Elevated Carbon Dioxide in a Semi-Natural Grassland

Plant production and vegetation composition during six years

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Abstract

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This thesis is concerned with the possible effects of a further increase in atmospheric carbon dioxide on vegetation in a semi-natural grassland. It is based on an experimental field study focusing on production responses and changes in vegetation composition.

A Swedish semi-natural grassland was exposed to ambient and twice ambient elevated CO_2 in open-top chambers during six consecutive growing seasons. Plots without chambers constituted a control treatment. The field had been grazed previously, but when the experiment started this was replaced by a cutting regime with one cut (0 cm above the soil surface) each year in early August. Leaf-level photosynthesis and stomatal conductance, biomass production, and vegetation composition were studied.

The first three growing seasons showed a 30 to 60 % increase in above-ground biomass at harvest under elevated CO_2. In later years there was no difference in above-ground biomass between the treatments, but differences between treatments increased below ground. Throughout the experiment, elevated CO_2 increased leaf-level photosynthesis and tended to decrease stomatal conductance. Nitrogen concentration in stems and leaves was lower at elevated CO_2.

CO_2 effects on vegetation composition were small in comparison with effects of time and/or weather, but increased towards the end of the experiment. Legumes may benefit most from elevated CO_2, which also tended to increase diversity (Shannon index) in summer, but decrease it in spring.

Changes in the weather (especially precipitation) were highly directional over time. This means that weather effects are confounded with effects that are time-dependent (such as a possible succession after a change in management, or an increasing nutrient deficit). However, weather effects did appear to be decisive.

Keywords: global change, photosynthesis, stomatal conductance, biodiversity, point-intercept, root/shoot ratios, grasses, forbs, legumes.

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Förhöjd koldioxid i en gammal betesmark.

Växtproduktion och vegetationssammansättning under sex år.

Sammanfattning

I denna avhandling studeras vilka effekter en fortsatt ökning av koldioxidhalten (CO_2) i atmosfären kan ha på vegetationen i en gammal betesmark. Detta har jag studerat i ett sexårig fältförsök (1995-2000) där växtproduktionen och vegetationsförändringar har stått i centrum.

I en gammal betesmark utanför Uppsala fördubblades koldioxidhalten i plastcylindrar som var öppna upptill (s.k. open-top chambers, OTC; behandling Elevated). Samtidigt användes samma sorts cylindrar även utan ökad koldioxid (behandling Ambient), samt kontrollringar av samma storlek, men utan cylinder (behandling Control). Området har en lång historia av bete (senast hästbete), men försöksfältet stängslades in när försöket påbörjades, och betet ersattes med en skörd om året (i början av augusti), varvid vegetationen klipptes ner ända till markytan. Jag studerade fotosyntes på bladnivå och stomatakonduktans (klyvöppningarnas genomsläpplighet för bl. a. CO_2 och vatten), samt växtproduktion och vegetationssammansättning.

Under de första tre åren ledde en högre koldioxidhalt till en 30- till 60-procentig ökning av ovanjordisk biomassa. Under senare år har jag inte hittat någon skillnad mellan behandlingarna i biomassa över markytan, men rotproduktionen började skilja sig alltmer mellan behandlingarna. Ökad CO_2 ledde till högre fotosynteshastighet och lägre stomatakonduktans under hela försöksperioden. Kvävehalten i de ovanjordiska växtdelarna var lägre under förhöjd CO_2.

Artsammansättningen påverkades i liten utsträckning av förhöjd CO_2 jämfört med vädret och/eller tiden, men koldioxideffekten ökade mot försökets slut. Baljväxterna kan vara den grupp som gynnas mest. Ökad CO_2 ledde till högre diversitet (uttryckt som Shannon-index) i juli, men lägre i april/maj.

Nederbörden ökade från år till år, 1999 undantaget. Därför är det mycket svårt att skilja vädrets påverkan från effekter som har ett samband med tiden (exempelvis en möjlig succession på grund av den ändrade hävden, eller en tilltagande näringsbrist). Ändå finns det tecken på att vädereffekten är mycket viktig. Det är alltså mycket möjligt att de indirekta effekter som ökad CO_2 kan tänkas ha som växthusgas visar sig vara mycket viktigare än de direkta effekterna i detta ekosystem.

Contents

Appendix

Papers I-IV

This thesis is based on the following papers, which I refer to in the text using their Roman numerals:

I. Marissink, M., R. Pettersson & E. Sindhøj. 2002. Above-ground plant production under elevated carbon dioxide in a Swedish semi-natural grassland. *Agriculture, Ecosystems and Environment* in press.
II. Sindhøj, E., A.-C. Hansson, O. Andrén, T. Kätterer, M. Marissink & R. Pettersson. 2000. Root dynamics in a semi-natural grassland in relation to atmospheric carbon dioxide enrichment, soil water and shoot biomass. *Plant & Soil* 223: 253-263.
III. Marissink, M. & M. Hansson. Vegetation composition of a Swedish semi-natural grassland during six years of elevated CO_2. *Journal of Vegetation Science* in press.
IV. Marissink, M., R. Pettersson, E. Sindhøj, O. Andrén & M. Hansson. Plant production and species composition in a semi-natural grassland during six years of elevated CO_2. Manuscript.

Agriculture, Ecosystems and Environment, *Plant & Soil* and *Journal of Vegetation Science* kindly permitted reproduction of papers I, II and III, respectively.

Introduction

Global change

Since environmental issues first hit the political and media agenda in the 1960s, the focus of environmental research has shifted from direct and immediate problems, although not necessarily uncomplicated ones, to more diffuse ones where cause and effect may be separate in space and time. During the 1990s, global change became one of the hot topics for researchers, journalists and environmental conservationists alike. The term global change has thus been used with varying interpretation in a great variety of contexts. Sometimes it is used as a synonym for climate change, sometimes it is applied to changes in atmospheric chemistry. The Global Change and Terrestrial Ecosystems (GCTE) core project of the International Geosphere Biosphere Programme (IGBP) advocates an even wider use by combining both these factors and adding land use changes (Walker and Steffen 1999). Increasingly, changes (declines) in biodiversity are considered to be one of the components of global change as well as a result of it (IGBP 1997, Walker and Steffen 1999).

Although global change has many faces, climate change has received most attention (Walker and Steffen 1999). The Intergovernmental Panel on Climate Change (IPCC, established in 1988 by the United Nations Environment Programme UNEP and the World Meteorological Organization WMO) use the term climate change to signify any change in climate over time, whether due to natural causes or as a result of human activity (IPCC 2001). This contrasts with the usage by the United Nations Framework Convention on Climate Change, in which climate change means a change of climate which is attributed directly or indirectly to human activity that alters the composition of the global atmosphere and which is in addition to natural climate variability observed over comparable time periods (art.1, UNFCCC 1992). According to the IPCC, there is no doubt that the world’s climate is changing (Baede *et al.* 2001). The instrumental record, i.e. direct measurements of environmental variables, shows various changes in temperature, precipitation, snow cover, sea level, land- and sea-ice extent and atmospheric and oceanic circulation patterns (see fig. 1).

Climate models, such as energy balance models (EBMs), (atmospheric) general circulation models (GCMs) and atmosphere-ocean general circulation models (AOGCMs) are used to assess impacts of various forcing agents on the climate and to distinguish any trends from the background noise (Mitchell *et al*. 2001). The direct climate forcing by variations in solar irradiance during the solar cycle is relatively well determined, but indirect effects, for example through ozone changes, are still poorly understood (Hansen *et al*. 1998, Harrison and Shine 1999). Estimates of volcanic forcing are still rather crude (Hansen *et al*. 1998). The climate change observed in the latter half of the twentieth century is unlikely to be caused by these natural forcings and background variability alone (Tett *et al*. 1996, 1999, 2000, Hegerl *et al*. 1997). Models including anthropogenic climate forcing, viz. greenhouse gases (GHGs) and (sulfate) aerosols, give the best approximation of the actual situation (Hegerl *et al*. 1997). GHGs are well measured and give a strong positive (warming) forcing by absorbing long-wave radiation emitted from the earth, thus capturing energy that would otherwise be radiated into space. This forcing is, at least partly, counteracted

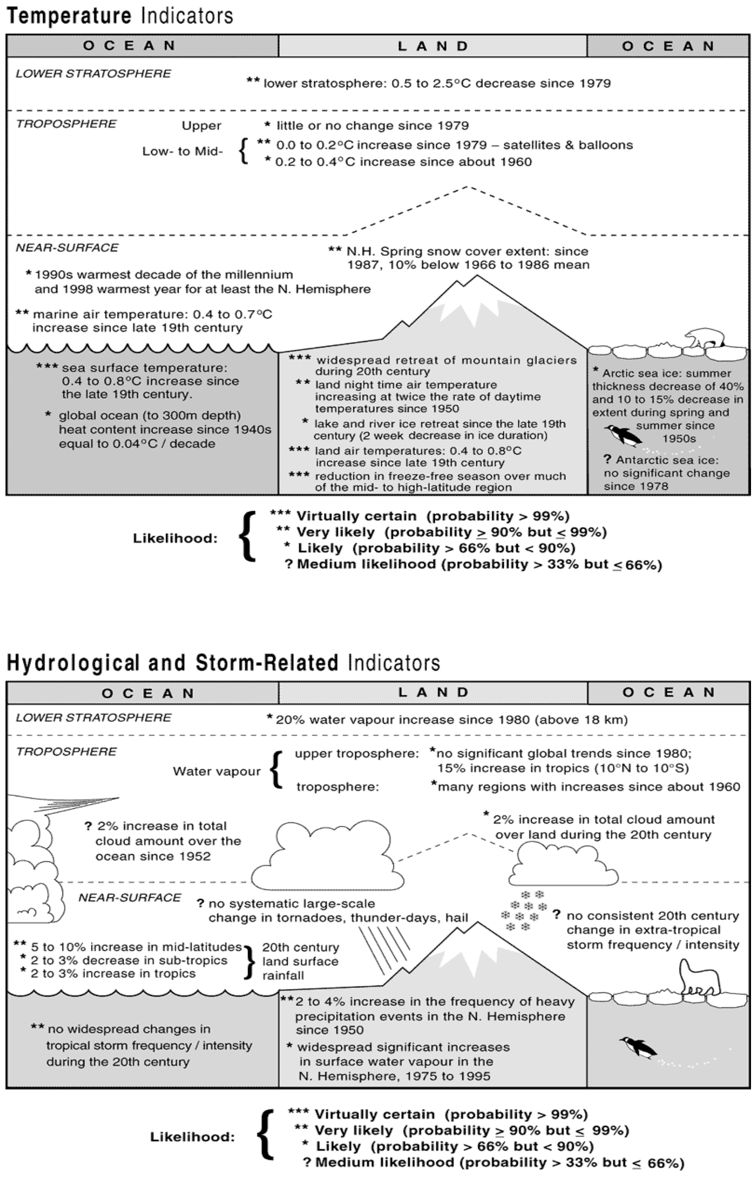

Fig. 1. Climate change: schematic of observed variations of the temperature, hydrological and storm-related indicators. From IPCC 2001.

by other, less well understood and measured, negative anthropogenic forcing agents, such as aerosols, anthropogenic clouds and land-use changes influencing albedo, evapotranspiration and surface roughness (Hansen *et al*. 1998).

GHGs are the best understood aspect of climate change, and among these carbon dioxide (CO_2) has received most attention. It was first recognised in 1896 as a possible driver of climate change by the Swedish physicist Arrhenius in the context of the then lively debate on probable causes of the Ice Age (Arrhenius 1896).
CO_2 is a rather weak climate forcing agent compared to other GHGs such as CH_4, N_2O and chlorofluorocarbons, but since its atmospheric concentration is much higher than that of the other GHGs, CO_2 is considered the most important, contributing about 60% of the total forcing brought about by anthropogenic changes in GHG concentrations (Ramaswamy *et al.* 2001). It should be noted, however, that the relation between CO_2 concentration and temperature is not as straightforward as this, since changes in CO_2 concentration may as well be brought about by climate change as be a cause of it (Fischer *et al.* 1999).

Atmospheric carbon dioxide and the global carbon cycle

Atmospheric carbon dioxide

The first continuous measurements of atmospheric carbon dioxide started in 1958 on Mauna Loa, Hawaii. The measurements show a steady increase in the mean annual concentration, superimposed on a clear seasonal pattern (fig.2). The CO_2 concentration in the middle layers of the troposphere has increased from 315.98 parts per million by volume (ppmv, equals µmol mol^{-1}) in 1959 to 370.9 ppmv in 2001, an increase of 17.4% (Keeling and Whorf 2002). Measurement series from many other places confirm this trend and also show that the seasonal variation increases further north (Conway *et al.* 1994, Thoning and Tans 2000, Keeling and Whorf 2001).

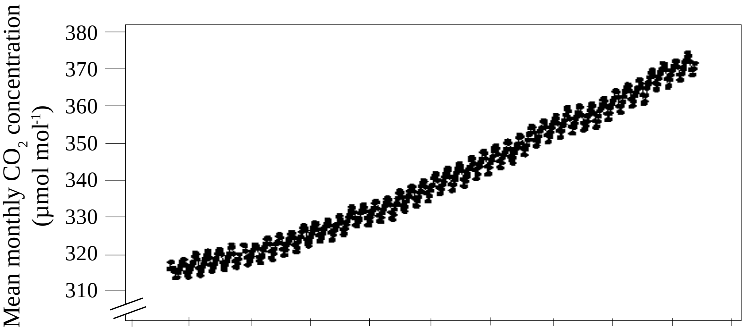

Fig. 2. Mean monthly concentrations of atmospheric CO2 measured at Mauna Loa, Hawaii, since 1958. Note that the y-axis is cut at 305 µmol mol^{-1}. From Keeling and Whorf 2002.

Analyses of air bubbles extracted from Antarctic ice cores, representing air samples from the year 1000 up to 1978, show that the start of the recent increase of atmospheric CO_2 was approximately concurrent with the beginning of industrialisation (ca 1800).

Before that, CO$_2$ levels had first been rather stable between 275 and 285 µmol mol^{-1} for several centuries, and then between the years 1550 and 1800 somewhat lower (Etheridge *et al*. 1996, 1998). Another ice core, dating back as far as 420 000 years, revealed a pattern of rapidly increasing CO$_2$ concentrations to about 280 - 300 µmol mol^{-1} at the end of glaciations, and slowly decreasing CO$_2$ concentrations towards ca 180 µmol mol^{-1} during glaciations (Barnola *et al*. 1999, Petit *et al*. 1999). (See fig. 3.)

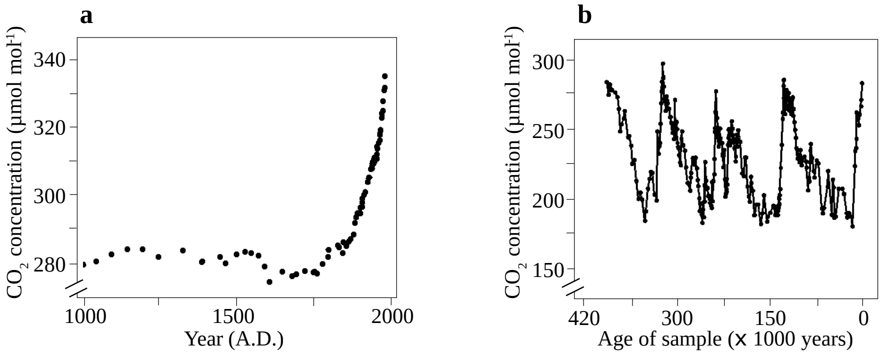

Fig. 3. Concentrations of carbon dioxide in air from ice cores as indication of previous atmospheric conditions. Note that the y-axes are cut. Modified after a) Etheridge *et al*. 1998, b) Barnola *et al*. 1999.

The recent increase in CO$_2$ levels is mainly due to CO$_2$ emissions from fossil fuel burning and land use changes. From 1750 to 1998, fossil fuel combustion and cement production caused about 270 Gt (gigaton; 1 Gt = 1 Pg) of carbon to be released to the atmosphere, half of which has occurred since the mid-1970s (Marland *et al*. 2001). Calculations show that land-use changes between 1850 and 1990 contributed another 124 Gt of carbon, 87% of which was due to deforestation, mainly in the tropics (Houghton 1999). The release of CO$_2$ to the atmosphere has increased dramatically during the second half of the 20th century (fig. 4).

