Effects of Elevated Atmospheric CO_2 on Soil Organic Carbon Dynamics in a Mediterranean Forest Ecosystem

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Effects of Elevated Atmospheric CO_2 on Soil Organic Carbon Dynamics in a Mediterranean Forest Ecosystem

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Propositions

1. Elevated CO_2 has no impact on litter chemistry of Mediterranean oak species experiencing high CO_2 level. Consequently, litter turnover is not affected by high CO_2 in this type of Mediterranean ecosystems.

   this thesis

2. Differences in litter quality of different plant species are more significant for global C and N cycles than impacts of elevated CO_2 on litter quality.

   this thesis

3. Under field conditions, decomposition of high-quality litter is not necessarily faster than that of low-quality litter.

   this thesis

4. Increased C sequestration in soil is more likely to come about through increased plant production than through increased residence of litter to decompose.

   this thesis

5. Because of the slow turnover of carbon in the soil, CO_2 spring sites are better tools for studying the impact of elevated CO_2 on the biogeochemistry of C and N in terrestrial ecosystems than experiments in which CO_2 concentration is manipulated.

   this thesis

6. Pools of elements are more meaningful than concentrations of elements in terrestrial ecosystems.

7. Research on the impact of global change on ecosystems should adhere to the principle “the longer the better” and/or “from cradle to grave”

8. To reduce the risk of global warming, we should minimize CO_2 emissions. At the same time, it would be better to ***green*** our planet and to ***maintain*** (and to increase) its biodiversity.

9. One problem is that when scientists make a single hypothesis, they become attached to it.

10. The statement "Potential effects of climatic change and increased CO_2 level are difficult to predict" by van Breemen and Heal is true.

    van Breemen N. and Heal O.W. (1991) Decomposition and accumulation of organic matter in terrestrial ecosystems: A key processes in biogeochemistry and environmental change. In: van Breemen N. (Ed.) Decomposition and accumulation of organic matter in terrestrial ecosystems: Research priorities and approaches (pp. 1-4). Ecosystems Research Report, 1, Commission of the European Communities.

11. I agree with Körner who stated "Basic research deserves support, even if there is no obvious current application".

    Körner Ch. (1995) Towards a better experimental basis for upscaling plant responses to elevated CO_2 and climate warming. Plant, Cell and Environment 18, 1101-1110.

12. The only real mistake is the one from which we get nothing.

13. The Dutch practice of travelling by bicycle might, to some extent, mitigate CO_2 emissions.

14. Internationally, Wageningen is well-known for its university and agricultural institutes, not for the city itself.

Stellingen behorend bij proefschrift van F.G. Raiesi ***Effects of elevated atmospheric CO_2 on soil organic carbon dynamics in a Mediterranean forest ecosystem***, Wageningen, 23 June 1998.

Fayez Raiesi Gahrooee (1998) **Effects of elevated atmospheric CO_2 on soil organic carbon dynamics in a Mediterranean forest ecosystem.** PhD dissertation, Laboratory of Soil Science and Geology, Wageningen Agricultural University, The Netherlands, pp. 166.

Abstract

Elevated atmospheric CO_2 has the potential to change the composition and dynamics of soil organic matter (SOM) and consequently C and N cycling in terrestrial ecosystems. Because of the long-lived nature of SOM, long-lasting experiments are required for studying the effect of elevated CO_2 on soil organic matter dynamics. Therefore, the study of ecosystems that have been exposed to long-term enhanced CO_2 concentrations is highly desirable for better understanding feedback mechanisms between litter production, litter quality, soil organic matter decomposability and the atmospheric CO_2 level. This work deals with the effect of enhanced atmospheric CO_2 on chemical composition and C and N mineralization in a leaf litter-soil organic matter continuum around a mineral CO_2 spring in a Mediterranean woodland ecosystem. Leaf litter from *Quercus cerris* L., *Quercus pubescens* Willd. and *Smilax aspera* L., and soil samples from the forest floor (F and HA layers) and 0-10 cm mineral soil were taken at elevated and ambient CO_2 concentrations, and analyzed for chemical composition (C, N, lignin, cellulose, polyphenols). C and N mineralization in plant litter and soil samples were determined using litterbag and laboratory incubation methods.

Elevated CO_2 affected neither chemical composition nor elemental ratios of leaf litter. The C mineralization rate during litter decomposition was not affected by elevated CO_2, in accordance with the absence of a CO_2 effect on litter quality. Leaf litter produced at high CO_2 had a higher N mineralization during the initial stage of decomposition period. This difference, however, disappeared at the end of the incubation. *Q. pubescens* had a higher litter quality than *Q. cerris*, and subsequently *in vitro* faster C and N mineralization rates, but litter decomposition under field conditions did not differ significantly between the two species.

Total C contents in the forest floor were higher at elevated CO_2, but not so in the 0-10 cm mineral soil. For the three layers, total N contents and C/N ratios were not affected by elevated CO_2. Total C and N pool sizes in the forest floor were doubled by elevated CO_2, but such effects were not seen in the 0-10 cm mineral soil. The C mineralization rates of the three soil layers of the areas exposed to elevated CO_2 did not differ from those of the areas under ambient conditions. Although N immobilization in the F and HA layers from the elevated CO_2 plots was lower, that of the 0-10 cm A horizon was not affected by high CO_2.

The increase in the organic carbon pool of the forest floor in the absence of an effect of elevated CO_2 on litter quality and decomposability can be explained by increased biomass production under elevated CO_2. Under elevated CO_2 soil N pools also increased, but the rate of N immobilization in forest floor was lower than that under ambient CO_2. This study of long-term CO_2 effects casts some doubt on the common view that elevated CO_2 changes litter quality of plants, and thereby slows down decomposability of litter and N release. Because species composition has a strong influence on C and N cycles than elevated CO_2, effects of increasing atmospheric CO_2 on species composition may be more important to feedbacks between CO_2 concentration and soil organic matter than the CO_2 effect on litter quality of a given species.

***Key-words*:** soil organic matter, climate change, litter quality and decomposability, mineral CO_2 springs, Mediterranean ecosystems.

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Fayez Raiesi Gahrooee

Wageningen, June 1998

To:

Javaher and Maryam
Assadollah and Ali Akbar
Tahereh and Armine
My teachers

Contents

Chapter 1

General Introduction

INTRODUCTION

The global atmospheric CO_2 concentration has been increasing from 280 ppm prior to the Industrial Revolution to 360 ppm at present. The CO_2 level is expected to increase further by at least another 50% in the middle of the 21st century (Goldewijk et al., 1994; IPCC, 1995; Schimel, 1995). The annual increase in the global atmospheric CO_2 concentration is caused by burning of fossil fuel (5.8-6.2 Gt carbon annually) and deforestation (1.5-3 Gt carbon annually), the latter mainly in the tropics. The imbalance between global C sources (i.e. fossil fuel and deforestation) and known sinks (i.e. ocean uptake and atmospheric increase) suggests the presence of an unknown C sink (the so-called "missing CO_2") of about 0.4-4 Gt per year, which is probably trapped in soils and vegetation (Gifford, 1994; Schimel, 1995).

The increased CO_2 concentration and the related increased greenhouse effect is expected to lead to an increase in global temperature of 1.5-4.5 °C by the end of the next century (Mitchell et al., 1990). Therefore, rising atmospheric CO_2 has raised much public concern. Elevated atmospheric CO_2 and global temperature may have many consequences for terrestrial ecosystems, ranging from short-term physiological effects (Eamus and Jarvis, 1989; Poorter, 1993; Luxmoore et al., 1993) to long-term alteration in pools of soil organic matter (SOM) and nutrients (O'Neill and Norby, 1996; Hungate et al., 1997). The processes and feedbacks caused by increased atmospheric CO_2 concentration that may affect the functioning and structure of terrestrial ecosystems, and the global C cycle are summarized in Figure 1.1. The objective of this thesis is to contribute to our understanding of the effects of CO_2 on the dynamics of SOM. In this introductory chapter I will first review the literature concerning this subject, next formulate the research questions addressed, and finally outline the structure of the thesis.

