Oecologia (1994) 99:343–351

ORIGINAL PAPER

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Long term effects of naturally elevated CO_2 on mediterranean grassland and forest trees

Received: 20 February 1994/Accepted 12 May 1994

Abstract We investigated the carbon supply status in species-rich mediterranean plant communities growing in a bowl-shaped 1-ha "CO_2 spring" area near Sienna, Italy. A geothermic "lime-kiln" has provided these communities, for as long as historical records are available, with pure CO_2 that mixes with ambient air at canopy level to daytime means of 500–1000 ppm CO_2. Immediately outside the spring area similar plant communities are growing on similar substrate, and in the same climate, but under ca. 355 ppm CO_2. We found no evidence that plants in the CO_2 spring area grow faster, flower earlier or become larger. However, we found very large differences in tissue quality among the 40 species studied inside and outside the spring area. Depending on weather conditions, the mean concentration of total non-structural carbohydrates (TNC, sugars and starch) in leaves of herbaceous plants was 38–47% higher in the spring area. Fast growing ruderals growing on garden soil inside and outside the spring area show the same response. Among trees, leaves of the deciduous *Quercus pubscens* contain twice as much TNC inside as outside the vent area, whereas evergreen *Q. ilex* leaves show no significant difference. TNC levels in branch wood paralleled leaf values. TNC in shade leaves was also higher. Elevated CO_2 had no effect on the sugar fraction, therefore differences in TNC are due to starch accumulation. Leaf nitrogen concentration decreases under elevated CO_2. These observations suggest that the commonly reported TNC accumulation and N depletion in leaves growing under elevated CO_2 are not restricted to the artificial conditions of short-term CO_2 enrichment experiments but persist over very long periods. Such an alteration of tissue composition can be expected to occur in other plant communities also if atmospheric CO_2 levels continue to rise. Effects on food webs and nutrient cycling are likely.

Key words Carbohydrates · Global change · Natural CO_2 springs · Leaf nitrogen · Photosynthesis

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Introduction

Our current knowledge about the long-term responses of natural vegetation to increasing atmospheric CO_2 concentrations is very poor, since only a few field studies with elevated CO_2 have used wild plants growing in natural ecosystems (Körner 1993) and so far only four of these have lasted for more than two seasons (arctic tundra: Grulke et al. 1990; salt marsh: Leadley and Drake 1993; prairie: Owensby 1993; alpine grassland: Körner et al. 1994). Nothing is known about long-term responses to CO_2 of undisturbed perennial herbs, shrubs and trees in their natural environments, although such wild plants, including grassland plants, contain 99% of the global biomass carbon pool compared to the 1% of biomass carbon stored in the agricultural crops and managed pastures of the world, whose CO_2 responses are much better understood (e.g. Wittwer 1984; Acock and Allen 1985; Idso et al. 1991).

It has long been known that, in the short term, elevation of ambient CO_2 concentration can increase the rate of photosynthesis in C3 plants and stimulate growth, provided other resources are not seriously limited (Wittwer 1984; Strain and Cure 1985; Woodward et al. 1991; Bazzaz and Fajer 1992). However, in the longer term and under natural conditions, plants may be unable to invest the increased amount of photo-assimilates into new structural biomass, thus

leading to deposition and accumulation of assimilates as starch or sugars in tissues. If this happens, feedback regulation may lead to downward adjustment of photosynthetic capacity (Wulff and Strain 1982; Azcon-Bieto 1983; Scheidegger and Nösberger 1984; Stitt 1991, Bazzaz and Fajer 1992). In a recent review of the effect of elevated CO_2 on photosynthesis, Long and Drake (1992) showed that downward regulation of photosynthesis was often, but not always, observed in experiments with elevated CO_2. Even in cases where some adjustment followed exposure to elevated CO_2, actual photosynthetic rates measured at elevated CO_2 remained higher than those measured at ambient CO_2 concentrations, a phenomenon also documented for whole plant canopies (Drake and Leadley 1991).

Thus, CO_2 stimulation of carbon gain can occur in plants even after several seasons of exposure to elevated CO_2. However, provided moisture and light are not becoming increasingly limiting, the ultimate degree of growth stimulation by elevated CO_2 in natural vegetation will be set by the speed of mineral nutrient recycling, even though CO_2 fertilization may improve nutrient acquisition initially. Experiments on managed soils or the addition of fertilizer may exhibit ranges of CO_2 effects opposite to those to be expected in the majority of natural ecosystems (Woodward 1992; Körner and Arnone 1993). Therefore it is doubtful whether available experimental observations reflect realistic future CO_2 responses of natural vegetation, just as we do not know if short-term growth stimulation by elevated CO_2 will lead to greater carbon sequestration by the biosphere (Eamus and Jarvis 1989; Körner 1993).

Vegetation that has been growing for many years in naturally CO_2-enriched atmospheres, such as those around "CO_2 springs," provides a unique opportunity to address these questions. In these areas carbon dioxide of geological origin is continuously released to the atmosphere through natural vents and this produces local increases in the atmospheric CO_2 concentrations such as may be expected in a future CO_2-enriched world (Miglietta and Raschi 1993). These conditions are ideal for assessing responses and even micro-evolutionary adaptation in natural vegetation after periods of exposure to elevated CO_2 concentrations that could never be simulated experimentally.