Of the carbon thus released between 1850 and 1998, 176 Gt (i.e. 43%) accumulated in the atmosphere, and the remainder was taken up by oceanic and terrestrial (the so-called missing sink) sinks in approximately equal amounts (Bolin *et al*. 2000 and references therein).

Carbon pools and annual fluxes

The total carbon content of the atmosphere (the atmospheric carbon pool) is estimated to be about 750 Gt, which is the same order of magnitude as the carbon contained in all terrestrial vegetation (about 610 Gt). Soils and detritus contain another 1580 Gt, which gives a total for the terrestrial pool of approximately 2190 Gt. The oceanic carbon pool is by far the largest at almost 40 000 Gt, of which 3 Gt is contained in marine biota,1020 Gt in the surface ocean, 38 100 Gt in the intermediate and deep ocean, 150 Gt in surface sediments, and up to 700 Gt is dissolved organic carbon. (Schimel *et al*. 1996.) The bulk of the carbon on earth, over 75 Pt (75 million Gt) is found in the lithosphere (Falkowski *et al*. 2000), inactive in terms of the global carbon cycle. Fossil fuels, finally, contain about 4130 Gt of carbon (Falkowski *et al*. 2000).

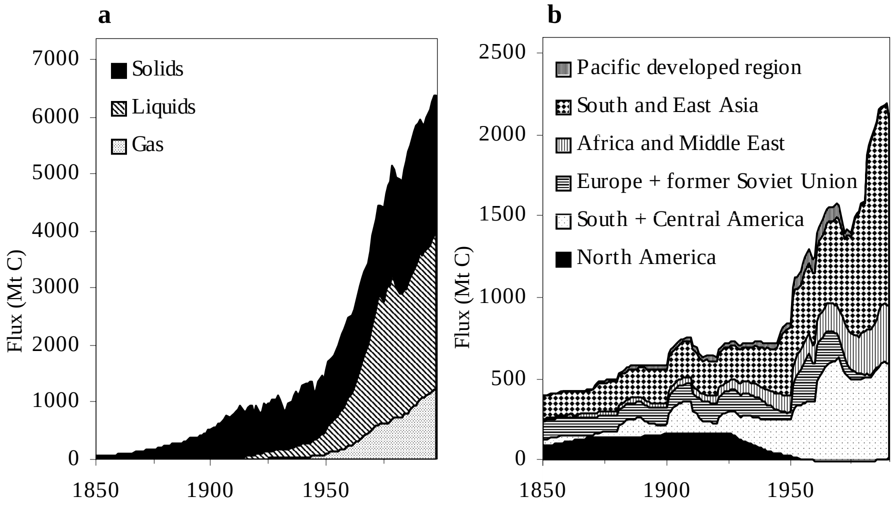

Fig. 4. Annual carbon fluxes to the atmosphere a) as a result of fossil fuel consumption, and b) as a result of land use changes. Note different scales on y-axes. Data from a) Marland *et al.* 2001, b) Houghton 1999.

The main natural fluxes between compartments are the primary production/respiration flux between atmosphere and terrestrial biosphere (120 Gt per year) and the physical exchange of carbon between atmosphere and the oceans (90 Gt per year). These fluxes are approximately in balance on a yearly basis. Other natural fluxes, such as those due to sedimentation, volcanism and weathering, have much less impact on the atmospheric carbon pool and are at least an order of magnitude smaller than the current anthropogenic perturbation (Prentice *et al.* 2001).

Between 1989 and 1998, human-induced fluxes of carbon to the atmosphere averaged 7.9 Gt per year, of which 1.6 Gt was due to land-use change. Of this carbon, 3.3 Gt per year accumulated in the atmosphere and 2.3 Gt was taken up by the ocean. The residual 2.3 Gt per year were stored in terrestrial ecosystems (the “missing carbon sink”) (Bolin *et al.* 2000). Terrestrial ecosystems are thus of major importance in global change research and have become of political interest also after the acceptance of the Kyoto Protocol (UNFCCC 1997), in which countries are encouraged to develop their carbon sinks to offset their CO_2 emissions and thus meet their targets. This means that, according to the Protocol, carbon sequestered through afforestation and reforestation (the land use, land-use change and forestry, or LULUCF, sector, Noble *et al.* 2000) in a country since 1990 can be accounted in the carbon budget of that country. Recently the Marrakesh Accords (UNFCCC 2001) extended the LULUCF sector with four additional activities that may be accounted, viz. forest management, cropland management, grazing land management and revegetation. This (initial) focus on forests is understandable, given the amount of carbon per area stored in the vegetation. However, table 1 shows that other ecosystems can be considerable sinks as well if soil carbon is included in the analysis (WBGU 1998).

Climate change and the global carbon cycle

Global warming will affect the carbon cycle in various ways, through both physical and biological processes. CO_2 uptake by the oceans will be reduced because of a

Table 1. *Carbon pools in vegetation (above- and below-ground living and dead mass) and soils (O-horizon, mineral soil to 1 m depth, and colocated peatlands) in different ecosystems and climate regions. Modified after WBGU 1998 and Dixon* et al. *1994.*

	C pool (Gt)			**Area**	**C density (t ha^{-1})**		
	Vegetation	Soil	Total	**(10^6 ha)**	Vegetation	Soil	Total
High-latitude forests	88	471	559	1372	64	343	407
Russia	*74*	*249*	*323*	*884*	*83*	*281*	*365*
Canada	*12*	*211*	*223*	*436*	*28*	*484*	*511*
Alaska	*2*	*11*	*13*	*52*	*39*	*212*	*250*
Mid-latitude forests (incl. Scandinavia)	59	100	159	1038	57	96	153
USA (ex Alaska)	*15*	*26*	*41*	*241*	*62*	*108*	*170*
Europe	*9*	*25*	*34*	*283*	*32*	*90*	*120*
China	*17*	*16*	*33*	*118*	*144*	*136*	*280*
Australia	*18*	*33*	*51*	*396*	*45*	*83*	*129*
Low-latitude forests	212	216	428	1755	121	123	244
Asia (ex Russia/China)	*41*	*43*	*84*	*310*	*132*	*139*	*271*
Africa	*52*	*63*	*115*	*527*	*99*	*120*	*218*
South and Central America	*119*	*110*	*229*	*918*	*130*	*120*	*249*
Grasslands	75	559	634	3500	21	160	181
Tropical savannas	*66*	*264*	*330*	*2250*	*29*	*117*	*146*
Temperate grasslands	*9*	*295*	*304*	*1250*	*7*	*236*	*243*
Croplands	3	128	131	1600	2	80	82
Wetlands	15	225	240	350	43	543	686
Tundras, alpine grasslands	6	121	127	950	6	128	134
(Semi) deserts	8	191	199	4550	2	42	44

lower solubility of CO_2 at higher temperatures, and increased vertical stratification in the oceans will reduce the amount of exchange between surface and deep water, thereby reducing oceanic uptake even further, but also reducing release of oceanic CO_2 to the atmosphere. Warming and altered rainfall patterns may lead to a higher primary production and thus higher carbon sequestration in terrestrial ecosystems, yet on the other hand it may lead to higher respiration and release of carbon that is now sequestered in soil organic matter (Prentice *et al.* 2001). There is still much uncertainty about the forcings and feedbacks involved, and most of it falls well beyond the scope of this thesis.

Direct effects of elevated CO_2 on plants

Involved mechanisms

The direct effects on plants of a rise in atmospheric CO_2 have not received nearly as much media and public attention as the role of CO_2 as a greenhouse gas (Körner 2000). Yet (practically) all life on earth is based on the carbon plants sequester from atmospheric CO_2, and the 17% increase in this resource during the 40 years of measuring at Mauna Loa (and the over 30% increase since pre-industrial time, or the probable doubling compared to pre-industrial levels by the end of this century (Prentice *et al.* 2001)) may very well have far-reaching consequences that are as important as a possible climate change.

The key process to understand when studying plant (and therefore ecosystem) responses to elevated CO_2 is photosynthesis. In photosynthesis, plants use (sun) light energy to combine water and atmospheric CO_2 into carbohydrates ($C_nH_{2n}O_n$), producing oxygen (O_2) as a by-product. The net reaction is the same for all plants, but three different photosynthetic pathways exist. The vast majority of plants use the so-called C-3 pathway, in which CO_2 initially is fixed into 3-phosphoglyceric acid (3-PGA). A small fraction of plants, mostly but not exclusively monocots, initially fix CO_2 into a 4-carbon acid; their photosynthetic pathway is consequentially known as the C-4 pathway. This 4-carbon acid is then transferred from the mesophyll cell into a bundle sheath cell, where the remaining photosynthetic processes take place. Although this pathway requires extra energy, it is more efficient than the C-3 pathway in fixing CO_2 at high temperatures, because the spatial separation between the initial CO_2 fixing and the main photosynthetic processes inhibits photorespiration (see below). The third photosynthetic pathway, first found in some *Crassulaceae* and therefore called crassulacean acid metabolism (CAM), is basically a variant of the C-4 pathway, often found in succulents, in which CO_2 fixing and the remaining photosynthetic processes are separated in time rather than in space, with fixing taking place at night. The C-4 and CAM photosynthetic pathways are especially effective at high temperatures and in drought situations. Not surprisingly, all species from the cool-temperate Swedish semi-natural grassland studied in this thesis are C-3 plants, with the exception of the succulent *Sedum acre*, that uses the CAM pathway at least occasionally when water-stressed (Kluge 1977, Schuber and Kluge 1981).

The first effect of elevated carbon dioxide takes place before the actual photosynthesis by affecting stomatal aperture. Evolution has favoured terrestrial plants with leaves having an epidermis that is relatively impermeable but contains pores, the so-called stomata, through which gas exchange can take place. This enables them to take up CO_2 yet prevents unnecessary water loss through leaves (Ludlow 1982). The opening of stomata is regulated by osmotic pressure in the so-called guard cells that border them (Von Mohl 1856). Stomatal aperture is affected by a number of environmental factors, such as water status of the plant, relative humidity, wind speed and light, as was discovered already by Francis Darwin (1898). However, a CO_2 deficiency in the leaf and in the stoma sometimes appears to affect its opening even stronger, causing the stoma to open even at low humidity (Kappen *et al*. 1994). Therefore it is widely accepted that elevated CO_2 reduces plant transpiration (Körner 2000). Consequently, elevated CO_2 is generally found to lead to an increase in water use efficiency (WUE) (Eamus 1991, Jackson *et al*. 1994), for which often the instantaneous transpiration efficiency, which is the ratio of the rate of assimilation to the rate of transpiration, is used as an approximation (Eamus 1991). Some studies have shown a positive effect of elevated CO_2 on plant growth during years with water stress only (for example Owensby *et al*. 1999), and others have found higher soil moisture under elevated CO_2 (Niklaus *et al*. 1998). However, there are many processes involved, many of which are species-specific, and the resulting picture, especially at a community level, is far from clear yet (Jones and Jongen 1996, Körner 2000).

Another effect of elevated CO_2 on photosynthesis is its suppression of photorespiration. Photorespiration occurs because the enzyme ribulose bisphosphate carboxylase (rubisco) can catalyse two different reactions. It was first discovered to catalyse the fixation of CO_2 by combining it with ribulose-1,5-bisphosphate to form two molecules of 3-PGA, thus playing a crucial role in photosynthesis. However, rubisco can also catalyse the oxidation of ribulose-1,5-bisphosphate, that is, the reaction in which it is combined with O_2 to form phosphoglycolate and one molecule of 3-PGA. The phosphoglycolate can in its turn be converted into serine, whereupon CO_2 is released. Thus photorespiration hampers net photosynthesis in two ways: first by having CO_2 compete with O_2 for the rubisco available, and second by the release of CO_2 from phosphoglycolate. Rubisco has a much higher affinity for CO_2 than for O_2, but since the atmospheric O_2 concentration is more than 500 times higher than the CO_2 concentration, a considerable part of the reactions catalysed are oxidations. This holds especially at higher temperatures, when the ratio of dissolved O_2 to CO_2 in the chloroplasts is higher. Therefore the C-4 pathway, where rubisco is present only in the bundle sheath cells with their relatively high CO_2 concentration, is efficient in warmer climates. Elevated atmospheric CO_2 decreases the ratio of O_2 to CO_2 in the chloroplasts, thereby decreasing photorespiration in C-3 plants (Amthor 1994).

Plants eventually show an acclimation of photosynthesis to elevated CO_2 in most studies (Gunderson and Wullschleger 1994, Drake *et al*. 1996). This is partly because the plant simply cannot handle all the carbon provided (the sink strength is limiting photosynthesis), partly because the plant produces relatively less rubisco, or rubisco activity is lower (Gunderson and Wullschleger 1994, Drake *et al*. 1996, Rogers *et al*. 1998). The mechanisms behind photosynthesis acclimation are not fully understood. They may involve changes in leaf morphology (Gunderson and Wullschleger 1994, Bowes *et al*. 1996), inhibition of rubisco (Bowes *et al*. 1996, Rey and Jarvis 1998), a build-up of structural and non-structural carbohydrates in the leaf (Conroy and Hocking 1993, Ceulemans and Mousseau 1994, Rey and Jarvis 1998, Ghildiyal *et al*. 2001, Vu *et al*. 2002) that may suppress the expression of several photosynthetic genes (Drake *et al*. 1996), and decreased stomatal density (Gunderson and Wullschleger 1994, Drake *et al*. 1996). Leaf age and position (Osborne *et al*. 1998) and time of year (Bunce 2001) may also play a role. For photosynthesis at the canopy rather than leaf level, still other mechanisms might be involved, such as a reduction in canopy leaf area. However, this does not seem to be the case (Drake *et al*. 1996). Acclimation of photosynthesis is not found in all studies (Drake *et al*. 1996) and may be species-specific (Ceulemans *et al*. 1997). Furthermore, studies that used small pot sizes generally show more acclimation than those with larger pots (Drake *et al*. 1996), probably due to water stress, physical root constrains or N-limitation. The importance of controlled N-supply when studying acclimation was stressed by Pettersson and McDonald (1994), as acclimation may otherwise be an artefact of more rapidly increasing N-stress under elevated CO_2 (see also Ceulemans and Mousseau 1994). The question of acclimation is not answered yet, and the answer may well have a large impact on plants as a sink of CO_2 and thus on the future global carbon cycle (Schulze and Caldwell 1994).

Single-plant studies

Körner (2000) shows how the main focus of biological elevated-CO_2 research initially was on single plant studies. These studies were often carried out on plants grown in pots under laboratory conditions (see for example Hunt *et al*. 1991, 1993, Pettersson & McDonald 1992). Poorter (1993), in his review of studies on 156 plant species, reports an average growth stimulation (measured as harvested biomass at elevated CO_2 in relation to that at ambient CO_2) of 37%. However, there are large intra- and interspecific differences and the growth stimulation caused by elevated CO_2 ranges from -37% for the C-4 grass *Andropogon glomeratus* to +313% for the C-3 tree *Banksia serrata* and even +395% for one of the studies on the legume *Glycine max* (which had an average stimulation of 71%). Also Hunt *et al.* (1991, 1993 - note that the 1991 paper is included in Poorter's review) show large interspecific differences in biomass stimulation by elevated CO_2 in British herbaceous species, ranging from 0 to 82%. They come to the conclusion that the higher growth stimulation is found in competitive species (as defined by Grime 1974). Poorter (1993) finds that C-3 plants are more stimulated (41%) than C-4 plants (22%) and the few CAM plants studied were stimulated by only 15%. Further, within the C3-group, nitrogen fixing plants were more stimulated than others (50 and 41%, respectively), crops more than wild herbs (58 and 35%, respectively) and fast growing wild species more than intermediate or slow growing ones (54, 38 and 23%, respectively).

Single-plant are badly needed in the understanding of the mechanisms behind plant responses to elevated carbon dioxide, and they offer a reference when studying plant responses under natural conditions. However, they do not give all information needed to understand what will actually happen with plants, be it in managed or natural systems, as the atmospheric carbon dioxide concentration continues to increase. Predictions of future vegetation responses will, according to Körner (2000) benefit if experiment limitations of time, space and complexity are overcome. Where the first two of these are financially determined and hence unfortunately often outside the individual scientist's influence, an appropriate level of complexity, that is, including species interactions, can and should be allowed if the aim is to understand ecosystems. Both Körner (2000) and Jones *et al.* (2000) therefore strongly advocate in situ field experiments or, if that is not possible, studies of complex model communities.