ELEVATED CO_2 AND LITTER QUANTITY

The immediate effect of elevated CO_2 on terrestrial plants may be an increase of net primary production (NPP) and, therefore, increased storage of C in plants, both above and below-ground (Fig. 1.1). Changes in the atmospheric CO_2 level *directly* influence plant physiology and growth rate, especially in C_3 plants. Elevated atmospheric CO_2 concentrations increase net primary production (NPP) by 30-40% in species with a C_3 photosynthetic pathway and by 25% in species with a C_4 photosynthetic pathway

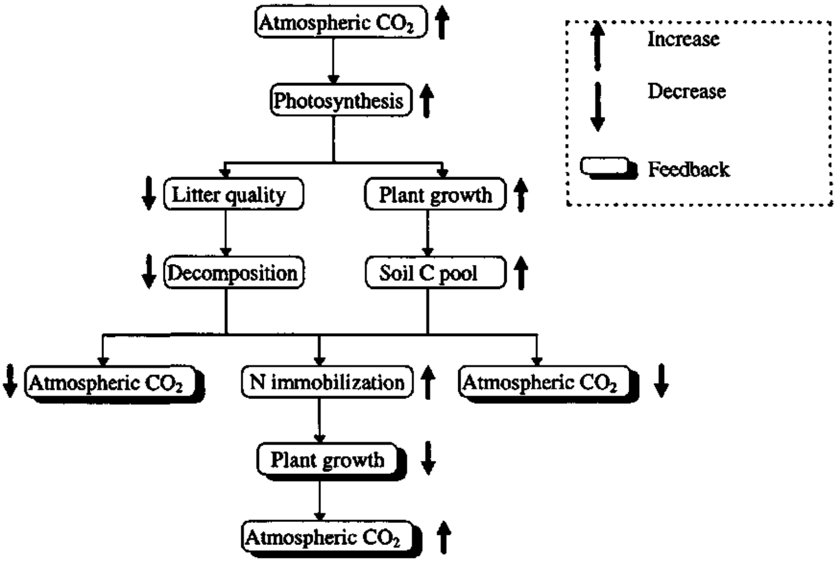

Figure 1.1 Scheme showing the interlinkage and feedbacks between elevated CO_2, plant production and soil organic matter (SOM), in terrestrial ecosystems. The effects of elevated temperature on each process and feedback are not given.

(Kimball,1983; Mooney et al., 1991; Poorter, 1993). Plenty of variation, however, occurs among species, so that the stimulating effects of elevated CO_2 on NPP varies from nil to strong (Amthor, 1995). Because the more responsive C_3 plants make up about 95% of the earth's plant species, the effect of elevated CO_2 on the global C and N cycle may be appreciable.

Elevated CO_2 may increase net primary production, provided that other growth factors, such as N and water availability, are not limiting (Mooney et al., 1998). However, Idso and Idso (1994) demonstrated that the relative effect of elevated CO_2 on plant growth rates is much higher when environmental conditions for plant growth are less than optimum. Yet, it is doubtful whether increased net primary production (NPP) is maintained under continued exposure to elevated atmospheric CO_2, especially in N-poor ecosystems. This is largely because plants growing at continued elevated CO_2 may adapt to the steadily increasing CO_2 concentration (Miglietta et al., 1995) and because the soil may become depleted in the N necessary for further enhanced stimulation of photosynthesis, particularly in natural ecosystems.

Through above and below-ground plant debris, photosynthesized CO_2-C ultimately reaches the soil where it is largely decomposed to CO_2, and partly stored temporally as soil organic matter. Increased litter input may lead to accumulation of carbon in the soil as soil organic matter (SOM), provided that other factors regulating decomposition processes such as temperature and soil moisture do not counteract this effect. Increased SOM pools may eventually provide a negative feedback to the atmospheric CO_2 level. However, global air temperature will increase simultaneously with increasing atmospheric CO_2 concentration. Increasing temperature has the potential to accelerate the decomposition rate, and therefore causes less storage of SOM in the soil. The net effect of these two opposing processes is rather uncertain, and further investigations are required. In a model artificial tropical ecosystem, for instance, litter production at elevated CO_2 was significantly higher (38%) than that measured at ambient CO_2 (Körner and Arnone, 1992). The net CO_2 effect, however, was not a greater C accumulation in the soil, because plant growth at elevated CO_2 resulted in a greater C turnover in the soil via enhanced microbial activity induced by increased root exudation (Körner and Arnone, 1992).

ELEVATED CO_2, LITTER QUALITY AND DECOMPOSITION

Decomposition and accumulation of soil organic matter are affected not only by the quantity of plant material, but also by the quality of decomposing litter. Plant materials (i.e. leaf litter, roots debris, rhizodeposition) are the main source of organic C and N in the soil. The quality of plant materials or "resource quality" depends on the types and amounts of the chemical constituents of plant materials, which are used by soil microorganisms as a source of energy and nutrients, especially N. The chemical constituents are mainly lignin, cellulose, soluble carbohydrates and polyphenols, and N in proteins and enzymes. These constituents and their ratios to N such as C/N, lignin/N, polyphenol/N are often used as litter quality indicators. Litter quality is recognized as a major regulating factor in litter decomposition (Swift et al., 1979). There are two factors that positively or negatively affect litter quality. For a given species, litter quality is primarily dependent on the availability of soil N, where lower N content or lower soil N accessibility is often associated with low contents of N and high contents of lignin, and higher C/N and lignin/N ratio in litter (Berg et al., 1987; Cotrufo et al., 1995). For example, birch litter produced at high N supply decomposed faster than litter produced at low N supply during the first months, in accordance with its high quality. However, this difference disappeared afterwards (Cotrufo et al., 1995). Similarly, Scots pine litter from a fertilized trial decomposed faster than litter from an unfertilized trail, but the difference in decomposition rate disappeared after about 3 years (Berg et al., 1987).

Enhanced CO_2 may *indirectly* influence the chemical composition of plant material and thereby the rate of decomposition (Fig. 1.1). The effects of elevated CO_2 on litter quality at the ecosystem level are difficult to assess. Changes in litter quality of plants grown under elevated CO_2 should be evaluated before measuring litter decomposition in terrestrial ecosystems. This means that we have to know whether a given species is responsive to CO_2 level or not. There has been an ever-increasing number of publications on the effect of elevated CO_2 on litter quality and subsequent decomposition in terrestrial ecosystems. The data obtained so far vary remarkably, ranging from no impact to considerable changes in chemical composition of litter. One factor that may help to explain this variation is the duration of the CO_2-enriched experiments. For the sake of simplicity, two type of experiments may be distinguished: (1) short-term CO_2 experiments with a length less than a plant life cycle and (2) long-term CO_2 experiments with a length of more than a plant life cycle.

Short-term experiments

Plant material grown at short-term elevated CO_2 (often <5 years) has been reported to have higher contents of non-structural carbohydrate (Allen et al., 1988), of lignin (Melillo, 1983; Field et al., 1992; Cotrufo et al., 1994) and of secondary metabolites (polyphenolics, Lambers, 1993; Peñuelas et al., 1996, 1997), and lower contents of nutrients, in particular nitrogen (Allen et al., 1988; Cotrufo et al., 1994, 1998; Boerner and Rebbeck, 1995; Torbert et al., 1995). Litter quality parameters such as C/N ratio, and lignin/N ratio of plant tissues have been found to be higher in elevated atmospheric CO_2 as a direct consequence of increased C fixation (Bazzaz, 1990; Coûteaux et al., 1991, 1996; Cotrufo et al., 1994). High-CO_2-grown plant material often decomposes more slowly than that grown at ambient CO_2 because of higher lignin contents and C/N ratios (Coûteaux et al., 1991, 1996; Cotrufo et al., 1994; Boerner and Rebbeck, 1995; Ball, 1997).

