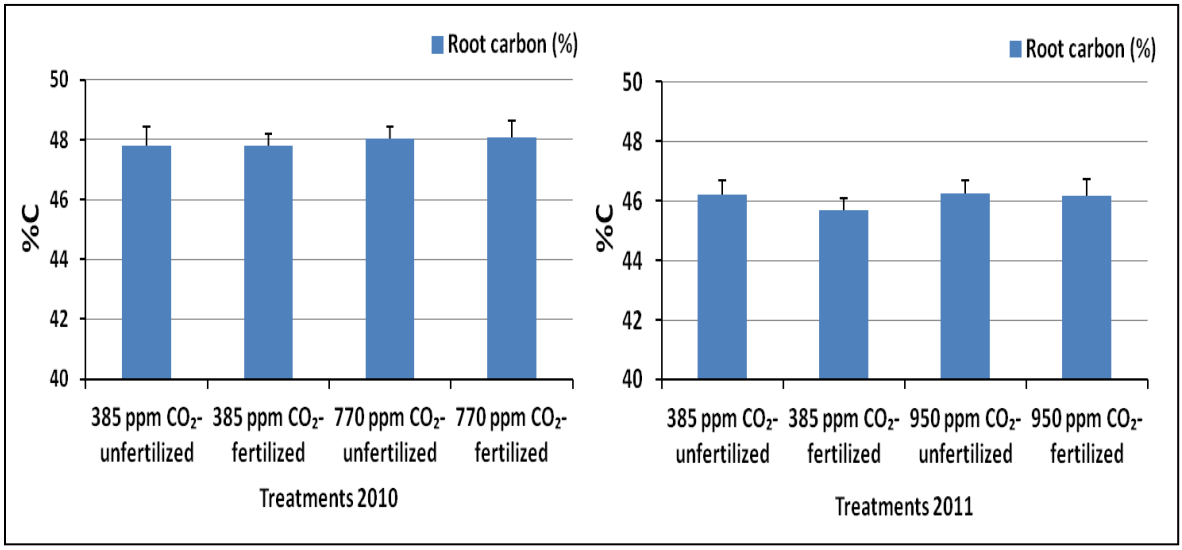

Fig. 4.3.15: Mean values of the root C content of beech after the first (left) and the second growing season (right).

4.3.2.7 Leaf C/N ratio

The C/N ratio in the leaf varied significantly from 26.6 - 55.3% in 2010 and from 21.0 - 44.9% in 2011 (Tab. 4.3.11). In both growing seasons, the lowest value belonged to fertilized plants grown in ambient air (Fig. 4.3.16). Fertilization caused a significant decrease in the C/N ratio. Elevated CO_2 increased this ratio in both years (Tab. 4.312).

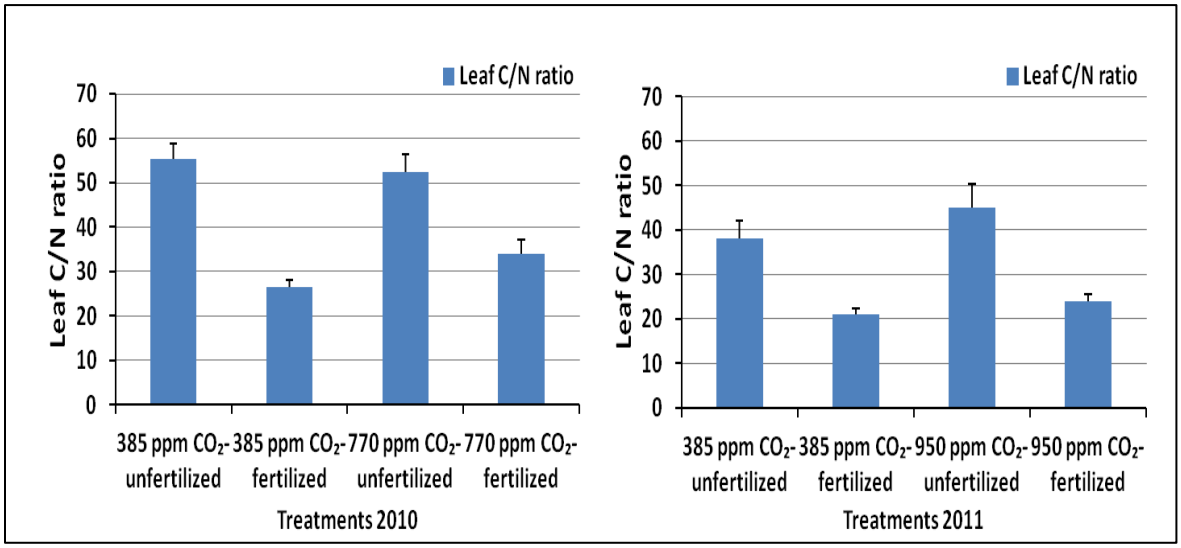

Fig. 4.3.16: Mean values of the leaf C/N ratio of beech after the first (left) and the second growing season (right).

Tab. 4.3.11: Mean (± st.dev.) C/N ratio in leaf, stem and root of beech at the end of both growing seasons.

Treatments of beech	C/N ratio (Mean ± st.dev.)		
	Leaf	Stem (without bark)	Root
(2010)			
385 ppm CO_2 -unfertilized	55.31 ± 3.52	230.77 ± 20.11	80.32 ± 6.60
385 ppm CO_2 -fertilized	26.60 ± 1.54	52.04 ± 4.13	43.79 ± 6.17
Elevated CO_2 -unfertilized	52.50 ± 3.92	227.32 ± 26.36	83.68 ± 8.07
Elevated CO_2 -fertilized	34.11 ± 3.15	72.77 ± 8.42	52.83 ± 11.1
(2011)			
385 ppm CO_2 -unfertilized	38.03 ± 3.96	190.24 ± 25.76	83.29 ± 7.09
385 ppm CO_2 -fertilized	21.04 ± 1.35	37.95 ± 2.40	28.27 ± 2.78
Elevated CO_2 -unfertilized	44.90 ± 5.49	200.87 ± 27.30	76.80 ± 5.46
Elevated CO_2 -fertilized	24.00 ± 1.52	38.80 ± 4.41	32.47 ± 1.97

Tab. 4.3.12: Statistical strength of the effects of fertilization and CO_2 concentrations on the C/N ratio of leaf, stem and root between variants of beech; * p≤0.05; ** p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		C/N ratio		
		Leaf	Stem	Root
CO_2 concentration	2010	**	n.s.	*
	2011	***	n.s.	n.s.
Fertilization	2010	***	***	***
	2011	***	***	***
Interaction effect between CO_2 level and fertilization	2010	***	*	n.s.
	2011	*	n.s.	***

4.3.2.8 Stem C/N ratio

The C/N ratio in stems varied substantially among treatments in both experimental years (Tab. 4.3.11). The differences of the mean values between treatments were in tendency the same in both growing seasons (Fig. 4.3.17). Fertilized plants grown under different CO_2 concentrations (ambient air/elevated) had a significantly lower C/N ratio. But the effects of the CO_2 concentration on the C/N ratio were statistically insignificant (Tab. 4.3.12).

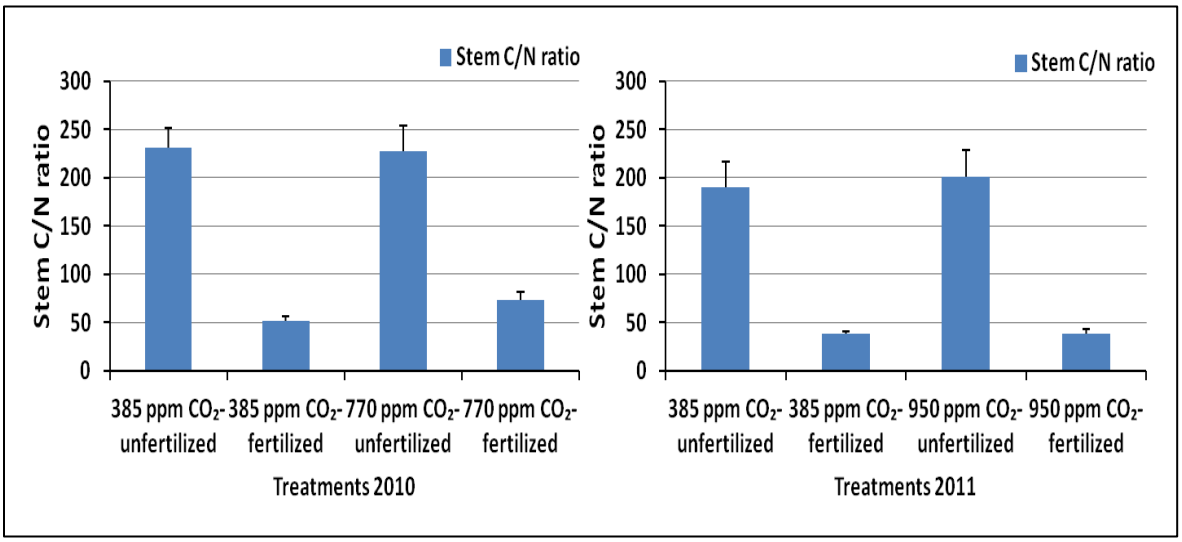

Fig. 4.3.17: Mean values of the stem C/N ratio of beech after the first (left) and the second growing season (right).