Experiments including plant interactions, especially grassland communities

Experiments in which the plants are not grown in isolation differ greatly in complexity and degree of resemblance to real-world systems. On the one extreme are artificial communities consisting of different numbers of species (including single-species communities) that are used when plant diversity, number of functional groups or species richness is one of the experimental variables rather than an outcome of a natural process (see for example Craine *et al.* 2001, Niklaus *et al.* 2001, Reich *et al.* 2001a) or when differences between species grown in monocultures are of interest (Reich *et al.* 2001b). The other extreme are situations in which the vegetation is naturally used to high levels of CO_2 because of growing near a natural CO_2 spring (Koch 1993, Miglietta *et al.* 1993, Cook *et al.* 1998, Tognetti *et al.* 2000). In between, in order of increasing resemblance to reality, are controlled artificial communities composed to resemble natural ones (Gifford and Rawson 1993, Körner *et al.* 1993, Díaz *et al.* 1998, Jones *et al.* 1998, Volk *et al.* 2000), transplanted natural vegetation (monoliths), for example grasslands, turfs and bogs in climate facilities (Arnone and Bohlen 1998, Heijmans *et al.* 2001), controlled climate facilities built in situ (Vourlitis and Oechel 1993, Jones *et al.* 1993, Oechel *et al.* 1994), open-top chambers (OTCs) enclosing existing vegetation (for descriptions see Rogers *et al.* 1983, Vourlitis and Oechel 1993, Jones *et al.* 1993, Jäger and Weigel 1993; see also citations below and chapter 2 in this thesis and the included papers) and free air CO_2 enrichment (FACE) installations (for descriptions see Hendrey *et al.* 1993, Wall and Kimball 1993; see also citations below). For this thesis, OTCs and FACE are the most important experimental setups.

Open-top chambers and free air CO_2 enrichment both have advantages and disadvantages of conceptional and practical nature, although many find FACE to be the best technology (McLeod 1993, Schulze *et al.* 1999). OTCs interfere with the microclimate to a much higher extent than FACE. This means that temperature inside an OTC can be several degrees higher than outside, wind speed can be lower or (because of fans blowing in CO_2) higher, and hence humidity can be lower or higher. OTCs also tend to be rather small compared to a FACE setup. Moreover, the chamber wall hinders the free exchange between OTC and surroundings of for example herbivores, pollinators and seeds. This may, however, be an advantage as well as a disadvantage. Further, compared to FACE, OTCs are very cheap in construction and maintenance. FACE has a much lower impact on the microclimate, but it is not absent and should not be overlooked. FACE installations are usually rather large, which enables studies of larger-scale ecosystems such as forests, however it makes replication more difficult, especially in small-scale landscapes. The high initial and maintenance costs of FACE make an experiment with proper replications very expensive.

Mooney *et al.* (1999) summarise 14 long-term (i.e. over one year) studies on elevated CO_2 in herbaceous systems (five studies used OTCs, one FACE) and 12 on forest systems (seven OTC, two FACE). Herbaceous studies were carried out on tundra(Alaska, USA), wetland (Maryland, USA), tallgrass prairie (Kansas, USA), cultivated pastures (New South Wales, Australia; New Zealand; France; Switzerland; and Quebec, Canada) and (semi-)natural grasslands (Switzerland, UK, Italy and California, USA). The sites vary greatly in mean temperature, precipitation, history and species composition. I will come back to these studies in more detail in the Discussion chapter of this thesis; here it suffices to say that elevated CO_2 caused above-ground biomass to increase by an average of 14%, but the differences between systems and years were large, with responses ranging from decreases to +85%.

Why study grasslands?

Grassland vegetation, especially in temperate regions, contains only little carbon when compared to forests (see table 1). Yet grasslands have become popular ecosystems in elevated-CO_2 research (Mooney *et al.* 1999). The rationale for this is a practical one: in grasslands, ecosystem processes can be studied at a small spatial scale. Relatively small plots can be representative and contain many different species. Moreover, species composition of a grassland can show large non-directional variation between years (Herben *et al.* 1993, Van der Maarel and Sykes 1993), giving a possibility for the vegetation to change comparatively fast when influenced by external factors. An experimental study of the effects of elevated CO_2 on for example vegetation dynamics of an old-growth deciduous temperate forest would be a practical impossibility.

But not only for practical reasons is the study of grasslands of interest. From a carbon cycle point of view, grassland soils are of great importance, containing many times more carbon than the vegetation itself (see table 1). In combination with the economic importance of grasslands (Nagy *et al.* 1997) and the considerable proportion of land area they cover (about one fifth of the total land area of the world (Hadley 1993), about half of which are temperate grasslands (Sala 2001)) this makes grassland responses to elevated CO_2 of major import for future global change developments.

A third reason for studying grasslands concerns biodiversity. Although grasslands are not as rich in species as for example tropical rainforests on either a global or a per area base (Mooney *et al*. 1995), in places they may contain a large part of the local biodiversity. In Sweden, more than two thirds of the total number of threatened vascular plant species can be found in the agricultural landscape, with grasslands having the greatest diversity (Naturvårdsverket 1993, Gärdenfors 2000). Therefore grassland responses to elevated CO_2 are of interest for nature conservation.

Aims and scope of the Nåntuna CO_2 project and this thesis

The Nåntuna CO_2 project (see next chapter) has its origin in the scarceness of long-term studies in natural or semi-natural ecosystems (Körner 2000) by the mid-1990s, and the differences in responses between the systems that had been studied (for example Owensby *et al*. 1993, Oechel *et al*. 1994). Given the data that were available at the time, it was hard, impossible even, to predict the future of ecosystems in Sweden under elevated CO_2. Swedish forests were already being studied (see for example Roberntz 1998), but there were at least two reasons to also study other ecosystems, in this case a semi-natural grassland: the carbon source/sink behaviour of these systems, and the effects on the biodiversity they contain. Therefore, the Nåntuna CO_2 project had two major objectives:

- to study the effects of elevated CO_2 on the carbon cycle in a semi-natural grassland, and
- to study the effects of elevated CO_2 on vegetation composition and plant biodiversity in a semi-natural grassland.

In this thesis, I concentrate on the latter objective and on the production part (especially above-ground production) of the former. My colleague Erik Sindhøj has described below-ground production, decomposition and carbon balances in more detail in his thesis (Sindhøj 2001) and the papers therein.

Hence, the questions I will ask (and hopefully answer) in this thesis, are the following:

- will elevated CO_2 stimulate leaf-level photosynthesis under field conditions?
- if so, will this result in higher standing biomass?
- will the effect of elevated CO_2 decrease over the years?
- will elevated CO_2 be more beneficial for fast-growing and leguminous species?
- if so, will this result in vegetation changes?

Material and methods

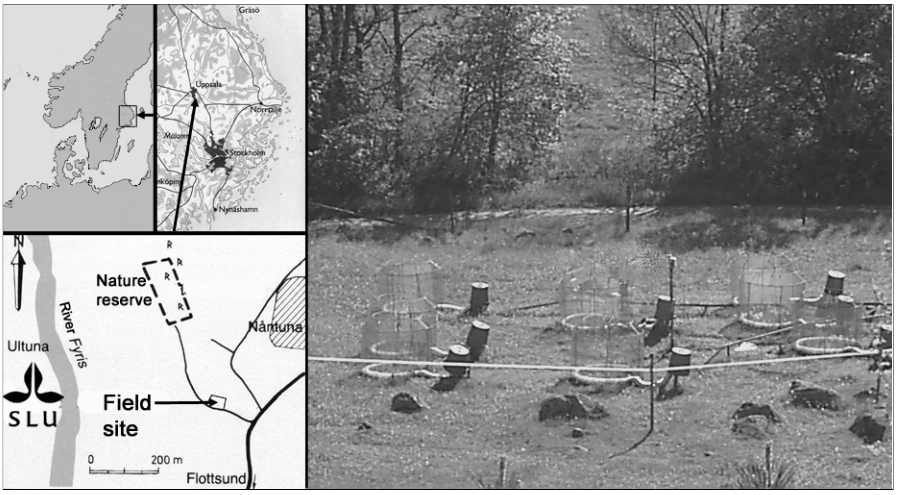

Photo: Erik Sindhøj

Fig. 5. The Nåntuna field site and its location.

The Nåntuna field site

The field experiment was conducted at Nåntuna, about 5 km south of Uppsala in Central Sweden, 59°48'N, 17°38'E, 10 m a.s.l. on a south western facing mild (less than 5 °) slope off the river Fyris opposite the Ultuna university campus (fig. 5). The mean annual temperature is 5.5 °C and the mean annual precipitation 527 mm (average values for 1961–1990; see table 2).

The soil was classified as a eutric cambisol and the upper 30 cm was a loam consisting of about 22 % clay, 47 % silt, 18 % sand and 13 % gravel. The organic matter content of the upper 30 cm was about 7.5 % and the mineral nitrogen content (NH_4^+ and NO_3^-) fell between 1.2 and 2.0 g m^{-2}, when soil bulk density of 1.3 g cm^{-3} and 20 % stoniness are assumed.

Historical maps show that this site has been grazed since at least 1720, but probably much longer. The first trace signs of settlement in the district is an Iron Age grave field. During this period (around 2000 years ago) the climate got colder and wetter and a more permanent agriculture gradually replaced burn-beating (slash and burn). Therefore it is probable that hay-making, grazing and cultivation have been practised at the site for at least 2000 years. Near the experimental plot, traces of small ancient patches of cultivated land have been found, probably dating back to the Middle Ages. On maps from 1760 and 1863, the experimental plot is denoted as a pasture situated alongside a narrow path very near the farmyards in the old hamlet Nåntuna. During the summer, the main stock (cattle herd) grazed the forest 1 km south of the hamlet while pastures near the farmyards were grazed by the livestock needed daily, such as horses and probably cows.

Table 2. *Monthly precipitation (Ppt), in mm, and average temperature at 1.5 m height (Temp) in ºC. Normal denotes the 30-year average for 1961-1990. Data from Ultuna weather station, about 1 km from the experimental site.*

	1995		**1996**		**1997**		**1998**	
Month	Ppt	Temp	Ppt	Temp	Ppt	Temp	Ppt	Temp
Jan	42.4	*-3.4*	7.3	*-5.1*	11.3	*-2.7*	66.9	*-0.6*
Feb	41.6	*0.2*	18.8	*-8.8*	42.6	*-0.7*	28.3	*0.6*
Mar	42.9	*1.3*	25.8	*-1.9*	16.4	*1.3*	28.6	*-1.1*
Apr	97.9	*3.3*	14.0	*7.9*	21.9	*3.5*	34.3	*3.7*
May	39.8	*8.8*	57.7	*7.9*	57.6	*8.5*	47.8	*9.9*
Jun	47.4	*15.4*	44.2	*14.4*	95.1	*15.6*	98.3	*12.7*
Jul	33.8	*16.9*	61.5	*15.1*	38.3	*18.1*	83.9	*15.6*
Aug	10.9	*16.7*	29.3	*17.8*	58.9	*19.5*	54.8	*13.7*
Sep	79.8	*11.1*	14.1	*9.1*	58.5	*12.0*	43.4	*11.7*
Oct	26.0	*8.6*	23.3	*7.7*	52.3	*4.1*	58.5	*5.2*
Nov	27.6	*-1.6*	110.8	*2.2*	54.3	*1.0*	22.7	*-1.5*
Dec	11.9	*-8.6*	24.7	*-4.1*	43.4	*-1.0*	59.3	*-0.9*

	1999		**2000**		**Normal**	
Month	Ppt	Temp	Ppt	Temp	Ppt	Temp
Jan	56.8	*-3.0*	23.3	*-1.4*	33.8	*-4.4*
Feb	28.2	*-2.8*	11.4	*-0.9*	24.6	*-4.6*
Mar	43.5	*0.9*	18.9	*1.2*	25.5	*-1.1*
Apr	82.9	*6.5*	22.7	*6.1*	29.3	*3.9*
May	15.4	*9.3*	39.7	*11.8*	32.8	*10.2*
Jun	32.6	*16.3*	45.7	*13.8*	45.9	*15.0*
Jul	11.9	*18.9*	153.3	*15.6*	70.5	*16.3*
Aug	32.8	*15.1*	Experiment		66.4	*15.1*
Sep	68.4	*14.5*	finished		57.0	*10.8*
Oct	25.6	*7.1*			49.5	*6.4*
Nov	9.2	*3.9*			50.6	*1.2*
Dec	100.4	*-2.9*			41.1	*-2.8*

When the experiment started, the experimental plot was enclosed and grazing was replaced with a cutting regime with one cutting per year (see below).

There are about 30 to 40 species of vascular plants per square metre and in total I have found about 50 species in the plots (see appendix to paper III). When not restricted by grazing, grasses typically constitute 30–50 % of the above-ground dry biomass at the end of the growing season. *Agrostis capillaris*, *Poa pratensis* and *Festuca rubra* dominate among grasses, and the most common forbs are *Achillea millefolium*, *Carum carvi*, *Stellaria graminea*, *Plantago media* and *Taraxacum* sect. *Ruderalia* (hereafter referred to as *Taraxacum*). *Trifolium repens* and *Lotus corniculatus* are the prevailing legumes; *Trifolium media* and *T. pratense* also occur. The only annuals found are *Myosotis stricta*, *Erophila verna* and *Veronica verna* and these occurred in small numbers. Several of the species present are indicators of unfertilised grasslands that have been in cultivation for an extended period (Larsson and Ekstam, 1987). See appendix A for a species list. (Nomenclature according to Krok and Almquist 2001)

Experimental setup

OTCs and monitoring of climatic variables

The experiment started in spring 1995 when eight open-top chambers made of polypropylene (height 1 m, diameter 1.5 m) on a steel framework and four chamberless control rings (height 5 cm, diameter 1.5 m) were placed in the field. Four replicate blocks were placed along the slope of the field and three treatments (Elevated, Ambient and Control) were assigned in a randomised block design. From April to October each year, four of the chambers (Elevated) continuously received CO_2-enriched air (700 ± 100 µmol mol^{-1}). The other chambers received ambient air (350 ± 20 µmol mol^{-1}; Ambient). The air was blown in by fans through a plastic drainage pipe with a diameter of 15 cm, laid on the ground inside the chamber along the wall (fig. 6). The four rings constituted the control treatment (Control).

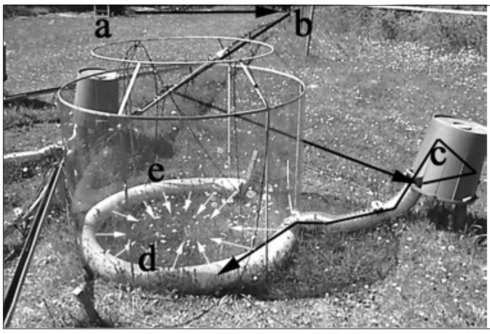

Photo: Mark Marissink

The CO_2 supply consisted of commercially available tubes (a), from which pure CO_2 was supplied at a rate adjusted by a separate needle valve (in shed above b) for each Elevated chamber. It was mixed with outside air blown in by a fan (c) and led into the chamber by a drainage pipe (d). Every second hour an air sample was taken (e) for automatic infrared CO_2 analysis. Steps c-e apply to Ambient chambers as well.

Fig. 6. Air and CO_2 supply in an open-top chamber.

The carbon dioxide level in each chamber, as well as outside the chambers, was monitored every second hour by an infrared gas analyser (SBA-1 OEM, PP Systems, Hitchin, Herts, England). Photosynthetically active radiation (PAR) inside one chamber and outside was measured with a Li-190 SB quantum sensor (Li-Cor Inc., Lincoln, NE, USA); the results indicating a reduction in PAR of about 10 % inside the chamber. Air temperature was measured continuously inside and outside the chambers and showed a mean temperature increase in the chambers of about 2 ºC. Soil temperature, measured at 10 cm depth, was increased by 0 to 1 ºC. All measurements were stored in a data logger (CR10, Campbell Inc., Logan, Utah, USA).

From 1997 to 1999, soil moisture was measured using a Time Domain Reflectometry (TDR) method with a Tektronix 1502B cable tester connected to a PC (for details, see Sindhøj, 2001). Additional weather data come from the nearby weather station at Ultuna, about 1 km from the experimental field. Table 2 and figure 7 show weather data from 1995 to 2000 from the Ultuna station, as well as average values for the period 1961–1990.