Other short-term pot experiments, however, showed little or no impact of elevated CO_2 on chemical composition of plant residues (Curtis et al., 1989; Norby et al., 1995; Owensby et al., 1993; Kemp et al., 1994; Taylor and Ball, 1994; Randlett et al., 1996; O'Neill and Norby, 1996; Hirschel et al., 1997). Recently, Ball and Drake (1997) indicated that the polymeric composition (e.g. lignin, cellulose and hemicellulose), and C/N ratio of senescent litter of *Spartina patens* (a C_4 grass) grown at elevated CO_2 (ambient + 340 ppm) did not differ from that grown at ambient CO_2. However, the C/N ratio in *Scirpus olneyi* (a C_3 sedge) was higher at elevated CO_2, whereas the contents of lignin, cellulose and hemicellulose in litter remained unaffected. Franck et al. (1997) reported that enhanced CO_2 slightly increased the C/N ratio in *Lolium* roots, while it decreased the C/N ratio in *Avena* shoots, both C_3 grasses.

The absence of a CO_2 effect on litter quality was reflected in litter C decomposition and N mineralization. For example, Torbert et al. (1995), Henning et al. (1996) and Randlett et al. (1996) found that enhanced CO_2 did not affect the C turnover rate of plant residue derived from CO_2-enriched treatments. Norby et al. (1995) and O'Neill and Norby (1996) reported that elevated CO_2 did not influence the mass loss rate of yellow poplar (*Liriodendron tulipifera* L.) litter after 18 and 24 months field incubation. Also in a litter bag experiment with litter produced under elevated CO_2, elevated CO_2 did not affect *in situ* decomposition rates of litter in native plant communities from lowland calcareous grassland and lowland wet tropical forest ecosystems (Hirschel et al., 1997). Henning et al. (1996) found a non-significant change in cumulative N mineralization in sorghum stem and soybean leaf from an enriched CO_2 treatment. Because of a lack of CO_2 effect on potentially mineralizable N, the increased N mineralization disappeared with time. Some studies reported that elevated CO_2 did not influence cumulative N mineralization and net N mineralization rates of litter incubated at elevated CO_2 (Cotrufo et al., 1994; Ross et al., 1995; Randlett et al., 1996; Henning et al., 1996). In contrast, Torbert et al. (1995) reported significantly higher net N immobilization during 59 days of incubation for cotton plant residue derived from a 550 ppm CO_2 treatment, in spite of the absence of an effect of elevated CO_2 on C decomposition.

Long-term experiments

Very few CO_2 experiments lasted longer than a plant life cycle. The only long-term studies about CO_2 effects on the chemistry of plant materials were carried out around natural CO_2 springs in Central Italy (Körner and Miglietta, 1994; Jones et al., 1995; Miglietta et al., 1995; Bettarini et al., 1995). In contrast to plants used in short-term experiments, plants growing around mineral-CO_2 springs in central Italy often did not show a reduction in the concentration of leaf N, e.g. *Erica arborea* L. (Miglietta et al., 1995; Bettarini et al., 1995), *Agrostis canina* L. and *Scirpus lacustis* L. (Miglietta et al., 1995) and leaf C/N ratio of *Arbutus unedo* L. (Jones et al., 1995). Leaves from several herbaceous and ruderal species, and of *Quercus pubescens* L. growing at elevated CO_2 around mineral CO_2 springs tended to have higher contents of non-structural carbohydrate (mainly starch) and lower contents of nitrogen, but such changes were not seen in *Quercus ilex* L. leaves (Körner and Miglietta, 1994). Rather, the concentration of N in ruderal leaves was higher at elevated than at ambient CO_2. The large majority of these data, however, refer to green tissue. Leaf litter usually has a different chemical composition from fresh leaves due to withdrawal of nutrients during senescence. No data has been reported for senescent litter around natural CO_2 springs.

Sources of uncertainty

The existing contrast between short- and long-term studies on possible impact of elevated CO_2 on litter decomposability may indicate that some factors, such as plant species and available soil resources (Franck et al., 1997), type of experimental set-up (field vs. controlled conditions, O'Neill and Norby, 1996), and length of exposure should be taken into consideration when generalizing this type of data to C and N cycling at the ecosystem level. There are three known reasons for the existing controversies:

First, in some experimental designs, effects of elevated CO_2 on litter quality are based on chemical analysis of green leaves. Because quantities of various chemical components differ between green and senesced litter (O'Neil and Norby, 1996), predictions based on green leaves will not be useful in studies of soil organic matter dynamics. In yellow poplar (*Liriodendron tulipifera* L.), N concentration in green leaf at elevated CO_2 was lower than that at ambient, but the C/N ratio of naturally abscised leaf litter was not affected by elevated CO_2 (O'Neill and Norby, 1996). Arp and Berendse (1993) stated that the efficiency of N withdrawal from the senescing leaves may decrease at elevated CO_2, and the efficiency is often species-dependent. Decreased efficiency of N re-immobilization may sometimes explain why in some species there is little or no effect of CO_2 on the chemistry of senescent litter.

Second, most of the information available on effects of CO_2 on litter quality was obtained in short-term greenhouse experiments, and it is not known whether CO_2 effects on litter chemistry observed in short-term experiments are sustained over a longer period of exposure. Markkola et al. (1996) grew Scots pine seedlings in unfertilized forest soil at ambient and elevated CO_2, and observed a reduction in the needle N concentration by CO_2 enrichment in the first year (previous-year needle), but such a CO_2 effect was not detected in the second year (the current year needle). Similarly, Verburg (1998) showed that the C/N ratio of *Betula* litter grown under elevated CO_2 in southern Norway increased during the first year of experiment, whereas such an increased C/N ratio was not observed after two years exposure to 560 ppm of CO_2.

Third, part of the contrasting information may result from sampling plant tissues at different stages of development (e.g. phenological differences), because the concentration of various constituents may change with the stage of developmental (cf. Peñuelas and Estiarte, 1998). In case of trees, age may be an important factor. Over 90% of published data was obtained from growing of seedlings and saplings at high CO_2 levels. Seedlings and saplings are more responsive to elevated CO_2 than mature trees (Lee and Jarvis, 1995). Globally, ecosystems contain more mature trees than seedlings and saplings, so that CO_2 effect on litter quality of large trees is more relevant for the global C cycle. The absence of any CO_2 effect on litter quality of some species could be due to either increased nutrient uptake by roots (Day et al., 1996) or downregulation of photosynthesis at elevated CO_2 (Ryle et al., 1992; Grulke et al., 1993; El Kohen et al., 1993; Miglietta et al., 1995; Oechel and Vourlitis, 1996). In N-limited ecosystems, direct uptake of organic nitrogen via the absorption of amino acids (Keilland, 1994) or via non-mycorrhizal fungi (Chapin et al., 1993) may keep litter quality constant, because inorganic N is not replenished for further root uptake in these ecosystems.

ELEVATED CO_2 AND SOIL ORGANIC MATTER

Soil is the largest store of organic carbon in the biosphere, estimated at 1300-1600 Gt C, or 65-80 % of the total biospheric organic carbon (Schlesinger, 1993; Goldewijk et al., 1994; Goudriaan, 1995; Schlesinger, 1995; Houghton, 1995). Therefore, soil organic matter (SOM) plays an important role, both in the global carbon cycle and in nutrient cycles of terrestrial ecosystems. Potentially, small changes in quantity and quality of SOM caused by changes in atmospheric CO_2 can influence environmentally relevant soil properties associated with SOM, such as the capacity of the soil to supply nutrients and store water. CO_2-induced changes in quality of plant litter and SOM slow down the mineralization rate and thus also the accessibility of major nutrients. On the other hand, enhanced CO_2 reduces the transpiration and therefore may increase soil moisture, decreasing the time period that decomposition and mineralization in surface soils are limited by water availability. Because the CO_2 concentration is already one to ten times higher in the soil atmosphere than in the ambient air, it is unlikely that elevated CO_2 affects directly chemical processes occurring in the soil.

