Impacts on plant communities by elevated CO_2 concentration

Master's thesis, 20 credits.

by

Miia Liisa Persson

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Preface

This master's thesis, 20 p, completes my master's degree in biology and environmental protection within the programme of natural resources at the Swedish University of Agricultural Sciences in Uppsala. Commissioned by Elforsk AB in Stockholm, the thesis was written during the spring of 2005 in cooperation with the Department of Environmental Assessment at the Swedish University of Agricultural Sciences in Uppsala. The subject falls under Elforsk's investigation of the possibility to store carbon dioxide under ground in deep saline aquifers in the future. My task was to find plant indicators on elevated carbon dioxide concentration. Possible plant indicators could then be used in detecting leakages of stored carbon dioxide.

It's been very interesting and exciting to work with this thesis. The subject is closely related to the problems we have today with increasing emissions of greenhouse gases and the reality of these problems has been with me during the whole process of this thesis. The feeling of being apart of something and to make a difference gives you even greater motivation to do your best. With this I would like to give a great thank to Elforsk AB for giving me the chance to do this thesis and for sponsoring my trip to Italy where the vegetation analysis was done. I would also like to thank Ulf Grandin for giving me help and guidance when ever I needed to and Federico Selvi at the University of Florence for helping me with the plant identification. Franco Miglietta at IBIMET-CNR in Florence, who came up with the research area, arranged the accommodation and warned me about the poisonous snakes, thank you.

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Abstract

Our climate is in change. Carbon dioxide (CO_2) levels this high, as the present CO_2 concentration ($\approx$370 ppm), have not been seen for the past 420 000 years. The cause for this is a combination of industrial CO_2 emissions, the burning of fossil fuels and emissions arising from land use changes, such as deforestation and cultivation of virgin lands *etc*. An approach to this problem could be storage of CO_2. Deep saline aquifers have the greatest storage potentials in the Nordic countries. Yet, it will probably take one or two decades before the technique for storage of CO_2 will be applicable in a greater extent in Europe. A condition for storage of CO_2 is to find storage places that guarantee that eventual leakage will have no essential significance in a time period of a couple of hundred years.

The aim of this thesis is to find plant indicators on elevated CO_2 concentration that could be used in detecting leakages of stored CO_2. A comparison was done between plant communities near a CO_2 spring in Italy that were exposed to elevated CO_2 concentration and plant communities in a control area with ambient CO_2 concentration regarding the percentage cover of vascular plants. Ellenberg indices of soil pH (R), light (L), soil moisture (F) and nitrogen content (N) were used to estimate and compare the environmental conditions and vegetation preferences of the two study areas. Shannon-Wiener- and evenness indices were calculated for the two study areas. Ordination method, Correspondence Analysis (CA), was used to illustrate differences, similarities and gradients in the collected data material.

There were no significant differences between the CO_2 area and control area regarding cover or Shannon-Wiener- and evenness indices. However, there were significant differences in Ellenberg indices for soil pH and nitrogen content between the CO_2 and control area. The ordination showed a strong correlation with the first axis in the CA-diagram and the F, N and R-indices, consequently creating a gradient between them. Although some significant differences were found between the areas, the general conclusion is that no clear differences can be found between an area with elevated CO_2 concentration and another with ambient CO_2 concentration when looking at the vegetation composition alone.

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1 Introduction

Our climate is in change. The mean surface temperature of the earth has increased with 0.6°C the past century (Sindhöj 2001). Studies show a rising of the sea surface and a worldwide reduction of glacier extent. Heavy rainfalls and extreme weather conditions are becoming more and more common in some parts of the world. Much of this is believed to be a consequence of the rising emissions of greenhouse gases we experience today. Carbon dioxide (CO_2) is considered to be the most important greenhouse gas due to its relative abundance. Atmospheric CO_2 has increased about 30% in the last 200 years and will likely continue to rise in the near future. This is largely due to a combination of industrial CO_2 emissions, the burning of fossil fuels and emissions arising from land use changes, such as deforestation and cultivation of virgin lands *etc*. Although CO_2 levels have varied considerably throughout history, levels this high, as the present CO_2 concentration ($\approx$370 ppm), have not been seen for the past 420 000 years (Sindhöj 2001).

Greenhouse gases absorb infrared radiation (heat) emitted from the earth, that otherwise would radiate back into space. In other words, CO_2 works roughly in the same way as the glass in a greenhouse. Without the natural greenhouse effect of the atmosphere, life on earth would be impossible – with a surface temperature almost 35°C colder than it is today (http://www.internat.environ.se/documents/pollutans/climate/vaxthuse.html). Today's rising of the concentration of greenhouse gases is intensifying the greenhouse effect and, as mentioned above, will affect the heat-balance at the earth's surface.

The concern about the rising atmospheric CO_2 concentration along with the many predictions of climate change, led the UN Framework Convention on Climate Change to draft the Kyoto Protocol in 1997. This was the first global effort to address the climate change by reducing national greenhouse gas emissions, in particular CO_2, to levels existing prior to 1990. This means a reduction of 5.2 % for the industrialized countries (Gode et al. 2004). The Kyoto Protocol came into force the 16th of February 2005. The first obligation period extends until 2012 and is only a first step towards a sustainable climate. After this, higher demands have to be set for the emission reductions to be able to prevent a global warming.

1.1 Storage of CO_2

A reduction of emissions of CO_2 can be achieved either by reducing emissions (sources), or by increasing activities that absorb CO_2 (sinks), *e.g.*, planting trees. There is also a completely different approach to this problem: storage of CO_2.

In 1991, IEA (International Energy Agency) started a research and development programme about greenhouse gases – IEA Greenhouse Gas Research and Development Programme (IEA GHG). The main activities within this programme concerned methods for reduction of greenhouse gases, especially CO_2. An area that has been given a lot of time is storage of CO_2.