Field management

When the experimental field was enclosed at the beginning of the experiment, horse grazing ceased to occur. Instead a cutting regime was established. The vegetation was

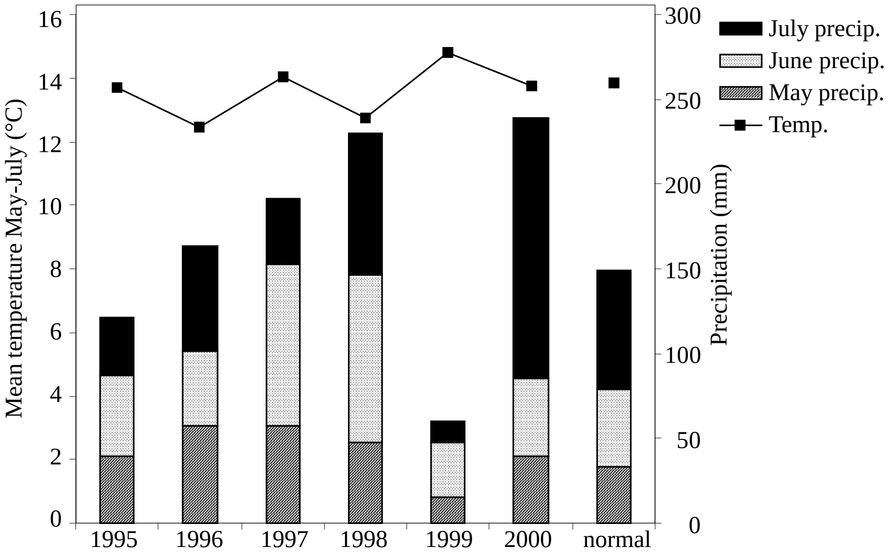

Fig. 7. Mean temperature and precipitation for three months preceding the harvest, and long-term average values (1961-1990). Data from Ultuna weather station, about 1 km from the field site.

cut once a year from 1995 to 2000, during the first days of August. The cutting was at ground level, and also rosette leaves such as those of *Plantago media* were harvested. The rationale behind this rather severe cutting regime was the following: in order to be able to calculate correct values of production and carbon sequestration, as well as root/shoot ratios, the entire above-ground production of a given area had to be known. This area would be a considerable part of each plot, so space limitations did not allow a difference between harvesting for measurement purposes (see below) and field management harvesting.

Of course this management shift has consequences of its own. However, continued grazing was not possible, nor was a more realistic grazing-mimicking regime. A more common approach would have been to cut the vegetation about 5 cm above the soil surface. However, apart from the difficulty this would have meant to assess total plant production, this might well have impacted on the vegetation even more, given the severe horse-grazing regime it would replace.

The experimental field was not fertilised, nor was it irrigated before or during the experiment.

Non-destructive above-ground production measurements

I measured leaf-level photosynthesis on mature leaves of *Taraxacum* and *Plantago media* (not in 2000) (2 – 3 leaves per species and plot), the only species present in each plot with a leaf size on which measuring was practicable, using ADC LCA2 (1996 and 1997) and LCA4 (1997 – 1999) (ADC, Hoddesdon, Herts, England) and CIRAS-1 (2000) (PP Systems, Hitchin, Herts, England) photosynthesis measuring devices. Measurements were carried out at saturating natural light levels only (insolation more than 700 µmol $m^{-2}s^{-1}$, typically between 1000 and 1500 µmol $m^{-2}s^{-1}$, sunny days). I measured photosynthesis on five occasions in 1996, seven in 1997, five in 1998, five in 1999, and three in 2000.

I conducted separate measurements of stomatal conductance of *Taraxacum* and *P. media* from 1996 using a Delta-T Porometer AP4 (Delta-T Devices Ltd, Burwell, Cambridge, England) which enabled us to use smaller-leaved species than was possible with the photosynthesis measuring equipment. Therefore, from 1997 I included *Trifolium repens* as well. In 1996 stomatal conductance was measured on five occasions, in 1997 on seven occasions, in 1998 only twice, due to unfavourable weather conditions, in 1999 also only twice, and three times in 2000.

Destructive above-ground production measurements

Each year the experiment was harvested in the first week of August by cutting the vegetation at the soil surface. During the first two years, the harvested central 0.25 m^2 was then divided into four groups, namely *P. media*, *Taraxacum*, other forbs, and grasses. To be able to identify effects specific to legumes, seven groups were used from 1997: *Trifolium repens*, other *Trifolium*, *Lotus corniculatus*, *P. media*, *Taraxacum*, other forbs, and grasses*. The material was then dried at 70 °C for at least 72 hours. After drying all samples were weighed and then ground. Nitrogen concentration was determined for each sample in an Elemental Analyzer NA 1500 (Carlo Erba Strumentazione, Strada Rivoltana 20090 Rodano, Italy). In 1996 a more comprehensive analysis was carried out, including N, P, K, Ca, Mg, Mn, S and Na.

During 1998 I took small (0.01 m^2) random harvest samples on June 2, June 23 and July 14, outside the central 0.25 m^2 of each plot in order to study changes during the growing season.

Outside the central 0.25 m^2, but in the plots and before cutting the vegetation, leaves were collected from *P. media* (1995-1998) and *Taraxacum* (1996-1998) for determination of specific leaf area (SLA). For each plot, a total leaf area of about 200 cm^2 of each species was collected. I determined leaf area using a Li-Cor Model 3100 area meter (LI-COR Environmental Division, Lincoln, NE, USA), then dried the leaves at 70 °C for at least 72 hours and determined leaf biomass.

At harvest time I collected, from each plot, seeds from *P. media* (1995, 1996), *L. corniculatus* (1996, 1997), *Ranunculus bulbosus* (1995-1998) and *Carum carvi* (1995-1997). I later counted these seeds and weighed the samples.

Vegetation composition

Vegetation composition in the central 50×50 cm of each plot was assessed twice a year, in April/May and late July. The first assessment was made in April, 1995, the last just before the final harvest in late July, 2000. I used a point-intercept method (Levy & Madden 1933, Goodall 1952), using a 4 mm thick pin and a 9×9 grid. All

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* To be exact, during the first three years 2×0.25 m^2 of the harvested material was used for further analysis. Half of this was divided into groups as described, whereas the other half was dried and weighed directly. There were only very small differences between these two samples per plot, and I will not use the non-divided samples in this thesis. However, they were taken into account in Paper I, which explains some slight differences between values in this paper and in the thesis.

species touching the pin were recorded for each maze in the grid. Thus, for each plot a species has an occurrence between 0 and 81.

I also analysed vegetation composition on a biomass base. To do this I divided the harvested material into different functional groups as described above.

Below-ground measurements (in short)

Below-ground responses to elevated CO_2 were studied mainly by my co-worker Erik Sindhøj, who has given a detailed account of the methods used (Sindhøj 2001). Here I will confine myself to a brief description.

From April 1996, minirhizotrons were used to study root responses. These were transparent plastic tubes, 4 cm in diameter and 35 cm long, which were inserted into the soil in each plot at a 45^o angle. Using an endoscope, roots crossing a grid painted on the upward facing side of the tube were counted six to fourteen times throughout the growing season.

Because of the limited size of the plots, repeated root sampling by means of taking soil cores was not a viable option. Nylon mesh ingrowth bags (mesh size 1 cm) were used during the 1999 and 2000 growing seasons, two in each plot. The bags were installed in April 1999 and removed in connection with the above-ground harvest early in August, then new bags were installed in April 2000 and also removed at harvest in August. In 1999, the roots were washed in order to separate the roots, which were then scanned for measuring length, dried and weighed as described above; in 2000, they were only washed, dried and weighed.

Statistics

The measured values for photosynthesis and stomatal conductance were averaged for each plot; the different species were analysed separately. For each year and species I performed a repeated measurements analysis (Proc MIXED in SAS, SAS Institute Inc, Cary, NC, USA, 1989–1996) in which block and treatment (or block, chamber and CO_2) were the explanatory variables, and the different days throughout the growing season constituted the repetitions. To allow for effects of within-day changes of temperature, I also included leaf temperature in some analyses, as well as its interaction with treatment, but this never gave significant results.

The other production data were also analysed using repeated measurements analysis, but that in this case the years constituted the repetitions. For analysis of root dynamics, see Sindhøj (2001).

The vegetation data were analysed by categorising the species data and dividing the biomass data into functional groups (grasses, legumes and non-leguminous forbs), whose values, both in absolute and relative terms, were then analysed in the way described above. The point-intercept data were also analysed in principal component analyses (PCA). The first axes of the PCAs were then analysed with the above univariate methods.

Unless stated otherwise, I used a significance level of 5%.

Plant production

Biomass production

Above ground

During the first three years of the experiment, CO_2 had a positive and increasing effect on above-ground biomass harvested in early August, but I did not find any CO_2 effect from the fourth year onward (fig. 8). Differences between years were significant for all treatments, but to a variable degree. Averaged over the treatments, biomass was significantly higher in the three wettest years than in 1996 and 1999 (table 3). In the Elevated treatment, only the years 1997 and 1999 differed significantly (p = 0.0102), whereas in the Ambient treatment 1998 had a significantly higher above-ground production than all other years except 2000, and 2000 was significantly higher than 1995 and 1996 ($p < 0.0001$). In the Control treatment, the three wettest years of the study (1997, 1998 and 2000) showed a significantly higher production than 1996 ($p = 0.003$). Also the year x treat interaction was significant ($p = 0.0173$), indicating that responses to CO_2 (and chamber) differed over time..

I did not find any significant differences in above-ground biomass for the first two sampling dates during the growing season in 1998 (0.01 m^2 samples). The third sampling, however, showed a significant difference (p=0.0005) between Elevated and the other treatments, with Elevated producing 2.5 times as much as Ambient and three times as much as Control (fig. 9). Yet at harvest in early August, with 0.25 m^2 samples, there was no significant difference in above-ground biomass between the treatments (see above). Since there does not seem to be a biological or weather-related explanation to these results, and since the biomass estimates at elevated CO_2 on the third sampling occasion are more than twice as high as the highest biomass I ever found at harvest using a larger sampling area, my conclusion is that these results indicate that the 0.01 m^2 samples were simply too small to give reliable data.

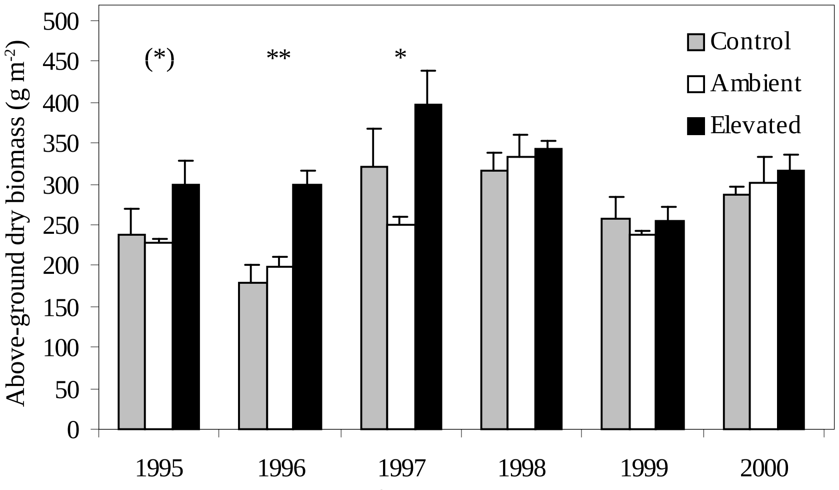


Fig. 8. Above-ground dry biomass (g m^{-2}, + 1 S.E) at harvest (early August) in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: ** $p < 0.01$; * $0.01 < p < 0.05$; (*) $0.05 < p < 0.1$.

Table 3. *Above-ground dry biomass (g m^{-2}) at harvest (early August), averaged over all treatments, and standard errors. Values with the same letter are not significantly different (*$p > 0.05$*).*

Year	Biomass	SE	
1995	255.17	16.14	ab
1996	226.10	18.41	a
1997	322.27	26.43	c
1998	330.87	11.38	c
1999	249.63	9.98	a
2000	301.59	11.96	bc

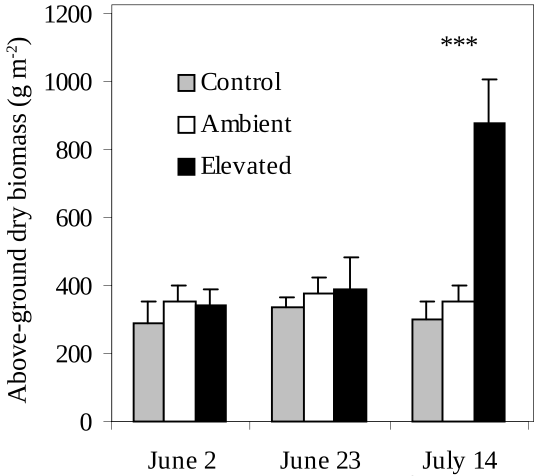


Fig. 9. Above-ground dry biomass (g m^{-2}, + 1 S.E) at three dates during the 1998 growing season in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC; 0.01 m^2 samples. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: *** $p < 0.001$ (but see text for details).

Below ground (in short)

Sindhøj (2001) shows that elevated CO_2 had a positive and increasing effect also on below-ground production as studied in minirhizotrons (fig. 10). This effect lasted throughout the experiment, although it was smaller in the extremely wet growing season of 1998. Root counts went down faster as well under elevated CO_2, except after the very dry summer of 1999 after which extremely low soil moisture may have inhibited decomposition.

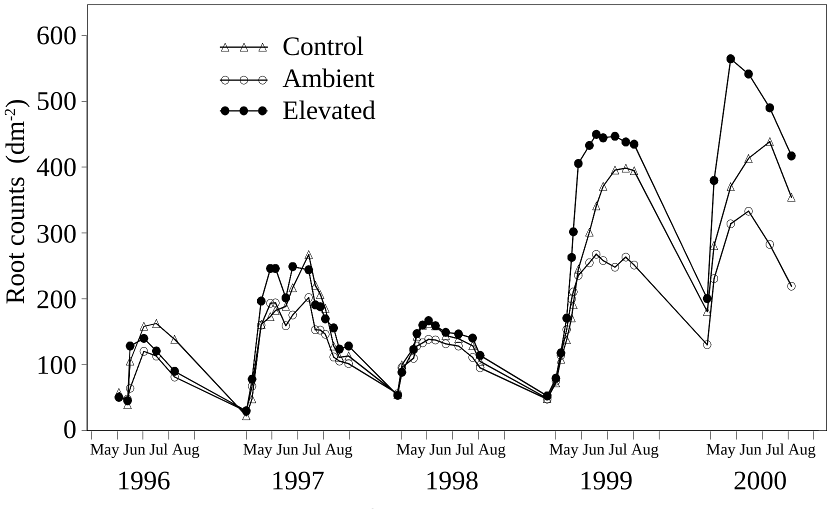


Fig. 10. Total root counts (roots per dm^2) up to 20 cm depth observed with minirhizotrons in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC (adapted from Sindhøj 2001).

For the two years they were used, ingrowth cores showed a large (+ 98%) and significant increase in root production in the Elevated treatment when compared to Ambient (fig. 11). Root production was 80% higher in 2000 than in 1999. For both years, root ingrowth biomass showed a good correlation with the increase in minirhizotron root counts, which justifies using root counts as estimates of root production during the years when no direct root biomass measurements were taken.

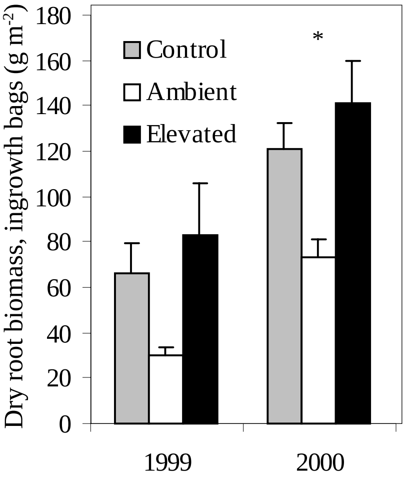


Fig. 11. Root dry biomass (g m^{-2}, + 1 S.E) from ingrowth cores in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC; taken in early August. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: * p < 0.05 (adapted from Sindhøj 2001).