Numerous studies have concentrated on CO_2 effects on plant growth but few on chemical composition of litter, and far less on SOM itself. Most importantly, it is not known whether effects of CO_2 on litter quality and decomposition are eventually reflected the soil organic matter dynamics. Several studies have reported a lack of significant changes in total soil C and N contents and C/N ratio associated with CO_2 enrichment (Rogers et al., 1992; Körner and Arnone, 1992; Zak et al., 1993; Wood et al., 1994; Johnson et al., 1994; Rice et al., 1994; Arnone and Körner, 1995; Ross et al., 1995, 1996; Niklaus and Körner, 1996; Hungate et al., 1996; Dhillion et al., 1996). For example, Hungate et al. (1996) observed that surface soil C pools were not affected significantly by elevated CO_2 after four growing seasons of exposure of intact annual grasslands to ambient+350 ppm CO_2. They used ^{13}C isotope signature as a tracer to evaluate the input rate of newly-assimilated C in the soil, and found that the impact of elevated CO_2 on soil C pool was not detectable, because soil C pools are large and heterogeneous compared to the annual plant input.

In addition to the effect of quantity and quality of plant materials on decomposition processes in the soil, the flux of labile C from roots to the soil may play an important role in both SOM pools and decomposition. Elevated CO_2 may increase rhizodeposition through increased C allocation to below-ground components (Goudriaan and de Ruiter, 1983, Lekkerkerk et al., 1990; Kuikman et al., 1991; Körner and Arnone, 1992; Dìaz et al., 1993; Zak et al., 1993; Rogers et al., 1994; Darrah, 1996). Increased rhizodeposition stimulates microbial activity, and therefore may accelerate the decomposition of organic matter (Körner and Arnone, 1992; Zak et al., 1993). The net effect of such interacting processes might be a small increase in C sequestration by terrestrial ecosystems in the long-run. However, Körner and Arnone (1992) stated that in terrestrial ecosystems under elevated CO_2 increased C uptake does not necessarily play a role in C sequestration, since elevated CO_2 will likely cause faster C turnover through the stimulation of soil microbes caused by enhanced root turnover. By contrast, Goudriaan and de Ruiter (1983), Lekkerkerk et al. (1990) and Kuikman et al. (1991) hypothesized that the decomposition of native soil organic matter is retarded due to preference of soil microbes for easily decomposable compounds (rhizodeposition) induced by elevated CO_2. This hypothesis is valid only if sufficient nutrients are available (van Breemen and van Dam, 1993; Gorissen, 1996), because increased microbial activity may lead to increased turnover of soil C when nutrient availability is relatively low, as indicated by the work of Körner and Arnone (1992) and Zak et al. (1993).

One problem with most experiments on effects of elevated CO_2 on litter quality and decomposability and subsequent carbon storage in the soil is that they use laboratory incubations which mimic only part of the field conditions. For example, in most experiments the complexity of the decomposer food web soil (including soil micro, meso and macro-fauna) is very low (Tian, 1992; Coûteaux et al., 1991). Coûteaux et al. (1991) observed that the litter decomposition rate of two-year-old sweet chestnut grown under elevated CO_2 (C/N:80) for two years was lower than the litter decomposition rate of plants grown under ambient conditions (C/N:43) without the inoculation of decomposers. However, with increasing diversity of soil organisms, litter with higher C/N ratio from elevated CO_2 units decomposed faster (30%) than litter with low C/N ratio from ambient CO_2. Coûteaux et al. (1991) attributed the faster decomposition to a change in microflora composition, especially due to increased white-rot fungi activity, and to the stimulation of microflora by the soil fauna.

Thus, to get a better insight in the role of soils as sink and/or source of the atmospheric CO_2, and to obtain a more overall understanding of SOM dynamics at the future elevated CO_2, it is important to study the processes occurring in the whole system, and to know long-term feedbacks between ecosystem's components (Canadell et al., 1996; Berntson and Bazzaz, 1996).

LONG-TERM STUDIES AROUND MINERAL CO_2 SPRINGS

From the forgoing one may conclude that for a comprehensive understanding of the effects of elevated CO_2 on plant litter quantity and quality, and ultimately on SOM dynamics, long-lasting experiments at the ecosystem level are needed. Ecosystems that have been exposed to long-term enhanced CO_2 concentration are particularly relevant for better understanding feedback mechanisms between soil organic matter decomposability and the atmospheric CO_2 level. Such ecosystems are found only in the vicinity of natural CO_2 springs. Species growing around these CO_2-emitting springs have been exposed to high CO_2 level for decades to centuries (Miglietta and Raschi, 1993). So, the possibility exists to test long-term effects of elevated CO_2 on quality of senescent leaf litter and C and N cycling by studying the vegetation near natural CO_2 springs. Another advantage of natural CO_2 springs is that we can study CO_2 effects on C and N cycling in a natural environment where below-ground feedbacks are in operation. On the other hand, such sites usually suffer from a number of limitations such as the lack of true replicates and controls, high spatial and temporal variability in CO_2 concentration, the possible admixture of toxic gases, and uncertainty about the history of CO_2 concentrations (Miglietta and Raschi, 1993; Amthor, 1995). Large spatial variability in soil properties may also frustrate research around natural CO_2 springs. Yet, CO_2 springs are used as experimental sites to study the response of plants and ecosystems to elevated CO_2 (Miglietta et al., 1993a; Amthor, 1995), and should be similarly useful for soil studies.

JUSTIFICATION AND AIMS OF THE RESEARCH

Given the considerable uncertainties sketched above, and the fact that most research aimed at experiments to test and quantify the hypothetical effects of increased CO_2 on terrestrial ecosystems consists of experiments that rarely last longer than a few years, essential research questions are:

"*Will elevated CO_2 affect litter quality and decomposability of mature plants growing in N-limited ecosystems which have been exposed to elevated CO_2 for many years?*" and

"*Are CO_2 effects on litter quality reflected in soil C dynamics in a natural environment?*"

The answers to these mentioned questions require long-term exposure of an ecosystem to elevated CO_2. As Amthor (1995) stated in his review "*Analyses of soil C pool sizes and qualities near natural CO_2 springs may be the best available measures of effects of long-term elevated $[CO_2]_a$*[1] *on litter production and decomposition. Any experiment that can be conducted, using any technique, is of short duration compared to the time scale of soil C turnover in many (or perhaps all) ecosystems. And, the 'step change' in $[CO_2]_a$ that is*

---

[1] The atmospheric CO_2 concentration.

used in experiments may cause different plant and ecosystem responses to $[CO_2]_a$ than the responses that are occurring with the present gradual increase in global $[CO_2]_a$."

The aim of this research is to quantify the long-term effects of elevated CO_2 on soil organic matter in the proximity of one of the natural CO_2 springs in Central Italy. I hypothesise that the natural forests subjected to naturally elevated CO_2 for a long-term period (more than a decade and/or century) around CO_2 springs will show the following patterns with increasing ambient CO_2 concentration (e.g. increasing distance from the CO_2 source):

(i) decreasing leaf litter (substrate) quality and decreasing rates of decomposition of leaf litter.
(ii) increasing pools of surface litter and of SOM
(iii) increasing residence times of surface litter and SOM and
(iv) decreasing nutrient accessibility

I tested these hypothesises by using a leaf litter-soil organic matter continuum. This continuum would help to track changes in biogeochemical cycles from "newly-formed" substrate (young C) to "native" soil organic matter (old C).

