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Carbon dioxide emissions at an Italian mineral spring: measurements of average CO_2 concentration and air temperature

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Abstract

Emissions of carbon dioxide from vents at the Bossoleto mineral spring in Central Italy have been calculated to exceed 12 t day^{-1}. This emission leads to enhanced atmospheric concentrations of CO_2 over an area of more than 3000 m^2. The vent gas is over 99% pure CO_2, with a characteristic isotopic signature that is totally depleted in ^{14}C. At night, concentrations at the bottom of the bowl-like depression can increase to levels approaching 75%. In the morning, this high concentration of CO_2 is associated with a rapid temperature increase of over 10°C before the CO_2 disperses. This site is being used in a number of studies of the response of plant communities to long-term enhanced CO_2 concentrations. The problem of defining CO_2 concentrations in these studies was approached by comparing estimates determined by gas analysis measurements and isotopic analysis of leaf material. The isotopic method used ^{14}C as a tracer, integrating effective concentration over the life of a leaf by calculating from the ratio of ^{14}C measurements of plant material growing near the spring and at a control site. The estimates obtained using isotopic analysis of leaf material were similar to gas analysis measurements obtained during the day. This suggests that plants at this site are responding to the concentrations during the day, rather than the much higher night-time concentrations, making the system useful for biological research.

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1. Introduction

Sites with natural emissions of carbon dioxide provide an opportunity for research into long-term effects of CO_2 on biological systems. The potential of such sites has only recently been realised, yet the presence of CO_2 vents is relatively widespread in regions of geological activity. A survey of CO_2 vents in Italy (Miglietta et al., 1993a) identified several sites well suited for research on biological systems. The Bossoleto site in Central Tuscany was considered most promising because the CO_2 concentrations during the day are at physiologically relevant levels, averaging between 600 and 1200 μmol mol^{-1} over a large area (more than 3000 m^2). In addition, concentrations of atmospheric pollutants such as H_2S or SO_2 in the vent gas are low. Research at this site has been in progress since 1992 (Miglietta et al., 1993a). Results obtained from this site (Miglietta et al., 1994) and at similar sites in Italy (Miglietta and Raschi, 1993; Badiani et al., 1993; Miglietta et al., 1993b) have produced results that are consistent with those obtained in short-term experiments (Woodward and Bazzaz, 1988; Jarvis, 1989; Eamus and Jarvis, 1989; Acock, 1990; Woodward et al., 1991).

1.1. The Bossoleto site

The Bossoleto site in Central Italy (43°17′N, 11°35′E) is located approximately 40 km south-east of the city of Siena in Tuscany. The site is a tree-fringed bowl-like depression with the main vent located at the bottom (Fig. 1). The almost pure (more than 99 %) CO_2 emitted from the vent accumulates to high concentrations in stable nocturnal conditions. On most mornings the presence of the CO_2 at the bottom of the bowl is clearly apparent, with a visible refractive boundary at the interface between the air and the denser CO_2. This is often accompanied by a layer of condensed water droplets (mist) floating at the boundary, between 2 and 3 m above the bottom of the bowl. The effect of these variations in CO_2 concentration on plant growth is unknown. Definition of the average CO_2 concentration for plant material growing at the site is difficult because the high night-time concentrations occur when stomata tend to be shut, and indeed they would induce stomatal closure in most plants (Jones, 1992). There is therefore a need to obtain an estimate of the effective average CO_2 concentration. One approach is to use the plants as integrating sensors using the naturally occurring isotopic signature of CO_2 from the vent as a tracer. At the Bossoleto site, the vent CO_2 is totally depleted in ^{14}C, making it a suitable tracer for the calculation of effective average concentration of vented CO_2 absorbed by plants.

This paper describes the development of CO_2 gradients and the interaction with air temperature during the day at the Bossoleto site. CO_2 concentrations measured directly using gas analysis are compared with indirect estimates obtained using isotope analysis of carbon from plant material collected at the site.