One of the obvious shortcomings of working with plants in CO_2 springs is the fact that none of the sites is truly replicated and finding adequate controls is difficult (Miglietta et al. 1993). Similar problems are encountered by ecologists working on islands, high mountains or other peculiar places in the landscape, and they almost always occur when natural gradients of climatic or edaphic conditions are used in functional ecology. However, the great realism of such natural experiments compared to laboratory studies or manipulative experiments have caused them to become one of the major components of ecological research.

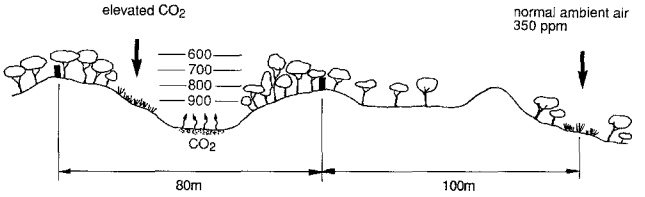

Fig. 1 The Bossoleto CO_2 spring and the control site near Rapolano Terme, central Italy

In the case of the study presented in this paper, we have been particularly fortunate in three respects. First, the CO_2 spring causes CO_2 enrichment to occur over a very large area, thus exposing to elevated CO_2 vegetation of more than 70 species, each represented by many individuals in various places. Plants are not bound to certain holes, gulleys or otherwise microclimatically peculiar niches in the landscape. Second, at the plant level, the atmosphere is virtually free of phytotoxic pollutants. Third, within a distance of only about 100 m, we found a largely undisturbed area with almost identical vegetation growing on a similar substrate, with similar exposure and climate under normal ambient CO_2 levels (Fig. 1), thus, partially reducing the intrinsic statistical shortcomings. In addition, we base our conclusions on a relatively large set of quite different species.

In this field survey we use the accumulation of total non-structural carbohydrates in leaves and young branches as a measure of a possible imbalance between source activity (photosynthetic CO_2 uptake) and sink activity (e.g. structural growth) under elevated CO_2. In addition, leaf nitrogen concentration was measured as an indicator of reduction in protein content and downward regulation of photosynthesis.

Materials and methods

Site description

The CO_2 spring Bossoleto is located in the vicinity of the village Rapolano Terme near Siena, in central Italy. This spring is shaped like a large circular bowl and was formed when an underground cavern collapsed several hundred years ago. CO_2 emission at this site has locally been known (primarily through reports of fatal accidents) at least back to the early part of this century, but possibly dates back much longer, given the large travertine deposits from geothermal water. An early note on Rapolano Terme was published by DeLaunay (1899), and a balneological description of the geothermal "bicarbonic" springs is given by Francalanci (1959). Almost pure CO_2 is emitted from several vents located at various points within the Bossoleto basin (Fig. 2) and creates a steep gradient of CO_2 concentration. Actual levels of atmospheric CO_2 within the spring area vary with windspeed and convective turbulence, but they are consistently above normal ambient levels under all climatic conditions. Mean daytime levels of CO_2 on raised banks, where most of the natural vegetation occurs, normally range between 500 and 1000 ppm, thus simulating a situation forecast to occur globally within the next 200 years. Although traces of hydrogen sulphide have been detected accompanying the CO_2 venting at the surface, this gas is largely dissolved in water vapour, is rapidly oxidised when

in contact with air, and finally is greatly diluted (Miglietta et al. 1993). Accordingly, gas-chromatographic determinations of H_2S concentrations made in air samples collected at the spring, showed that levels were already below detection limits of 0.1 ppb when CO_2 concentrations were around 5000 ppm (close to the vents; F. Miglietta, unpublished).

The natural flora of Bossoleto includes more than 70 plant species of 39 families with a marked predominance of hemicryptophytes in typical mediterranean grassland associations (F. Selvi, personal communication). Half of the basin is forested (mainly with *Quercus ilex*). The study presented in this paper was made on plants growing on raised banks of the Bossoleto bowl where air and CO_2 are already well mixed and where species diversity is highest. A small abandoned garden plot that was recently used for a soybean experiment provided space for numerous ruderal species that are also found in fields outside Bossoleto.