CO_2 effects on total primary production

When root counts are taken as an indication of root biomass production, and are combined with the above-ground harvest biomass data, it becomes clear that the effect of elevated CO_2 lasted throughout the entire experiment, with the possible exception of 1998, which was the only growing season without any water deficit (Sindhøj 2000). However, the effect changed over time. During the first years, up to 1997, most of the extra production occurred above-ground. During the last two years, on the other hand, most extra C was invested in root growth. Figure 12 gives an indication of this, using the ratio of counted roots divided by above-ground biomass at harvest. Nitrogen depletion and a large water deficit in 1999 are two possible causes for this observed change, which I will discuss below.

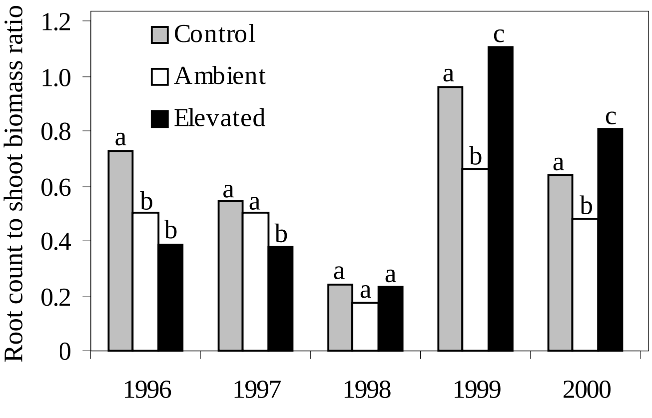


Fig. 12. Root count to shoot biomass ratios in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC; used as an analogue of root/shoot ratios. Means with different letters within a year are significantly different (p < 0.05) (adapted from Sindhøj 2001).

Above-ground nutrient concentrations

Table 4 shows the results from the nutrient analysis after two growing seasons. Plants grown at elevated CO_2 contained lower levels of most elements analysed, but the differences between species and plots were large. Nitrogen constituted the only significant difference for all four species or species groups analysed, and phosphorus and sulphur showed near-significant differences.

Table 4. *Nutrient concentrations in above-ground biomass at harvest (early August, 1996) after two growing seasons in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC. Standard errors shown in italics.*

	N mg g^{-1}		P mg g^{-1}		K mg g^{-1}		Ca mg g^{-1}	
Plantago media								
Control	12.00	*0.603*	3.11	*0.510*	25.25	*0.150*	55.55	*1.15*
Ambient	10.88	*0.700*	2.91	*0.231*	25.70	*1.96*	50.25	*2.95*
Elevated	9.05	*0.741*	2.62	*0.155*	23.28	*1.06*	42.95	*5.50*
Taraxacum sect. Ruderalia								
Control	12.83	*0.225*	3.01	*0.091*	31.83	*2.50*	30.43	*0.785*
Ambient	11.30	*0.227*	3.01	*0.201*	29.43	*0.610*	30.85	*1.85*
Elevated	10.15	*0.452*	3.03	*0.280*	29.83	*1.72*	31.55	*0.936*
Other forbs								
Control	13.43	*0.873*	3.21	*0.335*	20.88	*1.00*	17.53	*1.63*
Ambient	12.25	*0.742*	2.90	*0.158*	21.70	*0.342*	14.60	*0.977*
Elevated	10.35	*0.562*	2.53	*0.157*	19.45	*0.417*	14.63	*0.675*
Grasses								
Control	10.95	*0.650*	2.10	*0.888*	15.23	*0.319*	7.40	*0.750*
Ambient	10.98	*0.613*	1.97	*0.064*	16.05	*0.666*	7.25	*0.396*
Elevated	9.60	*0.235*	1.83	*0.050*	15.32	*0.890*	6.80	*0.470*

	Mg mg g^{-1}		Mn mg g^{-1}		S mg g^{-1}		Na µg g^{-1}	
Plantago media								
Control	3.04	*0.110*	0.04	*0.007*	2.09	*0.020*	33.45	*8.05*
Ambient	3.44	*0.620*	0.05	*0.007*	2.05	*0.110*	40.55	*8.64*
Elevated	2.68	*0.250*	0.05	*0.008*	1.63	*0.126*	32.98	*6.60*
Taraxacum sect. Ruderalia								
Control	3.82	*0.252*	0.09	*0.014*	1.59	*0.103*	581.45	*254*
Ambient	3.57	*0.503*	0.10	*0.011*	1.36	*0.064*	548.50	*285*
Elevated	3.54	*0.358*	0.09	*0.012*	1.25	*0.116*	503.75	*263*
Other forbs								
Control	2.75	*0.085*	0.16	*0.028*	1.22	*0.027*	59.63	*14.7*
Ambient	2.32	*0.225*	0.11	*0.016*	1.13	*0.021*	60.08	*16.4*
Elevated	2.37	*0.077*	0.13	*0.018*	0.96	*0.025*	79.65	*35.0*
Grasses								
Control	1.57	*0.131*	0.26	*0.035*	1.06	*0.070*	35.43	*7.25*
Ambient	1.51	*0.165*	0.29	*0.037*	1.06	*0.309*	38.18	*7.15*
Elevated	1.33	*0.074*	0.29	*0.058*	1.01	*0.030*	45.70	*10.3*

Repeated measurement analysis of the nitrogen data for the harvests from 1996 to 2000 (for *Trifolium repens*: 1997 to 2000) showed a significant treatment effect only in grasses. The interaction between treatment and year was significant only for *T. repens*. This interaction was almost significant for forbs ($p = 0.0563$), but this may well have been an artefact of the change in sampling from 1997, when legumes were first separated from non-leguminous forbs. If I exclude the 1996 harvest from the forbs analysis, the interaction is no longer significant ($p > 0.1$). The factor year is significant in both forbs (both in- and excluding the 1996 data), grasses and *T. repens*. Within-year significances are shown in figure 13.

Analysing the CO_2 effect as a two-level factor rather than treatment with three levels and using the same repeated measurements analysis as above, I found a significant CO_2 effect for all three groups analysed. Also the interaction between CO_2 and year

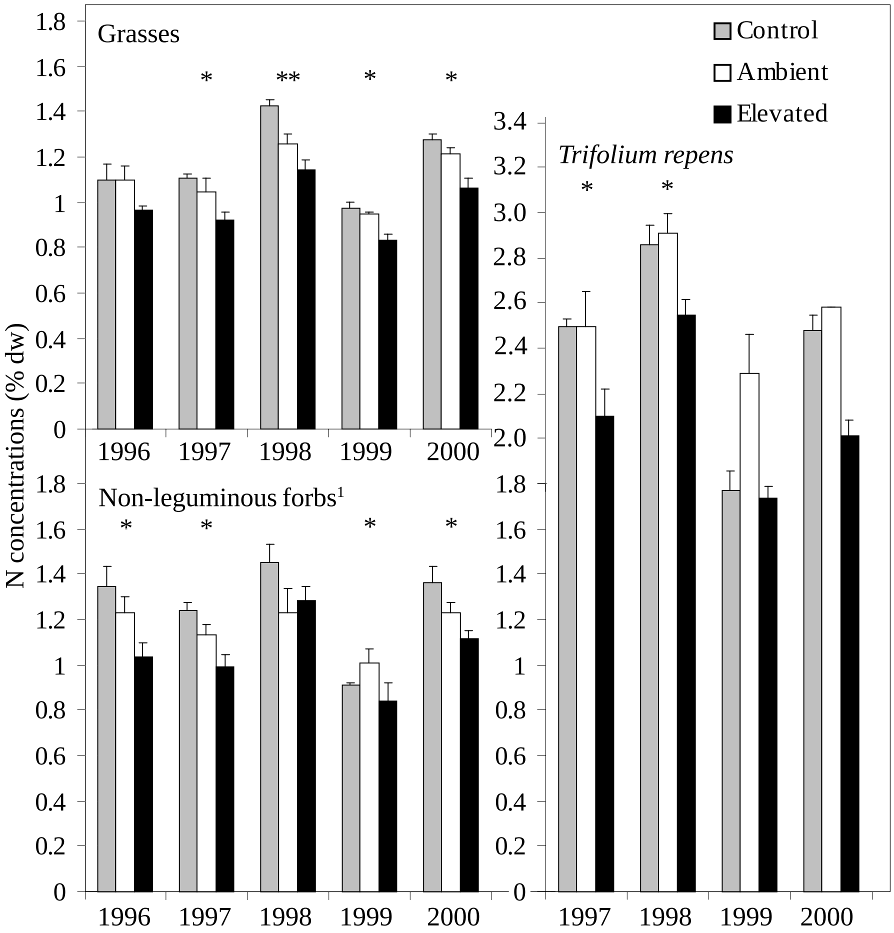


Fig. 13. Nitrogen concentrations (in % of dry mass) of above-ground biomass at harvest (early August) in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: ** $p < 0.01$; * $0.01 < p < 0.05$.

[1] This category includes legumes in 1996 data.

was significant for *T. repens*. The chamber effect, analysed in the same way, was significant only in grasses, both as a main effect and in interaction with year.

Some other production-related measurements

Specific leaf area

The effect of CO_2 on specific leaf area (SLA) was small. Over the course of all years measured, neither *Plantago media* nor *Taraxacum* showed a significant response. *Taraxacum* showed a significant difference between years, whereas *P. media* did not. There was no interaction between treatment and year. For each year, SLA in *P. media* was lower at elevated than at ambient CO_2. However, this difference was significant only in 1996 (fig. 14).

Seed mass

Ranunculus bulbosus, the only species for which seed mass was studied during four years, showed a significant treatment effect over these four years (repeated measurement analysis). However, this treatment effect seems to be brought about by a combination of chamber and CO_2 effects rather than being a consequence of CO_2 *per se*. Hence, for the two single years in which there was a significant difference between treatments, 1995 and 1997, only the difference between Control and Elevated was significant (fig. 15). Differences between years were significant, but there was no interaction between year and treatment.

Carum carvi was studied for three years and showed a significant chamber effect during the first year (fig. 15). Over all years, however, treatment effects were not significant, but differences between years were.

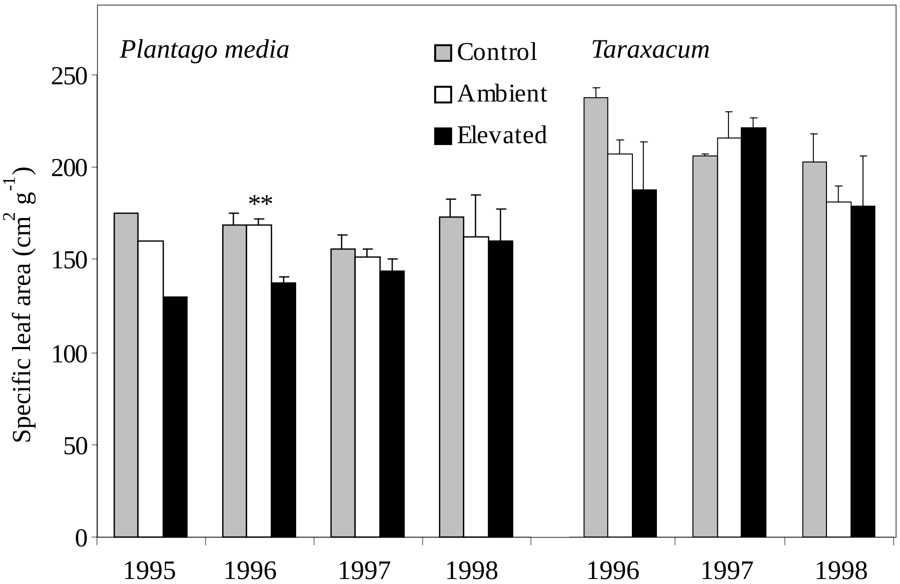


Fig. 14. Specific leaf area (SLA) at harvest (early August) for two species in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: ** $p < 0.01$.

The other two studied species, *Lotus corniculatus* and *Plantago media*, each studied during two years, did not show any significant differences or even non-significant trends (fig. 15). Thus, it may be so that seeds of different species respond differently to elevated CO_2, which should be taken into account when predicting future vegetation.

Production processes

Photosynthesis

Repeated measurement analysis shows that leaf level photosynthesis was significantly higher at elevated CO_2 for both species measured (*Plantago media* and *Taraxacum*) and all years but one; the exception being the extremely and continuously wet growing season of 1998. For both species, photosynthesis decreased significantly throughout each growing season, but the slope of this decrease does not seem to be affected by CO_2. In other words, there is no interaction between treatment and day of year (with the possible exception of 1997, but due to technical problems data for this growing season are scarce and no firm conclusions should be drawn from these). For each measurement occasion on which I found a significant treatment effect, photosynthesis at elevated CO_2 was significantly higher than in the ambient treatment (fig. 16).

There does not seem to be a trend in leaf-level photosynthesis over the years. Measured photosynthesis was higher in 1998 to 2000 than in the previous years, but this is an artefact that disappears if measurements for a specific week of the year are compared. Measurements taken during the same week of the year

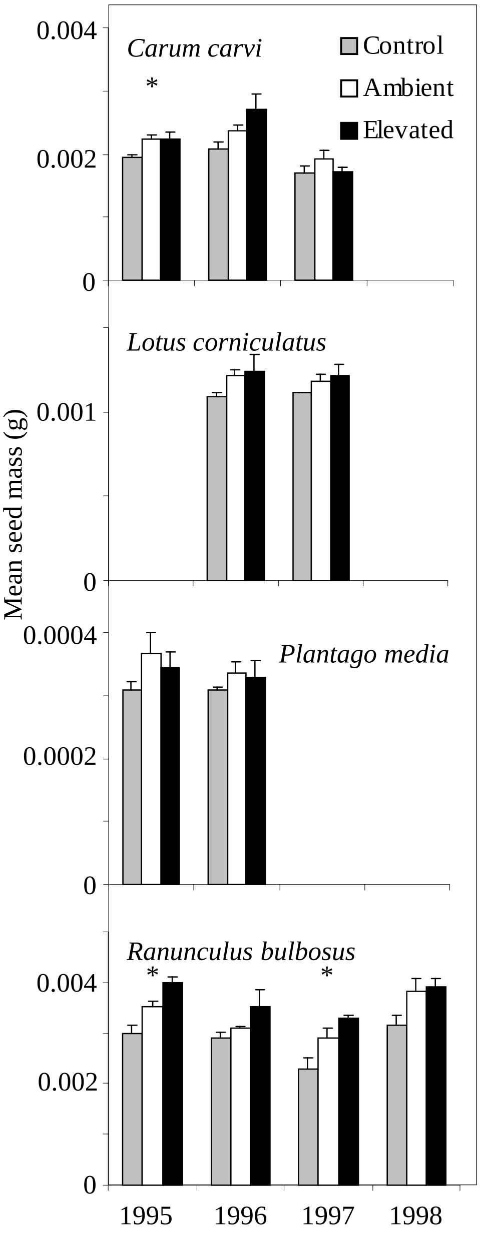
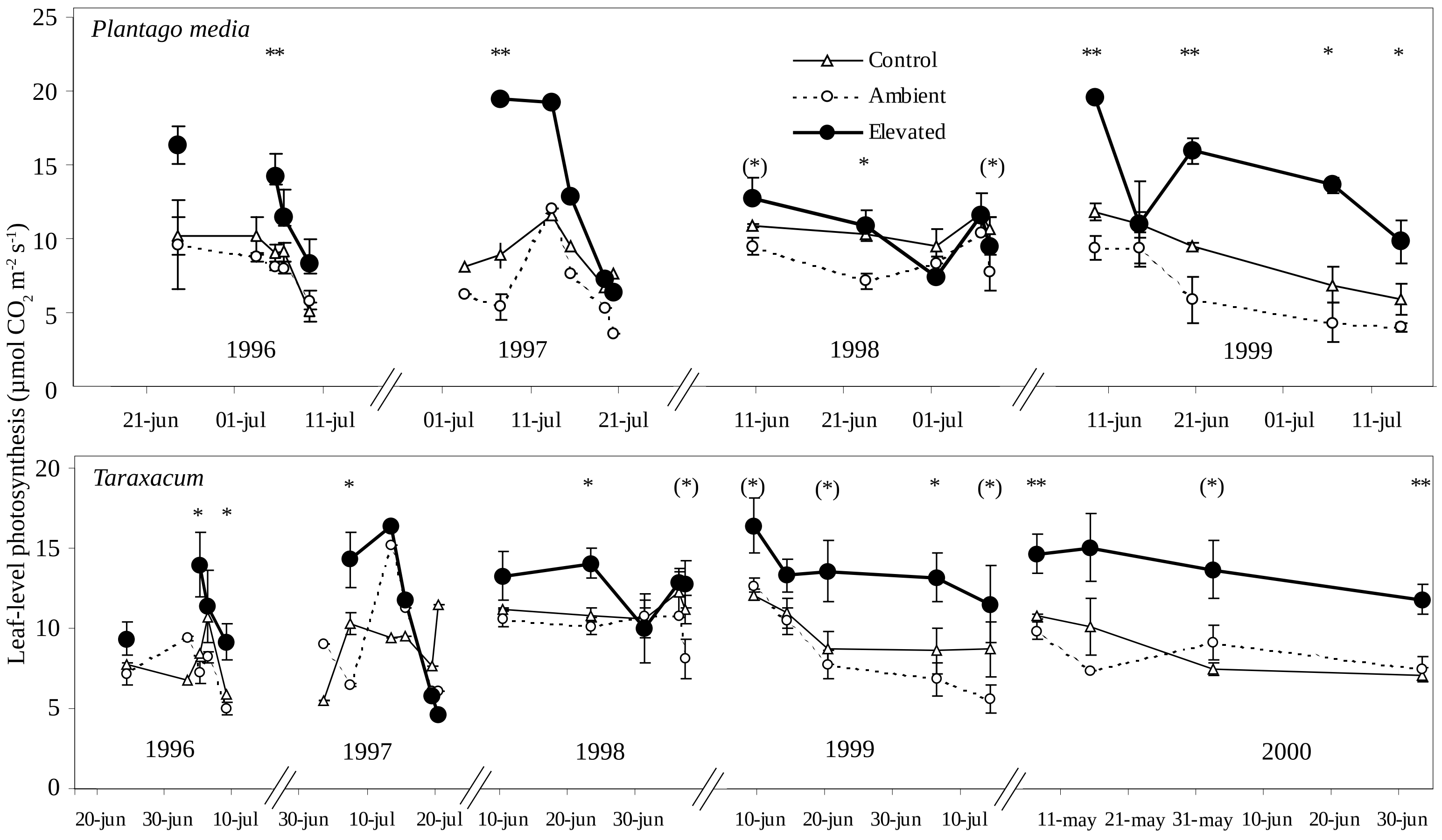


Fig. 15. Mean seed mass (gram per seed) for four species in OTCs under ambient and twice ambient elevated CO_2 concentrations and in controls without OTC. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect: * $p < 0.05$.