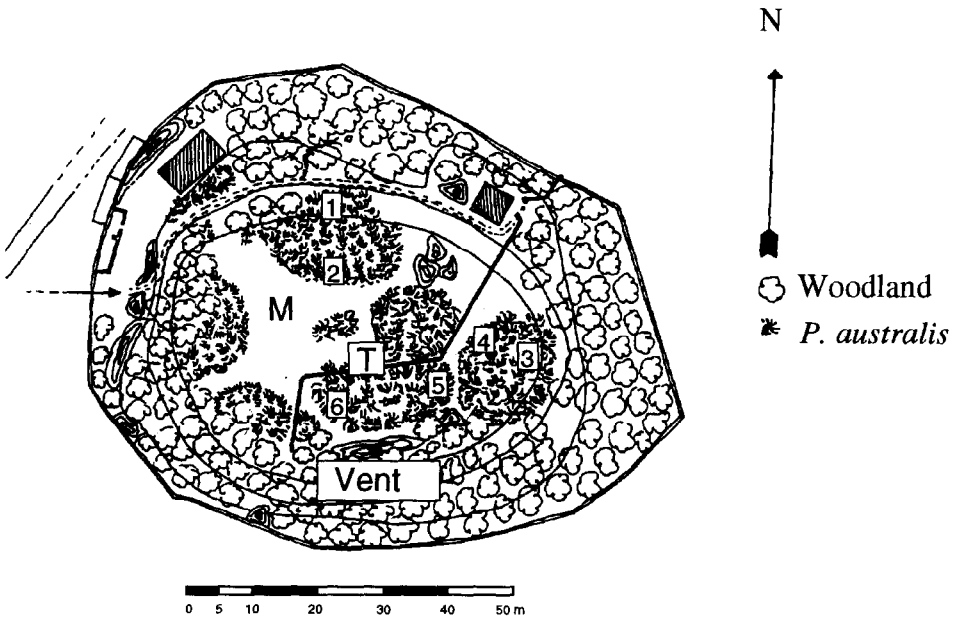

Fig. 1. Il Bossoleto. The main CO_2 vent is in a cave under the position marked Vent. The position marked M shows the location of the mast used for measuring the CO_2 profile and that marked T for the temperature gradient. Contour lines represent 4 m height. Main vegetation types are shown. Numbers (1–6) indicate the sites used for collection of leaf material for carbon isotope analysis.

2. Methods

2.1. Atmospheric carbon dioxide concentration (gas analysis)

A carbon dioxide profile was measured over a 24 h period commencing at 17:00 h on 30 July 1992 (all times are Central European Time). Small air pumps were supported at five heights in a vertical array above the ground (0.2–2.1 m) at a location at the bottom of the bowl (Fig. 1, M). CO_2 concentrations at the site range from 0.04 to 75% (v/v) over a 24 h period. Standard IR gas analysers cannot be used to measure CO_2 concentrations over this range as their response saturates at high concentrations. Air was pumped to a collection point near the edge of the site. An air sample (1 ± 0.05 ml) was extracted from each line using a syringe, at intervals during the measurement period. CO_2 concentrations were measured by injecting the gas sample into a circulating closed gas analysis system that incorporated a CO_2 gas analyser (LI-6250, Li-Cor, Lincoln, NE) operating in absolute mode (accuracy ±0.2 μmol mol^{-1}). The volume of the system was modified by using different leaf chambers (Li-Cor LI-6200 Portable Photosynthesis System) to act as buffers. At night, with high concentrations, the system volume was 4.16 dm^3, and a system volume of 0.43 dm^3 was used at other times. Background CO_2 concentrations in the CO_2 analyser during measurement were equal to ambient concentrations at the top of the bowl

ranging from 0.04 to 0.1% v/v (400–1000 μmol mol^{-1}). Tests at the start and end of the sequence of measurements indicated that leakage from the system was negligible. The CO_2 concentration ([CO_2]) was calculated from the dilution of the air sample in the closed system, and results are reported as a volume percentage. Errors in these measurements are estimated to be approximately ±5% of the calculated value.