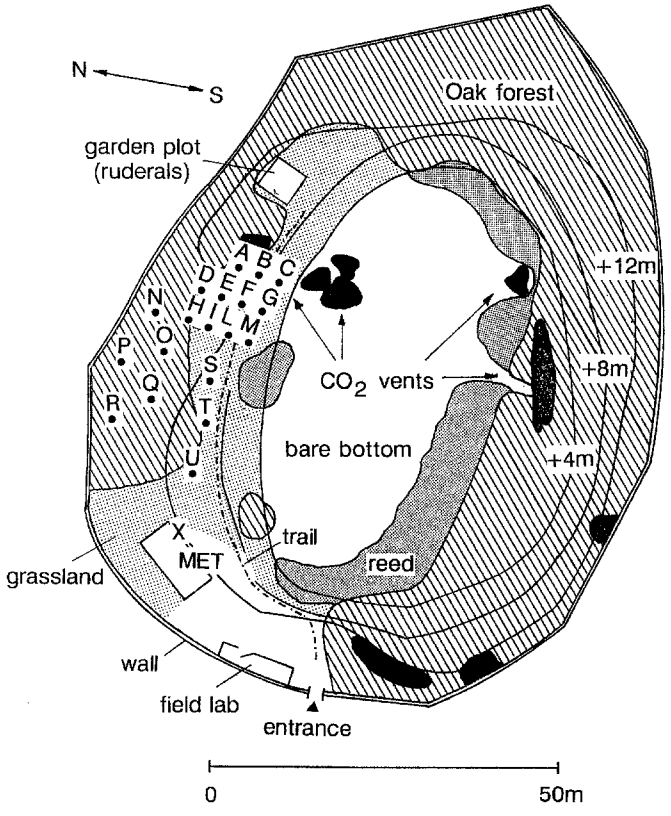

Fig. 2 The Bossoleto basin (*Black* areas of geothermic CO_2 vents, letter *A–X* positions where measurements of CO_2 concentration were made). Plants were sampled in the zone *D–U*, mainly near *E*, *F*, *I*, *L*, *S*

Except for the garden plot, the continuous soil layer in the grassland and forest sites is of the rendzina type, has a depth of only 5–10 cm, and rests on travertine bedrock. As usual in such calcareous areas, roots penetrate crevices to much greater depth. Except for the local greenhouse effect at the bare bottom of the Bossoleto bowl, where a lake of pure CO_2 may build up during calm periods, weather data measured at vegetation level did not differ significantly from those reported from the nearest weather station (Fig. 3). The 2–3 K differences in temperature recorded in early April are due to lower night-time temperatures at the reference site, where clear and calm weather led to a local temperature inversion. At a distance of about 100 m west of the Bossoleto site (Fig. 1), an area with similar exposure and morphology and a similar plant association was chosen as control site. Soils within the Bossoleto basin had higher humus content (but were shallower), were slightly more acid (pH 6.2 vs. 7.6) and contained greater amounts of soluble nitrogen, phosphate and potassium than the control site (Table 1). Based on these data, the interior of the spring zone is rather richer in key nutrients than the area outside, although this is not reflected in the appearance of the vegetation. Soil moisture was similar and high in both sites due to rainy weather in the weeks preceding our sampling campaign.

Monitoring of CO_2 concentration

CO_2 concentrations have been continuously monitored at two points within the spring since November 1992. Air is sampled at 10-min intervals and sampling points are automatically altered every other day. CO_2 concentrations are measured and logged with a single-beam, self-zeroing infrared gasanalyzer (IRGA; EGM-1,

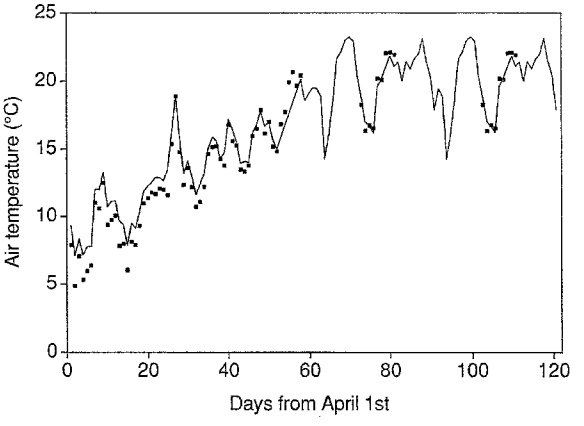

Fig. 3 Air temperatures (2 m) during spring 1993 measured in the Bossoleto basin (*MET* in Fig. 2) and at Poggio S. Cecilia farm, situated at the same elevation 3 km from the village of Rapolano Terme (5 km from Bossoleto). Temperature sensors for the two stations were cross-calibrated in the laboratory

Table 1 Chemical analysis of top-soils. Samples of Bossoleto soils were taken in the proximity of locations D, E, H and I in Fig. 2, where the calcareous grassland community occurs ($n = 3$)

	Control	Bossoleto	Significance of difference
pH (in KCl)	7.6 ± 0.1	6.2 ± 0.6	ns
Nitrogen (in KCl extract, mg/100 g)	1.5 ± 0.1	2.3 ± 1.0	ns
Phosphate (P_2O_5) (Na-bicarbonate extract, pH 8, µg/g)	19.5 ± 3.1	41.0 ± 4.3	$P < 0.05$
Potassium (K_2O) (Ammonium acetate extract, mg/100 g)	22.1 ± 4.2	58.3 ± 2.6	$P < 0.01$
Organic matter (%)	11.5 ± 2.7	40.5 ± 6.8	$P < 0.01$

PP-Systems, Hitchin, UK). Additional measurements were made at the various plant sampling areas with a second mobile IRGA of the same type during the field survey (1-min logging interval). During the same period, CO_2-sensitive diffusion tubes (Dräger, Lübeck, Germany) were exposed at various places within the sampling area, including tree crowns (Fig. 2). These tubes provide an estimate of average CO_2 concentrations integrated over a maximum time of 8 h. Repeated readings can be made by visual estimation of colour changes of a specific reactant along a graded scale and readings are corrected for temperature and atmospheric pressure.