STUDY LOCATION

The studied location is a Mediterranean forest ecosystem. Mediterranean ecosystems are a subject of concern in the IGBP-GCTE (the International Geosphere-Biosphere Programme - Global Change and Terrestrial Ecosystems) because of their vulnerability under global climate change (IGBP, 1994). The area is located in the neighbourhood of the village of Laiatico, approximately 35 km from Pisa, Italy (Fig. 1.2). The Laiatico area is at 225 m above sea level with an annual rainfall of about 830 mm. The site is situated along a gentle slope facing north-west. Carbon dioxide from thermal decomposition of carbonatic parent material, probably caused by volcanic activity (Miglietta et al., 1993a) is emitted naturally at the surface from several large and small vents along a narrow stream. The CO_2 from the vent is mixed with that from the air above the forest canopy. The CO_2 concentrations tend to decrease upstream, and there is a CO_2 gradient from ambient to about 600 ppm down to the bottom of the stream. The area is covered by a coppice oak-ash stand, containing typical Mediterranean mattoral forest species including *Quercus ilex, Q. pubescens, Quercus cerris, Fraxinus ornus, Pistacia lentiscus, Myrtus communis, Arbutus unedo* and other shrubs. The stand was last cut nearly 25 years ago. Bedrock in the Laiatico area is Tertiary marl from which a soil with calcareous subsoil and brown loamy-clay texture developed.

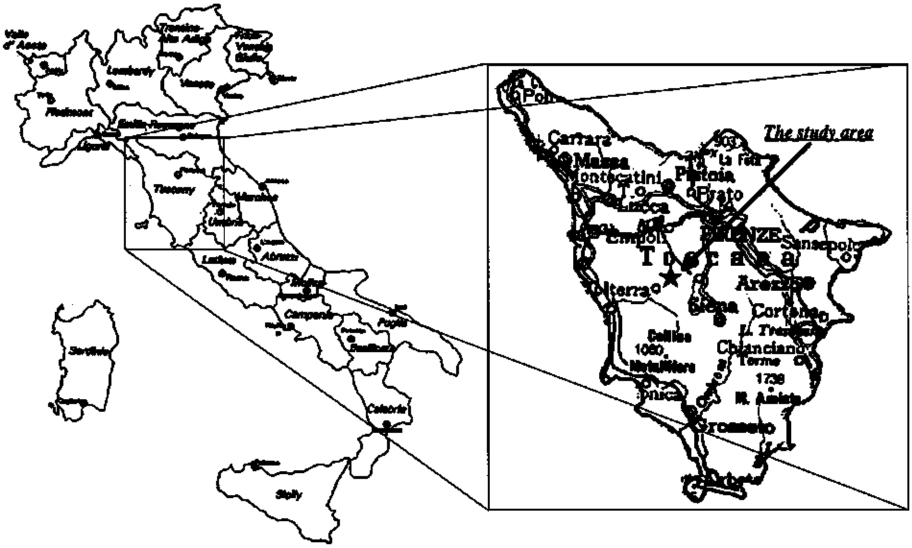

Figure 1.2 The location of the studied area, Pisa, Italy (43° 26'N, 10° 42'E).

OUTLINE OF THIS THESIS

After a short introduction on climate change and SOM, the second chapter deals with the measurement of the local CO_2 concentration at the site. The distribution of CO_2 level was estimated on the basis of ^{14}C content in twigs of C_3 evergreen *Quercus ilex* growing at the site, and the short-term temporal variability of the concentrations of the atmospheric CO_2 and trace gases, such as SO_2, H_2S, NO and NO_2, were measured by use of fully automated measuring systems.

The third and fourth chapters discuss the impact of elevated CO_2 on litter quality and its decomposition. An experiment was carried out on naturally senesced leaf litters collected from three species, namely *Quercus pubescens*, *Quercus cerris* and *Smilax aspera* growing at the site. Litter quality parameters measured in this study include C and N contents, non-structural carbohydrates such as acid detergent fibre (ADF), cellulose, lignin, and secondary compounds like polyphenols. The litter samples were incubated to measure C and N mineralization under controlled conditions. Chapter 3 deals with the quantification of CO_2 impact on litter quality and decomposability of litter of the two deciduous oak trees, while chapter 4 deals with the evaluation of CO_2 impact on litter quality and decomposability of the understory evergreen *Smilax aspera*.

Chapters 5 and 6 test and evaluate the effect of CO_2 distribution on SOM itself (older than one year). It is reasonable to link the belowground C (SOM) to aboveground C (litter) and test whether the effects that were found in leaf litter can be expected also in the decomposing soil C. The impact of elevated CO_2 on contents and pools of C and N, and C/N ratio of SOM is reported in the fifth chapter. The rates of C and N mineralization were evaluated by incubating samples from the forest floor and 0-10 cm mineral soil. Short-term decomposition data are presented in the sixth chapter.

Chapter 7 presents the general discussion about the overall effect of elevated CO_2 on dynamics of a plant leaf litter C-soil organic C continuum around the Laiatico CO_2 site, and some concluding remarks. Also it discusses the possible implications of the current results in the context of global climate change, and feedback mechanisms between the atmosphere, the plant growth, soil C and ecosystem functioning in the context of global climate change are speculated upon. Finally, a few suggestions are given for further research around mineral CO_2 sites.

Since this thesis is a collection of papers which have been submitted to different journals for publication, there are some repetitions and overlaps.

(References are give on page 135)

Chapter 2

CO_2 concentration and atmospheric trace gas mixing ratio around natural CO_2 vents in different Mediterranean forests in Central Italy

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CO_2 concentration and atmospheric trace gas mixing ratio around natural CO_2 vents in different Mediterranean forests in Central Italy

ABSTRACT

In an effort to determine the CO_2 concentration experienced by the vegetation around three natural CO_2 vents in Mediterranean forests in Central Italy, short term and long term integrating measurements were performed. To also characterize the atmospheric mixing ratios of concomitant trace gases such as SO_2, H_2S, NO and NO_2 with a high resolution in time, atmospheric gas measurements were performed by use of fully automated measuring systems. In short term measurements, distinct diurnal courses of the CO_2 gas mixing ratio with high fluctuations were determined at the Bossoleto and Laiatico sites (daily mean of CO_2: 1074 ppm and 795 ppm, respectively), but not at Solfatara despite high fluctuations (daily mean of CO_2: 797 ppm CO_2). Different temporal patterns of atmospheric CO_2 concentrations would be attributed mainly to temperature variations and the topographic situations at the sites.

CO_2 released from all vents contained variable amounts of the atmospheric sulphur compounds H_2S and SO_2, but no NO or NO_2. Hydrogen sulfide daily mean concentration amounted to 11 ppb, 22 ppb and 245 ppb at Bossoleto, Laiatico and Solfatara, respectively, SO_2 was present at low concentrations at the Bossoleto and Laiatico (daily mean of SO_2: 2 to 16 ppb), but with a maximum concentration of 134 ppb at the Solfatara site.

In addition to CO_2 short term measurements, long term (annual average) CO_2 concentrations were determined at the Laiatico site using a radioisotopic technique based on the known ratio of $^{14}CO_2$ activity of the vent to $^{14}CO_2$ activity of the ambient air. The estimates obtained from this isotopic analysis were in the same range as the daily mean CO_2 concentration determined by infrared gas analysis but tended to be some what lower.

The impact of elevated CO_2 on the fluxes of the radiatively active trace gases CH_4 and N_2O between the soil and the atmosphere were determined using the closed chamber technique. A net flux of methane from the atmosphere to the soil was observed at the vents at the Bossoleto and the Laiatico site (- 88 µg CH_4 m^{-2} h^{-1} and -230 µg CH_4 m^{-2} h^{-1}, respectively). CH_4 fluxes at the corresponding control areas were generally lower. While N_2O emission was only minute both at the vents and the corresponding control areas (11 µg N m^{-2} h^{-1}), irrigation increased the net flux of N_2O to atmosphere by a factor of 13 selectively from the soil exposed to elevated

CO_2. Apparently, elevated CO_2 at the vents interacts with the microbial consumption and/or production processes of CH_4 and N_2O.