2.2. Air temperature

Air temperature gradients were measured on 23 and 24 July 1992 with unshielded fine-diameter copper–constantan thermocouple wire (42 swg, 0.1 mm diameter. Dural Plastics, Melbourne, Australia). Voltages were measured using a data logger (CR21x, Campbell Scientific, Shepshed, UK) with a resolution of 0.5 μV. Temperatures were measured relative to a reference thermistor located under the wiring panel on the data logger and have an estimated accuracy of better than ±0.5°C. Data were recorded every 10 s, and 30 min averages were calculated and stored by the data logger. Thermocouples were placed at four heights ranging from 0.4 to 3.1 m above the ground surface near the centre of the bowl (Fig. 1, T).

2.3. Average CO_2 concentration (radioisotope analysis)

^{14}C measurements of plant material were used to calculate an estimate of the effective average CO_2 concentration for plant material integrated over the lifetime of the leaf. Bulk samples of leaf material from between 10 and 20 leaves of *Phragmites australis*(Cav.) Trin. ex Steudel were collected from six locations at the Bossoleto site (Fig. 1) in July 1992 and air dried. A single bulk sample of leaf material was collected from plants of the same species growing at a nearby control site that does not have CO_2 enrichment (43°19′N, 11°35′E). Isotope analysis was conducted on cellulose extracted from the leaf material avoiding possible contamination from carbonate on leaf surfaces. The cellulose was converted to pure CO_2 at the NERC Radiocarbon Laboratory (East Kilbride) and the sample sent for determination of ^{14}C using accelerator mass spectroscopy (AMS) measurements at the University of Arizona, NSF facility.

The effective CO_2 concentration can be calculated from measurements of the abundance of ^{14}C in plant material collected at the Bossoleto and a nearby control site with normal ambient CO_2 concentrations. We assume that CO_2 at the Bossoleto site can only come from two sources—the atmosphere or the vent. The sum of the fractions of CO_2 fixed by the plant coming from the vent (F_v) and from normal atmosphere (F_a) by definition must, therefore, equal unity:

$$F_v + F_a = 1 \tag{1}$$

Using a mass balance approach, the measured ^{14}C abundance in plant material M_p must equal the sum of fractions coming from the vent and the atmosphere weighted by the abundance of ^{14}C in each of these gases M_v and M_a assuming no discrimination (measurements of M are expressed relative to the 'Absolute International Standard Activity' and corrected for ^{14}C discrimination using measurements of ^{13}C

(Stuiver and Polach, 1977)):

$$M_p = M_a F_a + M_v F_v \tag{2}$$

This can be solved for F_a, as $M_v = 0$:

$$F_a = \frac{M_p}{M_a} \tag{3}$$

F_a can also be defined in terms of the CO_2 concentrations in normal atmosphere (p'_a) and the average biological concentration at the site (p_s):

$$F_a = \frac{p'_a}{p_s} \tag{4}$$

The value of p_s can then be calculated, substituting a value of 350 μmol mol^{-1} for p'_a:

$$p_s = 350 \frac{M_a}{M_p} \tag{5}$$

This equation was used to estimate average CO_2 concentration from the six samples relative to that collected at the control site. The error in the estimate of p_s is equal to the sum of the percentage measurement errors of the AMS determinations and is less than ±2%.