Plant sampling and analysis

Samples were collected on 21 May (1400–1600 hours true solar time, partly overcast weather) and on 22 May 1993 (once during dawn, and again between 1400 and 1600 hours, clear day). In total, 40 different plant species were sampled, most of which grew inside as well as outside the vent area. Mean plant height was determined in 13 grassland species of open habitats only. The phenological status of each individual was noted at the time of sampling. Leaf samples were taken from at least five different plants. Where leaves were big enough (mainly trees) samples were taken with a cork borer (14 mm diameter) to permit later determination of specific leaf area. Only healthy and mature leaves were sampled and dried in the field in a microwave oven for 2 min immediately after sampling. Laboratory comparisons between microwave and 60° C oven-dried leaf samples revealed no significant differences in total non-structural carbohydrates (TNC) but a slight trend for more rather than less TNC in microwave-dried samples. Leaf nitrogen content was determined in the same samples using a CHN analyzer (Model 932, LECO Instruments, St. Joseph, Michigan USA). Total TNC was determined following the procedure described by Wong (1990). About 7 mg ground samples (mixed per species) were boiled in distilled water for 30 minutes. Invertase and isomerase treated subsamples of the solution were then analysed spectrophotometrically for glucose using the sigma reagent (Hexokinase, Sigma, St. Louis, Missouri, USA). The remaining sample (including starch) was incubated with dialyzed Clarase (fungal α-amylase of *Aspergillus oryzae*, Elkhart, Indiana, USA). The filtrate was then treated as above and analysed for glucose. Each sample was analysed twice and two starch and glucose standards were used for each 96-sample micro-titer plate.

Results

CO_2 concentrations

In the long-term, mean CO_2 concentrations during daylight hours were consistently above ambient within the vegetated part of the spring area (slightly above twice current ambient levels; Fig. 4). Short-term measurements made by IRGA and by Dräger tubes were in good agreement and indicate similar CO_2 concentrations for both sampling days and long-term measurements (Table 2). CO_2 concentrations at the level of the grassland community were *c.* 100 ppm higher than those measured at tree crown level or in the forest understory. Nighttime CO_2 concentrations were substantially above daytime averages. Mean atmospheric CO_2 concentrations measured by diffusion tubes at the control site were close to expected ambient level (355 ppm), thus confirming the reliability of measurements made with the same system within the spring area.

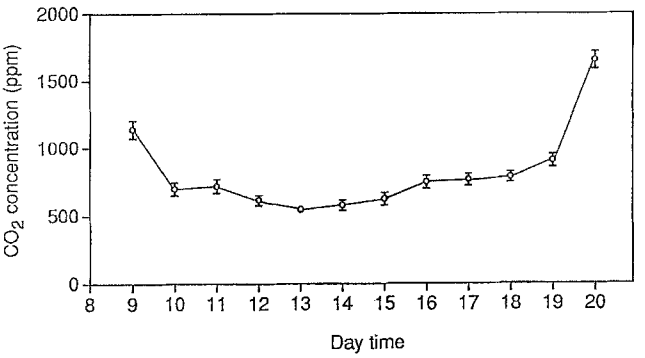

Fig. 4 Long-term means of the diurnal variation of CO_2 concentrations measured at point *X* in Fig. 2

Table 2 Average hourly CO_2 concentrations (ppm) measured by diffusion tubes in locations indicated in Fig. 2, 21 and 22 May 1993. Most plant material was collected in zones 2 and 3. Zone 1, values averaged over locations A, B, C (close to vents); zone 2, locations D, E, F, G, H, I, L, M; zone 3, locations N, P, R, S, T, U; zone 4, locations O, Q (near small side vents). Concomitant IRGA readings at sampling zone 1 were 645 ± 25 (day) and 1320 ± 45 ppm (night). The corresponding IRGA readings at zone 3 were 572 ± 9 and 1071 ± 26 ppm

True solar time	Zone within the Bossoleto basin			
	1	2	3	4
08–0900 hours	1785 ± 85	998 ± 48	513 ± 12	806 ± 106
10–11	1041 ± 143	676 ± 14	553 ± 4	846 ± 106
12–13	1291 ± 114	–	–	–
13–14	1276 ± 22	767 ± 10	–	–
14–15	1221 ± 48	727 ± 10	600 ± 9	1046 ± 156
15–16	1095 ± 56	746 ± 22	586 ± 7	1121 ± 63

Table 3 Mean total plant height (cm, $n = 5$)

Species	CO_2 low	CO_2 high	% Difference
Hippocrepis comosa	13	23	+ 77%
Centaurea alba	40	65	+ 63%
Arabis hirsuta	50	70	+ 40%
Convolvulus cantabrica	25	35	+ 40%
Helianthemum appeninum	20	23	+ 15%
Scabiosa columbaria	40	45	+ 13%
Plantago lanceolata	45	45	0%
Silene vulgaris ssp. *angustifolia*	45	45	0%
Globularia punctata	30	26	− 13%
Galium corrudifolium	48	40	− 17%
Sanguisorba minor	42	35	− 17%
Stachys recta	20	15	− 25%
Allium sphaerocephalon	50	20	− 60%
Mean ± SD	**36.0** ± 12.8	**37.5** ± 16.7	**+ 4%** (n.s.)