4.3.2.9 Root C/N ratio

The mean values of the C/N ratio ranged from 43.8 - 83.7% in 2010 and from 28.3 - 83.3 in 2011 (Fig. 4.3.18; Tab. 4.3.11). In both growing seasons the lowest ratio belonged to fertilized plants grown in ambient air. As with leaf and stem, the fertilization resulted in an increase of the C/N ratio in roots (Tab. 4.3.12). Only in 2010, a significant effect of elevated CO_2 concentration on the C/N ratio was detected.

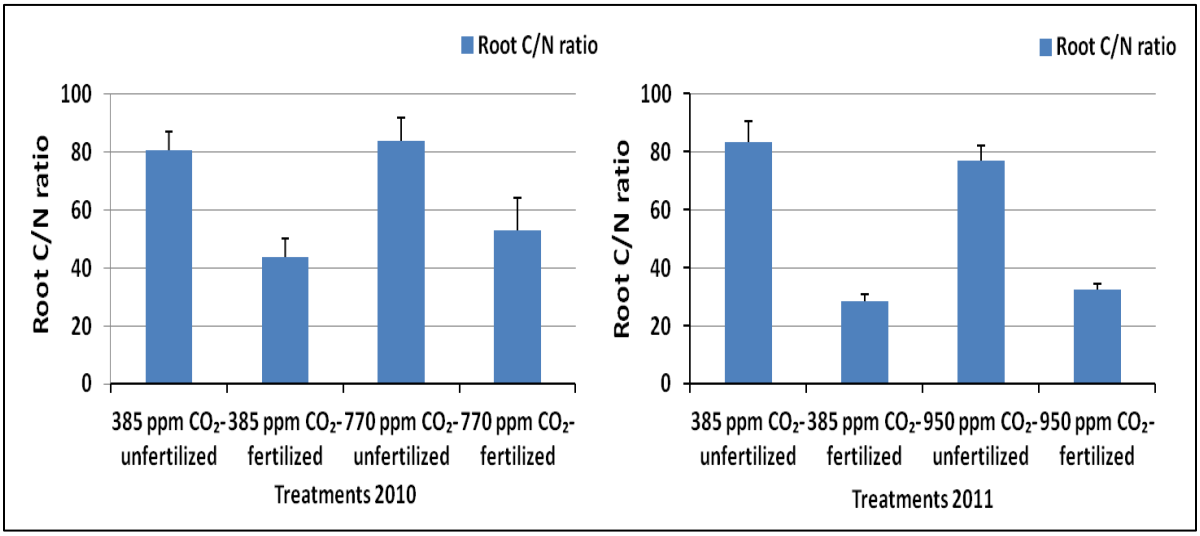

Fig. 4.3.18: Mean values of the root C/N ratio of beech after the first (left) and the second growing season (right).

4.3.2.10 Distribution of lignin in walls of vessels and fibers of beech

Cellular ultraviolet (UV) microspectrophotometry (UMSP) was used to topochemically detect lignin in vessel and fiber walls of beech.

Figure 4.3.20 illustrated the lignin distribution in vessel and fiber walls after the first growing season. Mean UV-absorbance values at 278 nm of vessel walls and fiber walls varied between the variants of treatments from 0.18 to 0.22 in 2010 and 0.18 to 0.21 in 2011(Fig. 4.3.19; Tab. 4.3.13).

In first growing season lignifications in both vessel and fiber walls was reduced by elevated CO_2. Under different CO_2 levels the mean UV-absorbance was less in fiber walls of fertilized plants than unfertilized plants (Fig. 4.3.19; Tab. 4.3.14).

The lignin content in vessel walls of fertilized plants was less than of unfertilized saplings but this effect occurred only between treatments that grown in ambient air. Interaction effect between CO_2 concentration and fertilization on the lignification of vessel walls was statistically significant (Fig. 4.3.19; Tab. 4.3.14).

In 2011, no significant differences were detected between the lignin distribution in vessel and fiber walls (Tab. 4.3.14).

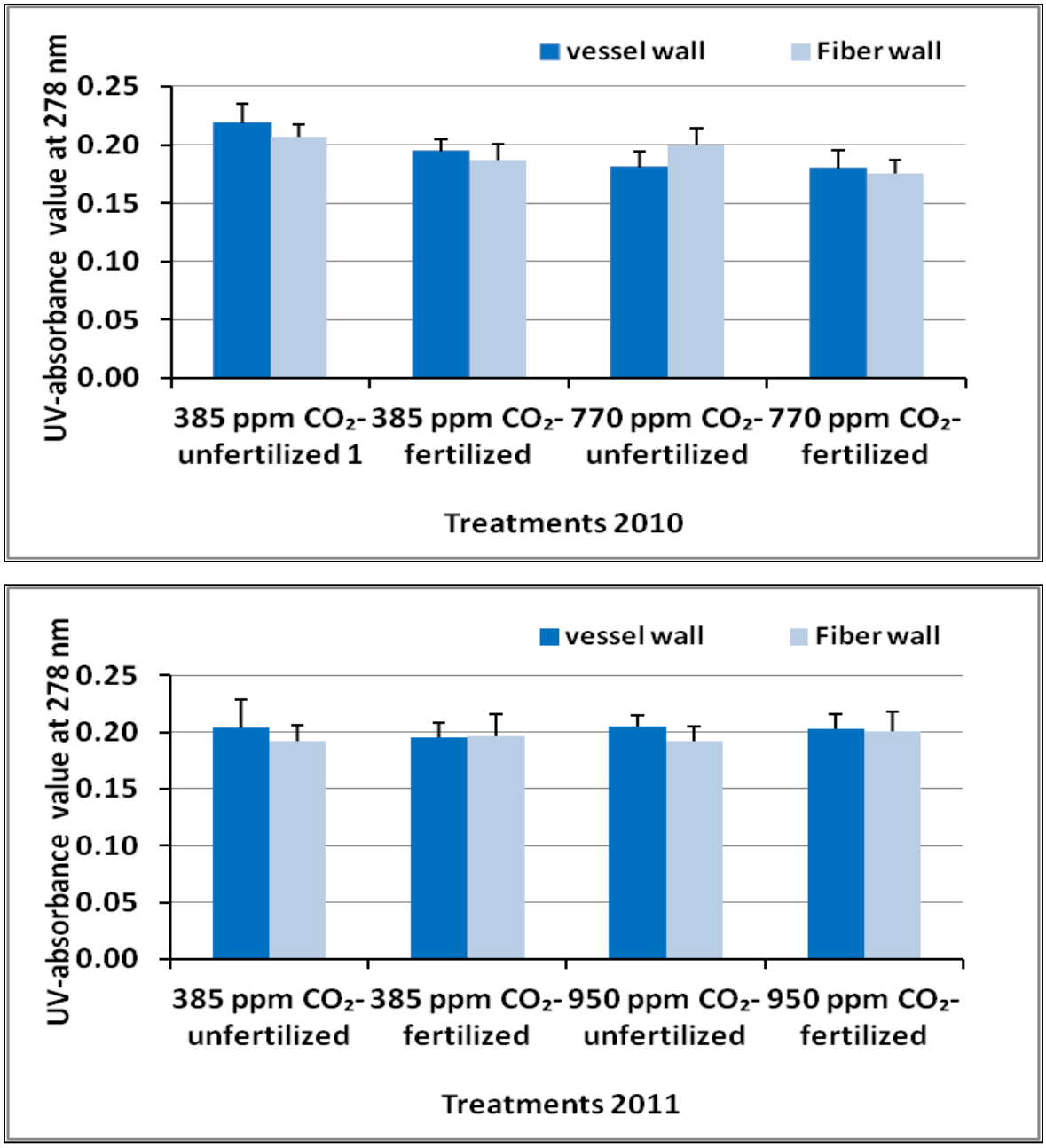

Fig. 4.3.19: Mean values of the UV-absorbance of vessel and fiber walls in beech after the first (up) and the second growing season (below).

Tab. 4.3.13: Mean (± st.dev.) of UV-absorbance of vessel and fiber walls in beech at the end of each growing season.