Fig. 16 (opposite page). Net photosynthesis at leaf level (µmol CO_2 m^{-2} s^{-1}) measured at saturating light levels on two species in OTCs under ambient and twice ambient CO_2 concentrations and in controls without OTC. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect per measurement occasion: ** $p < 0.01$; * $0.01 < p < 0.05$; (*) $0.05 < p < 0.1$.


Plantago media
Control
Ambient
Elevated
Leaf-level photosynthesis (µmol CO$_2$ m^{-2} s^{-1})
25
20
15
10
5
0
**
**
(*)
*
(*)
**
**
*
*
1996
1997
1998
1999
21-jun
01-jul
11-jul
01-jul
11-jul
21-jul
11-jun
21-jun
01-jul
11-jun
21-jun
01-jul
11-jul
Taraxacum
20
15
10
5
0
* *
*
*
(*)
(*)
(*)
*
(*)
**
(*)
**
1996
1997
1998
1999
2000
20-jun
30-jun
10-jul
30-jun
10-jul
20-jul
10-jun
20-jun
30-jun
10-jun
20-jun
30-jun
10-jul
11-may
21-may
31-may
10-jun
20-jun
30-jun

did differ significantly between years, but these differences did not show any trend and can probably be ascribed to the weather. In addition, note that the measurements from 1998, 1999 and 2000 are probably not as representative for the entire growing seasons as measurements from the first years, due to the extreme weather in these later years. This holds especially for 1998, when there were hardly any days sunny enough to carry out measurements, and 1999, when, due to drought, measurements had to be concentrated on a few non-representative plants that were not wilting.

For data from 1995 I refer to paper I.

Stomatal conductance

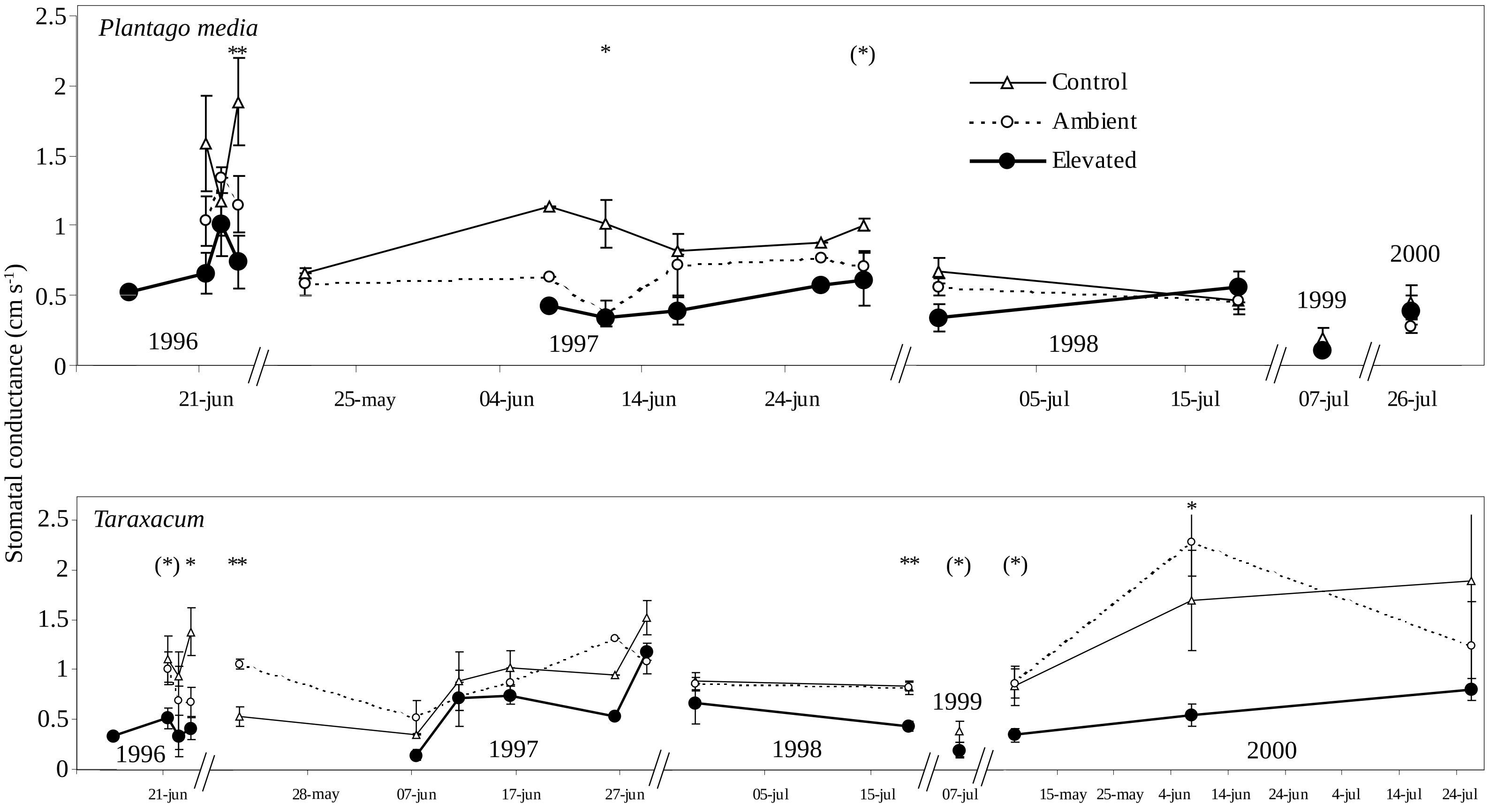
In comparison with photosynthesis, seasonal trends in stomatal conductance are far less clear (fig. 17). Moreover, the possible lack of representativeness I mentioned above may well be even more pronounced here. The results should therefore be interpreted with care.

Having said that, I did find significant treatment effects, using repeated measurements analysis for each year and species separately. However, these effects, and whether they were caused mainly by CO_2 or by the chambers, differed between years and species. Generally, stomatal conductance tended to be lower at elevated CO_2 than at ambient, and lower in OTCs than outside.

For *Taraxacum*, the species studied most intensively, I found that CO_2 significantly lowered stomatal conductance during 1998 and 2000. CO_2 did not have an effect during other years, either on stomatal conductance itself or on the change of stomatal conductance over the growing season (that is, the interaction between CO_2 and day of year was not significant). The chamber effect was significant in 1999, when plants in the Control treatment showed higher stomatal conductance than plants in the OTCs. In 1996 the OTCs significantly affected the change in stomatal conductance over the growing season, and in 1997 this effect was near-significant. The treatment effect was significant also in 1996, but when chamber and CO_2 effects were separated for that year, none of these factors was significant on its own. Four single measurement occasions showed significant differences (fig. 17), of which two can be ascribed to CO_2.

CO_2 did not cause any significant changes in stomatal conductance in *Plantago media* over any year. In 1998, however, the change in stomatal conductance over the growing season was significantly affected by CO_2 (but note that I have only two measurement occasions during this season). The treatment effect, which was significant in 1996 and 1997, was largely accounted for by chamber influences (which were near-significant in 1996 and significant in 1997, with neither year showing any significant or near-significant CO_2 effect). Treatment effect was significant on two single measurement occasions (see fig. 17), but then it was the OTCs rather than CO_2 causing the difference.

Fig. 17 (opposite page, continued on next page). Stomatal conductance (cm s^{-1}) measured at saturating light levels on three species in OTCs under ambient and twice ambient CO_2 concentrations and in controls without OTC. Note that for *Taraxacum* the horizontal scale for 2000 is smaller than for the other years. Error bars indicate 1 S.E. Stars indicate significance level of treatment effect per measurement occasion: ** $p < 0.01$; * $0.01 < p < 0.05$; (*) $0.05 < p < 0.1$.


Plantago media
Taraxacum
Control
Ambient
Elevated
Stomatal conductance (cm s-1)
0
0.5
1
1.5
2
2.5
1996
1997
1998
1999
2000
*
**
(*)
21-jun
25-may
04-jun
14-jun
24-jun
05-jul
15-jul
07-jul
26-jul
28-may
07-jun
17-jun
27-jun
15-may
25-may
4-jun
14-jun
24-jun
4-jul
14-jul
24-jul

Trifolium repens did not show a significant treatment effect on any single measurement occasion (note, however, that it was not measured on the two occasions on which *P. media* showed significant differences), but differences between treatments were significant over the1998 growing season. Due to absence of *T. repens* in some plots, especially Ambient, it is hard to say whether this effect was brought about by the OTC or by CO_2, or by a combination of both.

Putting Nåntuna into context - possible interpretations of the production study results

This six-year field study of plant production in a semi-natural grassland at elevated CO_2 clearly points out the importance of long-term experiments under natural weather conditions. Even after six years, it is hard to indisputably ascribe any of the changes observed to a single factor. Actually, it would have been much easier to draw firm conclusions had the experiment been stopped after three growing seasons. The value of those conclusions could, of course, be disputed.

It also becomes obvious that total plant production should be studied rather than just the conveniently located above-ground parts. Unfortunately, this is very laborious and often only above-ground production is used when comparing studies. In such comparisons, such as figure 18, it should be kept in mind that they may tell only half the production story.

Figure 18 shows the above-ground biomass stimulation found in the Nåntuna study in the context of that found in similar studies reviewed by

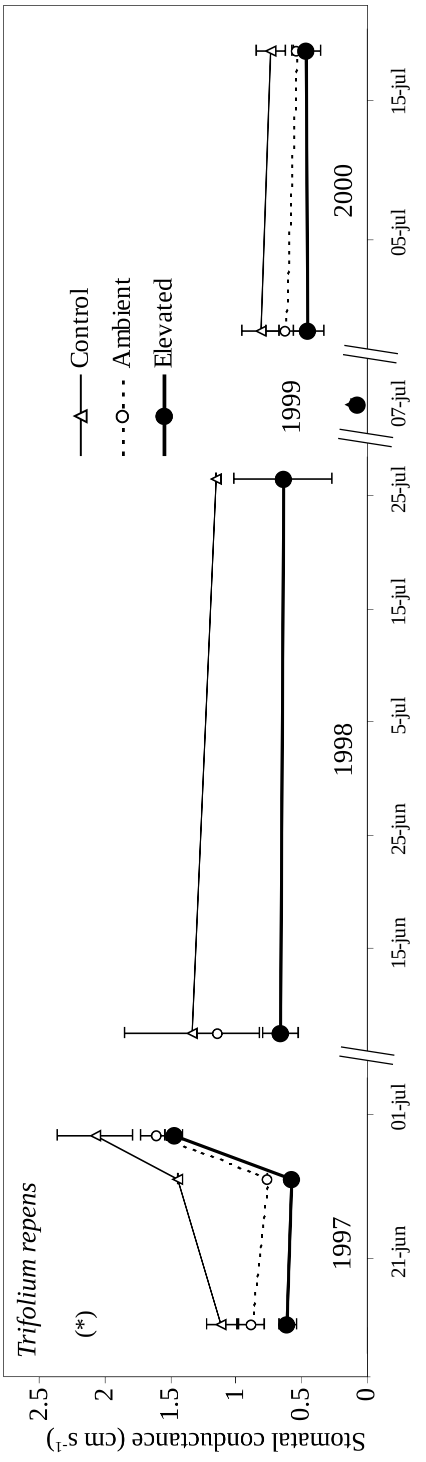


Fig. 17 (continued). Stomatal conductance (cm s^{-1}). Error bars indicate 1 S.E. Stars indicate significance level of treatment effect per measurement occasion: (*) $0.05 < p < 0.1$.

Koch and Mooney (1996). It appears that this study was carried out at a rather low-productive site, comparable in production to the California annual grassland (squares in fig. 18) studied by Field *et al.* (1996), and surpassing in production only the Swiss alpine sedge study by Körner *et al.* (1996). It should be noted, though, that production comparisons can be cumbersome because of different measurement variables (peak biomass, cumulative biomass) or harvesting techniques (cutting at ground level, leaving 5 cm of vegetation), as Koch and Mooney (1996) point out. Moreover, surprisingly often the method of biomass harvesting is not specified.

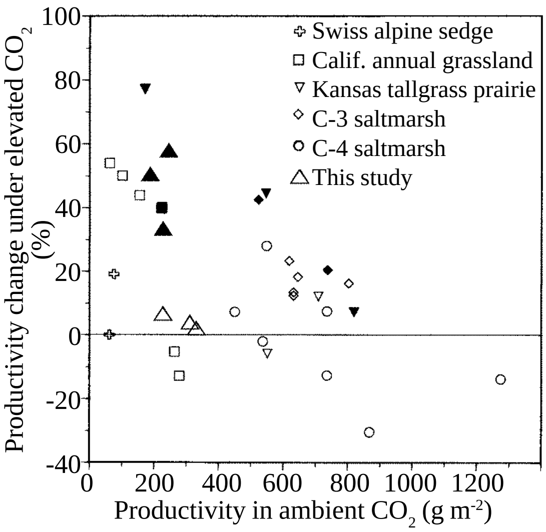


Fig. 18. Percentage change in above-ground production at elevated CO2 for six different herbaceous ecosystems. Solid symbols denote significant CO$_2$ effects. Adapted from Koch and Mooney (1996).

The stimulation of above-ground biomass by CO$_2$ I found during the first three years of the study fits well within the range shown in figure 18. The lack of response during the latter years is not unprecedented either. Owensby *et al.* (1999), who performed what to my knowledge is the longest lasting elevated CO$_2$ study in a natural grassland ecosystem, did not find a significant (at p<0.1) CO$_2$-related difference in above-ground production for four of the eight years studied. This was not a trend over time, however, as it might be in the Nåntuna study; but rather there was no response during wet years.

In the study by Owensby *et al.* (1999), root biomass generally shows the same pattern as above-ground biomass. That is, in wet years there was no CO$_2$ effect on roots, whereas in drier years there was (although CO$_2$ effects on roots become significant in wetter years than do effects on above-ground biomass). This makes an interesting contrast with the present study, in which the CO$_2$ effect on roots increased over time, whereas the effect on above-ground biomass disappeared. This may point to a nutrient depletion at this relatively low-productive site under a severe cutting regime. However, weather effects may also play a role.

