Plant Soil (2009) 323:7–19
DOI 10.1007/s11104-009-9936-x

REGULAR ARTICLE

Experimental setup of field lysimeters for studying effects of elevated ozone and below-ground pathogen infection on a plant-soil-system of juvenile beech (*Fagus sylvatica* L.)

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Received: 5 November 2008 / Accepted: 11 February 2009 / Published online: 27 February 2009
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Abstract An experiment, focusing on the effects of chronically enhanced O_3 regimes on young beech (*Fagus sylvatica*) and on the microbial rhizosphere community structure, was conducted from November 2002 to August 2006 in eight field lysimeters at the Helmholtz Zentrum München. The instrumentations of the lysimeters enabled the establishment of the water balance in the unsaturated zone and the assessment of the water uptake by plants. Further, the containment provided by the lysimeters made it possible to apply a root rot pathogen infection without contaminating the surrounding soil. A free-air fumigation system allowed to double the O_3 concentration in the air above four lysimeters relative to the ambient air. To avoid damage of the leaves the maximum O_3 concentration was limited to 150 nL L^{-1}. For nearly 70% of the time the set-point concentration was reached within 10%. In the final harvest the whole soil column was retrieved and a nearly complete data-set of above-ground and below-ground parameters became available.

Keywords Free-air ozone enrichment · Field lysimeters · $^{13}CO_2$ labelling · European beech · Phytophthora citricola infection

Responsible Editor: Per Ambus.

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Introduction

The global background ozone (O_3) concentrations have increased in the last decades and are predicted to persist at high levels (Ashmore 2005; The Royal Society 2008). Ozone is one of most widespread air pollutants and the impact of tropospheric O_3 on plants has been investigated for various agricultural crops and forest trees (Benton et al. 2000; Calatayud et al. 2002; Castagna et al. 2001; Fuhrer and Booker 2003; Karnosky et al. 2005; Manning 2005; Matyssek 2001; Matyssek et al. 2007; Oksanen et al. 2007; Reich 1987; Wittig et al. 2007).

The results from chamber studies with juvenile trees have been discussed by various authors (cf. Karnosky et al. 2005; Kolb and Matyssek 2001; Matyssek et al. 2007; Nunn et al. 2005; Samuelson and Kelly 2001).

To clarify the O_3 effects on the plant–soil-system a lysimeter study was set up with juvenile beech trees. The beeches were exposed for four years to ambient and elevated ozone concentrations respectively. In contrast to the chamber studies the beeches grew under field conditions providing a realistic span of climatic conditions and elevated O_3 concentrations (Pritsch et al. 2008; Schloter et al. 2005).

The lysimeter study was designed as an intermediate step between phytotron studies with 1- to 3-year old seedlings (Kozovits et al. 2005a, b; Luedemann et al. 2005) and studies on mature trees in the Kranzberg forest (Matyssek et al. 2005; Nunn et al. 2002, 2006). Lysimeter studies provide serveral advantages: while in the phytotron studies tree size is limited by container or pot size, the lysimeter soil volume is large enough to avoid restriction of plant growth due to insufficient below-ground space during the duration of the experiment. Additionally, there is access to the whole root system which is hardly possible for adult trees. A detailed spatially resolved below-ground sampling during the final harvest helped to get further insight into the possible effects of O_3 on soil processes. These are still not well-understood because the determination of below-ground parameters is hampered by their relative inaccessibility (Andersen 2003).

Lysimeters which are equipped with load cells and soil moisture probes in different depths allow for an exact determination of water balances and thus to access accurately the impact of O_3 on the evapotranspiration of the whole beech–soil-system, and to detect possible changes in stomatal behaviour that might occur under O_3 exposure (Paoletti and Grulke 2005).

Besides direct detrimental effects of O_3 on plants, many secondary O_3 effects such as the decrease or increase in plant fungal diseases have been observed which are not yet understood well (The Royal Society 2008). Our lysimeter experiment which combines the effects of chronic O_3 stress and a below-ground pathogen infection aims to yield a deeper insight on effects of the interaction of ozone exposure and biotic stress on the whole plant–soil system.

This paper describes the experimental set-up of the lysimeter study including the design and performance of the free-air O_3 fumigation system, the climatic conditions during the experiment and the potential of the lysimeters for global change research.

Material and methods

Lysimeters

The experiment was conducted at the out door lysimeter facilities of the Helmholtz Zentrum München, German Research Center for Environmental Health, (48°13′ N 11°36′ E, 490 m altitude) and used eight of the 48 lysimeters of the station.

Filling

The lysimeter vessels are made of stainless steel (V4A), they have a surface area of 1 m^2 and a depth of 2 m. As soil excavation inevitably involves destruction of soil structure, the most prevalent and recommended method to fill lysimeter vessels of this size is the undisturbed extraction of soil monoliths (Meissner et al. 2008; Unhold and Fank 2008). However, in forest soil contrasting to agricultural soils the heterogeneity due to remaining roots is usually very high. Therefore, in order to minimize heterogeneity between individual lysimeters, in this experiment the lysimeters were filled manually. In March 1999, soil (A- and B-horizon from a dystric Cambisol derived from Pleistocene Loess above tertiary sediments with a composition of 34% sand, 46% silt and 20% clay) was excavated in a beech stand at a forest site (“Höglwald”, 48°18′ N 11°05′ E, 540 m, Bavaria, Germany). The soil from each horizon was sieved separately (50 mm mesh) and then filled into the eight lysimeter vessels retaining the natural stratigraphy. During the filling, the soil was only slightly compressed by cautiously treading it down every 20 cm using soles with a coarse tread. After the filling the lysimeters were immediately placed at their final position in the lysimeter field. The area around the lysimeters was filled with the same soil. During the following 3 years the soil was exposed to ambient climatic conditions and was left untreated to allow natural compaction, stabilization, and development of a typical soil structure.