Treatments of beech	UV-absorbance value at 278 nm (Mean ± st.dev.)	
	Vessel cell wall	Fiber cell wall
(2010)		
385 ppm CO_2 -unfertilized	0.22 ± 0.02	0.21 ± 0.01
385 ppm CO_2 -fertilized	0.19 ± 0.01	0.19 ± 0.01
Elevated CO_2 -unfertilized	0.18 ± 0.01	0.20 ± 0.02
Elevated CO_2 -fertilized	0.18 ± 0.02	0.18 ± 0.01
(2011)		
385 ppm CO_2 -unfertilized	0.20 ± 0.03	0.19 ± 0.01
385 ppm CO_2 -fertilized	0.19 ± 0.01	0.20 ± 0.02
Elevated CO_2 -unfertilized	0.20 ± 0.01	0.19 ± 0.01
Elevated CO_2 -fertilized	0.20 ± 0.01	0.20 ± 0.02

Tab. 4.3.14: Statistical strength of the effects of fertilization and CO2 concentrations on the UV-absorbance of vessel and fiber walls between variants of beech; * p≤0.05; **; p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		UV-absorbance value at 278 nm	
		Vessel wall	Fiber wall
CO_2 concentration	2010	***	*
	2011	n.s.	n.s.
Fertilization	2010	*	***
	2011	n.s.	n.s.
Interaction effect between	2010	*	n.s.
CO_2 level and fertilization	2011	n.s.	n.s.

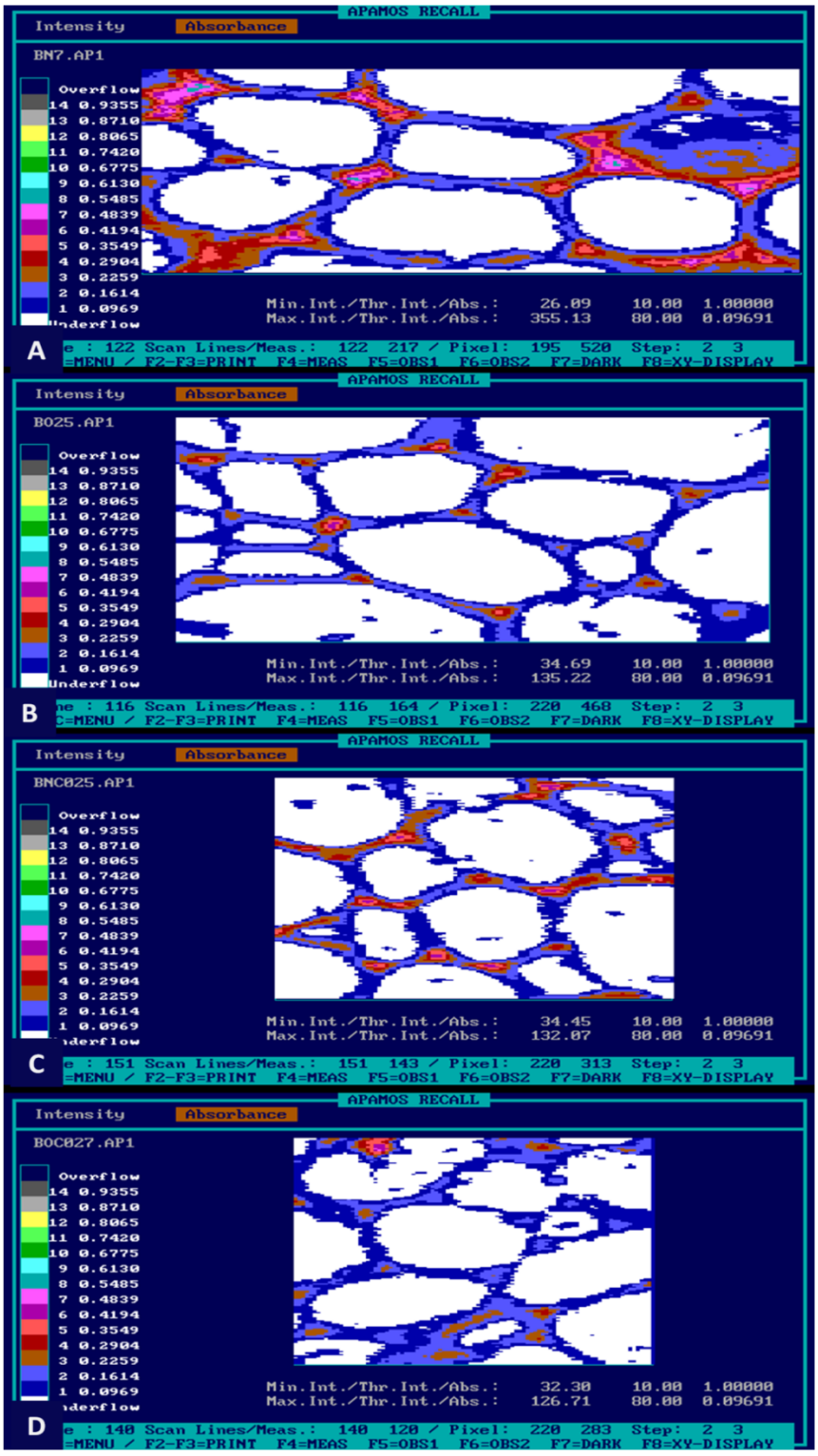

Fig. 4.3.20: UV-absorbance of vessel and fiber walls of different treatments of beech; (A) unfertilized-ambient air (control), (B) fertilized-ambient air, (C) unfertilized-770 ppm CO_2, (D) fertilized-770 ppm CO_2.

4.4 Anatomical analysis

4.4.1 Anatomical analysis of poplar

4.4.1.1 Ring width

The tree-ring width (RW) of poplar has been measured in two heights (A-B). In A (10 cm above ground), RW ranged from 1.70 - 2.20 mm in 2010 and from 0.78 - 1.26 mm in 2011 (Tab.4.4.1). The mean values differed between treatments in tendency in the same way in both growing seasons. Fertilized plants grown under different CO_2 levels (elevated - ambient air) had significantly wider rings than unfertilized plants (Fig. 4.4.1; Tab. 4.4.2). In contrast, elevated CO_2 had no significant effect on RW. Furthermore, there was no interaction effect between elevated CO_2 and fertilization in both of years.

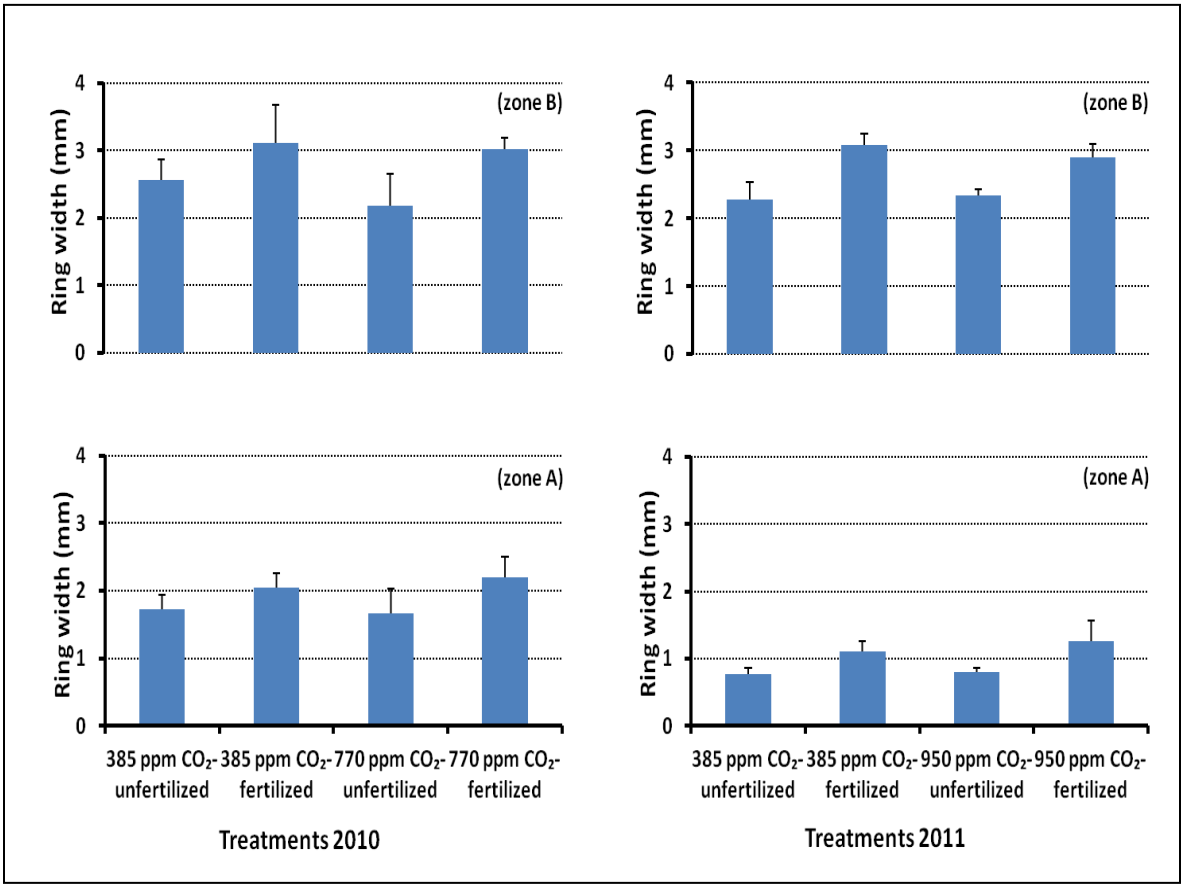

Fig. 4.4.1: Ring width (RW) of poplar along stems in the first (left) and the second growing season (right).