Plant size and phenology

Although not quantified in detail, mean plant height for 13 herbaceous species did not differ significantly between sites (Table 3), but some species, like *Hippocrepis comosa* and *Centaurea alba* were substantially taller in

Table 4 Total non-structural carbohydrate (TNC) content (%) in leaves of herbaceous species, and small shrubs grown in full sunlight (mixed samples of at least 5 individuals per site). 21 May 1993, 1400–1600 hours, partly overcast day, 22 May 1993, 1400–1600 hours, clear day

Species	CO_2 low		CO_2 high		Δ TNC (%)	
	Overcast	Clear	Overcast	Clear	Overcast	Clear
Arabis hirsuta	13.3	14.4	33.9	39.0	+ 155[a]	+ 171
Centaurea alba	8.9	13.8	17.3	21.1	+ 95	+ 53
Plantago lanceolata	11.0	20.9	20.3	21.9	+ 84	+ 5
Scabiosa columbaria	13.7	13.7	20.9	33.7	+ 53	+ 146
Teucrium montanum	14.2	15.3	19.8	17.7	+ 39	+ 15
Globularia punctata	17.3	14.8	23.5	28.3	+ 36	+ 91
Silene vulgaris ssp. *angustifolia*	8.4	8.8	11.1	11.7	+ 32	+ 33
Stachys recta	9.8	12.9	12.3	20.1	+ 26	+ 56
Sanguisorba minor	13.1	18.2	16.1	23.7	+ 22	+ 30
Helianthemum appeninum	12.1	12.3	14.6	19.0	+ 20	+ 54
Galium corrudifolium	20.4	19.5	23.8	23.2	+ 17	+ 19
Convolvulus cantabrica	9.4	–	10.6	–	+ 12	–
Allium sphaerocephalon	22.7	20.5	24.7	21.1	+ 9	+ 3
Echium sp. *italicum* af.	9.7	–	10.5	–	+ 9	–
Hippocrepis comosa	22.0	23.9	19.1	28.0	– 13	+ 17
Mean (complete pairs only)[b]	**14.4**[c]	**16.1**[d]	**19.8**[c]	**23.7**	**+ 38**	**+ 47**
± SD ($n = 13$)	4.8	4.2	6.0	7.1		

[a] Note: data are sorted by TNC difference for overcast weather

[b] t-Test for CO_2 effect: overcast $P = 0.004$, clear $P = 0.003$; t-Test for weather effect: low CO_2 $P = 0.093$, high CO_2 $P = 0.011$

[c] mean for all species, $n = 15$, CO_2 low 13.7 ± 4.8, CO_2 high 18.6 ± 6.5, $P = 0.004$

[d] The mean for 8 additional species sampled only at the control site on the clear day was 16.4 ± 5.0, thus indicating that the paired samples represent the community mean very well (*Carlina vulgaris* 11.1, *Convolvulus cantabrica* 15.5, *Echium* sp. *italicum* af. 10.2, *Helichrysium italicum* 17.3, *Lathyrus verna* cf. 16.1, *Mentha* sp. 26.2, *Scrophularia nudosa* 19.6, *Thymus serpyllium* aggr. 14.9)

Table 5 TNC content (%) in leaves of herbaceous plants grown in deep shade (for mixed samples per species, 21 May 1993, 1400–1600 hours, overcast day).

Species	CO_2 low	CO_2 high	Δ TNC (%)
Silene italica	1.1	4.9	(+ 360)
Parietaria officinalis	8.1	15.0	+ 85
Urospermum sp.	11.4	17.6	+ 54
Mean ± SD	**6.9** ± 5.3	**12.5** ± 6.7	**+ 81** ($P = 0.027$)

Table 6 TNC content (%) in leaves of ruderal species grown in full sunlight, clear day (22 May 1993, 1700–1800 hours)

Species[a]	CO_2 low	CO_2 high	Δ TNC (%)
Cerastium af. *arvense*	12.0	26.8	+ 123
Matricaria camomilla	14.9	25.5	+ 71
Erigeron sp.	17.1	26.9	+ 57
Convolvulus sp.	18.1	27.5	+ 53
Ranunculus bulbosus	18.9	28.1	+ 49
Daucus carota cf.	18.6	25.8	+ 38
Lathyrus verna cf.	23.2	30.4	+ 31
Rumex sp.	22.2	29.1	+ 31
Achillea millefolium	24.8	27.1	+ 9
Mean ± S.D. ($n = 9$)	**18.9** ± 4.1	27.5 ± 1.6	**+ 46** ($P < 0.001$)

[a] Non-flowering, young plants, except for *Matricaria* and *Ranunculus* which flowered

the CO_2 spring than in the control site, while others, like *Allium sphaerocephalon* and *Stachys recta* were smaller. The species that were taller within the spring area were not present in greater abundance nor were the smaller ones rarer. Hence, size differences do not seem to have affected species dominance. Phenology (shoot development, flowering) was not different between sites. Most species flowered at the time of sampling.