In July 2002, the upper soil horizon (Ah, approx. 30 cm) was replaced with fresh soil (Ah horizon) from a comparable forest site (Eurasburg) to provide a microbiologically active substrate for the trees. In November 2002, four nursery-grown beech saplings (*Fagus sylvatica* L., three years old, approx. 60 cm

high) were planted into each lysimeter (Fig. 1). The surrounding of the lysimeters was also planted with beech saplings in the same density to attain a homogenous beech stand (Fig. 2). During the four experimental growing periods tree samples were taken from these beeches and soil samples from outside the lysimeters so that the soil and plants in the lysimeters could be left undisturbed (Pritsch et al. 2008).

The lysimeters received natural precipitation. However, during the extremely hot and dry summer 2003 an additional irrigation had to be applied seven times (between June 26th and August 26th a total amount of 56 mm) to prevent shrinking of the soil columns and the detachment of the soil from the lysimeter tubes, which would have irreversibly changed water fluxes.

Maintenance of lysimeters comprised removal of weeds and control of occurrence of pests.

Instrumentation

The lysimeters were instrumented with temperature sensors, time domain reflectometry probes to measure the soil moisture and tensiometers to measure the soil water potential at five depths (30, 50, 80, 155, and 190 cm below soil surface). Further, with load cells the mass and the outflow of the lysimeters were measured with an accuracy of 100 g (corresponding to 0.1 mm precipitation) and 10 g, respectively. Measurements were performed every 10 min; the data were averaged over 1 h and transferred to the data base hosted at the Computing Centre of the Helmholtz Zentrum München. The outflow was collected in seepage tanks for chemical analyses like pH, conductivity, inorganic elements, and compounds, TOC and DOC. For a more detailed description of the lysimeter facility see Reth et al. (2008).

Meteorological data

Additional to the soil parameters, meteorological data (among others air temperature, relative humidity, precipitation amount and global radiation) were measured continuously at a meteorological station 100 m south of the experimental plot; means over 10 min were stored in the database of the research centre. The lysimeter and meteorological data set allow a rather accurate determination of the evapotranspiration rate.

Ozone fumigation system

The eight lysimeters of the experiment were arranged in two rows of four lysimeters. Each row was

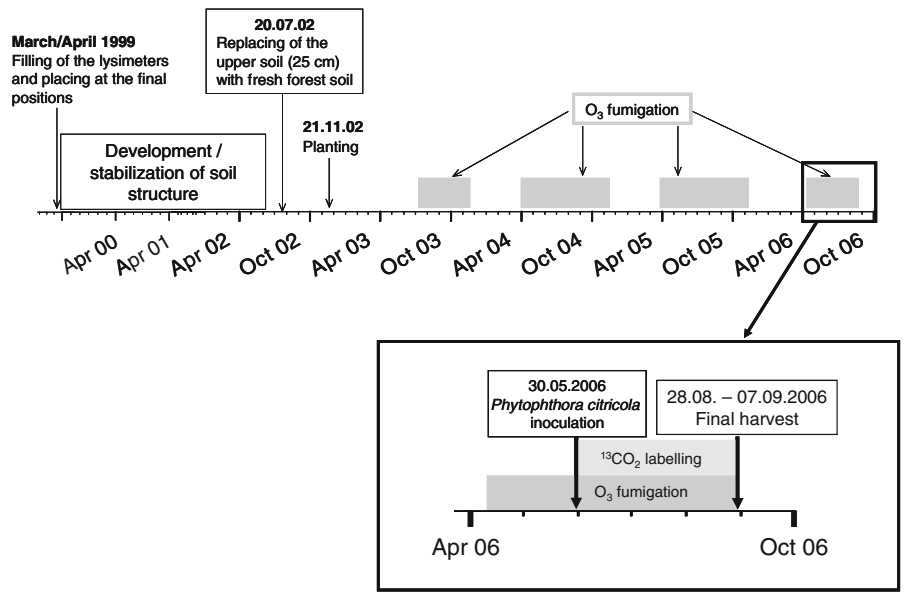

Fig. 1 Time line of the experiment. The upper scale shows the total period, the lower scale the experimental treatments during the vegetation period 2006