Sweden is represented in IEA by STEM, Statens energimyndighet, the energy authority of the state. In November 2001, STEM turned to Elforsk AB with an inquiry of cooperation with the energy sector about IEA's research of CO_2. The aim of the project was to give knowledge about how IEA's research of CO_2 storage was developing and to look at the possibilities of CO_2 storage in the Nordic energy system.

Deep saline aquifers (geological formations enclosed in the bedrock filled with brackish- or saline water) have the greatest storage potentials in the Nordic countries. The CO_2 is preferably stored as a supercritical fluid, which means that the CO_2 almost have the qualities of a fluid. Several aquifers are known within or near the Nordic countries. One aquifer is spreading from south-west of Sweden to northern Germany and another one from south-east of Gotland towards Kaliningrad, Russia. Eight aquifers have also been identified in Denmark and Norway.

It will probably take one or two decades before the technique for storage of CO_2 will be applicable in a greater extent in Europe. A condition for storage of CO_2 is to find storage places that guarantee that eventual leakage will have no essential significance in a time period of a couple of hundred years. The storage of CO_2 will also have to be at a reasonable cost and consistent with national and international laws and conventions. It is of great importance that this technique is environmentally sustainable and that the acceptance of the public is carefully investigated.

2 Background and literature review

2.1 Direct effects of elevated CO_2 concentration on plants

"*Every beginning biology student knows that photosynthesis will increase if you give a plant a "squirt" of CO_2 – given enough light, nutrients, water and suitable temperature. Logic tells us that if this is so, then more CO_2 in the atmosphere should mean more photosynthesis. This, in turn, should mean more yields or accumulated carbon in plants. This logic is fine for beginning biology; unfortunately, nature is not that simple*" (Norby et al. 2001).

The key process to understand when studying plant responses to elevated CO_2 is photosynthesis. In photosynthesis plants use sunlight 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). Small fractions 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 (Figure 1).
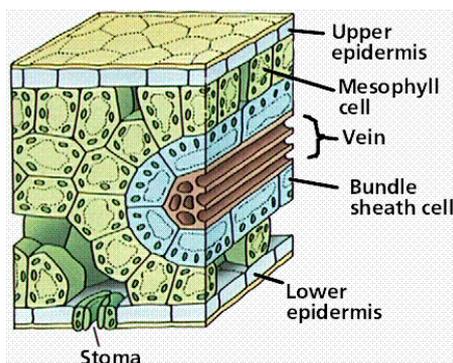
Figure 1. Cross section of a C_4 leaf (http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPS.html).

Although this pathway requires extra energy, it is more efficient than the C_3 pathway in fixing CO_2 at high temperatures and C_4 plants are thus able to capture CO_2 with minimal water loss (Marissink 2002). Since the rate of photosynthesis is saturated at prevailing ambient CO_2 conditions in C_4 plants, they generally respond less to elevated CO_2 concentrations than do C_3 plants (Lambers et al. 1998). 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. In the CAM photosynthetic pathway CO_2 fixing and the remaining photosynthetic processes are separated in time rather than in space, with fixing taking place at night (Marissink 2002).

Increasing CO_2 concentrations directly affects photosynthesis in several ways. One effect 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 (Marissink 2002). Normally rubisco operates at only about 25% of its capacity. Because of this, the rate of photosynthesis is very responsive to increases in CO_2 concentrations. At the same time, because O_2 competes with CO_2 for active sites on rubisco, higher CO_2 concentrations lead to decreased photorespiration and increased net carbon assimilation (Sindhöj 2001).

The capture of CO_2 by plants is usually associated with loss of water because both occur through the stomata (Figure 1). Stomatal opening and closing is affected by a number of environmental factors such as water availability, light, temperature and CO_2 concentration. The stomata generally close when exposed to elevated CO_2, which subsequently decreases the transpiration stream of water from the plant. Such stomatal closure has long been understood to decrease transpiration rates and improve plant water use efficiency, *i.e.*, loss of water relative to photosynthesis, leading to increased biomass production when water is limiting (Sindhöj 2001).

Plants grown under elevated CO_2 concentrations also show a trend of decreased tissue nitrogen concentration, particularly in the leaves. This decrease in nitrogen content per unit biomass suggests that elevated CO_2 concentrations increases plants nitrogen use efficiency, which is the rate of photosynthesis per unit leaf nitrogen. This is thought to be due, in part, to the increased efficiency of rubisco. Less rubisco is required at elevated CO_2 concentrations, this result in an increased efficiency with less amount and activity of rubisco. Since rubisco is a protein that normally accounts for 25-30% of the leaf nitrogen, this increased efficiency of rubisco is a major component of the lower nitrogen observed in the leaves (Drake et al. 1997). The decreased tissue nitrogen concentration is also partly due to an increased leaf concentration of total non-structural carbohydrates (TNC) under elevated CO_2 concentrations. The end result though, is the trend that plants grown under elevated CO_2 concentrations have higher C/N ratios (Sindhöj 2001).

There is another response of plants to elevated CO_2 concentrations that has a great potential impact on plant productivity. Plants balance their allocation of biomass to optimize their growth potential. When carbon is limited they allocate more material to leaves to capture more carbon. If nutrients are limiting they allocate more material to roots to "mine" more of these limiting nutrients. These allocation shifts have considerable consequences for plant

production. Increases in carbon uptake, as would occur under enhanced CO_2 concentrations, would result in increased growth underground, but not aboveground. It may be the case, however, that CO_2 levels expected for the next century may exceed plant capacity to effectively achieve these adjustments in allocation (Mooney et al. 1994).