3. Results and discussion

Average carbon dioxide concentrations in the bowl at the Bossoleto site exceed 0.1% (1000 μmol mol^{-1}) at most times of the day (Fig. 2). During the period of measurement, the lowest concentrations were observed in the mid-afternoon (15:30 h, about 0.1%). Late in the afternoon CO_2 concentrations rise (Fig. 2(a)). There is a ten-fold increase in average concentration over the 1 h period from 17:00 h, with concentrations in the bottom 1 m of the profile exceeding 1%. It is apparent from the shape of the profile at this time that dense CO_2-rich air is accumulating at the bottom of the bowl, creating a density and concentration gradient. During the night, more stable atmospheric conditions develop and concentrations build up to a maximum of around 75% at 07:00 h (Fig. 2(b)). There is a small reduction in concentration over the hour from 07:00 h, but the most rapid change occurs in the short period after 09:00 h, when the sun rises above the horizon and direct radiation is incident on the bottom of the bowl. The rapid period of dispersal between 09:00 and 10:00 h is followed by less dramatic reductions in average concentration through to the middle of the afternoon. Using the concentration profiles and the topographic map of the site to calculate the volume of the bowl it is possible to estimate a daily CO_2 emission from the vent. We estimate the volume of the bowl to be approximately 4000 m^3 up to a height of 2 m (above the base of the bowl) and that a daily emission of 12 t of CO_2 is the minimum required to give a CO_2 concentration of 75% in the morning. This estimate is calculated from the mass of CO_2 required to fill this volume over a 12 h period. It assumes that all the CO_2 emitted from the vent at night stays at the bottom

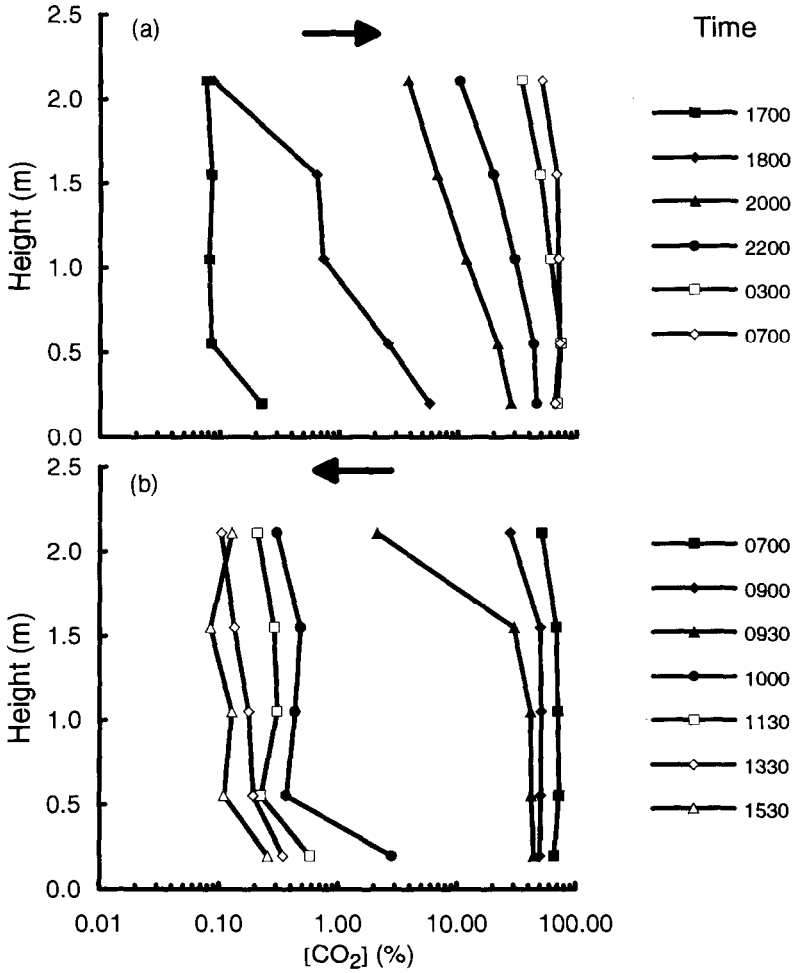

Fig. 2. Carbon dioxide concentration profiles for the Bossoleto site on 30 and 31 July 1992. (a) Build-up of $[CO_2]$ during the night. (b) Reduction of $[CO_2]$ during the day. (Arrows indicate time course.)

of the bowl with CO_2 displacing air with no mixing. As these assumptions are clearly simplifications, the emission from the vent is likely to be significantly higher than the estimate of 12 t day^{-1}.