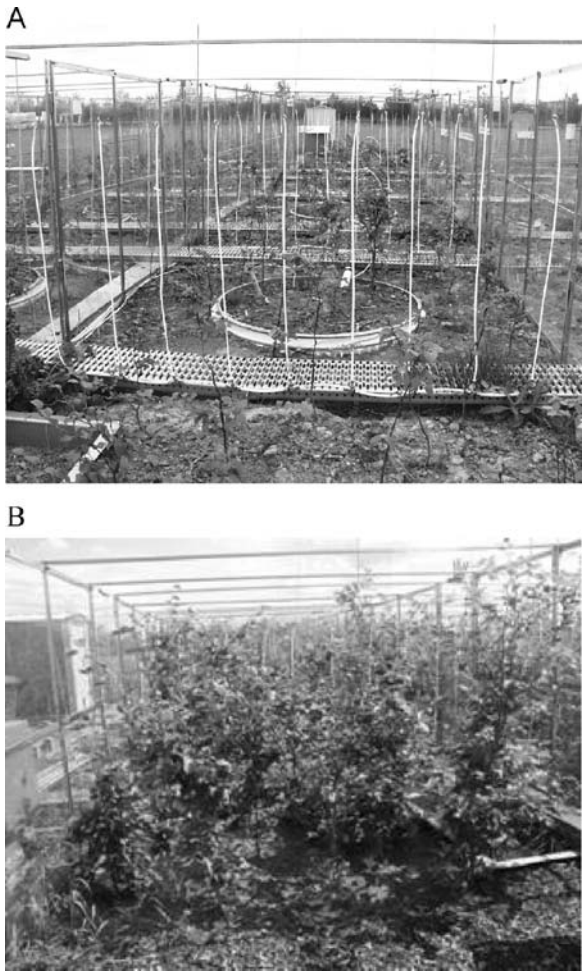

Fig. 2 View towards the northern lysimeter row. **a** Trees in 2003 were approximately 60 cm high—in the front the tube-curtain of the free air ozone enrichment system can be seen. **b** View in August 2005 when trees were up to 180 cm high

orientated in east–west direction. The canopy at the four northern lysimeters (N1 to N4) was exposed to twice ambient ozone levels (elevated) and the four southern lysimeters (S1 to S4) to ambient air (control, Fig. 3). To avoid air exchange between the lysimeter rows and to insure homogeneous fumigation, walls of acrylic glass 1.5 m high were installed both at the northern and southern border as well as between the two lysimeter rows.

To expose the trees on the northern lysimeter row to chronically ozone stress throughout the vegetation period a free air O_3 fumigation system (cf. Karnosky et al. 2001, 2007) for young trees was developed comparable to that used for fumigation of mature trees in the Kranzberg forest (Werner and Fabian 2002). The system was designed to maintain twice ambient O_3 concentration in the canopy of each lysimeter. To prevent leaves from acute ozone injury ozone concentration was restricted to an upper limit of 150 nL L^{-1} (Matyssek and Sandermann 2003).

Air (800 L min^{-1}) provided by four compressors was released through eight curtain-like tube-systems onto the experimental area (both on the fumigated and the control lysimeters). Each "tube-curtain" consisted of nine vertical arranged Teflon® tubes (6 mm diameter). Each tube had perforations of 1 mm diameter every 150 mm through which air was released. The length of the tubes and number of perforations were adapted to the height of the trees. The length of the tubes had to be changed from 1,000 mm at the beginning of the experiment to 1,800 mm at the end of the experiment and the number of the perforations from six to nine. The air volume of each row fumigated by a set of four tube-curtains increased from 30 to 54 m^3.

At the northerly four O_3-fumigated lysimeters, an ozone-oxygen-mixture was added continuously (1.7 L min^{-1}) to the air released through the curtains. The ozone was produced from oxygen by corona discharge using two voltage controlled ozone generators (OZ-500, Fischer, Germany). The oxygen was provided from an automatically switching two-bottle-system. The O_3 concentration of the ozone-oxygen-mixture was 0 to 3 µL L^{-1} depending on wind speed, on wind direction and on ambient O_3 concentration.

The ambient O_3 concentration was measured outside the lysimeter field and the O_3 concentrations for the control of the O_3 fumigation were measured in the centre of the eastern- and westernmost lysimeter at the height of the canopy. The actual O_3 concentrations at these three measuring points ("east", "west" and "ambient") were recorded every 10 seconds by three autonomous ozone analysers (CSI-3100, Columbia Scientific Industries, US) (O_3 A, Fig. 3). With these data a software (based on LabView, National Instruments, US) calculated the control voltage for the ozone generators. As the tube-curtains of the fumigation system were installed nearby both the easternmost and westernmost lysimeters, the two central lysimeters received the ozone by the wind tunnel effect of the acrylic glass walls.

Separately, the O_3 concentrations were measured at additional 10 measuring points by an O_3 analyser (CSI-3100, Columbia Scientific Industries, US): in