Plants eventually show an acclimation of photosynthesis after long-term exposure to elevated CO_2 concentrations in most studies. This is partly because the plant cannot handle all the carbon provided (the sink strength is limiting photosynthesis) and partly because the plant produces relatively less rubisco or rubisco activity is lower (Marissink 2002). Thus, this down-regulation of photosynthesis following long-term CO_2 enrichment has been linked with low nutrient availability (particularly nitrogen) and restricted root growth (Drake et al. 1997). However, in a study with plants from a CO_2 spring with nutrient supply and ample rooting volume, there was no evidence of a down-regulation of photosynthesis or a decline in rubisco content (Fordham et al. 1997).

The studies cited in the paragraphs above have led to further numerous investigations on how plant growth will be affected under an elevated CO_2 environment. Some studies have shown that the increased photosynthetic rate and water use efficiency observed in plants under elevated CO_2 concentrations often results in increased growth rates and biomass production (Bazzaz 1990). However, the response is species-specific and depends on, among other things, temperature, light, water and nutrient availability. There are many processes involved and the resulting picture, especially at a community level, is far from clear yet.

2.2 Effects of elevated CO_2 concentration on a community level

For two decades, scientists have been studying how elevated atmospheric CO_2 concentrations will alter plant growth. These studies are largely based on the responses of individually grown plants, but plants seldom grow in isolation in nature. They are rather members of natural or managed communities. Plants interact with their neighbours through the use of shared resources such as light, water, nutrients and pollinators, and through the modification of local microclimatic conditions such as humidity, temperature and wind speed. As plants grow and accumulate resources, they directly reduce the amount of resources available to neighbouring plants. As plants alter microclimate, they indirectly influence neighbours' resource acquisition and growth. The complex interactions among neighbouring plants, between plants and environmental conditions (resource availability and microclimate) have not yet been clearly resolved. Therefore, it is very hard to understand and predict how elevated CO_2 concentrations will effect plant-plant interactions and the structure and function of plant communities (Bazzaz et al. 1992).

As plants photosynthesise they deplete the CO_2 concentration at the leaf surface. These areas of depletion tend to be localised, as long as the mixing between the bulk atmosphere and the area around the leaf is adequate. Under these conditions, plants have little effect on the availability of CO_2 to the leaves of neighbouring plants. Thus, while plants may respond to CO_2 availability, they may not generally experience changes in CO_2 availability as a result of the activities of neighbours. Within dense stands of vegetation, however, there may be considerable resistance to gas exchange from the bulk atmosphere to individual leaf surfaces, and sub ambient CO_2 concentrations have been measured within dense stands. Increasing atmospheric CO_2 concentrations will not have the same effects on individually grown plants and dense stands, due to increased resistance to CO_2 diffusion to leaf surfaces within a complex stand. Furthermore, decreased airflow within a stand not only reduces the

rate of delivery of CO_2 to leaf surfaces but also the rate of water vapour loss from leaf surfaces. Although C_4 plants in dense stands may not become CO_2 limited, they might benefit from improved water vapour gradients within a dense canopy (Bazzaz et al. 1992).

Generally, studies show increased community level productivity and changes in community composition under elevated CO_2 atmospheres. The case is often that for C_3 species within a community, biomass increases as the CO_2 concentration increases. Conversely, the biomass of C_4 species is largely unaffected over broad gradients of light, soil moisture and nutrients. The result is that elevated CO_2 atmospheres under most environmental conditions increased C_3 species' representation within the community relative to that of C_4 species (Bazzaz et al. 1992).

2.3 Effects of elevated CO_2 concentration on different plant functional groups

Because of the great variability in CO_2 responsiveness of species and their genotypes it is rather difficult to make predictions for responses of complex plant assemblages. One way would be to group species into functionally meaningful groups of species with similar responses. One group is the nitrogen fixing species, often legumes. The responses to elevated CO_2 concentrations often depend upon the nutrient availability, and many studies have shown that nitrogen fixing species usually have a stronger biomass response to elevated CO_2 concentrations than non fixing species (Reich et al. 2001). Yet little work has been done on wild species at naturally low levels of nitrogen availability. The nitrogen fixation is controlled by water and phosphate availability and carbohydrate supply to nitrogen fixing bacteria. However, even under dry conditions legumes have been shown to profit from increased nitrogen fixation rates since CO_2 enrichment increases drought tolerance of legumes (Grünzweig et al. 2003).

Differences in responses to elevated CO2 concentrations between families like grasses and legumes have seldom been compared directly. Experiments under controlled conditions have shown that monocots are generally less stimulated than dicots. However, differences between grasses and legumes have been less clear *in natura*. C_3 grasses were more stimulated than legumes in Mediterranean grassland, tall grass prairies and alpine grassland, whereas no clear differences in responses to elevated CO_2 concentrations were found between plant types in calcareous grassland (Navas et al. 1997). Plants tend to show a functional equilibrium response in the sense that they reduce the site or amount of structures that produce a surplus of resources. Green-leaf fraction is reduced at elevated CO_2 concentrations because of excessive production of non-structural carbohydrates (TNC). Enhanced development and earlier flowering is common in dry ecotypes of annuals and can be an advantage in seasonally dry grasslands because more time and water can be used for grain filling (Grünzweig et al. 2003). Legumes have shown to be more branched under elevated CO_2 concentrations, despite a clear acceleration of senescence. The fruit and seed production were also slightly enhanced for the legumes. However, wild species from nutrient-poor ecosystems do not show such consistent responses to elevated CO_2 concentrations (Navas et al. 1997).

A variety of hypotheses have been developed about potential functional group differences in response to elevated CO_2 concentrations. One is that the intrinsic differences in photosynthetic biochemistry should lead to markedly greater responsiveness to elevated CO_2 concentrations for C_3 than C_4 plants. Another is as mentioned above; productivity in nitrogen fixing legumes may be more stimulated by elevated CO_2 concentrations than in non fixers, because the former should be less nitrogen limited. A series of related hypotheses

posit that increases in CO_2 or nitrogen supply should lead to a more pronounced growth increase in species of given strategies, habitats or growth rates. For example, C_3 grasses which are considered more disturbance adapted and nitrophilic should respond more to increase in CO_2 supply than C_4 grasses (Reich et al. 2001).