Tab. 4.4.1: Mean (± st.dev.) of ring width and of vessel characteristics of poplar during both growing seasons; zone A (10 cm above ground) and zone B (30 cm above ground).

Treatments of poplar	(Mean ± st.dev.)			
	RW (mm)	TVLA (%)	AVLA (µm²)	VD (n/mm²)
2010 (zone A)				
385 ppm CO_2 - unfertilized	1.70 ± 0.2	13.3 ± 0.7	672 ± 68.5	193 ± 15.8
385 ppm CO_2 - fertilized	2.00 ± 0.2	10.5 ± 1.4	558 ± 43.6	182 ± 24.4
Elevated CO_2 - unfertilized	1.70 ± 0.3	13.2 ± 1.2	659 ± 34.2	188 ± 14.0
Elevated CO_2 - fertilized	2.20 ± 0.3	12.5 ± 1.0	655 ± 52.8	186 ± 9.20
2010 (zone B)				
385 ppm CO_2 - unfertilized	2.60 ± 0.3	10.7 ± 1.3	601 ± 80.1	174 ± 26.2
385 ppm CO_2 - fertilized	3.10 ± 0.5	10.2 ± 0.6	558 ± 41.6	175 ± 8.80
Elevated CO_2 - unfertilized	2.20 ± 0.5	12.8 ± 0.9	679 ± 55.7	181 ± 21.7
Elevated CO_2 - fertilized	3.00 ± 0.2	10.1 ± 1.6	549 ± 32.6	175 ± 20.6
2011 (zone A)				
385 ppm CO_2 - unfertilized	0.78 ± 0.08	19.2 ± 2.0	850 ± 59.9	221 ± 13.6
385 ppm CO_2 - fertilized	1.11 ± 0.14	17.7 ± 1.6	759 ± 56.1	230 ± 18.3
Elevated CO_2 - unfertilized	0.80 ± 0.07	22.4 ± 2.1	968 ± 71.1	229 ± 10.4
Elevated CO_2 - fertilized	1.26 ± 0.30	19.1 ± 2.1	827 ± 69.4	225 ± 12.3
2011 (zone B)				
385 ppm CO_2 - unfertilized	2.27 ± 0.27	15.5 ± 1.6	832 ± 99.9	178 ± 15.0
385 ppm CO_2 - fertilized	3.08 ± 0.18	14.6 ± 0.7	807 ± 29.2	174 ± 7.83
Elevated CO_2 - unfertilized	2.34 ± 0.09	17.3 ± 1.4	900 ± 59.4	182 ± 13.5
Elevated CO_2 - fertilized	2.90 ± 0.20	16.1 ± 1.3	892 ± 90.1	175 ± 10.8

Tab. 4.4.2: Statistical strength of the effects of fertilization and CO_2 concentrations on ring width and vessel characteristics between the experimental variants of poplar; * p≤0.05; **; p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of poplar			RW	TVLA	AVLA	VD
CO_2 concentration	2010	zone A	n.s.	*	*	n.s.
		zone B	n.s.	*	n.s	n.s.
	2011	zone A	n.s.	**	***	n.s.
		zone B	n.s.	***	**	n.s.
Fertilization	2010	zone A	***	***	**	n.s.
		zone B	***	***	***	n.s.
	2011	zone A	***	**	***	n.s.
		zone B	***	*	n.s	n.s.
Interaction effect between CO_2 level and fertilization	2010	zone A	n.s.	**	**	n.s.
		zone B	n.s.	*	*	n.s.
	2011	zone A	n.s.	n.s.	n.s.	n.s.
		zone B	n.s.	n.s.	n.s.	n.s.

4.4.1.2. Total vessel lumen area (TVLA)

The total vessel lumen area (TVLA) of poplar varied from 10.5 - 13.3% in 2010 (zone A) and from 17.7 - 22.4% in 2011 (zone A) (Tab. 4.4.1) and responded in tendency similarly to the different growth conditions (Fig. 4.4.2) in both experimental years. Elevated CO_2 had a significant positive influence (Tab. 4.4.2). Both fertilized and unfertilized poplar

trees grown under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) had at least by 10% higher values than tress grown in ambient air (385 µmol CO_2 mol^{-1}) in both years; this trend was observed along stems. In contrast, fertilized trees under different CO_2 concentrations (elevated/ambient) had a significantly lower TVLA in both years (Tab. 4.4.2). An interaction between elevated CO_2 and fertilization was only detected in the first year.

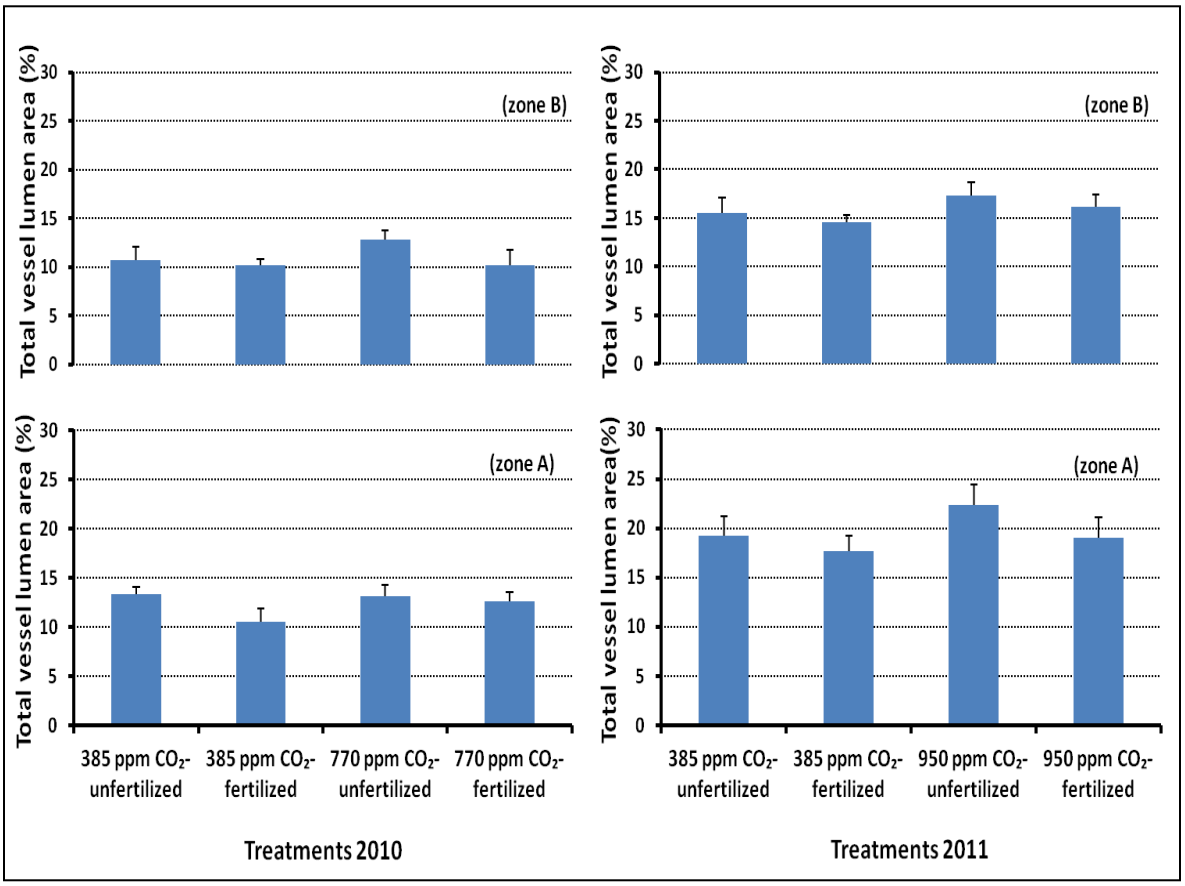

Fig. 4.4.2: Total vessel lumen area (TVLA) of poplar under different growth conditions after the first (left) and the second growing season (right).