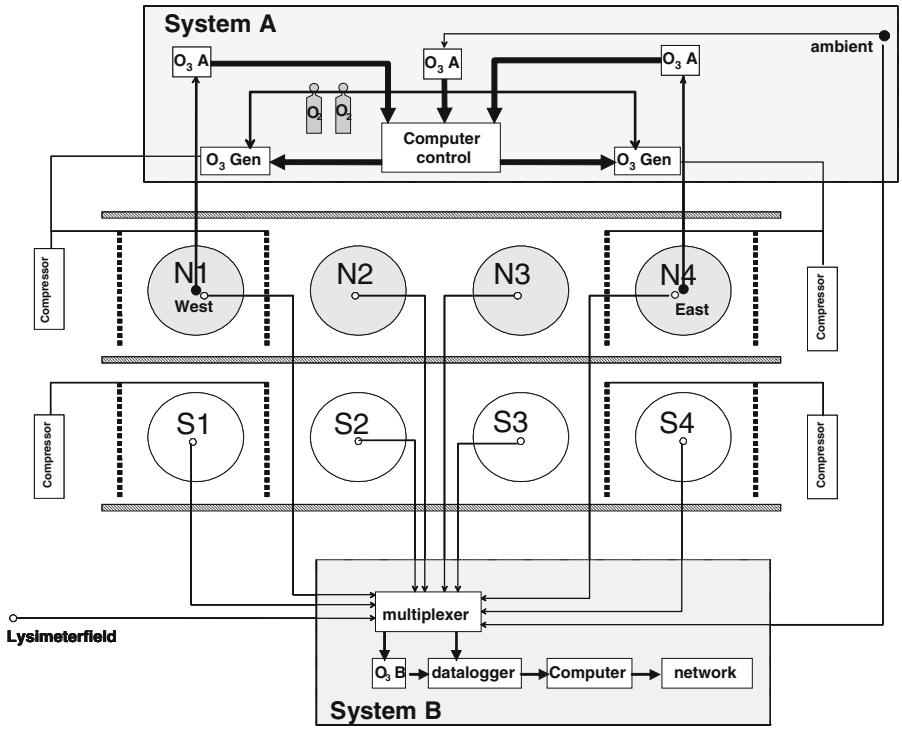

Fig. 3 Schematic sketch of the lysimeters and the free-air O_3 enrichment configuration. The System A is used for O_3 fumigation control and the System B for recording the O_3 concentrations in the centre of each lysimeter (*O_3 Gen* O_3 generator, *O_3 A* O_3 analyser for control system, *O_3 B* O_3 analyser measuring network). The O_3 fumigated lysimeters are arranged in east-west direction in the northern row (N1–N4, *grey*). The southern row provided the controls fumigated with ambient air (S1–S4, *white*)

the canopy above the each of the eight lysimeters, in the surrounding area and outside the lysimeter (ambient values). Every 50 min averages over 5 min were recorded in a data base.

Ozone fumigation started in June 2003 and was continued during daytime (sunrise to sunset) until December 2003. In 2004 and the following years, ozone fumigation started before bud break (beginning of May) and ended in 2004 and 2005 after completion of leaf senescence (end of October; see also Pritsch et al. 2008). In 2006 fumigation was stopped at the end of the experiment on August 28th when plants were harvested. During the fumigation periods O_3 fumigation was only briefly interrupted during sampling and measurements for safety reasons.

Pathogen inoculation

In order to stimulate a biotic stress, an inoculation with the root rot pathogen *Phytophthora citricola* was applied at two lysimeters with ambient O_3 and two lysimeters with elevated O_3 concentration. Many herbaceous and woody plants are susceptible to pathogens of the genus Phytophthora (Erwin and Ribeiro 1996; Oßwald et al. 2004). Previous studies showed that *P. citricola* infection could be very harmful for beech seedlings (Fleischmann et al. 2002, 2004, 2005). Inoculation was carried out after the leaves were fully developed on May 30th 2006. A suspension with *P. citricola* zoospores was given into three 40 cm deep holes per tree at the easternmost (N4, S4) and westernmost (N1, S1) lysimeters (Fig. 4) to avoid contamination of the "infection-control" lysimeters (for details see Fleischmann et al. 2009). Subsequently each lysimeter was irrigated with 12.5 L of deionised water to allow infection of the roots by the zoospores and the same procedure was performed on the following day. At the end of the experiment infestation of the roots was quantified by TaqMan real-time quantitative PCR (qPCR) (Fleischmann et al. 2009).

$^{13}CO_2$ labelling

Concomitant to the inoculation with the root pathogen, a $^{13}CO_2$ tracer experiment was started at all lysimeters to study carbon fluxes and allocation in the plant soil system. For this, the atmospheric CO_2 concentration in both treatments was enhanced by 75 μL L^{-1} with CO_2 derived from fossil-fuel burning

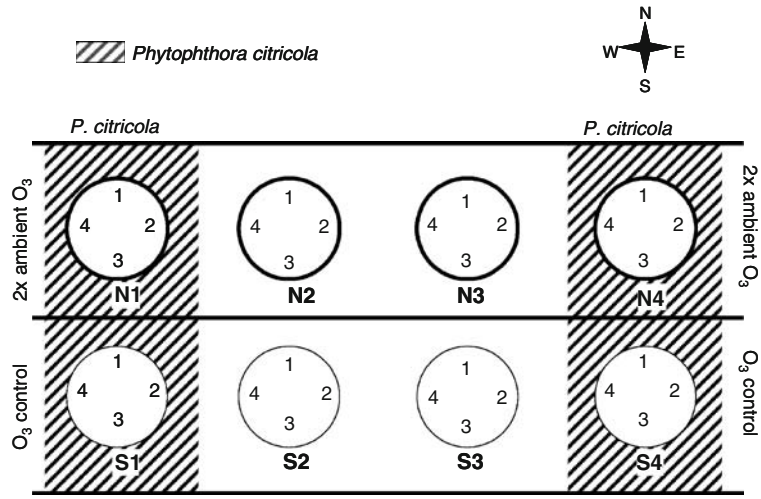

Fig. 4 Schematic view of the lysimeters and the different treatments in 2006: elevated O_3 (*thick circle lines*), ambient O_3 (*thin circle lines*), *Phytophthora citricola* inoculation (*coarse pattern*) on the lysimeters N1, S1, N4 and S4

(isotopic signature −47‰ V-PDB ^{13}C; Fig. 1) using the tubing of the O_3 fumigation system. CO_2 concentration was measured hourly in the centre of each lysimeter at the height of the canopy by means of photo-acoustic CO_2-controllers (7MB1300, Siemens, D). The average concentration of two lysimeters next to the vertical tubing (cf. Fig. 3) was used to control the inflow of tank-CO_2 into the tube-curtains via mass-flow controllers (MKS Andover, US) which were operated by a control program based on LabView (National Instruments, US). The addition of CO_2 was restricted to 75 μL L^{-1} to avoid strong alteration of photosynthesis or mitigation of the response to the elevated O_3 treatment (King et al. 2005). The labelling was continued until end of the experiment on August 28th 2006. Based on the $\delta^{13}C$ of CO_2 of the ambient air and of the tank-CO_2, −10‰ and −47‰ respectively, and the CO_2 concentrations (ambient and at the lysimeters) the daily mean $\delta^{13}C$ values of the CO_2 at the lysimeters were calculated.