Measurements of air temperature 0.5 m above the ground surface demonstrate an interesting phenomenon associated with the build-up and dispersal of CO_2 (Fig. 3). Every morning when high CO_2 concentrations are observed, there is an associated increase in air temperature in the zone of air with high CO_2 concentrations. The rapid increase in temperature is observed immediately after the sun rises above the horizon and direct radiation is incident on the air at the bottom of the bowl. Maximum temperatures occur immediately before the most rapid change in CO_2 concentrations

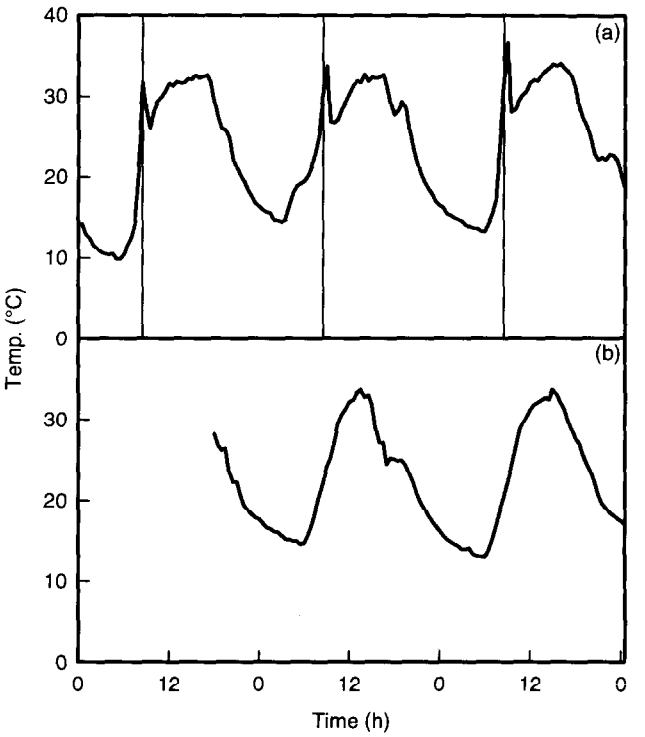

Fig. 3. Air temperature at the Bossoleto and control sites. Temperature was measured with a fine unshielded thermocouple 0.5 m above the soil surface. (a) Bossoleto site from 21 to 23 July. Vertical lines indicate the time that direct radiation was first incident at the bottom of the bowl. (b) Control site from 24 to 25 July. Data are 30 min means. Weather conditions at the sites were similar for duration of the measurements.

(Figs. 2 and 3). The maximum rate of temperature increase exceeded 0.5°C min^{-1} averaged over the 30 min measurement interval (Fig. 3). The maximum temperature varies from day to day, but appears to be correlated with the stability of the atmosphere during the night.

To investigate this observed phenomenon further, measurements of the temperature gradient were recorded on 23 and 24 July 1992. At night, a temperature inversion develops in the bowl. By 07:30 h, the inversion has disappeared and a strong temperature gradient is starting to develop. In the next 30 min, direct radiation became incident on the bottom of the bowl and air temperature increased more rapidly to develop a very marked temperature gradient. The timing and shape of this gradient provide strong evidence that the temperature increase is associated with the high CO_2 concentrations at the bottom of the bowl. The most rapid change in air temperature on 24 July occurred between 08:30 and 09:00 h, which was when the CO_2 started to disperse on that day. The period of most rapid temperature increase coincided with the time that direct radiation reached the bottom of the bowl (Fig. 3) and preceded the period of most rapid CO_2 dispersal (Fig. 2). It should be noted that the exact time of dispersal varied between days. After the high concentrations of CO_2 have dispersed,