4.4.1.3 Average vessel lumen area (AVLA)

The average vessel lumen area (AVLA), in response to the different growing conditions, showed the same pattern in both growing seasons (Fig. 4.4.3). In 2010 (zone A), AVLA ranged from 558 - 672 µm² and in 2011 (zone A) from 759 - 968 µm² (Tab. 4.4.1). In both growing seasons, unfertilized plants under elevated CO_2 (770/950 µmol CO_2 mol^{-1})

showed the largest AVLA whereas the smallest AVLA belonged to fertilized saplings in ambient air. In both years, AVLA increased significantly under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) but decreased under fertilization (Tab. 4.4.2). An interaction between elevated CO_2 and fertilization was detected only in 2010.

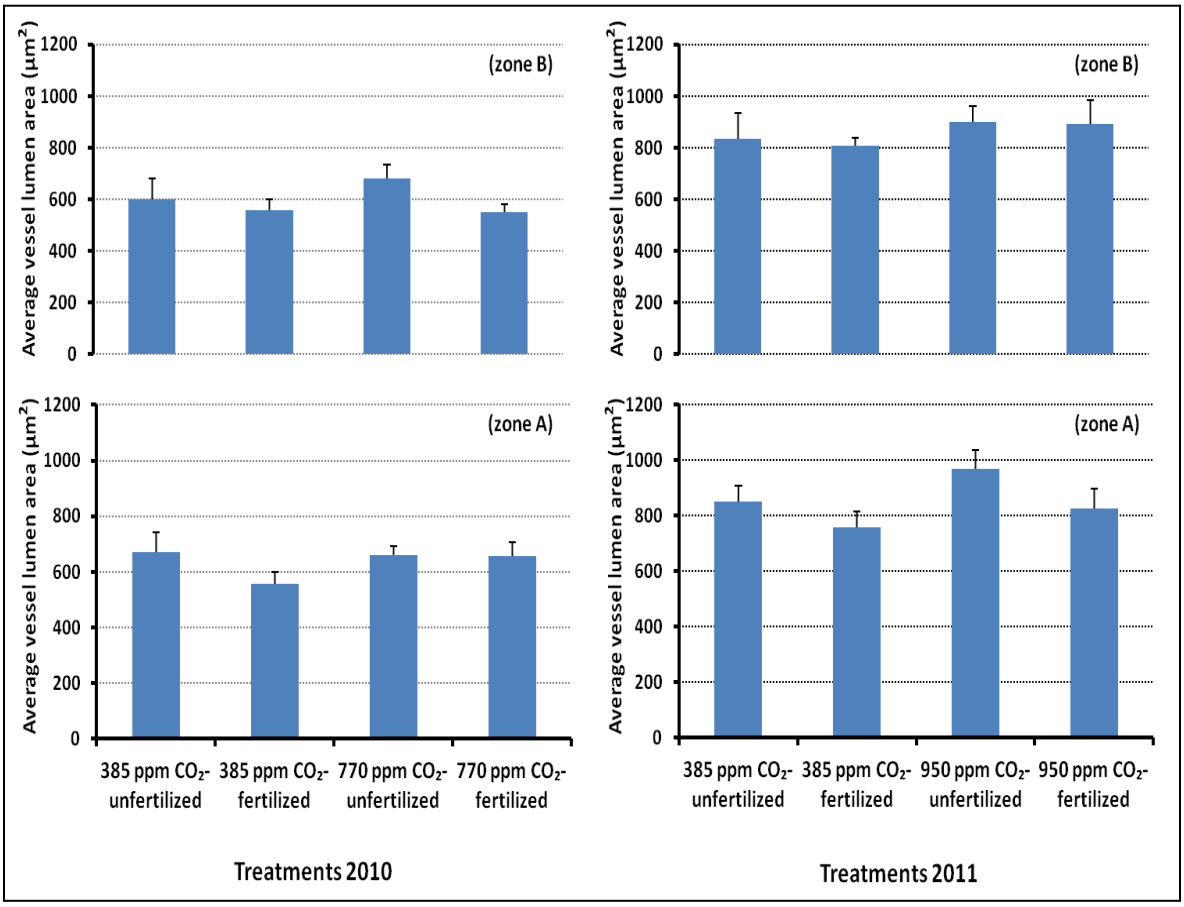

Fig. 4.4.3: Average vessel lumen area (AVLA) of poplar under different growth conditions after the first (left) and the second growing season (right).

4.4.1.4 Vessel density (VD)

In both growing seasons, vessel density (VD) did not significantly respond to any of the growth conditions (Fig. 4.4.4; Tab. 4.22). VD varied from 182 - 193 n/mm² in 2010 (zone A) and from 221 - 230 n/mm² in 2011 (zone B) (Tab. 4.4.1). Furthermore, there was no significant interaction effect between elevated CO_2 and fertilization (Tab. 4.4.2).

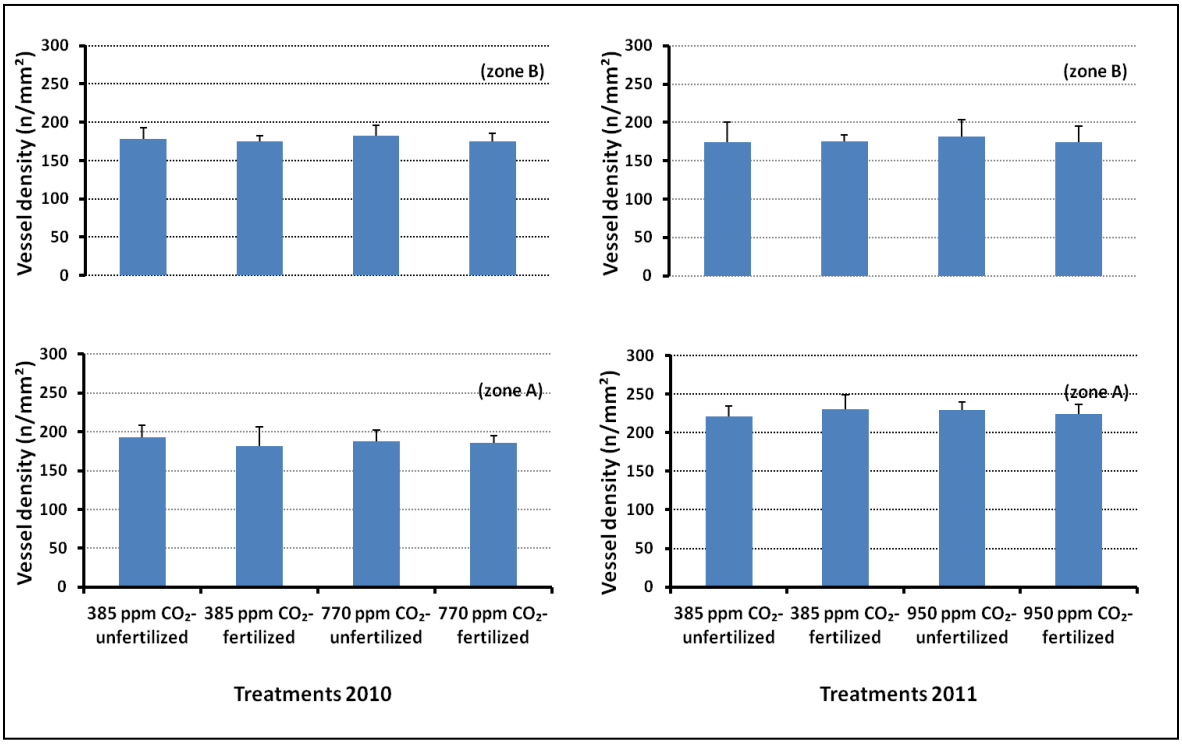

Fig. 4.4.4: Vessel density (VD) of poplar under different growth conditions after the first (left) and the second growing season (right).

4.4.1.5 Relationship between ring width and vessel variables

In both years, ring width (RW) and total vessel lumen area (TVLA) were negatively correlated with each other (Tab. 4.4.3). The same holds true for RW and AVLA although the correlation in 2011 was weak. In both years, the correlation between RW and VD was statistically insignificant. TVLA correlated strongly positively with AVLA and VD in both years.

Tab. 4.4.3: Correlations among ring width and vessel variables of poplar.

Treatments of poplar		RW	TVLA	AVLA	VD
2010	RW				
	TVLA	-0.38*			
	AVLA	-0.20	0.83**		
	VD	-0.33	0.48**	-0.05	
2011	RW				
	TVLA	-0.43*			
	AVLA	-0.36*	0.89**		
	VD	-0.24	0.45*	0.03	

4.4.2 Anatomical analysis of beech

4.4.2.1 Ring width

Ring width (RW) of beech ranged from 0.67 - 1.08 mm in 2010 and from 1.03 - 1.45 mm in 2011 (Tab. 4.4.4). Fertilized tress under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) had the widest tree ring (Fig. 4.4.5). In both years, elevated CO_2 induced a significant increase in RW but a significant effect of fertilization was detected only in 2011(Tab. 4.4.5). Also no interaction was found between CO_2 concentration and fertilization in both growing seasons.

