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METHODS

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Web-FACE: a new canopy free-air CO_2 enrichment system for tall trees in mature forests

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Abstract The long-term responses of forests to atmospheric CO_2 enrichment have been difficult to determine experimentally given the large scale and complex structure of their canopy. We have developed a CO_2 exposure system that uses the free-air CO_2 enrichment (FACE) approach but was designed for tall canopy trees. The system consists of a CO_2-release system installed within the crown of adult trees using a 45-m tower crane, a CO_2 monitoring system and an automated regulation system. Pure CO_2 gas is released from a network of small tubes woven into the forest canopy (web-FACE), and CO_2 is emitted from small laser-punched holes. The set point CO_2 concentration ($[CO_2]$) of 500 µmol mol^{-1} is controlled by a pulse-width modulation routine that adjusts the rate of CO_2 injection as a function of measured $[CO_2]$ in the canopy. CO_2 consumption for the enrichment of 14 tall canopy trees was about 2 tons per day over the whole growing season. The seasonal daytime mean CO_2 concentration was 520 µmol mol^{-1}. One-minute averages of CO_2 measurements conducted at canopy height in the center of the CO_2-enriched zone were within ±20% and ±10% of the target concentration for 76% and 47% of the exposure time, respectively. Despite the size of the canopy and the windy site conditions, performance values correspond to about 75% of that reported for conventional forest FACE with the added advantage of a much simpler and less intrusive infrastructure. Stable carbon isotope signals captured by 80 Bermuda grass (*Cynodon dactylon*) seedlings distributed within the canopy of treated and control tree districts showed a clearly delineated area, with some nearby individuals having been exposed to a gradient of $[CO_2]$, which is seen as added value. Time-integrated values of $[CO_2]$ derived from the C isotope composition of *C. dactylon* leaves indicated a mean (±SD) concentration of 513±63 µmol mol^{-1} in the web-FACE canopy area. In view of the size of the forest and the rough natural canopy, web-FACE is a most promising avenue towards natural forest experiments, which are greatly needed.

Keywords Elevated CO_2 · Exposure · Forest ecology · Global change · Stable carbon isotopes

Introduction

For the last 3 decades, there has been considerable scientific effort to determine plant responses to rising atmospheric CO_2 concentration ($[CO_2]$). Although the effects of elevated CO_2 and temperature on tree responses have been described extensively, there is a growing consensus that long-term forest tree research must be carried out under more realistic growth conditions and accommodate more diversified communities (Eamus and Jarvis 1989; Körner 1995, 2000; Ceulemans et al. 1999; Norby et al 1999). This is because mature forests typically have a closed canopy (with a leaf area index constrained by abiotic factors), a coupled plant-soil system (with microflora and fauna, and a steady-state nutrient cycle) and, in mixed forests, interspecific competition for resources. Such natural growth conditions and complexity of biotic interactions have not been fully explored in previous studies.

The recent advances with large-scale free-air CO_2 enrichment (FACE) experiments have partially resolved these issues. To expose plant systems to elevated CO_2 concentrations, the FACE technology uses no confinement structures but an array of vertical or horizontal vent pipes to release jets of CO_2-enriched air or pure CO_2 gas at the periphery of vegetation plots. It then relies on natural wind to disperse the CO_2 across the experimental area. Most of the FACE systems currently in operation utilize blowers or fans to inject CO_2-enriched air into the

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treatment area (see Lewin et al. 1994 for a description). It has been shown that in relatively young (~15–20 years) homogeneous forest plantations of intermediate stature (15–20 m in height), such FACE design allows good temporal and spatial control of CO_2 concentrations throughout the entire plot volume without significantly altering tree canopy microclimate (Hendrey et al. 1999).

Nevertheless, the responses of tall native forest canopies to elevated CO_2 remain hitherto unexplored despite major and ongoing advances in CO_2-enrichment technology. It is not known whether the heterogeneous nature of air turbulence and non-uniformity of wind profiles typically associated with the rough and complex canopy of tall mature forests would permit the utilization of conventional FACE technology. Moreover, the infrastructure necessary to extend the conventional FACE system to tall-stature forests would require several freestanding towers on large concrete bases or slender masts, substantially higher than e.g. a 30-m-high canopy, secured with guyed wires and anchors. Apart from prohibitive costs, the direct impacts of these structures on forest soil and vegetation, and the likelihood of mechanical damage to trees done by wires during windstorm call for alternative CO_2 exposure systems devised specifically for tall canopies. Two recent field studies have successfully used a FACE technique in which pure CO_2 gas is released as high-velocity jets from emission tubes (through numerous small perforations) positioned horizontally at the periphery of a FACE octagon (Miglietta et al. 2001; Okada et al. 2001). We adopted the “pure CO_2” approach and combined it with a line exposure system (cf. Tjoelker et al. 1994) to enrich in CO_2 the crowns of mature deciduous trees.

This paper describes a FACE system adapted for tall canopy trees (~30 m in height) and presents data on temporal and spatial variation of CO_2 concentrations obtained during the 2001 growing season. A construction crane constitutes the main “hardware” component in the canopy zone and is a core requirement for web-FACE.

Materials and methods

Site

We conducted this study in a highly diverse mixed forest stand located ~15 km south of Basel, Switzerland (47°28′ N, 7°30′ E; elevation 550 m a.s.l.). The forest is 80–120 years old, with tree heights between 30 and 35 m, a tree density (diameter ≥0.1 m) of 415 trees ha^{-1} and a stem basal area of 46 m^2 ha^{-1}. Leaf area index of the canopy in the experimental area is ~5 m^2 m^{-2} ground area. The stand used in this experiment is characterized by a dominance of *Fagus sylvatica* (L.) and *Quercus petraea* (Matt.) Liebl., with *Carpinus betulus* (L.), *Tilia platyphyllos* (Scop.), *Acer campestre* (L.) and *Prunus avium* (L.) present as companion species. In addition, the site has a strong presence of conifers [*Larix decidua* (Mill.), *Picea abies* (L.), *Pinus sylvestris* (L.) and *Abies alba* (Mill.)] not included in our test area.