Key-words: Climate change, Mediterranean ecosystems, elevated carbon dioxide, hydrogen sulfide, sulphur dioxide, methane, nitrous oxide, radioisotopic technique

Abbreviations: RT, room temperature; ppm, parts per million; ppb, parts per billion; pmC, percent modern carbon; PDB, Pee Dee Belemnite; GC, Gas chromatograph

INTRODUCTION

Within the context of global climate change plant ecophysiology plays an important role, since plants constitute the major energy input into the living world. Main elements of global change such as rising CO_2 and increased nitrogen deposition influence growth and allocation in plants. On a whole-plant level a series of biochemical adjustments is required within weeks up to years to achieve an acclimated state (Sage et al., 1989). At the community level, changes in competitive interactions under elevated CO_2 (Bazzaz, 1990; Bazzaz and McConnaughy, 1992) may result in quasi-stable states only after periods of years to centuries or more (Shugert et al., 1986; Davis, 1989). However, there is an urgent need to gain more information about physiological adaptation of plants to changes in atmospheric CO_2 gas mixing ratios over the entire life period, especially of long-lived species such as large trees. Many studies have been conducted in open-top-chambers (Ceulemans et al., 1995; Drake et al., 1996), FACE (Free Air Carbon Exposure Systems) systems (Nie et al., 1995; Nijs et al., 1996; Hebeisen et al., 1997) and glasshouses (Körner and Arnone, 1992). While these provide indications of short-term changes in plant physiology, there is a high risk that they do not reflect changes in plant properties related to long term growth under elevated CO_2.

The problem of time scale can be avoided by studying plants growing around the geothermal CO_2 vents as found in several places in Central Italy. The main advantage of using natural CO_2 vents is the fact that plants native to these sites should have adapted to elevated CO_2 over long periods of time even exceeding the life span of trees. However, high variability of CO_2 concentration with time and enrichment of other gases which may be toxic (e.g. sulfur dioxide and hydrogen sulfide) are disadvantages of natural CO_2 vents. For studying the response of plants growing around naturally elevated CO_2, it is therefore a major challenge to determine the CO_2 concentration experienced by the vegetation around the vents with a high resolution in time and space, and to characterize the atmospheric mixing ratio of concomitant trace gases (e.g. SO_2, H_2S, NO, NO_2).

Next to carbon, nitrous oxide (N_2O) and methane (CH_4) are the most important radiatively active trace gases in the atmosphere. At present, atmospheric concentration of N_2O is increasing at a rate of 0.27 ± 0.01 $yr.^{-1}$ (Khalil and Rasmussen, 1992) and atmospheric CH_4 concentration has nearly doubled during the last 100 years (Watson et al., 1990). Both, CH_4 and N_2O contribute to climate change (Rennenberg et al., 1995), but the magnitude of individual natural and man-made sources and sinks contributing to the global budget of N_2O and CH_4 is still highly uncertain. Especially the contribution of natural ecosystems, like forests, adapted to situations of CO_2 enrichment to the global CH_4 and N_2O is so far unknown, but is required to estimate the source/sink strength of forest soils for these trace gases with increasing CO_2 concentration in predictive models.

Therefore, the objectives of this study were (1) to characterize short-term variability of the CO_2 and trace gas mixing ratio experienced by the vegetation around CO_2 vents with a high resolution in time, (2) to estimate long-term CO_2 concentration using vegetation at the site as an integrator, and (3) to establish a first data base about the magnitude of N_2O and CH_4 exchange in forest soils exposed to elevated CO_2 for extended periods.

MATERIALS AND METHODS

Measuring sites

Field studies were performed at three sites in Central Italy with natural CO_2 vents, i.e. Bossoleto, Laiatico and Solfatara. The Bossoleto site has previously been described (Miglietta et al., 1993c; Körner and Miglietta, 1994; van Gardingen et al., 1995). It is near Siena (43° 17'N; 11° 36'E) and is covered by a typical Mediterranean forest mainly composed of *Quercus* spec. (*Q. ilex*, and *Q. pubescens*). The vent at Bossoleto occurs in a circular crater, 80 m in circumference and 20 m deep. Gas emissions occur from the bottom and on the flanks of the crater (Fig. 2.1). The control site is located in 5 km distance, with the forest stand, soil properties, and climate similar to the CO_2 enriched site (A. Raschi, unpublished data). The Laiatico site is situated in the neighbourhood of Volterra (43° 26'N, 10° 42'E). In contrast to the Bossoleto site, the Laiatico site is located on a gentle slope. The CO_2 emission comes from one major vent and a number of smaller ones in 5 m distances all located at the foot of the slope. About 200 m south-west of the vent, a control area with similar slope orientation, morphology, soil parent material, hydrology and vegetation was selected. The vent as well as the control area are covered mainly by different *Quercus* species. The Solfatara site (42° 30'N; 12° 08'E) near Viterbo has been described by Miglietta and Raschi (1993) and Miglietta et al. (1993c) (Fig. 2.2). At this site, several CO_2 vents of 5 to 40 cm in diameter are dispersed over an area of about 2 ha. The vents are visible only when covered with water. The area is dissected by several small creeks, creating seasonal swamps. The natural vegetation consists of different *Quercus* species, alders, wild roses, blackberries and brooms (Miglietta and Raschi, 1993).

Gas concentration measurements

Measurements of atmospheric CO_2 and trace gases (S_{total}, SO_2, NO and NO_x) were performed between May and June 1995 on clear sunny days without rain. All measurements were performed continuously using fully automated measuring systems at the control area and in the vicinity of the vent at each site (all times are Middle European Time). For this purpose air was drawn and guided through PTFA-teflon tubes to several gas analyzer. CO_2 was measured with an infrared gas analyzer (BINOS, Leybold-Heraeus, Hanau, Germany). The instrumental error was $\leq$ 60 ppm (using the 3000 ppm scale) or $\leq$ 12 ppm (using the 600 ppm scale). SO_2 was determined using a SO_2 specific monitor with UV fluorescence detection (AF 20 M, Antechnika, Karlsruhe, Germany) with a lower detectable range of 2 ppb. Analysis of total atmospheric sulphur compounds was performed with a flame photometric detector (FPD) (Sulfur Monitor Model 8450, Monitor Labs, incorporated, San Diego, USA) with a detection limit of 2 ppb. As previously reported for a number of CO_2 vents in Central Italy (Duchi et al., 1985), the sulphur emitted from the vents was dominated by H_2S. Therefore the difference between the concentration of the total atmospheric sulphur and the SO_2 concentration was attributed to H_2S. NO and NO_2 were measured using a chemiluminescens detector (AC 31 M, ANSYCO, Karlsruhe, Germany) with a detection limit of 0.35 ppb. To avoid photochemically conversion of NO_2 to NO, tubes were covered with black foam (Armaflex, Armstrong World Industries GmbH, Düsseldorf, Germany) and kept as short as possible.

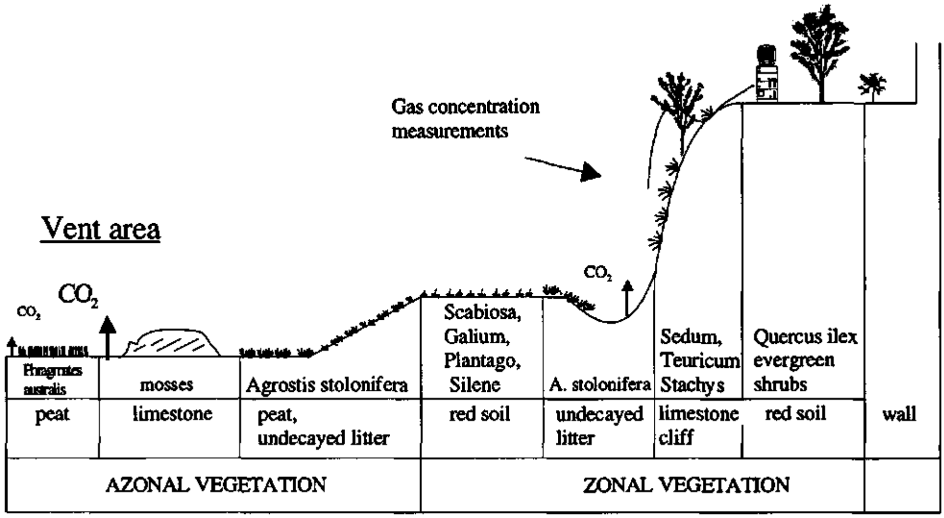

Figure 2.1. Cross-sectional diagram illustrating the relationship between topography and vegetation at the Bossoleto site (modified from Miglietta et al., 1993c).