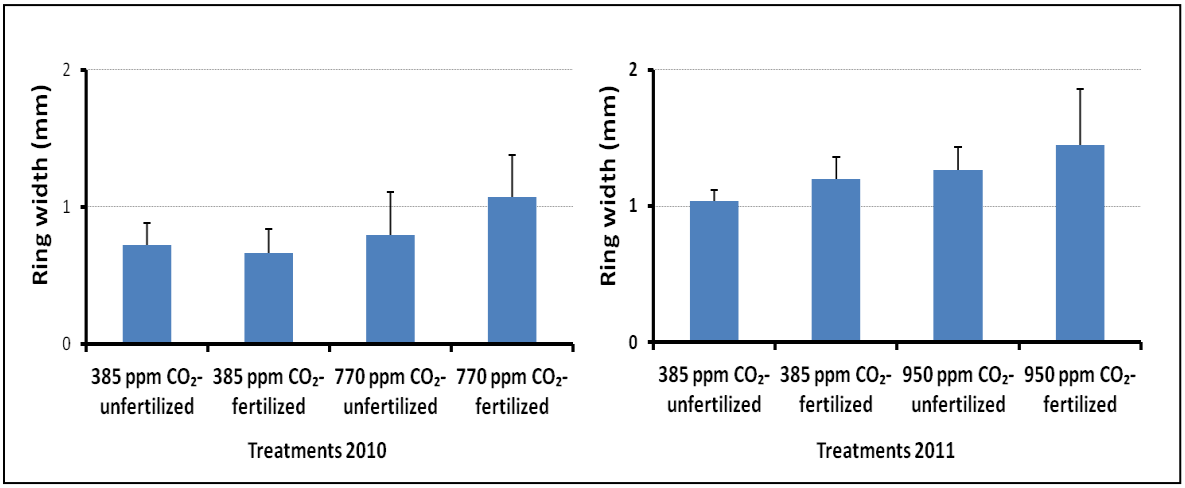

Fig. 4.4.5: Ring-width (RW) of beech in the first (left) and the second growing season (right).

4.4.2.2 Total vessel lumen area (TVLA)

The differences of the mean values of TVLA between trees under different growth conditions were in tendency the same in both years (Fig. 4.16; Tab. 4.4.4). The highest and the lowest value of TVLA belonged to the unfertilized variant under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) and to the fertilized variant in ambient air, respectively. These differences were highly significant. TVLA was significantly reduced by fertilization. In contrast, elevated CO_2 (770/950 µmol CO_2 mol^{-1}) caused a significant increase (Tab. 4.2.5).

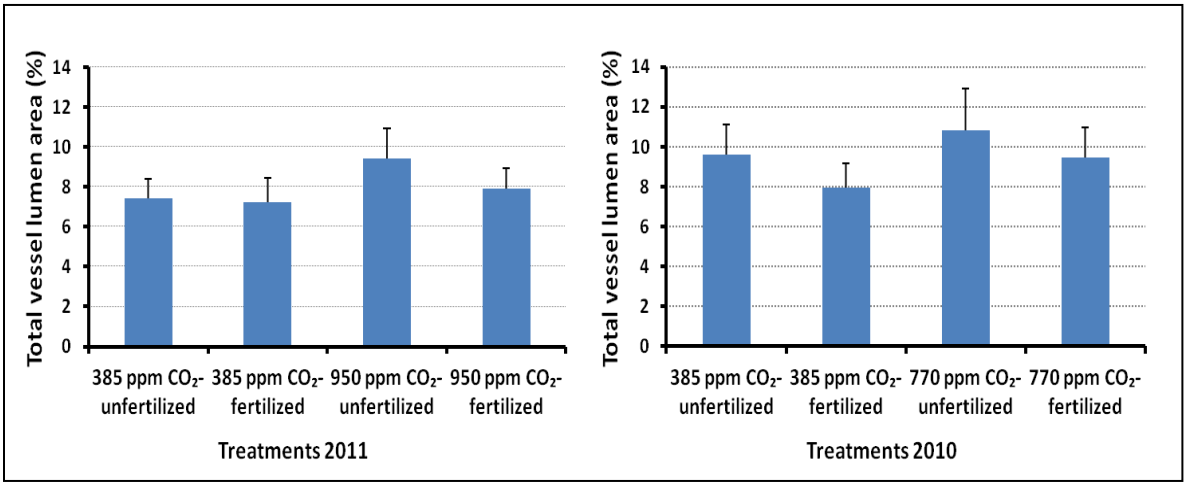

Fig. 4.4.6: Total vessel lumen area (TVLA) of beech in the first (left) and the second growing season (right).

4.4.2.3 Average vessel lumen area (AVLA)

AVLA under different growth conditions varied from 358 - 463 µm² in 2010 and from 413 - 514 µm² in 2011 (Tab. 4.4.4). The trend of AVLA among treatments was similar in both years (Fig. 4.4.7). Unfertilized plants under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) had formed the largest and fertilized plants in ambient air (385 µmol CO_2 mol^{-1}) the smallest AVLA. Nevertheless, these differences were insignificant. But a significant interaction effect on AVLA was detected between CO_2 concentration and fertilization in 2010 (Tab. 4.4.5).

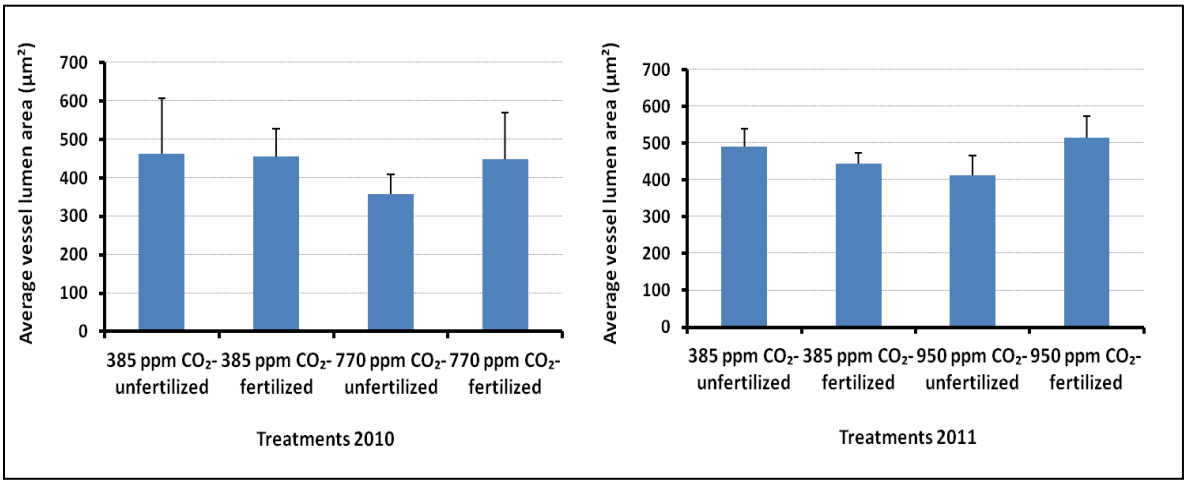

Fig. 4.4.7: Average vessel lumen area (AVLA) of beech in the first (left) and the second growing season (right).

Tab. 4.4.4: Mean (± st.dev.) of ring width and vessel characteristics between variants of beech in both growing seasons.

Treatments of beech	(Mean ± st.dev.)			
	RW (mm)	TVLA (%)	AVLA (µm²)	VD (n/mm²)
2010				
385 ppm CO_2 - unfertilized	0.72 ± 0.16	9.60 ± 1.50	463 ± 144.5	257 ± 139.7
385 ppm CO_2 - fertilized	0.67 ± 0.17	8.00 ± 1.20	454 ± 72.30	179 ± 41.10
Elevated CO_2 - unfertilized	0.80 ± 0.31	10.8 ± 2.10	358 ± 51.00	321 ± 116.8
Elevated CO_2 - fertilized	1.08 ± 0.30	9.50 ± 1.50	449 ± 119.7	221 ± 69.00
2011				
385 ppm CO_2 - unfertilized	1.03 ± 0.09	7.40 ± 1.00	490 ± 50.40	152 ± 33.60
385 ppm CO_2 - fertilized	1.20 ± 0.16	7.20 ± 1.20	443 ± 31.00	161 ± 24.00
Elevated CO_2 - unfertilized	1.26 ± 0.17	9.40 ± 1.50	413 ± 54.20	229 ± 46.20
Elevated CO_2 - fertilized	1.45 ± 0.41	7.90 ± 1.00	514 ± 59.60	148 ± 88.80

Tab. 4.4.5: Statistical strength of the effects of fertilization and CO_2 concentrations on the ring width and vessel characteristics between variants of beech; * p≤0.05; **; p≤0.01; *** p≤0.001; n.s., not significant.