The climate is a typical humid temperate zone climate, characterized by mild winters and moderately warm summers. The growing season of deciduous trees lasts from the end of April to early October (ca. 165 days). Mean January and July air temperatures are 2.1 and 19.1°C, respectively. Total annual precipitation for the region averages 990 mm, of which two-thirds falls during the growing season. Soils are of the rendzina type on calcareous bedrock (a silty loam with an accessible profile depth of ca. 30 cm and a pH of ~5.8 in the top 10 cm of the profile). Information about the site and the Swiss Canopy Crane is available at http://www.unibas.ch/botschoen/scc.

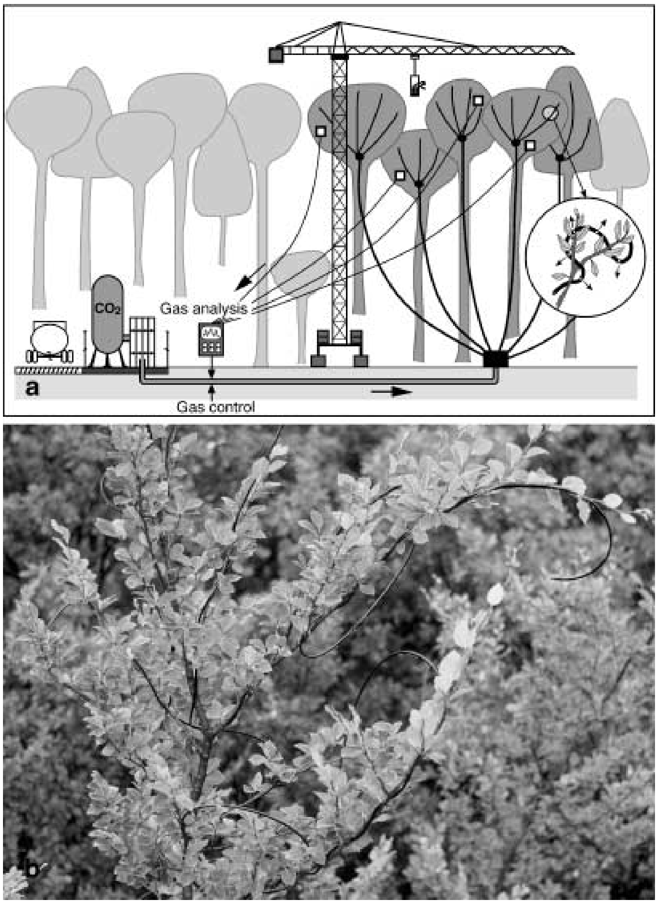

Fig. 1 **a** Diagram of the canopy CO_2-enrichment system at the Swiss Canopy Crane site. **b** CO_2-release tubes (with laser-punched holes) interlaced through the small branches of a beech tree, as shown by the enlargement in (**a**)

Description of the web-FACE system

The web-FACE system was made up of three parts: a computer-based data acquisition and control system, a CO_2 release system and a CO_2 concentration measurement system (Fig. 1a).

CO_2-release system

Food-grade liquid CO_2 was stored in a 22 m^3 insulated reservoir at 1.75 MPa and delivered to two heat-exchangers where it was vaporized. An electric heat-exchanger was used to supply additional energy for evaporating the liquefied CO_2 when the ambient air temperature was too low. The gaseous CO_2 was supplied, at a pressure of 450 kPa above ambient, to an array of two-way normally closed solenoid valves (EVT317, SMC Pneumatik, Egelsbach, Germany).

CO_2 gas was released from thin black plastic tubes woven into the crown of trees (in this case, 14 broad-leaved canopy trees, including 3 *Fagus*, 4 *Quercus*, 4 *Carpinus*, 1 *Prunus*, 1 *Acer* and 1 *Tilia*). Access to the canopy was provided by a 45 m freestanding tower crane (installed by helicopter in an ~8 m tree gap) equipped with a 30-m jib and a work gondola. Overall, a network of ~8.5 km of microtubing, designed originally for a conventional surface drip irrigation system (4.3 mm inside diameter, Drip Store, Escondido, Calif., USA), was installed in the canopy of treated trees (between 300 and 1,000 m of tubing per tree depending on crown size). Emission of pure CO_2 occurred through small laser-drilled holes (0.5 mm diameter) spaced at 30-cm intervals.

Within a tree crown, 5-m-long tube sections (plugged at one end) were wrapped around the primary axis of each major branch (diameter ≥3 cm) and interlaced through most first-order lateral branches as we proceeded from the proximal to the distal end (Fig. 1b). This resulted in a CO_2 delivery line system that mimicked each tree-specific canopy structure, with no particular focus on individual leaf position. The number of CO_2 emitters per crown volume (~25,000 holes over the whole CO_2-enriched canopy zone) was in the range of 10–20 holes per m^3 upper canopy (Fig. 1b). Based on our gas consumption data, we estimated that ~1 cm^3 pure CO_2 was released per perforation per second. The tube sections were then connected to manifolds (6-outlet drip head, Drip Store) attached at a lower position on larger branches. Unused outlets on each delivery manifold were closed with small valves. In some cases, extension tubes without any perforations were used in the vicinity of the manifolds to prevent a too high density of CO_2 emitting points. Each canopy tree was divided into a lower and upper layer, and all manifolds within a layer were connected with 8 mm (inside diameter) polyurethane tubing to a valve on the CO_2 release system. Two CO_2 supply lines were used per tree, thus allowing an independent injection rate for each layer. A computer program actuated separately the injection valves via 24-V DC solenoids. In addition, 14 control trees (representing the same species and number of trees per species) were chosen in remote areas and equipped with dummy tubing (no CO_2 added) following a similar procedure. All canopy installations were carried out under low wind conditions, allowing us to safely maneuver the round, 65-cm-diameter working gondola near any branch without the risk of wounding it.