The labelling setup was tested in 2005 in an open top chamber study. Because the added CO_2 accounted for only about 20% of the total concentration the labelling was relatively small. However, the isotopic

Table 1 Precipitation, temperature, and global radiation from 2003 to 2006 at the lysimeter station

	Year	Annual	Growing period (May to September)
Sum of precipitation (mm)	2003	546	267
	2004	742	364
	2005	876	533
	2006	873	422
Mean air temperature (°C)	2003	8.9	18.1
	2004	8.6	15.4
	2005	8.2	15.7
	2006	8.9	16.7
Integral of global radiation (MJ m^{-2})	2003	4,237	2,767
	2004	4,225	2,689
	2005	4,305	2,696
	2006	4,394	2,860

Given are annual values as well as values during the vegetation period (May to September)

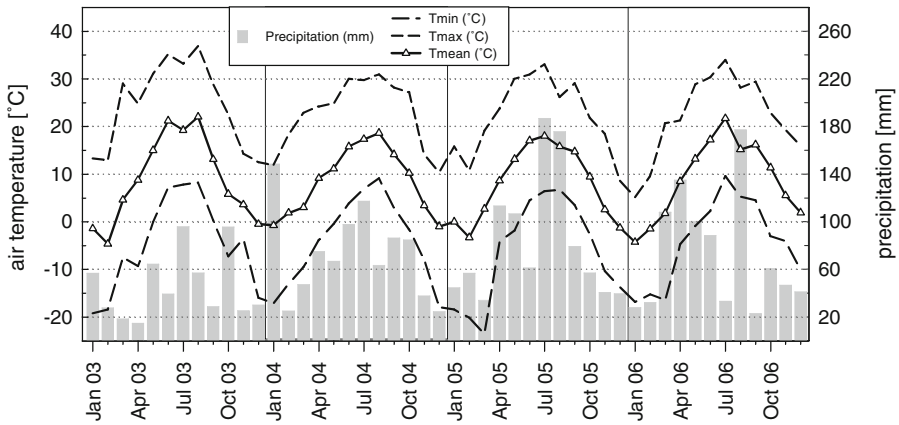

Fig. 5 Monthly amount of precipitation and mean (*triangles*, *solid line*), maximum and minimum temperature measured at the lysimeter station of the Helmholtz Zentrum München during the experiment (2003–2006)

signal could be recovered in different plant parts as well as in the soil microbial community and bulk soil (Esperschütz et al. 2009a, b).

Harvest

From August 28th to September 7th 2006, a complete harvest of the plants grown on the lysimeters was conducted.

Above-ground plants parts were cut off at the soil surface and separated into stems, axes, leaves (sun and shade) and buds. After harvesting the above-ground biomass the below-ground sampling was carried out by application of a newly developed Lysimeter Soil Retriever (LSR) technique for the first time (Seyfahrt and Reth 2008). The LSR allows sampling even in lower depths with minimal disturbance. The upper part of the soil column was cut into five disks each 20 cm thick beginning at the top of the lysimeters downwards. Samples were taken for different analysis from each disk. The soil in the lower part (from 100 to 200 cm depth) was dug out manually and then sieved to collect the roots quantitatively also in the deeper soil layer to assess complete tree biomass (above- and below-ground parts) (Winkler et al. 2009).

Results

Climatic conditions

During the four years of the experiment temperature and precipitation changed considerably. The year 2003 was extremely dry with only 546 mm annual

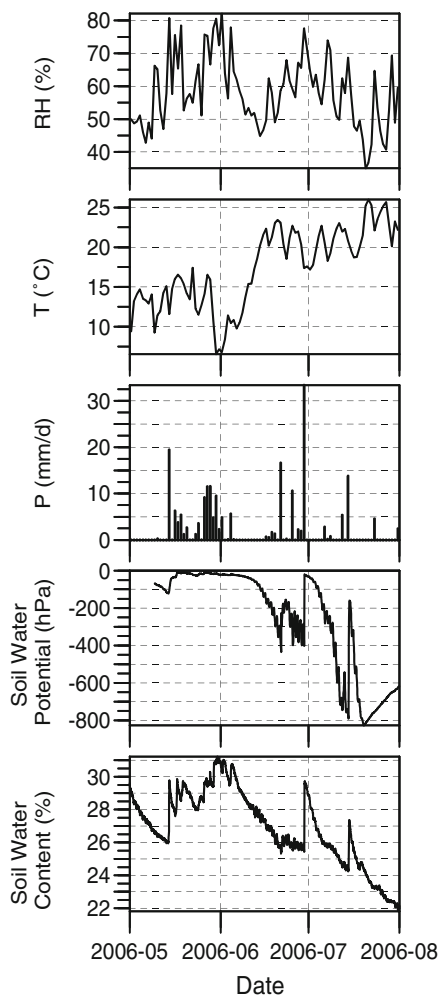

Fig. 6 Records of meteorological data: the daily sum of precipitation rate (*P*), the daily means of air temperature (*T*) and the relative humidity (*RH*) as well as lysimeter related data: hourly means of the soil water content and the soil water potential (Ψ_{soil}), measured at 30 cm depth in lysimeter S4