Treatments of beech		RW	TVLA	AVLA	VD
CO_2 concentration	**2010**	*	*	n.s.	n.s.
	2011	**	***	n.s.	**
Fertilization	**2010**	n.s.	*	n.s.	***
	2011	*	*	n.s.	*
Interaction effect between CO_2 level and fertilization	**2010**	n.s.	n.s.	n.s.	n.s.
	2011	n.s.	n.s.	***	***

4.4.2.4 Vessel density (VD)

VD varied from 179 - 321 (n/mm²) in 2010 and from 148 - 229 (n/mm²) in 2011. In both growing seasons, unfertilized plants under elevated CO_2 (770/950 µmol CO_2 mol^{-1}) had a highest VD (Fig. 4.4.8 Tab. 4.4.4). Nevertheless, a positive significant influence of elevated CO_2 only in 2011 was detected and differences between plants, grown under ambient air and trees under elevated CO_2 (770 µmol CO_2 mol^{-1}) were insignificant in 2010 (Tab. 4.4.4). VD decreased significantly by fertilization in both years. Furthermore, a significant interaction effect between CO_2 concentration and fertilization was determined in 2011 (Tab. 4.4.5).

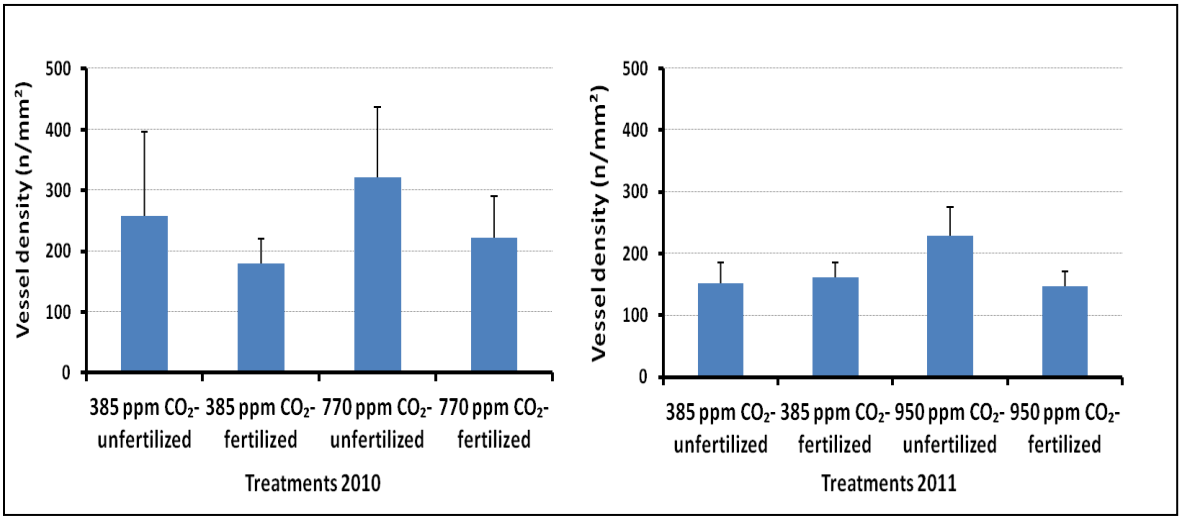

Fig. 4.4.8: Vessel density (VD) of beech in the first (left) and the second growing season (right).

4.4.2.5 Relationship between ring width and vessel variables

The same correlation trend between variables was found in both years (Tab. 4.4.6). However, no significant correlation was between RW and vessel variables. Instead, high correlations were found between vessel variables. Average vessel lumen area (AVLA) was highly negatively correlated with total TVLA and VD. Furthermore, a strong positive correlation was detected between TVLA and VD.

Tab. 4.4.6: Correlations between ring width and vessel variables of beech.

Treatments of beech		RW	TVLA	AVLA	VD
2010	RW				
	TVLA	-0.14			
	AVLA	-0.16	-0.46*		
	VD	-0.03	0.87**	-0.79**	
2011	RW				
	TVLA	0.07			
	AVLA	-0.14	-0.34*		
	VD	0.05	0.87**	-0.70**	

5. Discussion

Carbon dioxide is a natural component of the atmosphere and an essential factor for the growth of trees as nearly half of their bodies are composed of carbon. However, the CO_2-content in the ambient atmosphere has not been constant through time. Since around 1850, it has risen from about 270 ppm to around 350 ppm and will continue to do so in future (Burschel & Weber 1988). The scientific community, as soon as it became aware of the evidence of rising CO_2, started to study its effects on plants (Ceulemans & Mousseau 1994) resulting in an output of several thousands of scientific articles and approx. 120 reviews (Körner 2006). Despite this vast amount of CO_2-related papers being published so far, trees and shrubs are underrepresented. Nevertheless, a considerable but confusing variety of experimental results and a wide diversity of interpretations have become accessible.

The present study on beech and poplar saplings grown under elevated CO_2 in a greenhouse over two seasons has focused on the monitoring of physiological parameters, such as photosynthesis, transpiration and stomatal conductance, on the measurement of biomass of leaves, stem and roots and their content of nitrogen and carbon, and finally on vessel anatomical variables, such as average vessel lumen area. As the results are more coherent for beech than for poplar both tree species will not be considered in common but successively.

5.1 Physiological responses to elevated CO_2

5.1.1 Beech

In both study years, photosynthesis throughout the growing season was highest in saplings grown under elevated CO_2 whereas the influence of fertilization was ambiguous. Such positive effects of elevated CO_2 on the rate of photosynthesis have been repeatedly reported by, e.g., Leverenz et al. (1999), Overdieck et al. (2010), but also a contrary effect or no effect at all (Epron et al. 1995) was sometimes observed.

Also for fertilization, a positive effect under elevated CO_2 has been repeatedly described (e.g., Liozon et al. 2000).

The transpiration rate is strongly correlated with the stomatal conductance. For both variables, a clear effect of the different treatments on the saplings could be seen only in the second year when elevated CO_2 has resulted in a reduced stomatal conductance as also reported for beech by Saxe et al. (1998) and Leverenz et al. (1999). Again, a contrary or no effect on the stomatal conductance or on the transpiration of beech was observed by Heath and Kerstiens (1997) and Pontailler et al. (1994). The least stomatal conductance we found in unfertilized saplings grown under elevated CO_2. A reduced transpiration rate was measured by Saxe (1994). The rate of transpiration and stomatal conductance of our beech saplings were higher when fertilized as compared to unfertilized.

5.1.2 Poplar

The rate of photosynthesis, obtained in 2010, could not be brought into a meaningful context with other physiological results. In 2011, the physiological measurements, although not significant, showed an interesting signal over the vegetation period: From May to July, the saplings grown under elevated CO_2 were photosynthetically highly active but this positive effect disappeared towards the end of the vegetation period. Such behaviour was also observed by Radoglou and Jarvis 1990.

Also in this study, later during the growing season, saplings grown in ambient air showed a higher rate of photosynthesis than those grown under elevated CO_2. This phenomenon, known as ‘down regulation’, was described and explained by Gaurdillere and Moussea (1989) and Ceulemans et al. (1993) for other genotypes of poplar. There are various hypotheses put forward for this phenomenon (see the literature review). Gaurdillere and Moussea (1989) have been considering the size of the plant pots as one of the reasons whereas Ceulemans et al. (1993) in addition took a reduction of nutrient supply into account.

From July to the end of September, our fertilized saplings were photosynthetically more active than unfertilized saplings, that means, plants growing under unlimited nutrient

supply show a higher rate of photosynthesis (Volin & Reich 1996); a higher content of chlorophyll in the leaves of our fertilized saplings is a good argument for it.
All in all, transpiration and stomatal conductance remained unaffected by the differing experimental growing conditions. Such behaviour was observed by Volin and Reich (1996); admittedly, other studies with various genotypes of poplar under elevated CO_2 resulted in a reduced stomatal conductance (Bosac et al. 1995; Curtis et al. 1995).