Non-structural carbohydrates

In contrast to the inconsistent differences in size and the similar phenology of plants, leaf tissue composition differed significantly between sites (Table 4). In the grassland community, mean leaf TNC in controls ranged from 14% to 16% under overcast and clear weather, respectively, and reached 20 and 24% in the spring area. Thus, depending on weather, TNC was 38–47% higher at high than at low CO_2. TNC accumulation in leaves was substantially higher in bright weather both at elevated and ambient CO_2. TNC levels in herbaceous plants growing in deep shade were also increased under elevated CO_2 (Table 5).

The ruderal weeds growing on previously turned and fertilized garden ground in the CO_2-enriched area offered us the possibility to include inherently fast-growing species in TNC analysis. These species were expected to be able to invest more extra carbon in structural growth, in particular since they were growing on richer soils and under little competition. However, the analysis showed very high TNC accumulation also

Table 7 TNC content (%) in leaves of woody species, fully sunlit canopy 21 May 1993, 1600 hours, partly overcast day 22 May 1993, 1600 hours, clear day

Species	CO_2 low		CO_2 high		Δ TNC (%)		
	Overcast	Clear	Overcast	Clear	Overcast	Clear	(*P*)
Dry weight basis							
Quercus pubescens	11.2	11.0	19.9	21.1[a]	+ 77[b]	+ 92	(0.001)
Q. ilex	13.9	10.8	14.6	11.9	+ 5	+ 10	(0.420)
Mean ($n = 2$)	**12.6**	**10.9**	**17.2**	**16.5**	**+ 37**	**+ 51**	
± SD	1.9	0.1	3.8	6.5			
Leaf area basis							
Q. pubescens	9.0	9.4	19.9	20.5	+ 121	+ 118	(0.002)
Q. ilex	32.3	24.2	35.6	25.5	+ 10	+ 5	(0.085)
Mean ($n = 2$)	**20.7**	**16.8**	**27.8**	**23.0**	**+ 34**	**+ 37**	
± SD	16.5	10.5	11.1	3.5			

[a] Samples grown under 1% CO_2 collected around midday at the 10 km distant Castillioni CO_2-spring contained 21.8% TNC, indicating that TNC accumulation found here is close to saturation
[b] For the overcast day only mixed samples were available (no *P*)

in individuals of this group of species exposed to high CO_2 (28%), compared with those from outside the CO_2 spring (19%, Table 6).

Trees showed similar mean differences between sites as did grassland and ruderal species, but the difference was only due to the very pronounced enhancement of TNC in *Quercus pubescens* (+ 77%), whereas the evergreen *Q. ilex* showed no significant difference (Table 7). Per unit leaf area *Q. pubescens* had 118% more TNC under elevated CO_2 than under normal CO_2, and again the difference in *Q. ilex* was negligible (+ 5%). Absolute TNC values at elevated CO_2 were twice as high in *Q. pubescens* than in *Q. ilex* when expressed per unit dry mass, but, due to different SLA, were similar in both species when expressed per unit leaf area.

TNC levels in the xylem of *c.* 6-mm-diameter branchlets of *Q. pubescens* were 62% higher under elevated than normal CO_2, whereas again no differences were found in *Q. ilex* (Table 8). It should be noted that the evergreen *Q. ilex* was just about to commence new leaf and branch growth, whereas the deciduous *Q. pubescens* had sprouted about a month earlier. One might have expected levels of reserve pools would be different between species and more likely to be depleted in the deciduous species, the opposite of what the data show. Bark samples (including active phloem) showed no significant differences in TNC between either tree species and site.

In contrast to herbaceous species, low quantum flux densities dampened the difference between leaves of woody plants grown at ambient and elevated CO_2, with shade leaves of only one out of four species (*Hedera helix*) showing a significant TNC increase (Table 9).

TNC content increased during the day in a number of species (Table 10). In three herbaceous species mean afternoon/dawn differences in TNC content were + 59% at ambient and + 35% in elevated CO_2, and the same was observed in trees. It should be noted that these diurnal changes in TNC occurred at much higher absolute levels in plants exposed to elevated CO_2.

Table 8 TNC content (%) in branch wood and young bark (mixed samples of 5 branches, 22 May 1993, 1400–1600 hours)

Species	CO_2 low	CO_2 high	Δ TNC (%)
Wood[a]:			
Q. pubescens	4.4	7.0	+ 62
Q. ilex	10.9	10.0	− 8
Mean ± SD	**6.1** ± 4.1	**6.8** ± 3.3	**+ 11**
Bark[b]:			
Q. pubescens	8.0	8.0	+ 1
Q. ilex	11.9	11.3	− 5
Mean ± SD	**11.0** ± 2.7	**10.1** ± 1.8	**− 8**

[a] Wood (xylem only) was taken from twigs formed in 1991
[b] Bark was stripped from twigs formed in 1991

Table 9 TNC content (%) in leaves of woody species, from deep shade (21 May 1993, 1400–1600 hours)

Species	CO_2 low	CO_2 high	Δ TNC (%)
Hedera helix	10.8	18.8	+ 74
Ruscus aculeatus	12.0	13.0	+ 8
Q. ilex	9.0	9.5	+ 6
Q. pubescens	7.7	6.3	− 18
Mean ± SD	**9.9** ± 1.9	**11.9** ± 5.4	**+ 20**

CO_2 concentrations had no statistically significant effect on leaf sugar content, but there was a slight trend for higher values under elevated CO_2 (data not shown). The contribution of sugars to TNC in sunlit leaves ranges from 20 to 30% in herbaceous plants, and from 30 to 40% in the studied trees. In the shade, where TNC contents were generally lower, sugars contributed a higher fraction (up to 60% of TNC). Thus, the differences in TNC under the two CO_2 regimes were mainly due to different starch levels.