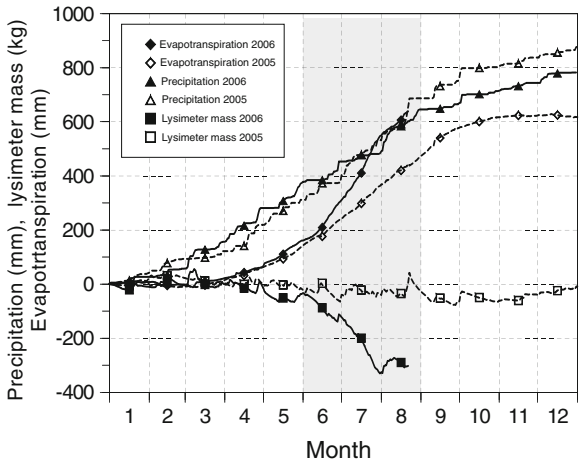

Fig. 7 Cumulative values of precipitation amounts, lysimeter mass changes and evapotranspiration rates for the year 2005 and 2006 for lysimeter N4. The decrease of the lysimeter mass in the summer 2006 is caused by an exceptional high water uptake by the plants during this period and not by precipitation rates or by lysimeter outflow (data not shown)

precipitation and 267 mm during the growing period, respectively (Table 1). The mean annual temperature was not very different from 2004 to 2006 but the summer 2003 was very hot and the mean temperature during the vegetation period was 18.1°C and was thus 2.7 K higher than in 2004 and was still 1.4 K higher than in 2006, the second warmest year. The summer 2004 was cool (15.4°C) and dry as precipitation was less than in 2005 and 2006. The winter 2004/2005 was very cold and in mid May 2005 temperatures still dropped below zero causing frost damage to young leaves. Mean monthly temperature between May and September 2005 never reached 20°C and precipitation was highest in summer 2005 (533 mm; Fig. 5). A relatively cold winter was followed by a warm growing period in 2006 with very contrasting monthly amounts of precipitation. In July 2006 precipitation was only 22 mm and compared to the corresponding month in previous years less than 50% in 2003 and even less than 20% of 2005. In contrast, in August 2006 almost 176 mm rain were recorded which was very similar to the amount measured in August 2005.

The annual amount of global radiation was highest in 2006 (4,394 MJ m^{-2}) as well as the insolation during the vegetation period May to September (2,860 MJ m^{-2}).

The records in Fig. 6 give an example of the lysimeter and the meteorological data and show the response of soil water content and soil water potential to precipitation events and water uptake by plants. During a dry and warm three week period in June 2006 the water content declined by approximately 5%. A precipitation event at the end of June increased both, the water content and, as expected, the water potential. The increase was only momentary and the soil water decreased again interrupted by excursions to higher values caused by smaller precipitation events in July until water potential reached the threshold value of the tensiometer (about 800 hPa). The increase of the tensiometer reading thereafter is artificial. The continuous decline of the water content in June and July was probably due to the intense water uptake of the beeches. This might be assumed

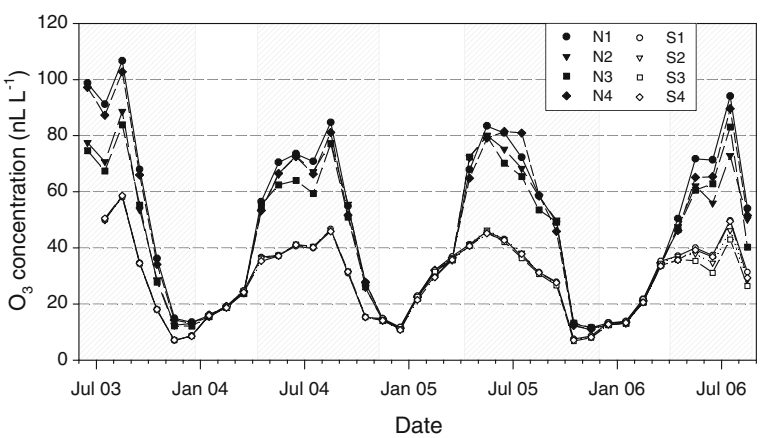

Fig. 8 Monthly means of daytime O_3 concentrations measured at the lysimeters (*open symbols* lysimeters under ambient O_3, *closed symbols* lysimeters under twice-ambient O_3)

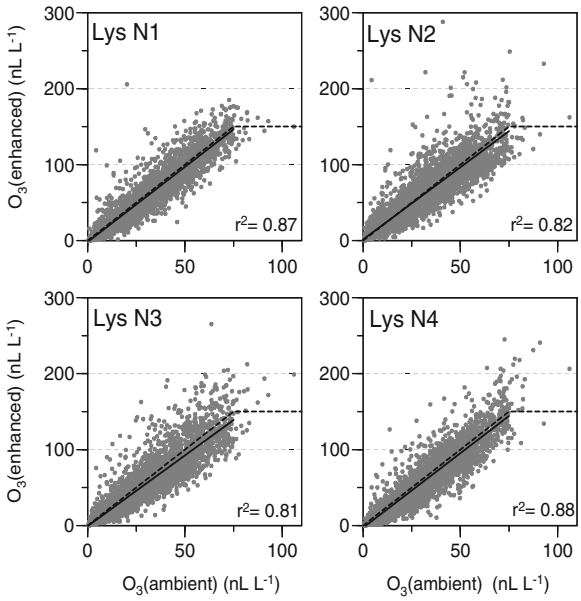

Fig. 9 Quality of free air ozone enrichment at the lysimeters. Shown are hourly means of elevated daytime O_3 concentrations versus the ambient daytime concentrations during the fumigation period 2005. The *solid line* shows the linear regression and the dashed line the desired twice ambient O_3 concentration (limited to 150 nL L^{-1})

because in June the air temperature increased by about 10 K (the level was kept in July) and the relative humidity tend to lower values which resulted in a higher vapour pressure deficit of the air and hence in higher transpiration rates. This is confirmed by the evapotranspiration rates calculated from the lysimeter and precipitation data (Fig. 7). From January 1st to August 22nd 2006 the evapotranspiration was much higher than in the same period in the year 2005, 638 kg-H_2O compared to 445 kg-H_2O.