CO_2-monitoring system

The temporal and spatial variability of CO_2 concentrations within the experimental plot area was determined using a customized 24-port sequential sampler and a non-dispersive infrared gas analyzer (IRGA) (LI-800, Li-Cor, Lincoln, Neb., USA). The analyzer has an accuracy of ±3% over a range of 200–2,000 µmol mol^{-1} and an output signal resolution of 0.012% of full scale (used without signal averaging). Twenty-four sampling lines were installed at different locations within the lower (n=6) and upper canopy (n=18) of the experimental plot (ca. two lines per tree), representing a ground area of ~550 m^2. All sampling air inlets were positioned at ~40 cm from any CO_2 release point, corresponding approximately to the mean distance between emission tubes and most leaves (visually estimated). Air from the CO_2-enriched canopy zone was continuously drawn by a vacuum pump (YP-70VC, ASF Thomas, Wuppertal, Germany) at a flow rate of 3 dm^3 min^{-1} through 35–45 m of black polyurethane tubing (4 mm inside diameter). Sampled air was then pumped sequentially from each port through a manifold of 24 three-way solenoid valves at a flow rate of 1 dm^3 min^{-1} and routed through the gas analyzer. Each channel was monitored for 20 s, allowing sufficient time to purge the measurement system. A given sampling line was thus scanned once every 8 min. CO_2 readings from the IRGA were monitored at 1-s intervals, and only the last reading was recorded (no running average). A span gas of 495 µmol CO_2 mol^{-1} air was analyzed during each sampling sequence to verify the stability of the IRGA.

On 17 August 2001, 12 additional sampling lines (each split into two intakes spaced ~4–5 m apart in the crown) were installed in the canopy of CO_2-enriched trees. These lines were then combined and connected to a single port on the monitoring system (hereafter referred to as control channel). The CO_2 concentration of composite air samples collected from these 24 new canopy locations was continuously measured for a 20-s period at 40-s intervals (i.e. the control channel was sampled alternately with channels that monitored the spatial distribution of CO_2). This sampling procedure considerably reduced the time period between two consecutive CO_2 measurements that could be used in the control program. Further from 13 September to 28 October 2001, CO_2 readings were recorded every 10 s from the control channel only.

In separate experiments, short-term fluctuations in CO_2 concentration were measured for 2 weeks in August 2001 by sampling canopy air at 28 m above ground in the center of the experimental area. Sampled air was drawn at a flow rate of 6 dm^3 min^{-1} through ~30 m of polyurethane tubing (4 mm inside diameter) connected to a high-resolution, fast-response CO_2 gas analyzer (LI-6252, Li-Cor; no signal averaging). These measurements corresponded to “near-instantaneous” CO_2 concentrations (integrated over a few seconds; see Nagy et al. 1994) that were analyzed at ~5 Hz and recorded at 1-s intervals on a portable computer. Additional CO_2 measurements were carried out to determine spatial variability within an individual crown. In this experiment, two sampling lines were installed at canopy height about 5 m apart within the crown of an oak tree, with air intakes located at ~35–40 cm from any CO_2-release tube. CO_2 concentrations were continuously measured and recorded every second over a 5-day period using two cross-calibrated LI-6252 gas analyzers.

Data acquisition and control system

Control signals and data logging are implemented using a custom control program run on an 80486 processor-based personal computer located in a field laboratory ~15 m from the CO_2-enriched area. In the center of the experimental plot, a control subsystem is enclosed in a weatherproof box, which also contains the CO_2-release and monitoring systems, an IRGA, two vacuum pumps, and digital boards used to drive the solenoid valves. The subsystem processes commands received by the main control computer through a wireless network and acquires CO_2 readings from the IRGA via a serial communication port. Additionally, the subsystem monitors the climatic data from a weather station installed above the canopy. Wind speed was measured at 42 m above ground using a cup anemometer (AN1, Delta-T, Cambridge, UK) mounted on the crane. The site was generally characterized by a NW predominant wind direction.

A simple feedback control system was used to regulate the release of CO_2. The average CO_2 concentration in the enriched zone was calculated at 8-min intervals (see monitoring system above) from CO_2 readings made at 24 canopy points and compared to a pre-defined target concentration. The duration of the pulse (maximum 2 s) used to drive each injection solenoid was then adjusted proportionally to the difference (in %) between the set point concentration and that measured in the canopy of CO_2-enriched trees. This generally resulted in pulses being reduced during calm periods and increased when conditions were windy. Initial values for the pulses were empirically determined for each release valve (i.e. each tree canopy layer). The set point CO_2 concentration was initially 550 µmol mol^{-1}, about 180–190 µmol mol^{-1} above ambient levels (Fig. 2b). However, based on our gas consumption during the first week of May, the target CO_2 concentration was subsequently reduced to 500 µmol mol^{-1} so that with given financial resources, CO_2 enrichment could be carried out over the entire growing season. Further, CO_2 release occurred during daytime hours only, as determined by measurements of photon flux density (PFD) made at canopy height using a quantum sensor (LI-190SB, Li-Cor) and a threshold value of 75 µmol m^{-2} s^{-1}.

Carbon isotope analysis

The pure carbon dioxide gas used to enrich the forest canopy was derived from fossil-fuel sources and, consequently, was more de-

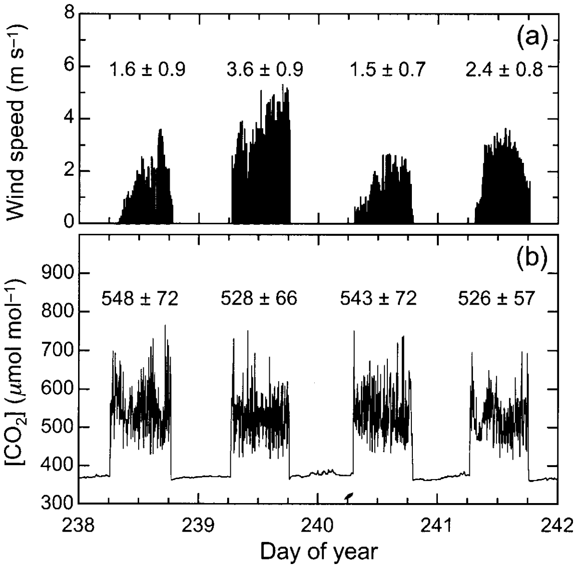

Fig. 2a, b Diurnal courses of wind speed and CO_2 concentrations for 4 representative days (26–29 August) during the 2001 growing season. **a** 10-min average wind speed (m s^{-1}) measured as wind run at the top of the crane (only daytime data shown). **b** 5-min average CO_2 concentrations (µmol mol^{-1}) calculated from 1-s measurements carried out at 28 m above ground in the center of the CO_2-enriched area. Numbers indicate mean (±SD) daytime wind speeds and CO_2 concentrations