Leaf nitrogen concentration

Leaf nitrogen concentration on a dry-matter basis was reduced under elevated CO_2 in most herbaceous grassland species and in *Q. pubescens* (Table 11). *Q. ilex* showed no difference. Ruderals from the garden plot within the Bossoleto basin had much higher nitrogen concentrations than those sampled on the fields around Bossoleto. On a TNC-free dry matter basis, differences in N concentration disappear in grassland species and *Q. pubescens* and become even more enhanced in the ruderals.

Table 10 Diurnal changes of TNC content (%) in leaves of three herbaceous plant species and two tree species (22 May 1993)

	CO_2 low			CO_2 high		
Species	08:00[a]	16:00	Δ TNC (%)	08:00[a]	16:00	Δ TNC (%)
Plantago lanceolata	7.7	20.9	+ 171	15.5	21.9	+ 41
Globularia punctata	15.3	14.8	− 3	21.5	28.3	+ 32
Scabiosa columbaria	8.2	13.7	+ 67	25.3	33.7	+ 33
Mean	**10.4**	**16.5**	**+ 59**	**20.8**	**28.0**	**+ 35**
± SD	4.2	3.9	$P = 0.266$	4.9	5.9	$P = 0.007$
Q. pubescens	7.7	11.0	+ 57	14.8	21.1	+ 43
Q. ilex	12.2	10.8	− 11	10.3	11.9	+ 16
Mean	**10.0**	**10.9**	**+ 9**	**12.6**	**16.5**	**+ 31**
± SD	3.2	0.1	$P = 0.755$	3.2	6.5	$P = 0.342$

[a] Leaves were sampled before they were reached by sun, hence, these numbers represent dawn values

Table 11 Leaf nitrogen concentration

	%N (d.m.)			%N (TNC free d.m.)		
Species	CO_2 low	CO_2 high	Δ N (%)	CO_2 low	CO_2 high	Δ N (%)
Mediterranean grassland species						
Arabis hirsuta	2.18	1.55	− 29	2.55	2.53	− 1
Plantago lanceolata	1.53	1.09	− 29	1.93	1.40	− 28
Helianthemum appeninum	2.40	1.91	− 20	2.74	2.36	− 14
Sanguisorba minor	1.65	1.32	− 20	2.02	1.73	− 14
Allium sphaerocephalon	1.96	1.67	− 15	2.46	2.12	− 14
Teucrium montanum	1.55	1.40	− 10	1.83	1.70	− 7
Globularia punctata	1.41	1.36	− 4	1.65	1.90	+ 15
Hippocrepis comosa	3.08	2.98	− 3	4.05	4.13	+ 2
Centaurea alba	2.47	2.52	+ 2	2.87	3.20	+ 12
Galium corrudifolium	1.65	1.75	+ 6	2.05	2.28	+ 11
Stachys recta	2.47	2.80	+ 13	2.84	3.50	+ 23
Mean ($n = 11$)	**2.03**	**1.85**	**− 9**[a]	**2.45**	**2.44**	**0**
± SD	0.53	0.64	$P = 0.061$	0.68	0.84	$P = 0.909$
Ruderals						
Convolvulus cantabrica	2.86	2.90	+ 1	3.49	4.00	+ 15
Achillea millefolium	3.30	3.66	+ 11	4.39	5.02	+ 14
Lathyrus verna cf.	3.00	3.85	+ 28	3.90	5.53	+ 42
Erigeron sp.	2.44	3.14	+ 29	2.94	4.30	+ 46
Matricaria camomilla	2.18	2.97	+ 36	2.56	3.99	+ 56
Daucus carota cf.	2.59	3.55	+ 37	3.18	4.78	+ 50
Cerastium af. *arvensis*	1.53	2.58	+ 69	1.74	3.52	+ 103
Ranunculus bulbosus	1.55	2.84	+ 83	1.91	3.95	+ 107
Rumex sp.	2.22	4.09	+ 84	2.85	5.77	+ 102
Mean ($n = 9$)	**2.41**	**3.29**	**+ 37**	**3.00**	**4.50**	**+ 50**
± SD	0.61	0.52	$P = 0.001$	0.87	0.87	$P < 0.001$
Trees						
Q. pubescens	1.97	1.68	− 15	2.24	2.13	− 5
± SD ($n = 5$)	0.04	0.09	$P = 0.049$	0.05	0.09	$P = 0.374$
Q. ilex	1.22	1.32	+ 9	1.36	1.50	+ 10
± SD ($n = 5$)	0.02	0.08	$P = 0.243$	0.02	0.10	$P = 0.198$

[a] The mean of the differences is − 10% and is significantly different from zero (one-tailed *t*-test $P = 0.046$)