Free air ozone enrichment

During all vegetation periods mean monthly ambient O_3 concentration during daytime was between 16 and 58 nL L^{-1}. The O_3 concentration in the enriched lysimeters was on average 1.7× ambient O_3 (Fig. 8). The slightly reduced enrichment compared to the target value "twice-ambient" was due to the upper limit of 150 nL L^{-1} and to interruption of the fumigation by researchers for sampling or experimental work during the vegetation period (Fig. 9). Hence, with the developed experimental fumigation plant twice ambient O_3 concentration was almost met.

The surrounding trees reduce possible O_3 gradients and as the O_3 concentration was measured in the centre of each lysimeter we can assume that O_3 concentration for all plants in the lysimeter was in the same range. During the fumigation period 2003, the mean monthly O_3 concentrations at the inner lysimeters (N2 and N3) were 20% lower than and the outer lysimeters (N1 and N4). Probably due to the more closed canopy in 2004 and 2005 this difference was only about 5% and 4%, respectively, but became again higher in 2006 (14%).

Cumulative seasonal values of ozone exposure can be expressed as AOT40 (accumulated seasonal exposure over 40 nL L^{-1}) or as sum0 (cumulative seasonal exposure during daylight). During the four growing periods of the experiment, the mean AOT40 doses on the enriched lysimeters ranged between 52.6 and 79.3 µL O_3 L^{-1} h and were on average 4.5 (±0.74) times higher than AOT40 values on the ambient O_3 lysimeters (Table 2). Comparable to the monthly average O_3 concentrations, the sum0 on the enriched lysimeters was on average 1.8 (±0.07)

Table 2 AOT40 and sum0 values throughout the vegetation periods between 2003 and 2006

	Treatment	2003		2004		2005		2006	
		1/4–31/10	18/6–31/10	1/4–31/10	18/6–31/10	1/4–31/10	18/6–31/10	1/4–31/10	18/6–31/10
AOT 40 (µL L^{-1} h)	Elevated	–	58.8	74.6	44.6	79.3	39.9	52.6	28.0
	Control	19.1	14.4	15.0	9.9	15.1	7.1	14.3	7.9
Sum0 (µL L^{-1} h)	Elevated	–	153.5	220.2	131.4	228.8	119.8	178.7	90.1
	Control	123.5	81.0	127.5	76.9	127.4	67.1	125.1	65.4

For better comparison of the O_3 exposure in 2003 which started in July the AOT40 and sum0 values for the period July 18th to October 31st are also given

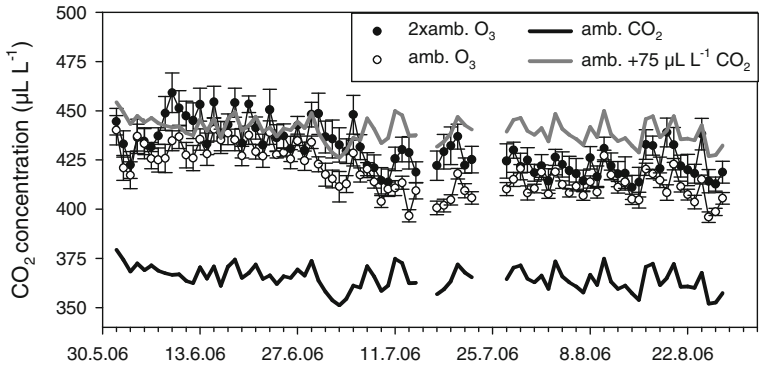

Fig. 10 Daily means of CO_2 concentration during day (8 A.M. to 6 P.M.) at the ambient O_3 lysimeters (*open symbols*, n=4±SE) and the elevated O_3 lysimeters (filled symbols, n=4±SE) as well as ambient CO_2 concentration (*black line*) and the desired concentrations of ambient +75 µL L^{-1} CO_2 during the $^{13}CO_2$ labelling in 2006

times higher than sum0 under the ambient O_3 conditions.

$^{13}CO_2$ labelling

The desired concentration of +75 µL L^{-1} during daylight hours (8 A.M. to 6 P.M.) was reached throughout the first 4 weeks of labelling. From beginning of July the CO_2 enhancement was approx. +50 µL L^{-1} in the ambient O_3 lysimeters and +60 µL L^{-1} in the elevated O_3 lysimeters, respectively (Fig. 10).

The estimated seasonal average $\delta^{13}C$ values during daylight hours (8 A.M. to 6 P.M.) were between −14.3‰ and −15.1‰ at the ambient O_3 lysimeters and between −15.2‰ and −16.5‰ at the elevated O_3 lysimeters (Fig. 11).

Pathogen infection

Results of quantitative real-time PCR analysis showed that infestation in the inoculated lysimeters occurred down to 40 cm depth with highest infestation in 0–20 cm depth (5.9 and 38.3 ng *P. citricola*-DNA per g root dry weight) (Fleischmann et al. 2009). But no differences in infestation were found between both O_3 treatments. As intended, no infestation was observed in the non-inoculated lysimeters (Fleischmann et al. 2009).