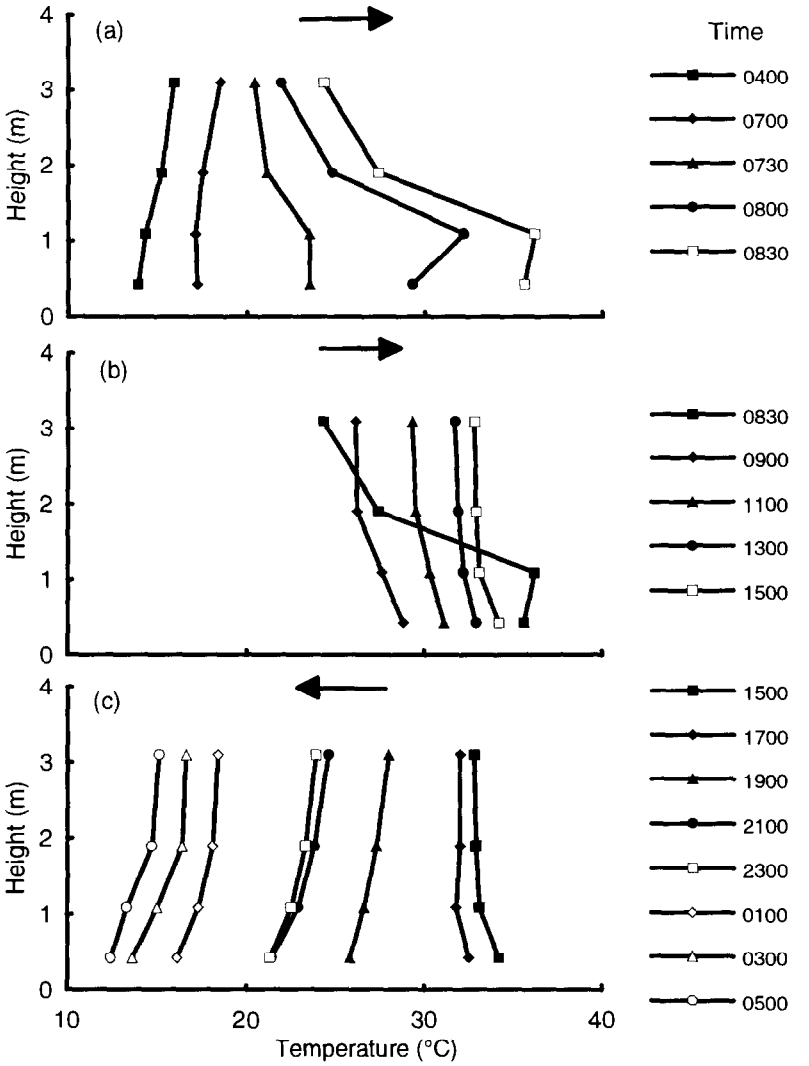

Fig. 4. Temperature profiles for the Bossoleto site on 23 and 24 July 1992. Temperatures were measured using fine unshielded thermocouples and are 30 min means. (Arrows indicate time course.)

the temperature gradient develops normally during the day (Fig. 4(b)) and at night, as temperatures decrease, a temperature inversion develops to a maximum at 05:00 h.

The temperature gradients observed at the Bossoleto site are not sufficient to disperse the CO_2 through buoyancy effects. Air containing 75% CO_2 will be 1.4 times as dense as air with 0.035% CO_2 at the same temperature and pressure. The temperature required to give an equivalent density would, therefore, be 1.4 times the ambient absolute temperature. Assuming an ambient air temperature of 20°C (293 K), this would require a temperature higher than 137°C (410 K) to disperse the CO_2

Table 1
Effective mean CO_2 concentrations (p_s) calculated from ^{14}C abundance measurements (M_p) of plant material of *Phragmites australis*

Location	M_p	p_s (μmol mol^{-1})
Control	1.108	(350)
1	0.529	734
2	0.400	971
3	0.557	697
4	0.452	860
5	0.355	1094
6	0.348	1116

The locations refer to those marked on Fig. 1, and the concentrations were calculated uising Eq. (5)

through the effect of buoyancy alone. Fortunately for the plants at the Bossoleto site, these temperatures are never observed and it appears that small localised eddies of wind are responsible for dispersal of the CO_2.