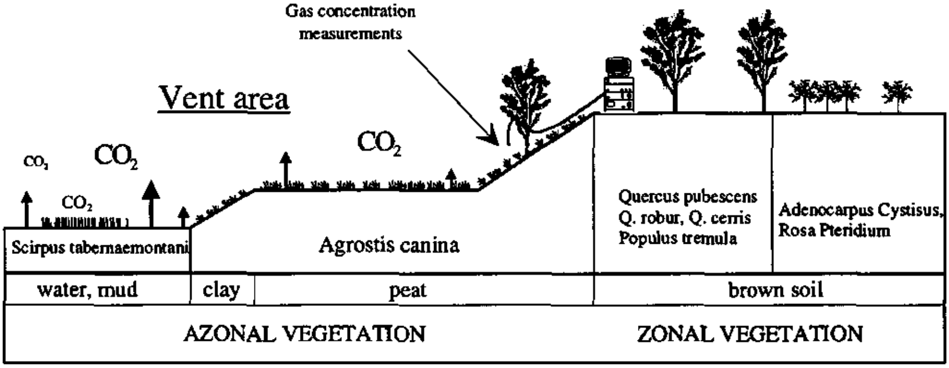

Figure 2.2. Cross-sectional diagram illustrating the relationship between topography and vegetation at the Solfatara site (modified from Miglietta et al., 1993c).

Immediately before measuring, calibration of the CO_2, SO_2, S_{total}, NO and NO_x analyzers were calibrated with pressurized standard gases (400 $\mu l\ l^{-1}$ CO_2, 10 $\mu l\ l^{-1}$ SO_2, H_2S and NO/NO_2, respectively, all Messer Griesheim, Neuried, Germany).

For the gas measurements the measuring system and data acquisition were separated. Atmospheric gas compounds were measured directly, digitized with an AD-converter (Opto 22 Automation Products GmbH, Bensheim, Germany) and data were transferred for computing with a RS 422 protocol. A special software UNIDAS (UNI-Data-Aquisitation-System), developed at the Institute of Forest Botany and Tree Physiology at Freiburg was used for continuous data acquisition.

Gas flux measurements

N_2O and CH_4 flux rates were measured at a control area and the vent of the sites described above. At the vent of Bossoleto a rainfall event was simulated by irrigating soil with 8 mm water. Gas flux measurements were performed using the closed chamber technique (Schütz et al., 1989). Chambers had a volume of 40 l, 42.5 l and 33.3 l, and covered a ground area of 0.25 m^2, 0.25 m^2 and 0.09 m^2, respectively. They consisted of an aluminium frame covered with plexiglass that were placed on top of a stainless steel rack which had been inserted into the soil. Gas samples from the closed chambers were taken every 40 minutes for 2 h several times a day from the chamber's atmospheres using the vacutainer technique. The air temperature inside the chambers was recorded at the time of sample collection. Samples were transported to the laboratory and analyzed for CH_4 and N_2O within 2 weeks by GC analysis. N_2O was determined using a Shimadzu GC 14A gas chromatograph equipped with an electron capture detector and a Hayesep N column (stainless steel 3 m, 1/8", 80/100 mesh) for N_2O-separation; CH_4 was determined using the flame ionization detector of the same GC, and a Hayesep Q column (stainless steel 3 m, 1/8", 80/100 mesh column) for CH_4-separation. The oven temperature was 60 °C, the detector temperature of the ECD and FID was 320°C. The carrier gas used for N_2O determination was N_2 at a flow rate of 60 ml min^{-1}. For CH_4 determination hydrocarbon-free synthetic air was used (60 ml min^{-1}). Calibration of the measuring system was performed routinely after 4 samples, using standard gases (Messer Griesheim, Neuried, Germany)

Long-term CO_2 concentration estimates

Assuming that the CO_2 assimilated by plants comes from two sources, the atmosphere (a) and the vent (v), the sum of the fractions of CO_2 assimilated by the plant from the vent (f_v) and from the atmosphere (f_a) must, by definition, be unity:

$$f_v + f_a = 1 \tag{1}$$

The ^{14}C in plant material M_p comes from the same two fractions weighted by the abundance of ^{14}C in each of the sources, M_v and M_a:

$$M_p = M_a \times f_a + M_v \times f_v \tag{2}$$

since $M_v = 0$,

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

Assuming that no isotopic discrimination takes place, f_a is also equal to the ratio of the normal ambient CO_2 concentration (p_a) at the sampling time to the average local concentration at the sampling point (p_s):

$$f_a = \frac{p_a}{p_s} \tag{4}$$

So, p_s can be calculated as:

$$p_s = p_a \times \frac{M_a}{M_p} \tag{5}$$

or

$$p_s = p_a \times \frac{(A^{14}C)_a}{(A^{14}C)_p} \tag{6}$$

in which $(A^{14}C)_a$ is the ^{14}C activity in the air, and $(A^{14}C)_p$ is the ^{14}C activity corrected for isotopic discrimination in plant. The ^{14}C activity is commonly expressed as percentage of the pre-bomb (1950) activity of ^{14}C or percent modern carbon (pmC) in the atmospheric background.

M_a/M_p shows the degree to which ^{14}C of the plant from ^{14}C from the ambient atmosphere was diluted by the ^{14}C-free CO_2 from the vent during photosynthetic production of plant C, and is considered as a diluting factor. The error in the estimate of the local mean CO_2 concentration (p_s) was less than ± 2 % illustrating a high reliability of the method in comparison to others (van Gardingen et al., 1995). Although the ^{14}C method is potentially reliable and precise, there are some uncertainties regarding the variation of ^{14}C abundance in the plant. Deciduous leaves are exposed to elevated CO_2 for a short-time, e.g. one growing season, while the CO_2 concentration may vary from year to year. In our study we sampled twigs, which will represent the mean effective CO_2 concentration experienced by the plant over a number of years.

Twigs from *Quercus ilex* L. grown at the Laiatico site and containing 10 annual growth rings were sampled 2.5 m above the ground in December 1995 and stored for about 2 weeks at RT. Twelve samples were taken from the vent area and five samples from the control area, oven-dried at 30 °C for one week and analyzed for ^{14}C and ^{13}C using the conventional ^{14}C age method (Mook and Streurman, 1983). The ^{14}C content is expressed as a percentage of a 1950 ("pre-bomb") standard air or as per cent modern carbon (pmC).
The carbon from the vent is virtually depleted in ^{14}C ($A^{14}C$=0.25 %), giving an apparent age of 48000 years (Miglietta, pers. comm.). This small amount of ^{14}C in the vent may originate either from the percolating water above the vent or from contamination by natural ^{14}C of ambient air.