pleted in ^{13}C than atmospheric air. Since the C isotope abundance of plant materials is directly influenced by the isotopic composition of the source air assimilated during photosynthesis, it is possible to use the isotope signal of plants grown in CO_2-enriched air as an indicator of the long-term mixing ratio of atmospheric CO_2 (365–370 µmol mol^{-1}) and tank CO_2 (i.e. time-averaged CO_2 concentration of the new source air; Marino and McElroy 1991; Cerling et al. 1993; Buchmann et al. 1996).

Cynodon dactylon (L.) Pers. (a C_4 grass) seeds were germinated in a growth chamber and the emergent, yellow sprouts were transferred to 80 small containers (0.1 dm^3 PE flasks; 10 seeds/flask) filled with a silty loam-sand mixture (supplemented with a slow release "full compound" fertilizer and watered to field capacity). One-third of the containers were installed at different locations within the upper crown of each CO_2-enriched tree. The remaining seedlings were distributed in the upper canopy of trees located at various distances from the CO_2-release zone. Seedlings were watered once a week (depending on rainfall) and harvested every second week (except for final harvest) by cutting all leaf blades in each container. Following harvesting, leaf material was dried and ground to a fine powder with a pestle in a mortar. One subsample (~1 mg) per container was analyzed for isotopic composition (δ^{13}C expressed in ‰ relative to PDB) at the Paul Scherrer Institute stable isotope facility using an isotope ratio mass spectrometer (DELTA-S Finningan MAT, Germany) with an elemental analyzer (EA-1110, Carlo Erba, Italy).

Discrimination (Δ) in C_4 plants is relatively insensitive to changes in c_i/c_a (the ratio of intercellular to ambient CO_2) that are typically associated with varying environmental conditions. We assumed that there were no substantial differences in environment between seedlings and, hence, used a constant value of 5.5‰ ("sun-drought" values for *C. dactylon*, reported by Buchmann et al. 1996) for Δ of all grass seedlings within and at various distances from the CO_2-enriched trees. The isotopic composition of the air surrounding each container (δ_{elev}) was calculated using respective grass δ^{13}C values. A mixing ratio model with the relative volume fraction and isotope ratio of its two CO_2 constituents (atmospheric and pure CO_2 gas) was then used to calculate a time-integrated value of CO_2 concentration as:

$$\delta_{\text{elev}} = \left[(c_{\text{elev}} - c_{\text{air}})/c_{\text{elev}} \times \delta_{\text{pure CO}_2}\right] + \left[c_{\text{air}}/c_{\text{elev}} \times \delta_{\text{air}}\right] \qquad (1)$$

where c_{elev} is the CO_2 concentration of the CO_2-enriched air, c_{air} is the atmospheric CO_2 concentration (assumed to be 370 µmol mol^{-1}), δ_{air} is the δ^{13}C of ambient air (assumed to be –8‰) and $\delta_{\text{pure CO2}}$ is the stable isotope signature of the added CO_2 gas ($\delta_{\text{pure CO2}}$=–31.0±1.7‰; n=21; determined for each tank filling). This annotation implies that c_{elev} may be equal to c_{air} (and therefore $\delta_{\text{elev}} \approx \delta_{\text{air}}$) in remote areas not influenced by the added CO_2. By rearranging Eq. 1 to solve for c_{elev}, we obtain:

$$c_{\text{elev}} = c_{\text{air}} \times \left(\delta_{\text{air}} - \delta_{\text{pure CO}_2}\right) / \left(\delta_{\text{elev}} - \delta_{\text{pure CO}_2}\right) \qquad (2)$$

Results

Short- and long-term control of CO_2 concentrations

The web-FACE system was operated from 2 May to 28 October 2001 during daylight hours, as determined by PFD levels at canopy height. The average gas consumption to CO_2-enrich the air in the canopy of 14 adult trees over the entire growing season was ~2 tons per day. Representative diurnal curves of 5-min average CO_2 concentration demonstrate the capability of the system to maintain the [CO_2] in the canopy near the target concentration of 500 µmol mol^{-1} (Fig. 2). Instantaneous (i.e. 1 s) mea-

Table 1 Proportion of web-FACE daytime CO_2 concentrations within ±10% and 20% of the target concentration of 500 µmol mol^{-1}, and CO_2 excursions >1,000 µmol mol^{-1} for different measurement period and frequency. "Grab" samples are 1-s measurements that corresponded to a CO_2 concentration integrated over a few seconds. One-minute means were calculated from the grab samples during the corresponding period

Period	Measurement frequency	Time interval	Fraction of CO_2 readings		
			±10% of target (450–550 µmol mol^{-1})	±20% of target (400–600 µmol mol^{-1})	>1,000 (µmol mol^{-1})
2 May–12 September	20 s each[a]	Grab sample	0.31	0.63	0.025
		8-min mean	0.66	0.88	<0.001
17 August–1 September	Continuous[b]	Grab sample	0.36	0.68	0.024
		1-min mean	0.47	0.76	0.006

[a] CO_2 measurements were performed at 18 upper canopy locations distributed within the CO_2-enriched zone

[b] CO_2 measurements were performed at 28 m above ground in the center of the CO_2-enriched zone