What complicates things is that there are substantial variations in response among species within groups. Another aspect to consider is the competition for nutrients, water, light *etc.* when plants are grown in mixed communities, since most of the studies and information available are based on monoculture experiments.

2.4 CO_2 springs

One way of providing a more realistic picture of long-term vegetation responses to elevated CO_2 concentrations is to examine specific characteristics or physiological traits in plants originating from a location with naturally elevated CO_2. At these few locations, where atmospheric CO_2 concentrations have been naturally elevated over evolutionary time scales, adaptations to this condition may be best developed. An example of such a location is a geothermal CO_2 spring. The theory behind many CO_2 springs is believed to be volcanic activity. There is a thermal decomposition of sedimentary calcareous bedrocks caused by the very high temperatures at the fractures. This decomposition produces CO_2 that vents to the surface through cracks in the bedrock and enter the atmosphere at openings in the ground, creating a CO_2 spring (Miglietta et al. 1993).

2.5 Aim

The aim of this thesis is to find plant indicators on elevated CO_2 concentration. To be able to find possible plant indicators, a comparison was done between plant communities near a CO_2 spring exposed to elevated CO_2 concentrations with plant communities in a control area with ambient CO_2 concentrations regarding the percentage cover of vascular plants.

3 Material and methods

3.1 Study area

The CO_2 spring, called "I Borboi", is located in the neighbourhood of the village Laiatico, approximately 35 km south-east of Pisa, Italy (Figure 2).
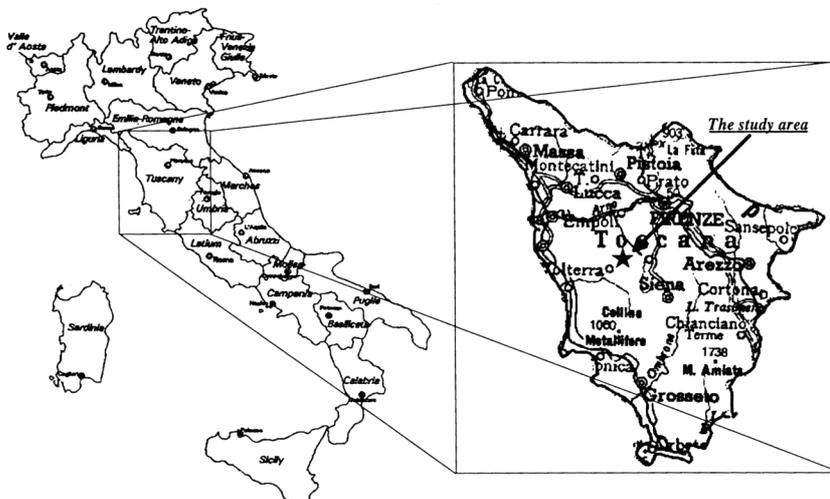
Figure 2. Location of the study area, Pisa, Italy (43º 26'N, 10º 42'E). (Gahrooee 1998).

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The Laiatico area is at 190-240 m above sea level with an annual rainfall of about 830 mm. The rainfall varies with a maximum in October and March and a minimum in July. The average yearly temperature is 15ºC and the max. and min. monthly temperatures are 33ºC and -4ºC respectively (Gahrooee 1998). The CO_2 enriched area extends over 0.7 ha and is situated along a gentle slope of about 40% facing north-west (Tongnetti et al. 2000). Carbon dioxide is emitted naturally at the surface from one major spring and a number of smaller ones 5 m separated along a narrow stream. The CO_2 from the spring is mixed with that from the air above the forest canopy. Figure 3 shows short-term CO_2 measurements at the CO_2 spring done by Gahrooee (1998). The highest CO_2 concentrations were found between 09:00 and 18:00 h with high fluctuations ranging between a CO_2 max of 2815 ppm and a CO_2 min of 209 ppm. The CO_2 concentrations tend to decrease upstream and the daily mean concentration is 795 ± 434 ppm (Gahrooee 1998).
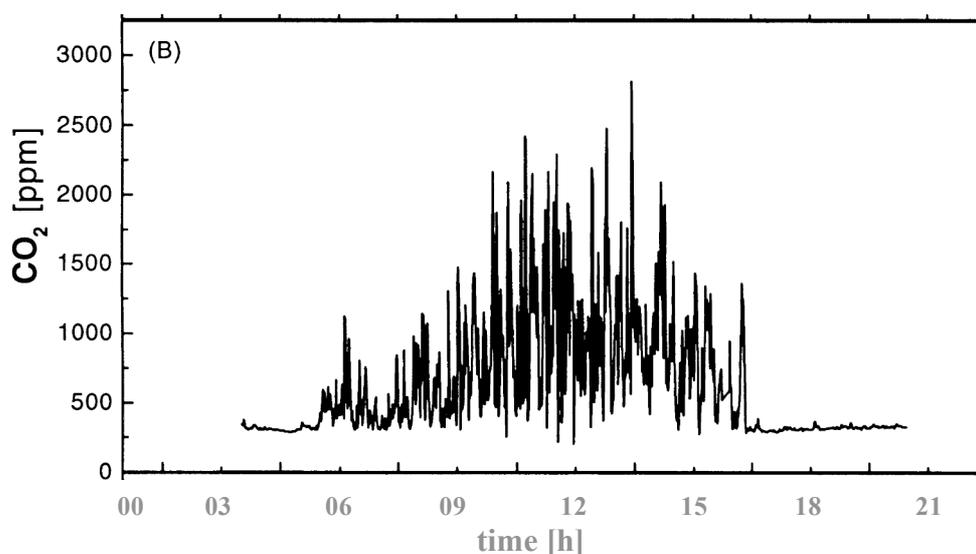
Figure 3. Atmospheric CO_2 gas mixing ratio in the vicinity of the CO_2 spring. CO_2 was measured with an infrared gas analyzer on a clear sunny day every minute continuously over *ca*. 15 h per day. The data set was smoothed by calculation of 5 min means (Gahrooee 1998).