In paper I I argue that the extreme weather is the possible cause of the lack in above-ground biomass response to CO$_2$ in 1998, rather than a nutrient depletion. Since then I have studied the system for two more growing seasons, but unfortunately these came with even more extreme weather (see fig. 7 for a slight indication).

To summarise:

- CO$_2$ stimulated leaf level photosynthesis
- CO$_2$ had a significant impact on plant biomass production throughout the experiment, except during the one growing season without any water deficit

- initially, the extra biomass production occurred mainly above ground; later, after an extremely dry season, exclusively below ground
- since the weather showed a directional change during the experiment, with one extreme exception, weather and time effects were largely confounded
- changes over time, such as the shift in CO_2-induced extra production from shoots to roots, if not confounded with weather effects, may either be caused by nutrient depletion or by changes in the vegetation following the change in management (see next chapter).

Vegetation composition

To analyse vegetation composition and how it changed over time and with treatments, I used two fundamentally different methods. On the one hand, I used the species data from the point-intercept measurements as they were. That is, every single species was a part of the analysis. With up to 50 species this calls for multivariate analysis, or other ways in which to make the information more accessible, such as the use of indices. On the other hand, I used biology rather than mathematics as a way of reducing the size of the data set by grouping the species into functional groups (grasses, legumes and other herbs). Responses of these functional groups could then be analysed by univariate statistics. An additional advantage of this latter method was that not only point-intercept measurements could be used, but also the biomass data from the harvests that were divided into functional groups.

PCA

I used principal component analysis (PCA) to analyse the point-intercept data in order to detect any patterns in species composition and its change over time. As an indirect gradient analysis, PCA is useful to detect gradients implied by (in this case) vegetation composition rather than using measured environmental variables as explanatory gradients, which is the case in direct gradient analyses instead. The measured “gradients” of time and CO_2 and possibly block could have been used in a direct gradient analysis of the present data; however, since I did not have exact data on for instance nutrient and water levels for each plot, I chose to use PCA.

Because of the so-called double-zero problem (two sites are not necessarily similar to each other because both lack a particular species; that is, shared presence of a species indicates similarity between sites, but shared absence does not), PCA is usually not a good description of reality if there are long environmental gradients present in the data set, along which species can be distributed unimodally. Instead, the gradients should be very short and the plots should share most species, differing rather because of the abundances (Legendre and Legendre 1998).

In the PCA that contains all years and both seasons (summer and spring), I found an explanatory value for axis 1 of about 30% (not shown). The first four axes, together explaining 65%, were all significantly correlated with season and year of experiment, and with no other measured or environmental variables. Since the difference between spring and summer vegetation assessments is quite obvious and of no interest *per se* in this study, separate analyses for spring and summer data may yield more insight into possible CO_2 effects. As there is some evidence that different investigators may get slightly different results in point-intercept analyses (Goodall 1952), and since another observer made the 1995 assessments, I have also performed analyses excluding the first year.

Spring data

Various analyses of the spring assessments showed a strong effect of year, but the effect of CO_2 was negligible. For the spring measurements, contrary to the summer

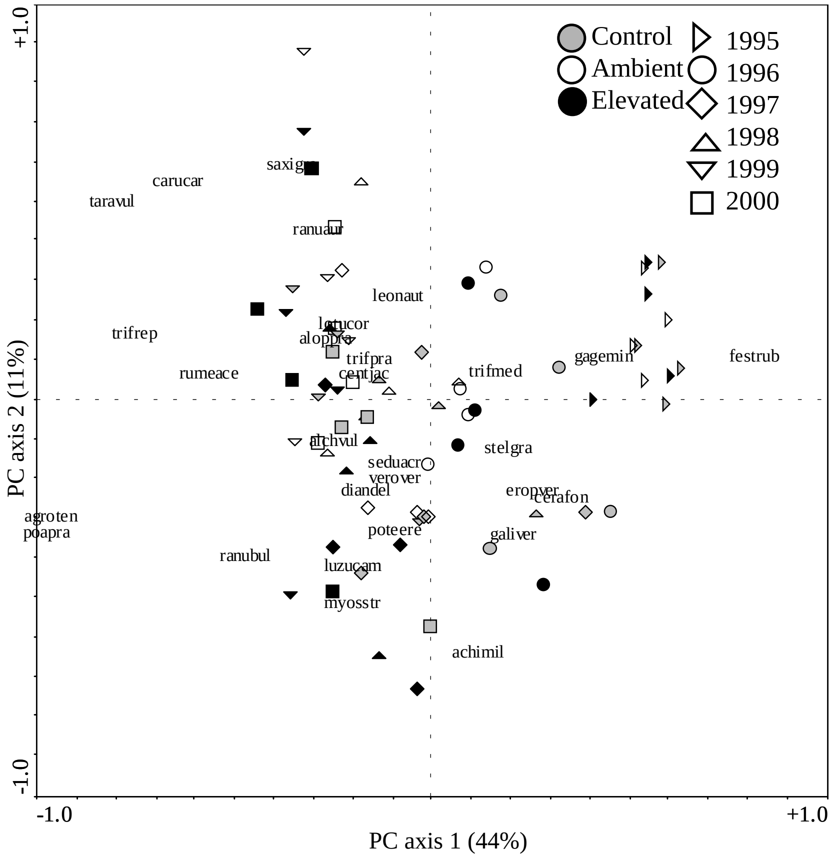


Fig 19. Species and plot scores of a PCA of the species composition in spring in OTCs at ambient and twice ambient CO_2 concentrations, and in controls without OTC. For reasons of clarity, some species in the central part of the graph have been left out. PCA scores were calculated using all species. See Appendix A for species list.

measurements, no other environmental or experimental variables than treatment and block were available.

Axis 1 of the PCA for all spring data explained 44% of the observed variation and was significantly correlated with year of experiment (fig. 19). However, none of the first four axes, which together explained over 70%, was correlated with CO_2. Axes 2 - 4 were significantly affected by block. In a similar analysis, but excluding 1995, the first axis explained about 30% of the variation and the first four axes together about 65%. Still, none of the first four axes showed a correlation with CO_2, but axis 1 was significantly correlated with year of experiment. All four axes showed a significant block effect.

When I analysed spring data for each year separately, axis 1 typically explained 35 - 40% of the observed variation (31% in 1996, 27% in 1999), axis 2 about 15 - 20% (24% in 1999) and axes 3 and 4 about 10% (17 and 15%, respectively, in 1999) each. In 1998, the third axis (explaining 15%) was significantly affected by treatment (both

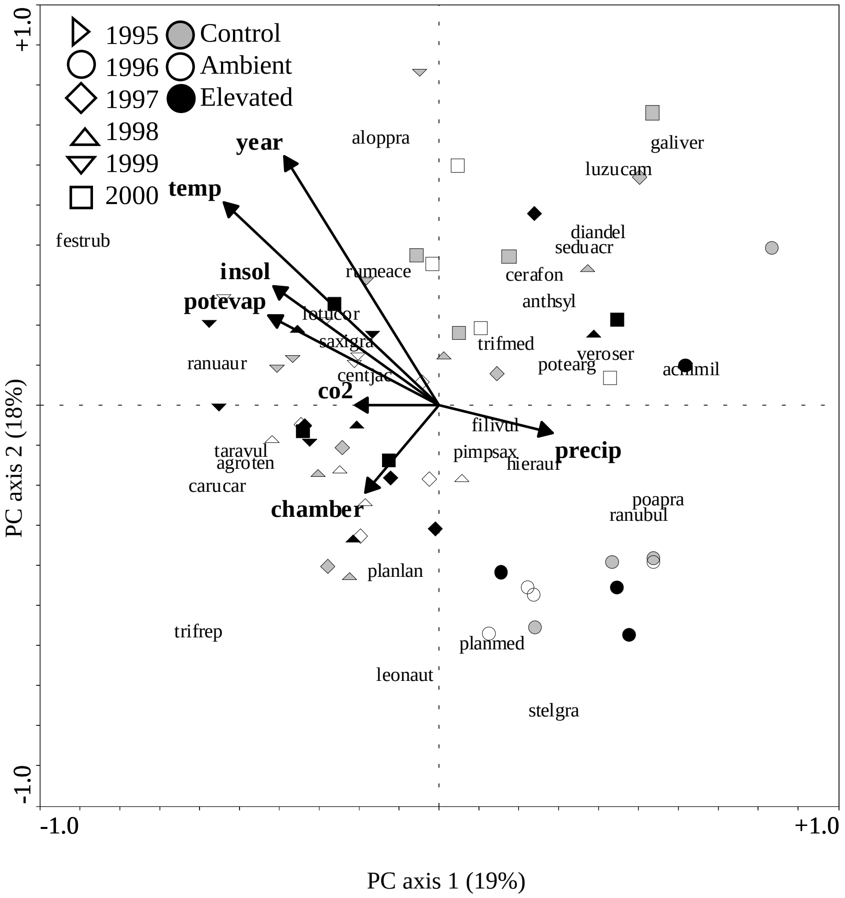


Fig 20. Species and plot scores and environmental variables on axes 1 and 2 of a PCA of the species composition in summer in OTCs at ambient and twice ambient CO_2 concentrations, and in controls without OTC, excluding 1995. For reasons of clarity, some species in the central part of the graph have been left out. PCA scores were calculated using all species. See Appendix A for species list; temp=mean seasonal temperature, insol=seasonal insolation, potevap=seasonal potential evapotranspiration, precip=seasonal precipitation.

the CO_2 and the chamber effect were significant), but in none of the other analyses did any of the first four axes show a significant treatment effect. Block had a significant effect on axis 3 (which explained 14%) in 2000, but on no other axis in any of the analyses.

Summer data

The picture shown by various analyses of the summer point intercept data is not easy to interpret. Analysed in the same way as the spring data, that is, excluding any explanatory variables other than year, block and treatment, the summer assessments too show a very strong time effect. There also appears to be a block effect, but the effect of treatment is much smaller and consists mainly of chamber effects. When 1995 is excluded from the analysis, the treatment effect becomes more significant

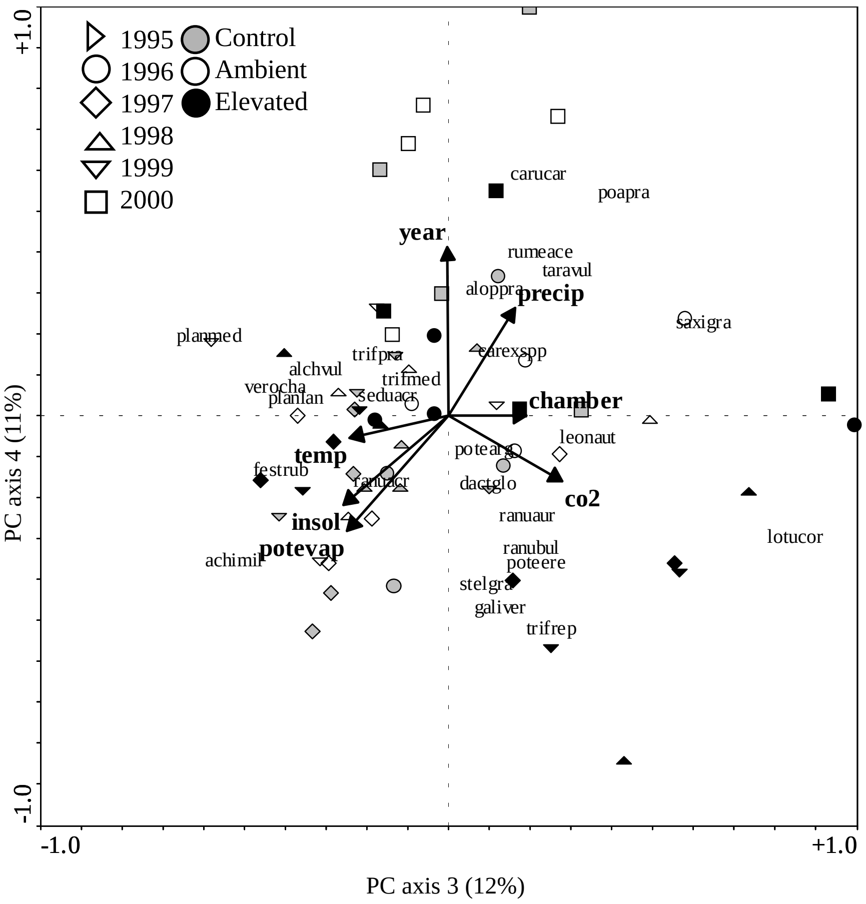


Fig. 21. As 20, on axes 3 and 4.

and the contribution of CO_2 more important. In the summer analyses, the factor year contains much information on seasonal precipitation, temperature, insolation and potential evapotranspiration which can be taken into account. Of course, some of these variables are highly correlated with each other, and some are highly confounded with the year of experiment, which makes it hard to separate their effects.

Axis 1 of the PCA for all summer data explained 23% of all variation, and the following axes contributed 15, 12 and 10%, respectively. The first axis was significantly correlated with five out of seven explanatory variables (treatment, block, and seasonal values for insolation, potential evaporation and precipitation), and showed an almost significant correlation with a sixth one (year of experiment). Treatment was also significantly correlated with axis 2, and all weather variables (which over the above also included the seasonal mean temperature) were significantly correlated with axis 3. However, when the treatment effect was separated into a chamber and a CO_2 effect, of these only the chamber effect showed significance (on axes 2 and 4). In an analysis excluding 1995 the first four axes explained 19, 18, 12 and 11% of the variation, respectively (fig. 20 and 21). In this analysis I excluded potential evaporation as an experimental variable in order to maintain enough degrees of freedom. All other

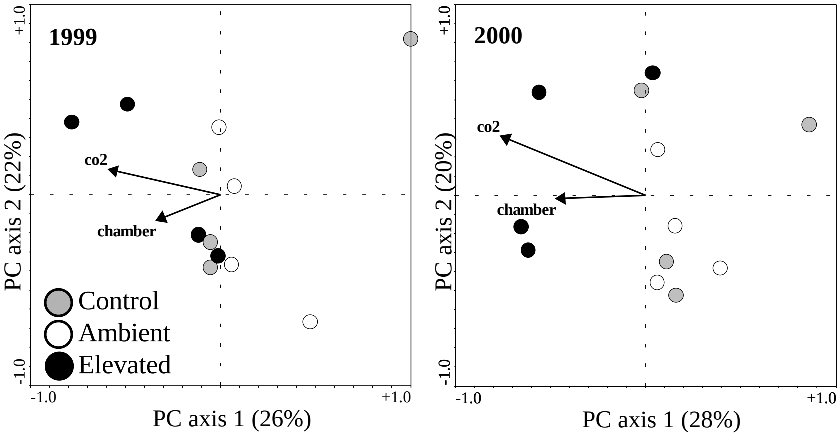


Fig. 22. Plot scores and treatment variables of PCAs of the species composition in summer in OTCs at ambient and twice ambient CO2 concentrations, and in controls without OTC.

weather variables, however, as well as year of experiment and block, were significantly correlated with axis 1, and treatment showed a near-significant correlation with both axis 1 and axis 2. Axis 3 was significantly correlated with all variables but precipitation, and axis 4 with all variables but block. When the treatment effect was separated into a chamber and CO_2 effect, chamber was most important for the first two axes, and CO_2 for the third and fourth. When I made year partial in the above tests, CO_2 tended to become slightly more significant, and chamber less so (see also Paper III).

For the PCAs of summer data for each year separately (in which no separating environmental variables were available), axis 1 typically explained between 25 and 35% of the observed variation, axis 2 about 20, axis 3 about 15 and axis 4 about 10%. In 1999 there was a significant relation between axis 1 (which explained 26%) and both treatment and block, and in 2000 the relation between axis 1 (which then explained 28%) and treatment was even more significant (fig. 22). In both years, it was CO_2 rather than a chamber effect that caused the significance. Other than that, there were significant relations between any of these variables and the lower axes only (axis 4 and treatment in 1997; axis 4 and block in 1996, axis 3 and block in 1998).

An interpretation of the principal components analyses

The PCAs showed that, apart from the obvious within-year difference between summer and spring measurements, the passing of time is the most important factor influencing vegetation composition in this experiment (fig. 19, 20). On the basis of the available data it is not possible to determine whether this time effect is in fact brought about by the change in management (from grazing to a severe cutting regime), by loss of nutrients from the system, or by other factors. More problematically, since the change in weather was largely directional over the course of the experiment, many weather effects are confounded with these time effects, so the effect of time may well be brought about by changes in the weather, at least partially.