5.2 Phenological responses to elevated CO_2

5.2.1 Beech

Various phenological parameters, such as height growth, leaf area and biomass, were measured at the end of each vegetation period. The height of the saplings although varying between the treatments was statistically not significantly different. This may be due to the large spread of the data acquired per treatment. At least Overdieck et al. (2007) mentioned a positive influence of elevated CO_2 on height growth of beech. Incidentally, comparing and generalizing the hitherto results for beech is not easy because of the differing experimental conditions and of differing genotypes of the study material.
The leaf area appeared to be unaffected by the various growth conditions. Admittedly, only measurements from one vegetation period were acquired. Other studies are giving evidence that the total leaf area was larger under elevated CO_2 than in ambient air (El Kohen et al. 1993; Epron et al. 1995) but the plants have been growing in the ground and not in a pot.
In general, it appears that beech under elevated CO_2 produces more biomass (El Kohen et al.1993; Overdieck 1993; Overdieck et al 2007) even if this is not persistently supported by our own results. This missing influence in our study can be due to the fact that half of the biomass consisted of roots which remained unaffected by the differing growth conditions (Spinnler et al. 2003). In our study, the increase in stem weight of saplings grown under elevated CO_2 was the most obvious but insignificant response. In both study years, the stems were heavier when grown under elevated CO_2 than in

ambient air but the above-ground biomass was significantly higher only in the first study year.

5.2.2 Poplar

The studies on poplar up to now are contradictory and based on numerous species or mixtures of species. Therefore, the different genotypes used should be considered as independent experimental variants. In these experiments, elevated CO_2 and fertilization often had a positive effect on the leaf area (Bosac et al. 1995; Simon et al. 1995; Curtis et al. 1995; Taylor et al. 2001; Gielen et al. 2001; Liberloo et al. 2005) but also a negative effect or no effect at all on the leaf area (Radglou & Jarvis 1990).
The height of poplar – a fast growing tree species – responded very intensely to fertilization, that is to say, fertilized poplar saplings were higher than unfertilized ones – independent from the CO_2 level. No effect of elevated CO_2 on the height of *Populus tremuloides* (Brown & Higginbotham 1986), *Castanea sativa* (EI Cohen et al. 1992) or *Betula pendula* (Petterson & McDonald 1992) was reported. But also a positive effect on the height of poplar due to elevated CO_2 was observed (Radoglou & Jarvis 1990; Ceulemans et al. 1995, 1996).
Other studies on poplar reported an increased biomass due to elevated CO_2 (Radoglou & Jarvis 1990; Ceulemans et al. 1995, 1996; Curtis et al. 1995; Liberloo et al. 2005). In our study, elevated CO_2 has presumably acted as a stress factor. The positive effect of fertilization on the biomass could, however, only be seen on the above-ground biomass but not for the roots.

5.3 Biochemical responses to elevated CO_2

5.3.1 Beech

A reduction of the nitrogen content in plants grown under elevated CO_2 has been observed very often in the past (Cotrufo et al. 1998; Poorter et al. 1997; Yin 2002; Ainsworth & Long 2005). Also in the saplings of this study, the N content was lower when grown under elevated CO_2. The reduction amounted to 10% in the leaves but

distinctly less in stems and roots. Similar observations were reported by Cotrufo et al. (1998). What is the reason for such reduction? The physiological mechanisms responsible for this phenomenon have not been definitely established, although a considerable number of hypotheses have been advanced to account for it (Taub & Wang 2008). In their review, the authors discuss and critically evaluate these hypotheses. One contributing factor clearly is dilution of N by increased photosynthetic assimilation of C. In addition, studies show strong evidence for a general decrease in the uptake of N per unit mass or length of roots under elevated CO_2. This decreased uptake appears to be the result both of a decreased N demand by shoots and of a decreased ability of the soil-root system to supply the plant with N. The best-supported mechanism for a decreased N supply is a decrease in the transpiration-driven flow of N in the soil due to a decreased stomatal conductance at elevated CO_2, although some evidence suggests that altered root-system architecture may also play a role. There is also limited evidence suggesting that under elevated CO_2 plants may exhibit increased rates of N loss through volatilization and/or root exudation, further contributing to lowering the N concentrations (Taub & Wang 2008).

As we know, elevated CO_2 causes an increase of the photosynthetic activity (Bazzaz 1990; Saxe et al., 1998; Ceulemans et al., 1999) that is why an elevated C content was observed in all segments of the saplings grown under elevated CO_2. In fertilized saplings, a higher N content was obvious in plant organs but the C content remained unaffected by fertilization.

Due to an increase of N by fertilization, the resulting C/N ratio was lower in fertilized saplings grown under different CO_2 concentration.

5.3.2 Lignification of beech in response to elevated CO_2

A significant effect of different treatments on lignification in beech was detected only in the first study year. In 2010 the lignification of the walls of vessels and fibres was lowered by elevated CO_2 whereas fertilization was negatively effective only in ambient air; both effects were significant only in the first study year. Up to now, the lignification under elevated CO_2 was measured in leaves and roots by Blaschke et al. (2002) who observed that lignification became more intense under elevated CO_2 only when the supply with N

was limited, that is to say, elevated CO_2 has no influence on fertilized saplings. The authors argue that saplings are growing faster under elevated CO_2 and therefore their total lignin content becomes higher. In contrast, we assume that the fertilized saplings under elevated CO_2 have grown faster and in consequence had less time for lignification.

5.3.3 Poplar

The results for poplar were highly inconsistent and did not reveal any plausible trend, except that the fertilized saplings exhibited a lower C/N ratio.

5.4 Anatomical responses to elevated CO_2

Numerous studies have dealt with the question of how plants respond to elevated CO_2. There is, however, little information on anatomical changes of the wood formed under elevated CO_2. Therefore, we observed and measured some anatomical features of beech and poplar under elevated CO_2.

5.4.1 Beech

The tree-ring widths in beech increased significantly under elevated CO_2. Such a positive effect on the volume of the stem was reported also by Overdieck et al. (2007) for broad-leave trees and by Telewski et al. (1999) and Ziche & Overdieck (2004) for conifers.

Between tree-ring width and the three vessel parameters, there was no significant correlation. But there was a negative association between AVLA (average vessel lumen area) and the other vessel parameters (vessel density (VD) and total vessel lumen area (TVLA)) and a positive association between TVLA and VD.

AVLA did not experience any significant changes by the various experimental growing conditions, although the smallest vessels in both study years occurred in unfertilized saplings grown under elevated CO_2 – even if not significant. Conversely, Overdieck et al. (2007) found reduced AVLA values under elevated CO_2.

Elevated CO_2 resulted in an increase of VD and TVLA; an increase of VD was also observed for oak by Atkinson and Taylor (1996). On the assumption of a constant AVLA and a positive correlation between VD and TVLA, the increase of TVLA – resulting from elevated CO_2 – together with an increasing number of vessels is explainable.
In both study years, VD and TVLA were reduced as a consequence of fertilization. The reduction of TVLA resulted from the reduction of VD.

5.4.2 Poplar

Elevated CO_2 did not at all influence the tree-ring width of poplar; the same result was observed for Norway spruce (Kostiainen et al. 2009). On the other hand, the vegetation period was shorter under elevated CO_2 (Ceulemans & Mousseau 1994). In contrast, fertilization resulted in wider tree rings and longer vegetation periods. That is why the widest and narrowest tree rings were observed in fertilized saplings grown in ambient air and in unfertilized saplings grown under elevated CO_2, respectively.
Between tree-ring width and the three vessel parameters a significantly negative association was observed. Thus, vessel density (VD) did not respond to differing growing conditions. The vessel lumen area has increased under elevated CO_2 just as Luo (2005) has described for *Populus nigra* and *P. euramericana*. Due to the positive association between AVLA and TVLA, TVLA increased with increasing AVLA.
Why did fertilization reduce AVLA and TVLA? TVLA and AVLA decreased with increasing tree-ring width caused by fertilization. In this way, it can be explained that with increasing tree-ring width the proportion of latewood increases as well and in consequence the number of small vessels increases.

6. Conclusion

The most relevant results in short are as follows:

6.1 Beech and elevated CO_2

No effect on

- ✓ Height growth
- ✓ Number of leaves
- ✓ Root dry weight
- ✓ Average vessel lumen area (AVLA)

but evoked

- ✓ Higher photosynthesis
- ✓ Lower in transpiration and stomatal conductance
- ✓ Lower in chlorophyll concentration index (CCI)
- ✓ Higher stem dry weight
- ✓ larger leaves
- ✓ Lower N content and higher C content of tissues
- ✓ Increase in ring width (RW) and vessel density (VD)
- ✓ Decrease in total vessel lumen area (TVLA)

6.2 Poplar and elevated CO_2:

No effect on

- ✓ Transpiration and stomatal conductance
- ✓ Chlorophyll concentration index (CCI)
- ✓ Number of leaves
- ✓ Height growth
- ✓ Ring width (RW) and vessel density (VD)

But evoked

- ✓ Less total biomass
- ✓ Larger leaves
- ✓ Increase in total vessel lumen area (TVLA) and average vessel lumen area (AVLA)

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