Soils of the area are developed from Tertiary marl and contain a 0.4 to 6.8 cm thick organic forest floor overlaying mineral A and B horizons. The soil pH (measured in H_2O solution) ranges from 6.5 in the topsoil to above 7.5 in the carbonate-rich subsoil. The texture is silty clay loam in the A horizon and silty clay in the B horizon.

The vegetation of the study location is representative of the major types of eu-Mediterranean and sub-Mediterranean ecosystems (Gahrooee 1998). The study area is a typical, semi-natural Mediterranean oak-ash coppice woodland which was last cut about 25 years ago. The main tree and shrub species are *Quercus ilex*, *Q. pubescens*, *Q. cerris*, *Fraxinus ornus*, *Pistacia lentiscus*, *Myrtus communis*, *Arbutus unedo* and *Smilax aspera*. The tree distribution is not uniform and the shrub layer is so dense that passage through the forest is locally difficult to impossible. Open areas in the woodland consist of scattered grass.

The control area was selected about 100 m upstream from the CO_2 spring. The environmental conditions are the same in these surroundings except for the CO_2 concentrations that are ambient.

3.2 Vegetation analysis

The vegetation analysis was carried out around the CO_2 spring in a radius of 25 m at six different plots of 2×2 m. All the plots were located in open areas in the woodland. In each plot three sub-plots of 0.5×0.5 were randomly selected, with the help of a calculator, and analysed regarding the percentage cover of vascular plants in the field layer. The same analyses were made at seven plots of 2×2 m in the control area. The vegetation analysis was carried out in a two week period in April (2-8/4 and 19-27/4). To get comparable results one day was spent around the CO_2 spring and the next day in a control area *etc*. The species identification was assisted by Federico Selvi at the University of Florence.

The proportion of species from the Leguminosae family (legumes = nitrogen-fixing) was calculated for the CO_2 area and the control area to investigate if these species have a stronger biomass response to elevated CO_2 concentrations than non-fixing species. The proportion of monocots and dicots was calculated for the CO_2 area and the proportion of monocots for the control area to investigate if monocots respond less to elevated CO_2 concentration than dicots.

3.3 Ellenberg indices

Ellenberg indices (R, L, F, and N) were calculated for every sub-plot around the CO_2 spring- and control area to be able to estimate and compare the environmental conditions and vegetation preferences of these areas.

Heinz Ellenberg (1913-1997) was a German botanist who published indicator values (Ellenberg values) for about 2720 central-European vascular plants. Each species are ranked from 1-9 for the following ecological factors: light (L), temperature (T), continentality (K), soil pH (R), nutrients/nitrogen (N), and others (salinity, heavy metal resistance). Soil moisture (F) is ranked from 1-12. These Ellenberg values are used to estimate environmental conditions at investigated areas by calculating Ellenberg indices:

$$\text{Ellenberg index} = \frac{\sum (x_i \times Ell_i)}{\sum x_i},$$

where x_i is the cover of species i and Ell_i is the Ellenberg value for species i.

One Ellenberg index of R, L, F and N was calculated for every study plot of 2×2 m in the CO_2 area and in the control area to decrease the heterogeneity of the data material. This was done by summing the percentage cover of plants in the three sub-plots and using that sum as the x_i in the equation. A mean value of the indices from the study plots were then calculated for R, L, F and N in the CO_2 area, and in the control area.

3.4 Shannon-Wiener indices

The diversity, *H*, and evenness, *J*, of the vegetation communities at the CO_2 spring and control area were calculated using the Shannon-Wiener index.

Ecologists use diversity indices to summarise number of species and their cover to one figure. The Shannon-Wiener index is calculated by the equation:

$$H = -\sum p_i \ln(p_i),$$

where H is the Shannon-Wiener index of diversity and p_i is the proportion of each species in the total sample of individuals. A high index value (H) means a high diversity but if the variation in cover between the species is large, the index value will be lower.

Usually an index for evenness (J) is presented together with the diversity index to describe how much the species composition differs from a composition where all the species have the same cover. This index for evenness is calculated by the equation:

$$J = H / H_{max} ,$$

where H_{max} is the highest possible H for the number of species in the area $H_{max} = \ln$ (number of species).

One Shannon-Wiener index and evenness index was calculated for every study plot of 2×2 m in the CO_2 area and in the control area to decrease the heterogeneity of the data material. This was done by summing the percentage cover of plants in the three sub-plots, getting a sum cover for each plot, which were used in the calculations.

3.5 Statistical methods

3.5.1 Multivariate methods

Ordination is a multivariate method that is used to find gradients in extensive data material. The method illustrates both graphically and numerically similarities and differences between samples. Every sample is characterized by a number of variables. In this study, every plant species is a variable. There are different types of ordination methods. Which one to use depends on the data material and the problem to solve. In spite of which method that is used, the end result is a diagram that shows the similarities (samples placed close to each other) and differences (samples placed far from each other) between the samples that are analysed.

The ordination method, Correspondence Analysis (CA), was used to illustrate differences, similarities and gradients in the collected data material. Correspondence Analysis assumes that species have one ecological optimum along each environmental gradient that together constitute the ecological niche for a species.

To identify possible gradients, the correlation, r, between Ellenberg indices and the scores of axis 1, 2 and 3 respectively was calculated.

To test for significant differences in cover, indices, ordination scores and proportion of legumes, monocots and dicots between the CO_2 area and control area, unpaired two-tailed t-tests were used.

4 Results

4.1 Vegetation analysis

In total 63 different taxa were found. Of these 48 were identified to species, two to family, three to genera and ten could not be identified at all although recognised as unique taxa (Appendix). Eighteen taxa out of the total 63 were found only in the CO_2 area, three of these

could not be identified (Appendix). Fifteen taxa were found only in the control area, two of these could not be identified.