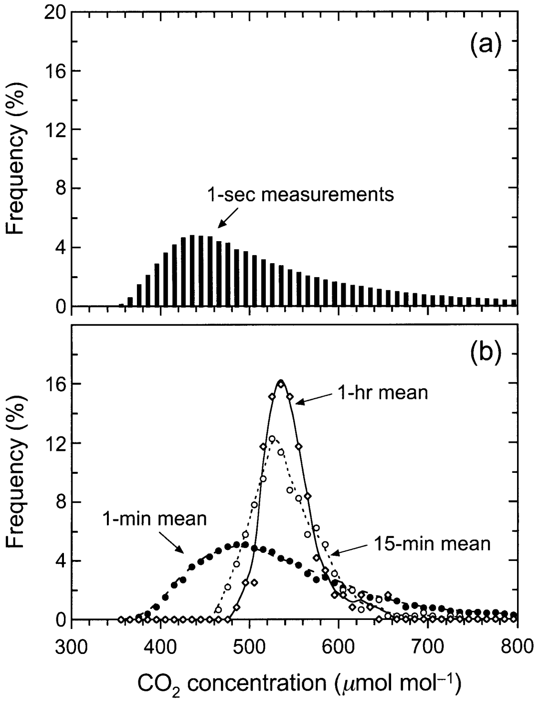

Fig. 3 **a** Frequency distribution of 1-sec measurements of CO_2 concentration performed at 28 m above ground in the center of the CO_2-enriched area during 2 weeks in August 2001. These data represent ~5-s averages of instantaneous CO_2 concentrations that are continuously analyzed (at 5 Hz) and recorded each second. The cumulative frequency corresponds to 93% of the total frequency. **b** Frequency distribution of 1-min, 15-min and 1-h average CO_2 concentrations calculated from the 1-s measurements. Each *bar* or data point represents the midpoint of a 10 µmol mol^{-1} CO_2 concentration class. Mean daytime wind speed during these CO_2 measurements ranged between 1.4 and 3.6 m s^{-1}

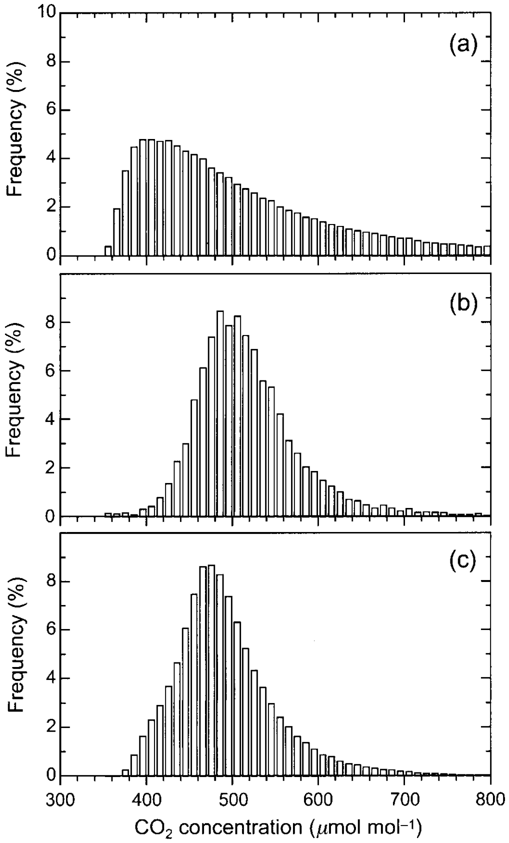

Fig. 4 **a** Frequency distribution of daytime CO_2 concentrations measured at 18 different (*upper*) canopy locations in the CO_2-enriched area from 2 May to 12 September 2001. Each sampling channel was sequentially monitored for 20 s, of which only the last 1-s CO_2 reading was recorded. **b** Frequency distribution of ~8-min average (i.e. one measurement cycle) CO_2 concentrations calculated from the data shown in **a**. **c** Frequency distribution of daytime CO_2 concentrations measured in a composite air sample (drawn from 24 sampling lines in the upper CO_2-enriched canopy) from 13 September to 28 October 2001. Each *bar* or data point represents the midpoint of a 10 µmol mol^{-1} CO_2 concentration class. In all three cases, the cumulative frequency shown is greater than 96% of the total frequency

surements of short-term CO_2 fluctuations carried out for a 2-week period indicated that daily mean CO_2 concentrations at 28 m above ground in the center of the CO_2-enriched area were ~8% above target (coefficient of variation=25–31%). As expected, a slightly better control of CO_2 concentration was achieved under moderate (~2–3 m s^{-1}) wind speed (Fig. 2). The frequency distribution of near-instantaneous CO_2 data was positively skewed, with a median CO_2 concentration of 497 µmol mol^{-1} (Fig. 3a). CO_2 concentrations lower than 400 µmol mol^{-1} were recorded in 7.8% of all measurements, whereas less than 2.4% of readings were above 1,000 µmol mol^{-1}. Fluctuations in CO_2 concentration that exceeded 1,000 µmol mol^{-1} were mostly short-lived (only ~3% of these excursions persisted more than 10 s). Increasing the signal average to 1-min, 15-min and 1-h intervals narrowed the frequency distribution of CO_2 data closer to the mean concentration (Fig. 3b). However, a greater integration period acts as a high frequency filter and masks the short-term variability of high [CO_2]. Based on measurements of near-instantaneous CO_2 concentrations, web-FACE controlled CO_2 levels within ±20% of the target concentration for 68% of the exposure time (Table 1). Furthermore, 1-min mean CO_2 concentrations remained within ±10% and 20% of the set point concentration for 47% and 76% of the daylight hours, respectively (Table 1).