Harvest

The total biomass of the individual trees was not affected by any treatment. This refers to both, to above-ground and to below-ground biomass (Winkler et al. 2009). However, as shown by Winkler et al. (2009) ozone had an impact on the vertical distribution of root biomass. The proportion of fine root biomass located in the uppermost 20 cm of the soil was higher under twice ambient O_3 (39±3%) than in the ambient O_3 regime (26±2%). In contrast, below 100 cm depth fine root biomass was significantly reduced by the twice ambient O_3 treatment (p=0.007).

Discussion

The lysimeter experiment was set up as the intermediate on the ontogenetic scale from young plants

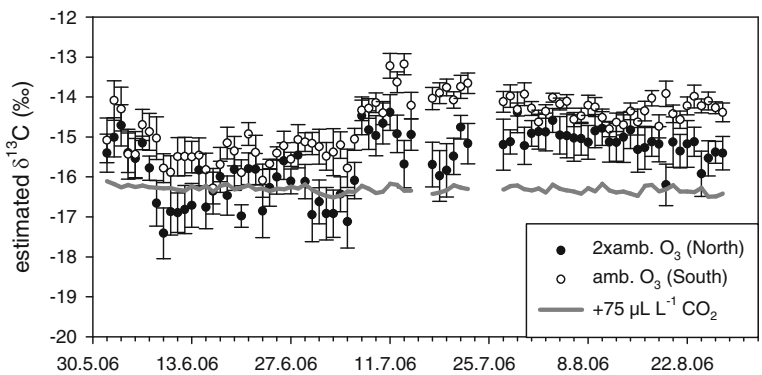

Fig. 11 Estimated $\delta^{13}C$ at the lysimeters under elevated O_3 (*filled symbols*) and at the lysimeters under ambient O_3 (*open symbols*). Values were calculated from daily average CO_2 concentration at each lysimeter (n=4). The *grey line* corresponds to the target $\delta^{13}C$ values

under controlled conditions in the phytotrons and glasshouse experiments to the adult trees in the field experiment at the Kranzberg forest.

Natural soil texture and aggregation was disturbed by excavation of the soil and filling of the lysimeters. However, at the end of the experiment soil bulk density in the eight refilled lysimeters was depending on soil depth between 1.0 and 1.7 g cm^{-3} and similar to the conditions at the natural forest site Höglwald (Gayler et al. 2009; Kreutzer and Bittersohl 1986). Probably the initial stabilization phase of 3 years of the soil was long enough for establishing a soil structure similar to the natural site. Luster et al. (2008) showed that already after an initial phase of one year refilled lysimeters can be used for most studies.

By the free air ozone fumigation system the trees were chronically exposed to elevated O_3 concentrations throughout almost four entire vegetation periods with very contrasting climatic conditions. The ozone fumigation was very close to the desired concentration of twice ambient O_3. The gradients in O_3 concentration between the four lysimeters were comparable to gradients that were observed also in other free air enrichment systems (Hendrey et al. 1993; Pepin and Körner 2002; Volk et al. 2003). The AOT 40 and sum0 values measured in 2004 at the lysimeters were within the same range as values achieved with a free air exposure system for adult trees in the Kranzberg Forest (50 km north of Munich; Löw et al. 2006; Matyssek et al. 2007).

The labelling with $^{13}CO_2$ in the last growing period of the experiment allowed tracing the photosynthetically fixed carbon in the plants and the carbon transfer to the microbes in the rhizosphere (Esperschütz et al. 2009a, b) and to the bulk soil. So far a continuous fumigation with ^{13}C depleted CO_2 under outdoor conditions was only done in FACE studies where the CO_2 enrichment was almost double the concentration we used in our experiment (Matamala et al. 2003; Pendall et al. 2001; Pepin and Körner 2002). The CO_2 concentration was slightly different between the two O_3 treatments due to technical reasons. This also led to the differences in the calculated isotopic ratios between both O_3 treatments. Furthermore, measurements of leaf gas exchange or soil respiration carried out in 2006 (data not shown) did not indicate that the higher CO_2 concentrations under double ambient O_3 were due to changes in photosynthesis or respiration.

The instrumentation of the lysimeters made it possible to establish the relationship between the water content and the water potential and to calculate the water fluxes in the water unsaturated zone. Having the lysimeter mass, the outflow from the lysimeter and the precipitation amount as the main components of the water balance, the water uptake by plants and the evapotranspiration rates can be assessed.

The volume of one lysimeter vessel allowed to grow up four trees to a height of almost two meters. The application of the root rot pathogen under field conditions was only possible by using the lysimeter setup because the closed soil compartment avoided the infection of the control trees.

During the first years of the experiment, the effect of the chronic ozone stress was not consistent under the different climatic conditions (Schloter et al. 2005; Pritsch et al. 2008). However, one has to take into account that the experiment integrates the response to elevated O_3 over four vegetation periods with different climatic conditions, and this may give a more uniform picture. The Lysimeter Soil Retriever technique enabled us to access the below-ground part of the biomass in the final harvest. Thus a nearly complete data-set of both the above-ground plant organs and the below-ground components such as roots, mycorrhiza, rhizosphere and the bulk soil became available. Furthermore, the effect of above-ground O_3 exposure and below-ground pathogen infection on the vertical distribution of roots could be described with a high spatial resolution.

The field lysimeters, usually widely used in hydrology, proved to be a valuable tool for ecological research such as investigation of the responses of a whole plant-soil-system to abiotic and biotic stress.

Acknowledgements This work was supported in parts by the German Research foundation (DFG) through the 'Sonderforschungsbereich' (SFB) 607. The authors would like to thank H.-D. Payer for his engagement to realise the experiment and O. Gefke, P. Bader, D. Schneider, M. Kugelmann and the technical staff from the Department of Environmental Engineering for their valuable support during the experiment.

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