There was a significant difference in the proportion of monocots and dicots in the CO_2 area (t-test, t_{11} = 8,89, $p < 0.0001$), with monocots having the highest proportion. There was also a significant difference in the proportion of monocots between the CO_2 area and the control area (t-test, t_{11} = -2.35, $p < 0.05$), with the control area having the highest proportion. There was no significant difference in mean cover or proportion of legumes between the CO_2 area and the control area (t-test$_C$, t_{11} = -1.26, $p > 0.05$, t-test$_L$, t_{11} = -1.33, $p > 0.05$).

4.2 Ellenberg indices

With the help of Ellenberg indices the environmental conditions of the CO_2 area and the control area were predicted (Table 1 and 2). According to the Ellenberg indices the vegetation composition in the CO_2 area indicated a calcareous soil with plants preferring often full light but also up to 70 % shade. The indices for soil moisture indicated soils ranging from dry to well moist and the nitrogen content was in between poor to medium rich.

In the control area the vegetation composition indicated a weak acid – weak basic to calcareous soil with often full light but also shade of 70 % according to the Ellenberg indices. The species indicated a soil moisture that was ranging from medium wet to dry and the nitrogen content of the soil was poor (Table 1 and 2).

The R-indices (soil-pH) and N-indices (nitrogen content) differed significantly between the CO_2 area and the control area (Table 1).

Table 1. Mean values, standard deviations and t-tests of Ellenberg indices.

	R-index (std)	L-index (std)	F-index (std)	N-index (std)
CO_2 area	7.9 (0.102)	7.0 (0.006)	5.7 (0.199)	3.9 (0.069)
Control area	7.6 (0.018)	7.1 (0.074)	5.3 (0.041)	3.5 (0.057)
t_{11}	2.75[*]	-0.835[ns]	1.27[ns]	2.73[*]

(* = $p < 0.05$, ns = not significant).

Table 2. Description of Ellenberg indicator values and indices relevant for this study (Ellenberg et al. 1991).

R7	weak acid to weak basic soil, plant never on strong acid soil
R8	often indicate calcareous soil
L7	medium light plant – often full light but also shade of 70 %
F5	plant often on medium moist soil and dry soil, not in wetlands
F6	In between F5 and F7
F7	plant only on well moist soil, not in wetlands
N3	nitrogen poor soil, plant more often on nitrogen poor soil than medium rich soil. Exceptionally on nitrogen rich soil.
N4	in between N3 and N5
N5	indicate medium rich nitrogen soil, plant seldom on nitrogen poor or rich soil

4.3 Shannon-Wiener indices

No significant differences were found in Shannon-Wiener indices and evenness indices between the CO_2 area and the control area (t-test$_{Sh}$, t_{11} = -0.153, p > 0.05, t-test$_{Ev}$, t_{11} = -0.211, p > 0.05).

4.4 Multivariate methods

For the first CA-axis there was a significant difference between scores of the sub-plots in the CO_2 area and the control area (t-test, t_{34} = -2.64, p < 0.05). The lower ranked axes did not differ significantly (t-tests, p > 0.05), although representing strong floristic gradients as indicated by high eigenvalues for both axis 2 and 3 (Figure 5).
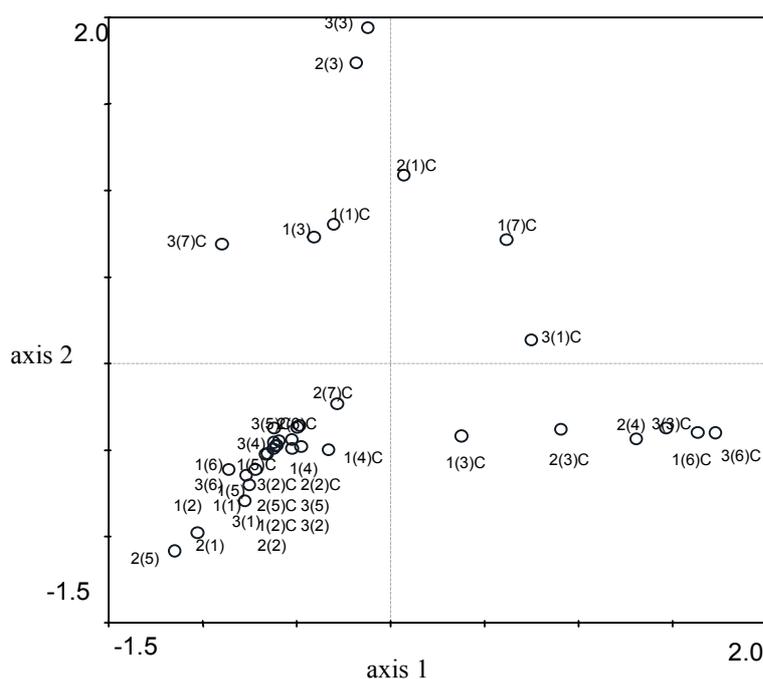
Figure 5.CA-diagram showing sub-plots. The numbers x(y) represent sub-plot (x) and plot (y) in the CO_2 area and x(y)C represent sub-plot and plot in the control area, *e.g.* 1(3)C is the first sub-plot in the third plot in the control area. Eigenvalues (expressing how much of the total variation that is explained by an axis) axis 1 = 0.600, axis 2 = 0.562 and axis 3 = 0.433.