The relative frequency of CO_2 measurements carried out at 18 different upper canopy positions (in ~8-min cycles) was surprisingly similar to the distribution of 1-s samples collected at the center of the CO_2-enriched area (Fig. 4a; Table 1). The asymmetry was, however, slightly more pronounced when considering multiple gas sampling points in the canopy, with the distribution showing a greater number of observations below 400 µmol mol^{-1}. Averaging CO_2 concentrations from those 18 sampling locations over each measurement cycle (i.e. ~8-min means) resulted in a near-normal distribution centered around 500 µmol mol^{-1} (Fig. 4b). These integrated CO_2 signals showed a frequency distribution quite similar to that of composite air samples (*n*=24 sampling locations)

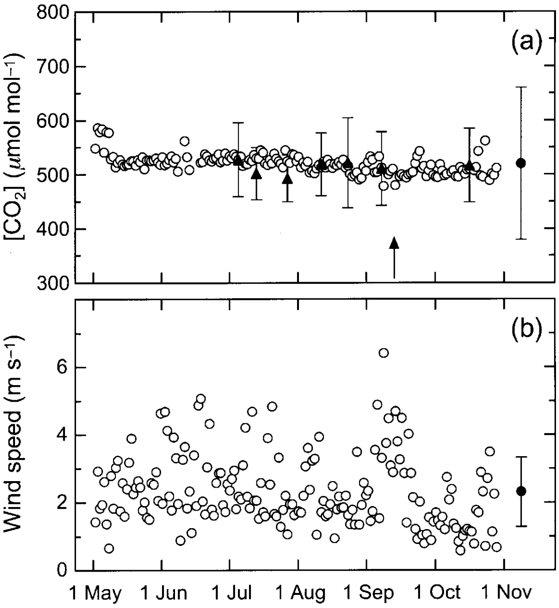

Fig. 5a, b Seasonal courses of daytime average CO$_2$ concentrations (µmol mol^{-1}) and wind speed (m s^{-1}) during the exposure period of 2 May–28 October 2001. **a** Mean CO$_2$ concentrations at canopy height, calculated from measurements at 17 different sample locations in the CO$_2$-enriched area (excluding one border tree in the north-east sector). The *arrow* indicates that on 13 September, a new sampling routine was implemented for controlling CO$_2$-enrichment. This consisted of a composite air sample drawn from 24 sampling lines, which was analyzed every 10 s; **b** mean wind speed measured at the top of the crane. *Filled circles with error bars* indicate the average (±SD) daytime CO$_2$ concentrations and wind speed across all CO$_2$-enrichment days. CO$_2$ values are missing for 9 days of exposure due to a faulty communication network that resulted in data loss. In **a** *filled triangles with error bars* indicate the average (±SD) CO$_2$ concentration calculated (Eq. 2) from leaf δ^{13}C of *Cynodon dactylon* seedlings distributed within the canopy of CO$_2$-enriched trees

obtained from the new fast track control routine, which was implemented on 13 September (Fig. 4c).

The average CO$_2$ concentration at canopy height calculated for each day of enrichment varied between 479 and 587 µmol mol^{-1} (Fig. 5a). Overall, the seasonal daytime mean [CO$_2$] (i.e. all upper canopy CO$_2$ measurements over the 179-day exposure period) was 520 µmol mol^{-1}. In the lower part of the exposed canopy (only a small fraction of total leaf area) which had a reduced tube density, the average daytime [CO$_2$] were generally less than the target concentration, ranging between 408 and 518 µmol mol^{-1} (data not shown). The current CO$_2$-enrichment installation has little effect on the lower understory given the ~30 m height of the forest, but it would be merely a matter of more tubes and money (for CO$_2$ gas) to include the deep shade zone. Our site was generally characterized by relatively high wind conditions (Fig. 5b). Daily mean wind speeds were in the range 0.6–6.4 m s^{-1}, with 29% of all wind measurements above 3 m s^{-1}. The mean daytime wind speed across all CO$_2$-enrichment days was 2.3 m s^{-1}.

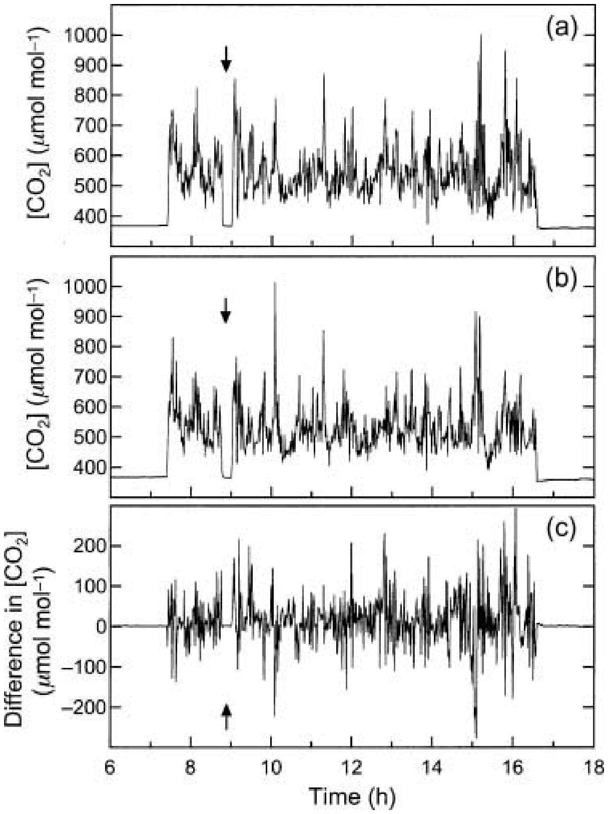

Fig. 6 CO$_2$ concentrations concurrently measured in **a** eastern and **b** western part of a tree crown located in the center of the CO$_2$-enriched zone on 17 September 2001. Sample intakes were established at canopy height, about 5 m apart. Data are 1-min average CO$_2$ concentrations calculated from 1-s measurements. **c** Difference in 1-min average CO$_2$ concentrations between the two crown locations. The *arrows* indicate a 15-min interruption in CO$_2$-enrichment carried out to determine the system response. Mean (±SD) daytime wind speed during these CO$_2$ measurements was 2.8±0.8 m s^{-1}

Spatial variability in CO$_2$

CO$_2$ measurements conducted ~5 m apart within an individual tree crown showed similar changes in [CO$_2$] with time and relatively homogeneous concentrations (Fig. 6). On that particular day (average wind speed=2.8 m s^{-1}), mean (±SD) CO$_2$ concentrations measured in eastern and western crown sectors were 543 (±89) and 529 (±81) µmol mol^{-1}, respectively. Given the heterogeneous nature of air turbulence at canopy height, there were several CO$_2$ fluctuations that differed in duration and magnitude between the two crown locations. However, the differences in CO$_2$ concentration between both sampling points were generally less than 50 µmol mol^{-1} (Fig. 6c). Long-term mean CO$_2$ concentrations in the upper crown of each individual tree in the CO$_2$-enriched zone ranged between 490 and 612 µmol mol^{-1} during the 2001 growing season (excluding one border tree in the north-east sector) (Fig. 7). When considering all sampling positions in both lower and upper canopies, long-term mean [CO$_2$] values were maintained within –19 and +22% of the target concentration.