The estimates of CO_2 concentration calculated from ^{14}C abundance measurements of plant material (Table 1) show a pattern of decreasing estimated effective concentration with distance from the vent (Fig. 5). The estimated values are similar to those measured during the day using gas analysis (Fig. 2) but tend to be lower. The likely reason for this discrepancy is the time-scale of the measurements. Atmospheric

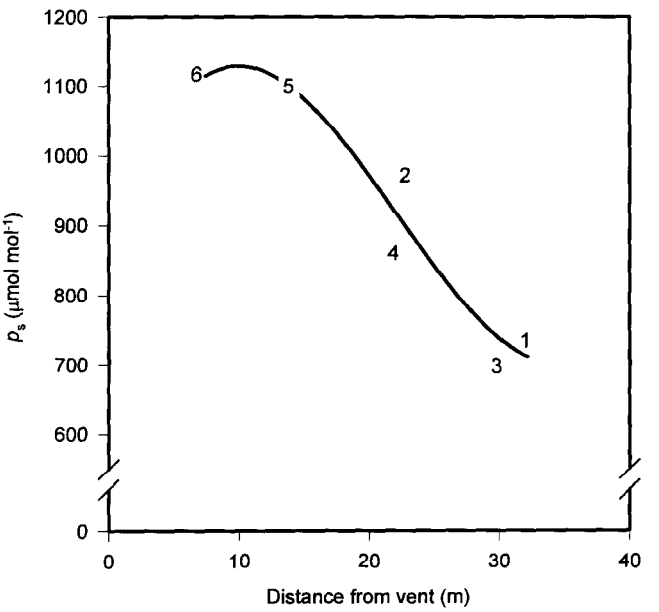

Fig. 5. Effective mean CO_2 concentrations (p_s) as a function of distance from the main CO_2 vent. Numbers represent sample locations (Fig. 1). The solid line is a third-order polynomial fitted to the data.

conditions influence the degree of mixing of air in the bowl, resulting in variation in average concentrations of CO_2 between days and seasons. The ^{14}C data are integrated over the lifetime of the leaf, whereas the gas analysis results are averaged over a period of a few minutes on only 1 day. The most important result from these measurements is that the value determined from the leaf material is much more closely related to daytime concentrations than to the daily average of CO_2 concentration at the site. The latter would exceed 10% over a 24 h period, but the isotope data clearly show that plants are mainly photosynthesising during periods with more physiologically relevant concentrations. This result is not too surprising, as photosynthesis will not occur during the night, because stomata are likely to close and light is required (Jones, 1992). A question does, however, remain regarding the effects of high CO_2 concentrations on plants during the night. The high concentrations of CO_2 at night, or, more importantly, the low oxygen concentrations, will inhibit aerobic respiration. As a result, products of anaerobic respiration such as ethanol may accumulate in many plant species (Crawford, 1984). Plants growing at the bottom of the bowl at the Bossoleto site may require specialised adaptations to survive these extreme conditions. This may explain why the bottom of the bowl is dominated by a monospecific community of *Phragmites australis*, a species adapted to short periods of anaerobic conditions in wetland communities (Crawford, 1989). In contrast, the plant communities above the region routinely flooded by high CO_2 at night are much more species rich and are similar in composition to that expected in the region.

4. Conclusions

Sites such as the Bossoleto in Italy present an opportunity for the study of long-term effects of CO_2 increase on natural vegetation. The variability in CO_2 concentration during the day and with distance from the vent create difficulties in defining the average CO_2 environment for plants in any study. The results obtained using ^{14}C as a natural tracer at the Bossoleto site demonstrate that this technique can be used to compare effective concentration between locations, with concentration tending to decrease with distance from the vent and with height. The concentrations calculated using this approach are close to those measured during the day using gas analysis techniques and have no relationship to a time-based average calculated from atmospheric concentrations over a 24 h period. This suggests that plants are responding to CO_2 concentrations during the day rather than to the much higher night-time concentrations.