There was a strong negative correlation between axis 1 and the F, N and R-indices (Table 3). Consequently, there is a gradient along the first axis that is strongly affected by first of all soil moisture (F), nitrogen content (N) and then soil pH (R). To see the width of the gradient the max and min values of the Ellenberg indices are presented in Table 3. Since the correlations were negative, sub-plots with high indices values are placed to the left in the CA-diagram. In total sub-plots in the CO_2 area have higher F- and N values, with only one sub-plot, 2(4), far to the right in the CA-diagram (Figure 5). The R-values are also generally higher to the left where most of the CO_2 sub-plots are. Along axis 2 there is a weak negative correlation with the R-indices (Table 3), where sub-plot 3(3) has the lowest R-value (Figure 5). The third axis showed no significant correlation with the Ellenberg indices.

Table 3. Correlation values for axis 1, 2 and the Ellenberg indices. Max and min values of Ellenberg indices.

	axis 1_r	axis 2_r	min Ell-ind	max Ell-ind
R	-0.737^{***}	-0.393^{*}	6.7	8.1
L	-0.319^{ns}	-0.218^{ns}	6.9	8.2
F	-0.810^{***}	-0.139^{ns}	4.0	6.0
N	-0.776^{***}	-0.306^{ns}	3.0	4.2

($*$ = $p < 0.05$, $***$ = $p < 0.0001$, ns = not significant)

5 Discussion

The aim of this thesis was to find plant indicators for elevated CO_2 concentration. Significant differences between the CO_2 area and the control area and possible plant indicators are discussed below.

5.1 Vegetation analysis

Drake (1997) is only one of many scientists who states that enhanced CO_2 concentrations increases the photosynthetic rate and water use efficiency which often results in increased growth rates and biomass production. This doesn't seem to be the case in the CO_2 area I studied since there were no significant differences in vegetation cover between the control area and CO_2 area. However, one thing to keep in mind is that plant responses to elevated CO_2 concentrations are species-specific and depends on, among other things, temperature, light, water and nutrient availability. Since most of the studies on plant responses to elevated CO_2 concentrations are carried out in chambers with controlled conditions and on monocultures, the resulting picture, especially on a community level, is far from clear yet. Another explanation for the lack of plant responses considering increased photosynthetic rate, could be the acclimation of photosynthesis that most studies show after long-term exposure to elevated CO_2 concentrations according to Marissink (2002).

The responses to elevated CO_2 concentrations often depend upon the nutrient availability, and many studies have shown that nitrogen fixing species (often legumes) usually have a stronger biomass response to elevated CO_2 concentrations than non fixing species (Reich et al. 2001). However, in this study there were no significant difference in the cover of legumes between the CO_2 area and control area. Yet little work has been done on wild species at naturally low levels of nitrogen availability and since the plants in my analysis indicated rather poor nitrogen levels it could be an explanation. However, one interesting observation was done regarding the presence of legumes in the CO_2 area. Five out of the six legumes that were present in the CO_2 area were found in the third plot that is located downstream of the spring. In Gahroee's (1998) site description of the CO_2 spring used in this analysis, he mentions that the CO_2 concentrations seem to increase downstream. If this is true, plot 3 is exposed to the highest CO_2 concentrations since it is the only study area downstream of the spring, and it makes the dominating presence of legumes in this area a possible plant response of elevated CO_2 concentration. However, since this was the only observation of dominating presence of legumes in the CO_2 area it can't be stated as a plant indicator of elevated CO_2 concentration.

Experiments under controlled conditions have shown that monocots are generally less stimulated than dicots (Navas 1997). In the present study, there was a lower proportion of monocots in the CO_2 area than in the control area, which could be a sign of less stimulation. If you compare monocots and dicots in the CO_2 area there was no sign of less stimulation of

monocots as these were dominating. However, the CO_2 area and control area are grasslands and a comparison between monocots and dicots could be misleading.

5.2 Ellenberg indices

Significant differences were found in Ellenberg indices between the two study areas. The vegetation composition indicated that the soil pH and nitrogen content were higher in the CO_2 area than in the control area. Plant responses on elevated CO_2 concentrations are dependent on nutrient availability (Reich et al. 2001) and a biomass increase (*i.e.* increase in cover) could be expected in the CO_2 area with its higher nitrogen content. However, even if the predicted nitrogen content in the soil was higher in the CO_2 area than in the control area, the nitrogen content was probably still too low to induce plant responses.

Soil pH is a result of the combined activities of soil organisms and abiotic soil chemical processes. In alkaline soils, like the one in the CO_2 area, there is an abundance of base cat ions, particularly calcium (Ca). The dominance of Ca, coupled with the tendency for alkaline soils to absorb CO_2 (g), results in the precipitation of $CaCO_3$ (calcite). Although in general, the presence of calcite in a system will support an alkaline soil solution, greatly elevated activity of CO_2 in the atmosphere can lead to acidic soil solutions (Essington 2004). The subsoil in the CO_2 area is carbonate-rich and has a higher soil-pH than the control area. However, the vegetation composition in the plot with the highest exposure of elevated CO_2 concentration, plot 3 in the CO_2 area, indicates the lowest soil-pH of all plots (Gahrooee 1998). According to Essington (2004) this could be a response of elevated CO_2 concentration, yet no indicator since this was the only observation of lower soil pH in the CO_2 area.

5.3 Multivariate methods

The high eigenvalues of the ordination showed that the three first axes were all important for describing differences in vegetation composition between the samples. However, as only the first axis showed a significant difference between the CO_2 area and the control area, it can be concluded that there are other gradients than the once considered in this study that are important for the distribution of species between the plots. The distribution of sub-plots along the first axis was partly explained by the Ellenberg indices. This axis was correlated with the F, N and R-indices, indicating clear gradients. However, inspections of the range of the considered Ellenberg indices show that the gradients are narrow, spanning about 1 unit.

There was no significant difference in soil moisture (F) between the CO_2 area and the control area, yet the ordination shows the strongest gradient with the F-index. This also indicates that there are other factors that influence the gradient more than the relation CO_2 area vs. control area.