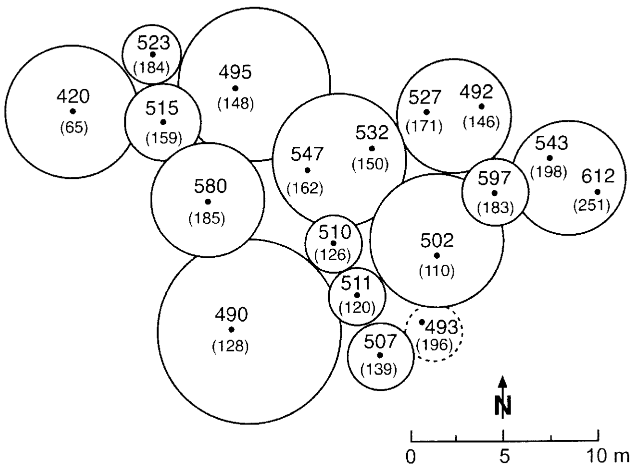

Fig. 7 Spatial distribution of the average (±SD) daytime CO_2 concentrations (μmol mol^{-1}) measured at 18 different positions in the CO_2-enriched area (tree crowns viewed from above) during the exposure period of 2 May–12 September. Measurements were carried out at canopy height (~28–32 m) in all 14 trees. The *dots* indicate the locations of the sample intakes. The sample location included in a *dashed circle* is installed at mid-canopy in the adjacent crown of a *Carpinus* tree whose main stem has forked. CO_2 concentrations at each canopy position are recorded every 8 min

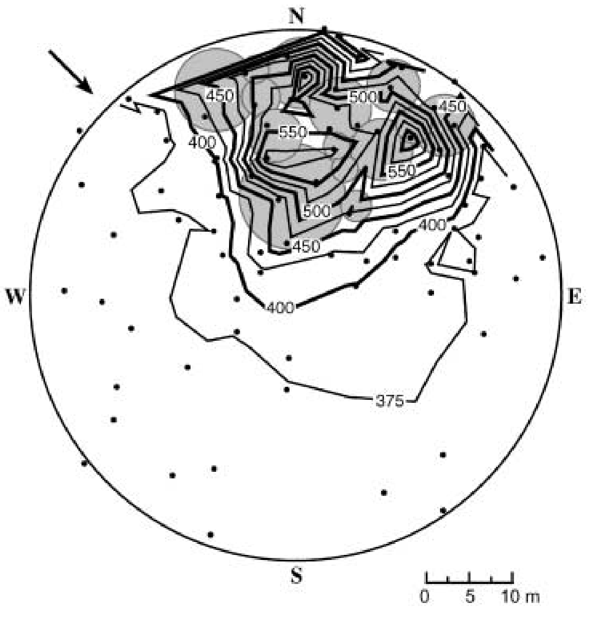

Fig. 8 Spatial distribution of time-integrated CO_2 concentrations (μmol mol^{-1}) calculated from leaf $\delta^{13}C$ of *Cynodon dactylon* seedlings (grown at canopy height from mid-June to mid-October in small plastic flasks attached to trees at different locations in the experimental area) and a mixing ratio model (Eq. 2). Plant material was harvested at ~2-week intervals (except final harvest; *n*=7) by cutting all leaf blades close to the base. The *dots* indicate the location of the flasks and the *shaded area* represents the CO_2-enriched trees. The *arrow* indicates the prevailing wind direction

The C isotope abundance of C_4-grass seedlings distributed within the CO_2-enriched canopy clearly reflected the ^{13}C-depleted signature of the pure CO_2 gas added. Using leaf $\delta^{13}C$ of grass seedlings and a mixing ratio model (Eq. 2), we calculated the time-integrated [CO_2] to which each seedling was exposed during the elevated CO_2 treatment. The spatial distribution of long-term mean CO_2 concentrations derived from isotopic measurements was very similar to that observed in air samples from 18 different upper canopy positions (Figs. 7, 8). There was also very good correspondence between the isotopically derived CO_2 mixing ratios and measured daily average [CO_2] on all harvesting dates (Fig. 5). On windy days, CO_2-enriched air was transported to neighboring trees by turbulence. This created a gradient of CO_2 concentration across the experimental area, which was mostly apparent in close-by individuals on the down-wind side of the plot (NW prevailing wind direction) (Fig. 8).

Discussion

Web-FACE and CO_2 concentrations

The FACE technology has been applied successfully to a wide variety of vegetation types with the exception of tall, natural forests. The Swiss Canopy Crane project was established, in part, to investigate the responses of adult canopy trees to elevated CO_2. Given the size and complexity of their canopy structure, we considered that the infrastructure necessary to extend conventional FACE system to tall trees would be too invasive and damaging for the soil and vegetation. Furthermore, the extensive damage caused by a severe windstorm to forests in France, Germany and Switzerland in December 1999 (which resulted in the loss of two trees in our experimental area) incited us to devise an alternative FACE system for tall forest canopies.

As recently demonstrated by Miglietta et al. (2001) and Okada et al. (2001), the emission of high-velocity pure CO_2 jets at the periphery of FACE plots provides rapid mixing of CO_2 with the ambient air and control performance similar to that achieved by conventional FACE. The web-FACE system described here uses this approach by releasing small jets of pure CO_2 gas from fine tubing (through multiple CO_2 emitting points) in the crown of each individual tree, adjacent to the leaves. Hence, we opted for a lightweight, flexible, UV-resistant line exposure system that could be supported by the trees themselves. In general, branch orientation was not affected by the tube installations, which were hardly visible at a distance of 10 m from the canopy. Further, the network of delivery and sampling tubes moved freely along with branches under windy conditions, without any apparent impact on the vegetation. Indeed more than a year after installation, there was no visible abrasion damage to the foliage or branches, and no increase in branch losses following storms, when compared with "tubeless" trees within the crane perimeter. The total tube surface areas were negligible in view of the canopy area. In fact, the tubes "disappear" within the leaf canopy, so that any significant microclimatic effects become hardly possible (Fig. 1b). Nevertheless, additional tubes have been installed on control trees. Overall, this relatively simple CO_2-exposure system successfully maintained long-term mean [CO_2] near the target concentration.