The rapid increase in air temperature in the morning appears to be associated with the presence of very high CO_2 concentrations at the bottom of the bowl and with processes leading to the eventual dispersal of the gas in the morning. The conditions present at the site immediately before and during the dispersal of the pool of high CO_2 at the bottom of the bowl deserve further investigation, with emphasis on the physical processes of energy balance and mass transfer.

Acknowledgements

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References

Acock, B., 1990. Effects of carbon dioxide on photosynthesis, plant growth, and other processes. In: B.A. Kimball, N.J. Rosenberg, L.H. Allen, G.H. Heichel, C.W. Stuber, D.E. Kissel and S. Ernst (Editors), Impact of Carbon Dioxide, Trace Gases, and Climate Change on Global Agriculture. ASA Special Publication. American Society of Agronomy, Madison, WI, pp. 45–60.

Badiani, M., Dannibale, A., Paolacci, A.R., Miglietta, F. and Raschi, A., 1993. The antioxidant status of soybean (*Glycine max*) leaves grown under natural CO_2 enrichment in the field. Aust. J. Plant Physiol., 20: 275–284.

Crawford, R.M.M., 1984. Anaerobic respiration and flood tolerance in higher plants. In: J.M. Palmer (Editor), The Physiology and Biochemistry of Plant Respiration. Cambridge University Press, Cambridge, pp. 67–75.

Crawford, R.M.M., 1989. Studies in Plant Survival. Ecological Case Histories of Plant Adaptation to Adversity. Blackwell Scientific, Oxford, 296 pp.

Eamus, D. and Jarvis, P.G., 1989. The direct effects of increase in the global atmospheric CO_2 concentration on natural and commercial temperate trees and forests. Adv. Ecol. Res., 19: 1–55.

Jarvis, P.G., 1989. Atmospheric carbon dioxide and forests. Philos. Trans. R. Soc. London, 324: 369–392.

Jones, H.G., 1992. Plants and Microclimate. A Quantitative Approach to Environmental Plant Physiology, 2nd edn. Cambridge University Press, Cambridge, 428 pp.

Miglietta, F. and Raschi, A., 1993. Studying the effect of elevated CO_2 in the open in a naturally enriched environment in Central Italy. Vegetatio, 104–105: 391–400.

Miglietta, F., Raschi, A., Bettarini, I., Resti, R. and Selvi, F., 1993a. Natural CO_2 springs in Italy: a resource for examining long-term response of vegetation to rising atmospheric CO_2 concentrations. Plant Cell Environ., 16: 873–878.

Miglietta, F., Raschi, A., Resti, R. and Badiani, M., 1993b. Growth and onto-morphogenesis of soybean (*Glycine max* Merril) in an open, naturally CO_2-enriched environment. Plant Cell Environ., 16: 909–918.

Miglietta, F., Badiani, M., Bettarini, I., van Gardingen, P.R., Selvi, F. and Raschi, A., 1994. Natural carbon dioxide vents as a resource for studying the long-term response of Mediterranean vegetation to elevated CO_2. In: J.M. Moreno and W.C. Oechel (Editors), Anticipated Effects on a Changing Global Environment on Mediterranean Ecosystems. Springer, Berlin, in press.

Stuiver, M. and Polach, H.A., 1977. Reporting of ^{14}C data. Radiocarbon, 19: 355–365.

Woodward, F.I. and Bazzaz, F.A., 1988. The responses of stomatal density to CO_2 partial pressure. J. Exp. Bot., 39: 1771–1781.

Woodward, F.I., Thompson, G.B. and McKee, I.F., 1991. The effects of elevated concentrations of carbon dioxide on individual plants, populations, communities and ecosystems. Ann. Bot., 67: 23–38.