The weak but significant correlation between axis 2 in the ordination and the R-index seems to be controlled by the third sub-plot in plot 3 (3(3)) in the CO_2 area. Sub-plot 3(3) is an outlier with vegetation composition indicating the lowest R-index and when 3(3) is removed from the calculations, the correlation between axis 2 and the R-index loses its significance.

6 Conclusion

The conclusion of this thesis is that no clear differences could be found between an area with elevated CO_2 concentration and another with ambient CO_2 concentration when looking at the vegetation composition alone. Yet, some interesting results were found in one of the study plots in the CO_2 area. This plot had high amounts of legumes and low soil pH although the highest exposure of CO_2. These results could not be stated as indicators but possible responses on elevated CO_2 concentrations since no similar results were found in the other plots in the CO_2 area. Another possible response was the lower stimulation of monocots in the CO_2 area compared to the control area.

Many studies show plant responses on elevated CO_2 concentrations, but on a physiology level and often on monocultures. To be able to predict plant responses on the elevated CO_2 concentrations we experience today, there has to be more studies done under uncontrolled conditions with natural vegetation. Environmental factors like the supply of water and nutrients are never controlled out in the field and plants very seldom grow in monocultures. Plant communities are very complex, and aspects that have to be taken in consideration when predicting responses to elevated CO_2 concentration are *e.g.* variations in response among species within the community and the competition for nutrients, water, light *etc*.

7 Future perspectives

Considering the detection of leakage of stored CO_2, I would like to seize the opportunity to mention another method that can be used for this purpose. This method, suggested by Franco Miglietta at the Institute for Biometeorology of the Italian National Research Council (IBIMET-CNR), uses stable carbon isotopes to detect leakage of stored CO_2.

The carbon on earth exists in three different isotopes: C^{12}, C^{13} and C^{14} whereas C^{12} and C^{13} are stable and C^{14} is unstable (radioactive). The current density (δ) of ^{13}C of the CO_2 in the atmosphere is -8 ‰ and the $\delta^{13}C$ of fossil fuels is about -30 ‰ (Miglietta 2005, pers.com). If you were to store CO_2 under ground, the $\delta^{13}C$ would be -30 ‰ since the CO_2 is from fossil fuels. Plants growing above the storage area experience a $\delta^{13}C$ of -8 ‰ in the atmosphere around them, but if there were to be a leakage of CO_2 from the storage below, the $\delta^{13}C$ in the atmosphere around the plants would increase since the CO_2 from the leakage mixes with the atmospheric CO_2. Under ambient CO_2 concentrations the $\delta^{13}C$ in plants is -28 ‰ (Miglietta 2005, pers.com) but if there is an increase of $\delta^{13}C$ in the atmosphere around them the $\delta^{13}C$ within the plants will also increase and indicate leakage. A mass spectrometer is used to measure the $\delta^{13}C$ of a plant which basically burns the plant sample, *e.g.* a piece of leaf, and then separates the isotopes (C^{12}, C^{13}) in the CO_2 with a magnet. To measure the $\delta^{13}C$ of a plant you only need about 1-2 mg of tissue which you dry and send to a laboratory to a cost of 10 $ per sample. To read more about stable carbon isotopes, visit this website: http://www.biology.duke.edu/bio265/sga/index.html

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8.2 Interview

Franco Miglietta at IBIMET-CNR, Institute for Biometeorology of the Italian National Research Council, 2005-04-14, Florence, Italy

8.3 Internet

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http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPS.html

http://www.internat.environ.se/documents/pollutans/climate/vaxthuse.html

9 Appendix

9.1 Species list in total

Lichens	Mosses	Vascular plants
Cladonia convoluta	*Campylium chrysophyllum*	*Astragalus monspessulanus*
Cladonia rangiformis	*Ctenidium molluscum*	*Brachypodium rupestre*
	Fissidens taxifolius	*Bromus sp.(erectus)*
	Pleurochaete squarosa	*Carex flacca (glauca)*
	Pseudoscleropodium	*Centaurea jacea*
	purum	*Centaurium erythraea*
	Trichostomum crispulum	*Coronilla minima*
		Dorycnium pentaphyllum
		Genista januensis
		Genista pilosa
		Gladiolus communis
		Globularia bisnagerica
		Hedysarum coronarium
		Hieracium pilosella
		Hieracium piloselloides agg.
		Hippocrepis comosa
		Juniperus communis
		Lathyrus aphaca
		Leontodon autumnalis
		Leucanthemum vulgare
		Ligustrum vulgare
		Lolium sp.
		Molinia arundinacea
		Myrtus communis
		Ophrys sphecodes agg.
		Orchidaceae
		Orchidaceae
		Phillyrea latifolia
		Pistacia lentiscus
		Poa sp.
		Polygala vulgaris
		Potentilla hirta
		Prunella vulgaris
		Rubia peregrina
		Scabiosa columbaria agg.
		Senecio erucifolius
		Sherardia arvensis
		Smilax aspera
		Stachys officinalis
		Teucrium chamaedrys
		Teucrium montanum
		Teucrium polium spp.capitatum
		Thymus longicaulis
		Trifolium ochroleucon
		Viola alba

9.2 Species only in the CO₂ area

Mosses	Vascular plants
Fissidens taxifolius	*Centaurium erythraea*
	Dorycnium pentaphyllum
	Hieracium pilosella
	Juniperus communis
	Lolium sp.
	Molina arundinacea
	Myntus communis
	Orchidaceae
	Phillyrea latifolia
	Poa sp.
	Scabiosa columbaria
	Sherardia arvensis
	Stachys officinalis
	Viola alba

9.3 Species only in the control area

Vascular plants
Globularia bisnagerica
Hedysarum coronarium
Hippocrepis comosa
Lathyrus aphaca
Leontodon autumnalis
Leucanthemum vulgare
Ligustrum vulgare
Orchidaceae
Pistacia lentiscus
Senecio erucifolius
Teucrium chamaedrys
Teucrium polium spp.capitatum
Trifolium ochroleucon