Pinter et al. (2000) reported that FACE designs, which emit pure CO_2 at the perimeter of the plots exhibited more variability in [CO_2] and had more difficulty maintaining a set point concentration than systems with blowers, particularly under conditions of low atmospheric turbulence. However, there is now compelling evidence that "pure CO_2" FACE systems using gas jets at high velocity can achieve adequate temporal and spatial CO_2 control in tree plantations and crop fields (Miglietta et al. 2001; Okada et al. 2001). In this study, the daytime measurements of [CO_2] have shown that the web-FACE technology was able to control CO_2 within ±20% of the target concentration for ~65% of the time. In comparison, the forest FACE at the Duke site achieved this performance for 62% of its 1995 operation season (Hendrey et al. 1999). Based on performance standards that use CO_2 averages over 1 min, the CO_2 control in web-FACE was less accurate that in conventional forest FACE, with 1-min means being within ±10% of target for 47% of the exposure time (compared with 69% for Duke forest FACE). However, 76% of the 1-min averages were within ±20% of the target concentration, which is very close to the "80% of the time" defined as an acceptable FACE performance by the scientific community (see Hendrey et al. 1999; Miglietta et al. 2001). Given the size of the trees, the web technology is definitely a most promising avenue towards the exposure of tall forest canopies to long-term CO_2-enrichment.

Since pure CO_2 is released in the vicinity of the foliage in our experimental setup, short-lived excursions in [CO_2] may occur more frequently and be of greater amplitude than in a conventional FACE system where prediluted CO_2 air is injected. As pointed out by Cardon et al. (1995), the frequency and duration of short-term fluctuations in [CO_2] may induce physiological responses that are significantly different from those observed under average CO_2 treatment. This is because several physiological processes have non-linear responses over a broad range of [CO_2] and time constant that vary from seconds to years (Eamus and Jarvis 1989; Körner 1995). In our study, CO_2 concentrations greater than 1,000 µmol mol^{-1} occurred in less than 3% of the measurements and only a very small fraction of these excursions exceeded 10 s. Hendrey et al. (1997) demonstrated that in wheat, photosynthesis is not significantly affected by CO_2 fluctuations that last less than 1 min. The dynamic responses of photosynthesis and stomatal conductance are generally longer in forest trees than in crop plants (Ellsworth et al. 1995; Saxe et al. 1998). This has led Hendrey et al. (1999) to suggest that the rapid (but short-lived) fluctuations in [CO_2] occasionally observed in forest FACE system have little effect on tree photosynthetic and stomatal responses. To our knowledge, the effects of short-term fluctuations in [CO_2] on forest tree physiology have not been determined.

All FACE systems depend primarily on adequate horizontal wind velocity to ensure adequate spatial uniformity and to prevent zones of high CO_2 concentrations. Conversely, high aerodynamic turbulence and wind gusts can reduce considerably the mean CO_2 concentration in the canopy by increasing the dispersion of CO_2 out of the enriched area. This would inevitably bring about a greater gas demand. Mean daily CO_2 use in web-FACE was ~2 tons over the first season of operation. This amount was similar on a ground area basis to other conventional FACE systems (Hendrey et al. 1999; Jordan et al. 1999). For example, the average CO_2 consumption measured at the Duke forest site (ring area=530 m^2) during a 133-day operating period was 260 kg h^{-1}, with CO_2 use ranging from ~70 kg h^{-1} on calm cloudy days to ~480 kg h^{-1} on clear windy days (Hendrey et al. 1999). For the Nevada Desert FACE facility (area=491 m^2 plot^{-1}) established in a *Larrea tridentata–Ambrosia dumosa–Lycium* spp. shrub community, mean daytime CO_2 use was ~150 kg h^{-1} plot^{-1} (based on data in Jordan et al. 1999). These values are in good agreement with our average CO_2 consumption of ~200 kg h^{-1} for an experimental area ~550 m^2.

Costs and experimental application

While applicable to 30 m tall natural forests, web-FACE uses a much simpler and less disturbing infrastructure to release CO_2 than conventional FACE systems. The principal hardware costs are represented by the canopy crane, which amounts to ca. US$ 100,000 including helicopter installation and manual concrete foundations (no trucks entered the forest; only 0.1% of the area within the crane periphery was sealed). Web-FACE does not require towers (or masts), blowers and associated large size plenum and vent pipes. Furthermore, the costs of CO_2 are roughly identical, approaching ca. US$ 90 per 100 m^2 at the current European CO_2 price of US$ 240 per ton. Accounting for all expenditure, web-FACE may budget equal to forest FACE with the added value of applicability to tall, diverse forests and free access to the canopy at any time for monitoring tree responses.

Our current FACE installation aims at testing individual tree responses; hence the different individuals of the several species are our replicates (including the century long history recorded in their growth rings). Despite the relatively large dimensions of this test system, soil feedback responses to CO_2-enrichment will remain limited. For an ecosystem approach, the CO_2-enriched canopy area of a forest of this stature would have to be at least 3–5 times larger, with replicate control and treated plots requiring replicate cranes and proportional costs. Clearly, the web-FACE technology could be extended to all trees within the crane perimeter (in our case: 62 adult trees over a 2,800 m^2 ground area). CO_2-release tubes could also be hung from the lower canopy branches to expose the understory (where a smaller hole density is required because of reduced atmospheric turbulence). Once realism seeps in, experimental ecological science becomes costly. However, this expenditure has to be seen in the light of the enormous sum of funds allocated to simple, comparatively cheap model systems, which in the case of a forest, will always leave us with substantial doubt about the basic question of whether the world's forests are still C-limited. Web-FACE indicates a practical avenue towards approaching such an answer in a multitude of environments.

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