Secondly, in this perennial grassland the effects of the plots themselves, whether caused by small-scale environmental heterogeneity or by original vegetation composition, or a combination of both, remained of importance throughout the experiment. (These effects are easily tested by including the interaction between treatment and block in the model. It is also easily recognisable if plots in the graphic outputs of the PCA are labelled individually.)

Treatment did have an impact on vegetation composition as analysed by PCA, but it was a rather small one. Both chamber and CO_2 had an effect. The CO_2 effect became more important towards the end of the experiment, which was clear when separate years were analysed by PCA and the results for different years compared (only shown for 1999 and 2000, fig. 22). This trend was also found by Vasseur and Potvin (1998) in a similar study, performed in southern Quebec during three years. Although it is not possible to compare these two studies directly because of differences in methodology, responses at species level do seem to differ between them (Paper III). This may point to the danger of generalising conclusions based on experiments performed on a specific site.

There was no detectable CO_2 effect, or indeed any treatment effect, on the PCA scores of the spring assessments.

Species diversity

A different approach to simplifying the complex data set that species abundances constitute is the use of indices. This has become particularly popular in biodiversity studies. Simple indices are, for instance, the number of species present, or the number of species per area. Of course there is more to biodiversity than just the number of species, but the other factors, such as genetic and ecosystem diversity, are beyond the scope of this thesis. More sophisticated indices also account for the relative abundance of each species. Typically a system with a given number of species is judged to be more diverse if the species are evenly distributed rather than if it is dominated by a few species, with the others occurring in very small numbers.

The use of only one figure to represent the species composition of an entire system obviously results in a large loss of information. Yet biodiversity indices facilitate comparisons between studies, treatments or ecosystems to find differences or similarities. They do not, however, give an explanation of the cause or even nature of these differences.

For this thesis, I have calculated the Shannon index (H’) to study how CO_2 affects biodiversity (see Magurran 1988 for details on calculations and statistics). This is one of the most widely used biodiversity indices. An additional advantage is that it does not draw as heavily on the most abundant species as does for instance Simpson’s index, another biodiversity index that is frequently used. Since the grid I used in the point-intercept measurements limited the maximum possible number of scored occurrences of our most abundant species to 81, Simpson’s index might give inaccurate results.

Figure 23 shows how the calculated Shannon indices varied between treatments and over time. In spring CO_2 had a negative effect on biodiversity, whereas in summer the effect was positive (see paper III for details). The chamber effect, if present

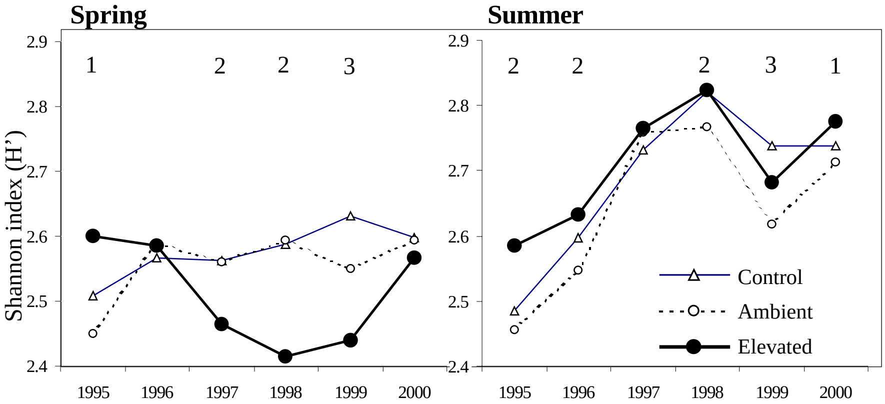


Fig. 23. Species diversity, calculated as the Shannon index, for point-intercept measurements in OTCs at ambient and twice ambient CO_2 concentrations and in controls without OTC. Figures give the number of significant pairwise comparisons (out of a maximum of 3) between treatments, if any.

at all, appeared to be smaller than the CO_2 effect. For all treatments, there appeared to be a positive trend in summer biodiversity (except for 1999), but no trend could be discerned for biodiversity in spring.

Again, various causes may be responsible for the trend of increasing summer biodiversity over time. It may be so that this really is a change with time itself, in which case it could be depletion of nutrients or a succession after the change in management that causes the vegetation to become more diverse. The deviation from this trend in the summer of 1999 could be explained by drought causing this season to have a low production, which resulted in fewer point-intercept hits irrespective of the underlying diversity. If I take this reasoning a step further, however, it may be so that the observed trend is really caused by the weather rather than by any change over time. The absence of a trend for the spring measurements may also point in this direction.

The discrepancy between spring and summer in the direction of the CO_2 effect is not explained easily. It might be the case that the differences in summer diversity actually mirror differences in production, since higher production allows for more hits in the point-intercept measurements and thus a higher diversity. However, this does not fit too well with the observed pattern over time, in which the year with the largest difference in above-ground production, 1997, was the only year that did not show any CO_2 effect on diversity, whereas the three years without differences in production did.

Leadley *et al.* (1999) did not find an effect of CO_2 on the Shannon index in their semi-natural calcareous grassland, harvested in June. They did find, though, that CO_2 increased the evenness of the vegetation. If this were the case in our field site as well, that might explain at least a part of the higher values for summer biodiversity at elevated CO_2. (Of course it still would not explain the discrepancy between spring and summer, unless we assume that this increase in evenness occurs during the growing season, but is not preserved into the next.) However, the evenness indices calculated from my vegetation data do not show such an increase, but rather the opposite: a small decrease in evenness at elevated CO_2 (not shown), but the point-intercept method I used may have serious drawbacks when it comes to calculating evenness (such as an overestimation of the evenness of species that occur in almost all quadrats).

Functional groups

Another popular way of working with complicated species data sets is to categorise the species into functional groups (or functional types). The concept of functional groups has been defined in many different ways (Gitay and Noble 1997), yet not as many as the functional groups themselves (Shugart 1997, Westoby and Leishman 1997). For my purposes it sufficed to divide the species into a few easily recognisable groups so that not only the point-intercept, but also the biomass data could be divided in this way. Since all plants belonged to herbaceous C3-species, the most evident division was between grasses and forbs. Bearing in mind that N may become important, I also separated legumes from other forbs. Another important division might have been the one between annual and perennial species. However, there were only very few annual species present, and only in very small numbers, and to separate these from the others would have been both time-consuming and error-prone in the case of the biomass samples. I performed the analyses of the point-intercept data with and without this division, and the results did not differ.

Again, the trend over time was much larger than any other effect. For the summer measurements, grasses increased dramatically and steadily, especially in biomass, but also in point-intercept hits (fig. 24), both in absolute (again with the exception of 1999) and in relative figures. As the other changes over time, this trend cannot indisputably be ascribed to a particular cause.

On the basis of biomass, none of the functional groups showed a significant response to CO_2, either in absolute or relative terms. Yet in point-intercept measurements, CO_2 was found to have a slight positive effect on legumes in summer. This effect was significant only in a repeated measurements analysis in which the possible effect of changing investigators was taken into account (i.e., if 1995 was excluded), but not in any single year.

Vegetation composition and CO_2 over time

Although the answers that the many ways to look upon and analyse vegetation composition yield are different in details, and sometimes more fundamentally, some more general conclusions are probably justified:

- the change in vegetation composition over time was much larger than any treatment effect;
- because precipitation showed a largely directional change over time, it was hard to distinguish between effects of weather and of nutrient status or succession following a change in management ;
- a possibly increasing CO_2 effect may be present, and it might express itself through a stimulation of legumes.

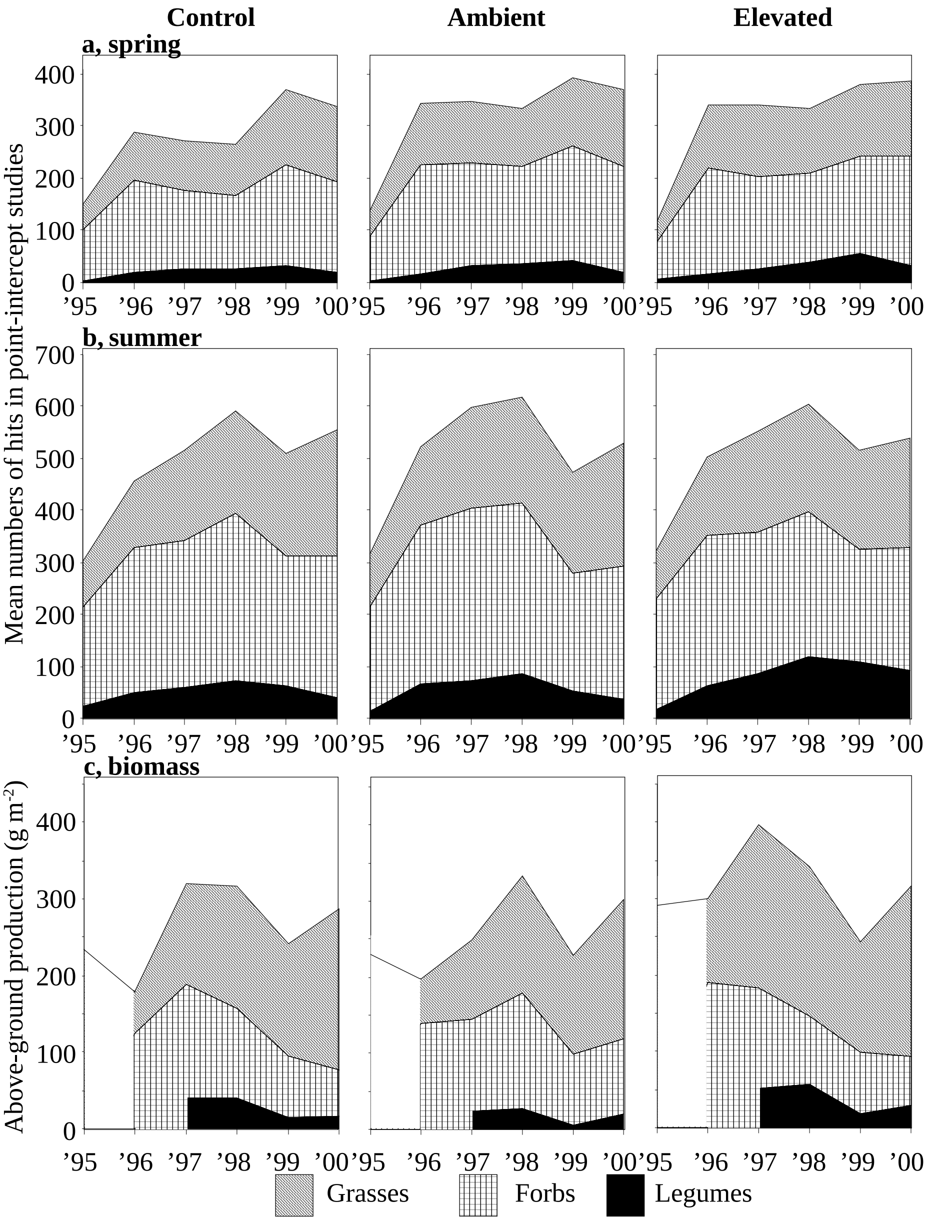


Fig. 24. Vegetation composition in OTCs at ambient and twice ambient CO_2 concentrations and in controls without OTC, divided into the main functional groups and measured as numbers of hits in point-intercept studies (a, spring; and b, summer) and as dry above-ground biomass (c, at harvest in early August).

Concluding remarks

In this thesis I have studied the direct effects of elevated CO_2 on plants in a semi-natural grassland ecosystem over the course of six growing seasons. Elevated CO_2 was found to affect the system throughout the experiment, but the magnitude and direction of the effect differed between different levels of system complexity.

On the leaf level, I found a stimulation of photosynthesis that persisted throughout the experiment.

Plant biomass production was stimulated by elevated CO_2 in all years but one, the exception being a year in which there was no water deficit during the growing season. Initially the extra production took place mainly above ground, but in later years there was a stimulation of roots, but not of above-ground biomass.

Under elevated CO_2, vegetation became less diverse in spring, yet more diverse in summer. The CO_2 effect on vegetation composition increased during the last years of the experiment. On the level of functional groups, however, only legumes seem to be (marginally positively) affected.

Although CO_2 effects may be large (above-ground biomass 1997) or small (vegetation composition), persistent (leaf-level processes; possibly below-ground biomass and vegetation composition) or transient (possibly above-ground biomass), it is clear that they play a limited role when compared to weather effects. That is, they play a limited role on a short time scale. Yet on a longer time scale, small but persistent effects may become more important than these short-time weather effects, since the CO_2 changes directionally over time, whereas the weather fluctuates.

But does it really fluctuate randomly?

I have studied only the direct effects of a change in atmospheric carbon dioxide, that is, only one of the aspects of global change. I have not studied the effects of climate change. Most scientists agree that the climate is changing, and many attribute this to the rise in carbon dioxide, among other things. Whether or not carbon dioxide or other anthropogenic factors are its cause, climate change will probably affect ecosystems such as the one I studied much more dramatically than carbon dioxide will do directly.

This makes it extremely difficult to predict future vegetation or plant production. Ideally, we would use climatic variables predicted by an AOGCM (see page 7) to manipulate the weather in experimental plots in order to resemble the weather at the CO_2 level of interest, taking into account that also the amount of climatic variation might or might not change. But in order to get reliable climatic responses from the climate models, plant responses should ideally be included in these models. After all, plants have a large regulatory effect on the weather, and on the atmosphere.

In other words, science has yet a long way to go. I hope this thesis has contributed with one small step. It has been a giant leap for a man, though.

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In his influential work *Plant Strategies and Vegetation Processes* (1979), Grime categorised plant strategies this way:

Intensity of disturbance	Intensity of stress	
	Low	High
Low	Competitors	Stress-tolerators
High	Ruderals	No viable strategy

At SLU, scientists, like plants, live in a changing and highly unpredictable environment. With stress being reciprocal to the amount of resources and disturbance being an inherent part of every reorganisation, it seems to me that the people at our department find themselves, in this analogy, in the lower right-hand cell where plants would not survive. And as if that wasn't enough, they have to put up with graduate students such as I.

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Photo: Mark Marissink

... och så är ingen avhandling komplett utan åtminstone en rejäl groda...

Appendix A: species list

Species (sorted after # summer occurrences)	**Hits/plot** (summer, 1): spring)	**Species** (sorted after # summer occurrences)	**Hits/plot** (summer, 1): spring)
Agrostis capillaris	71.8	Saxifraga granulata *	1) 2.7
Poa pratensis **	1) 50.2	Dianthus deltoides *	1.1
Festuca rubra	46.0	Cerastium fontanum	1.0
Trifolium repens	42.9	Luzula campestris	1) 1.5
Stellaria graminea	41.3	Carex spicata	0.9
Achillea millefolium	41.3	Potentilla erecta	0.8
Taraxacum	1) 41.2	Trifolium medium	0.6
Carum carvi	31.0	Potentilla argentea	0.4
Plantago media *	27.9	Dactylis glomerata **	0.4
Leontodon autumnalis	16.4	Festuca pratensis **	0.4
Lotus corniculatus	16.0	Pilosella officinarum	0.3
Galium verum	12.6	Hieracium umbellatum	0.2
Ranunculus bulbosus *	1) 21.3	Myosotis stricta	1) 2.3
Alopecurus pratensis	9.7	Phleum pratense **	0.2
Rumex acetosa	7.7	Galium boreale	0.1
Ranunculus auricomus	6.5	Allium oleraceum	0.1
Centaurea jacea *	5.9	Sedum acre	0.0
Ranunculus acris	4.6	Anthriscus sylvestris **	0.0
Veronica chamaedrys	3.9	Pimpinella saxifraga *	0.0
Trifolium pratense	2.3	Erophila verna	1) 4.9
Alchemilla monticola	2.3	Veronica verna	1) 1.3
Plantago lanceolata	2.3	Gagea lutea	1) 0.4
Veronica serpyllifolia	1.5	Gagea minima	1) 0.3
Filipendula vulgaris *	1.4		

* Indicator species for long-term cultivation and absence of fertiliser (Larsson & Ekstam 1987)

** Species that, when occurring in large numbers, indicate N-fertilisation (Naturvårdsverket 1987)

1) Species with higher occurrences in spring than in summer; spring occurrences given here.