Discussion

Our biometric and phenological observations suggest that there is little (if any) growth or developmental response to long-term exposure to elevated CO_2 in this mediterranean grassland community. However, the question whether or not biomass accumulation was greater remains to be quantified by destructive sampling of total plant biomass. Unpublished studies in the Bossoleto forest also suggest no change in annual branch growth in trees (S. Hättenschwiler et al.), and confirm the visual impression that plants look pretty much the same both inside and outside the CO_2 spring. Although more detailed studies on actual plant growth and biomass may reveal some species-specific responses to CO_2 (perhaps following Table 3), the present evidence strongly argues against a stimulation of biomass production at community level in the magnitudes that have been predicted from CO_2 enrichment experiments under horticultural growth conditions (e.g. Idso and Kimball 1992). Our observations are in line with available evidence from other mediterranean grassland systems (Williams et al. 1988; personal communications by Ch. Field, Stanford, and J. Roy, Montpellier), with the findings in tussock tundra in Alaska (Grulke et al. 1990), and our observations in alpine grassland (Körner et al. 1994), all of which indicate no or only minute biomass responses to elevated CO_2.

On the other hand, our data indicate important alterations in tissue composition in plants grown under elevated CO_2. Perennial grassland herbs and shrubs, ruderal weeds, understory plants as well as mature trees, altogether 40 species, of which 30 were studied at both sites, show substantially higher mean TNC levels inside than outside the CO_2 enriched area. This indicates (1) that plants have taken up significantly more carbon than could be invested in structural biomass, and (2) that downward adjustment of photosynthesis under high CO_2 was certainly not complete. (3) Surprisingly, ruderals on a nutrient rich site are not an exception, thus, suggesting that TNC accumulation under elevated CO_2 is not restricted to inherently slow-growing plants and/or poor soils. The probably low (if any) growth response but strong TNC response found here in "stress tolerators" and "ruderals" appears to fit the hypothesis suggested by Hunt et al. (1993) for British grassland species that stress tolerators and ruderals should be less responsive to CO_2 in terms of growth than "competitors".

The extent of TNC accumulation found here in natural plant communities compares well with values published for various greenhouse and growth chamber experiments, both with high and low nutrient supply (e.g. cotton, Wong 1990; rice, Rowland-Bramford et al. 1990; variety of wild herbaceous species, Poorter et al. 1992; a number of tropical plant species, Körner and Arnone 1992).

It has been known for over 100 years that plants accumulate more starch when exposed to elevated CO_2 (Saposchnikoff 1893). However, the data presented here, for the first time provide evidence that this phenomenon prevails in long-lived plants that have spent their full life under elevated CO_2, indeed, in plant populations, most of which may have been exposed to these life conditions for many generations. In other words, these findings indicate that carbohydrate accumulation in plants growing under elevated CO_2 is not reflecting a short-term imbalance of the carbon relations, but rather suggests an intrinsic inability of plants to dissipate the greater pools of assimilates accumulated under high CO_2. At least for some species, this may indicate that CO_2 saturation is already occurring at present ambient concentrations, and that the range of CO_2 concentrations to which these plants are particularly responsive has already been passed, as suggested by Polley et al. (1992).

Much has been written about the possible reasons for carbohydrate overflow reactions in plants in general and under elevated CO_2 in particular. Explanations largely fall into two categories, namely (1) limited sink activity (either via genotypic slow growth or resource limitation of growth), or (2) limited dissipation capacity for sugars from chloroplasts to the phloem (e.g. phosphorylation, diffusion pathways; Sharkey 1985; Schulze et al. 1991; Stitt 1991; Luxmoore 1991). We believe that the significance of the second group of factors has been underestimated, and we interpret our findings in favour of these cellular limitations. Ruderals accumulated similar or even more TNC, despite growing on comparatively rich soils and exhibiting much higher leaf nitrogen concentrations at the high-CO_2 site. Thus, fertilization does not preclude carbohydrate overflow responses under high CO_2 even in typically fast-growing species. Also for grassland species mineral nutrient supply in the soil was better rather than worse under high CO_2 (Table 1). Most of the reduction in leaf nitrogen concentration (except for *Plantago*) is due to a dilution effect caused by TNC accumulation (Sage et al. 1989). Since close correlations between leaf nitrogen content and photosynthetic capacity have been established (Field and Mooney 1986; Evans and Seemann 1989), the present data do not suggest a very pronounced reduction of photosynthetic capacity in leaves grown under elevated CO_2.

In conclusion, it appears rather uncertain whether wild plants such as those included in this survey will accumulate more biomass in a CO_2-enriched world, but it appears almost certain that the composition of their leaf tissue, and possibly also that of other tissues will be altered. The data collected in the Bossoleto CO_2 spring suggest that non-structural carbohydrate accumulation and N dilution in plants growing under elevated CO_2 is likely to become a reality in a CO_2-enriched world, and is not just an artefact of short-term CO_2 fertilization experiments.

If such changes in tissue quality occur in other wild vegetation under future atmospheric conditions, this will have far ranging effects on the food webs of the world and on soil processes. Findings that herbivores are indeed affected by such changes in food quality (Lincoln et al. 1993; Owensby 1993) are gaining even more weight.

Acknowledgements The authors wish to acknowledge F. Selvi and R. Körner for their help with field work, S. Pelaez-Riedl, M. Würth and B. Schäppi for help with TNC analyses and, in particular, A. Raschi for his enthusiasm and support.

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