Due to differences in the masses of carbon isotopes (^{12}C, ^{13}C, ^{14}C), isotopic fractionation occurs during photosynthesis, especially in C_3 plants. Therefore a correction for isotopic discrimination must be made. Assuming the $\delta^{13}C$ in the vent CO_2 is similar to the $\delta^{13}C$ in ambient CO_2 (-8.0 ‰) (Miglietta, pers. comm.), the isotopic discrimination can be estimated from ^{13}C contents in plant tissue, using the following equation (Stuiver and Polach, 1977),

$$(A^{14}C)_p = \left((a^{14}C)_p x(\frac{0.975^2}{(1+\frac{\delta^{13}C}{1000})^2}) \right) \qquad (7)$$

where $(a^{14}C)_p$ is the measured ^{14}C activity (%) and $\delta^{13}C$ is the ^{13}C content in plant tissues, relative to a standard. The $\delta^{13}C$ value (‰) was calculated from the measured carbon isotope ratios (R= $^{13}C/^{12}C$) of the twigs (p) and a standard (PDB) using the following equation,

$$\delta^{13}C = \left(\frac{R_p - R_{st.}}{R_{st.}} \right) \times 10^3 \qquad (8)$$

where $\delta^{13}C$ is the ^{13}C content of the twigs (p) and that of the standard (st.), and R is the mass ratio $^{13}C/^{12}C$ of the twigs or standard gas.

Statistical and geostatistical calculations

We used classical statistics and two-tailed *t* comparison to test the significance of differences in the abundance of ^{13}C and ^{14}C and the concentration of CO_2 between the vents and control areas. In addition, we used a geostatistical procedure to depict the CO_2 concentration map for the whole study area. To do so, first the spatial dependence of the CO_2 concentration was determined from a semivariogram. Semivariograms were calculated with the programme SPATANAL (Stein et al., 1994) and variogram models were fitted with the programme WLSFIT (Weighted Least Squares fitting) (Stein et al., 1994). The map was generated by ordinary kriging, using KRIGE (Stein et al., 1994).

RESULTS

Short-term CO_2 measurements

The results obtained from short-term CO_2 measurements at the CO_2 vents of 3 different field sites are shown in Figure 2.3. At all three sites, CO_2 concentration fluctuated strongly with time. Distinct diurnal courses of the CO_2 were observed at the Bossoleto and Laiatico site (Fig. 2.3A, B). At Bossoleto CO_2 concentrations fluctuated strongly from 04:00 h to 09:00 h (CO_2 max.: 8097 ppm, CO_2 min: 325 ppm) and remained at almost constant at 368 ± 54 ppm until 18:00 h. From 18:00 to 20:00 h CO_2 concentration increased again with high fluctuations, but did not reach the high morning values. The mean daily CO_2 concentration Bossoleto site was 1074 ppm. At the control area, the mean CO_2 concentration amounted to 292 ± 28 ppm. In contrast to the Bossoleto site, the highest CO_2 concentrations at the Laiatico site were found between 09:00 and 18:00 h with high fluctuations ranging between CO_2 max. of 2815 ppm and CO_2 min of 209 ppm CO_2. Almost constant CO_2 concentrations were found in the early morning (05:00 to 07:00 h) and in the late evening (18:00 to 22:00 h) (320 ± 15 ppm). The daily mean CO_2 concentration amounted to 795 ± 434 ppm, whereas mean CO_2 concentration of 282 ± 9 ppm was determined at a control area in close vicinity. At the vent of Solfatara a distinct diurnal course as observed at Bossoleto and Laiatico was not found (Fig. 2.3C). CO_2 gas mixing ratio showed high fluctuations over the entire measuring time ranging from high values of 2044 ppm to rather low gas mixing ratios of 316. The daily mean CO_2 gas mixing ratio amounted to 797 ± 343 ppm at the vent and to 325 ± 30 ppm at the control site.

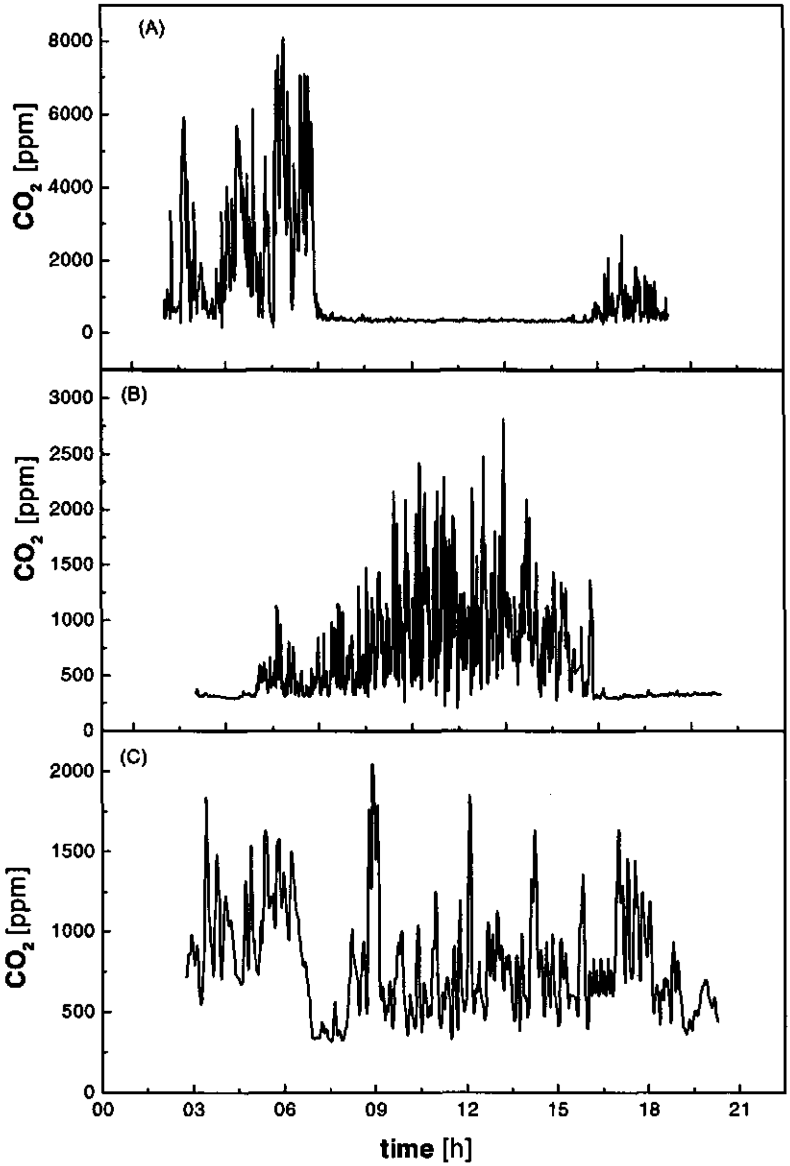

Figure 2.3. Atmospheric CO_2 gas mixing ratio in the vicinity of the CO_2 vent of three different sites in Central Italy: Bossoleto (A), Laiatico (B) and Solfatara (C). CO_2 was measured with an infrared gas analyzer. The measurements were performed on a clear sunny day every minute continuously over ca 15 h per day using fully automated measuring systems. The data set was smoothed by calculation of 5 min means.

Short-term measurements of H_2S, SO_2 and NO_x

At all sites H_2S and SO_2 were detected in considerable amounts (Fig. 2.4, 2.5). Atmospheric sulphur concentrations fluctuated strongly at a time scale of minutes, but still showed a distinct diurnal course. At Bossoleto and Laiatico but not at Solfatara, this diurnal course was similar to that of atmospheric CO_2. At Bossoleto (Fig. 2.4A, 2.5A) the atmospheric sulphur compounds fluctuated strongly in the morning until 09:00 h with mean concentration of 22 ppb (max. 83 ppb; min 2 ppb) for H_2S and 12 ppb (max. 23 ppb; min 5 ppb) for SO_2. Thereafter, the gas mixing ratio of both gases declines rapidly to almost constant values of 5 ± 1 ppb (H_2S) and 6 ± 1 ppb (SO_2) from 09:00 to 18:00 h. Subsequently, atmospheric mixing ratios of H_2S and SO_2 increased again to higher strongly fluctuating values. Whereas the H_2S gas mixing ratio (9 ± 4 ppb) did not reach the high level observed during the morning hours, SO_2 gas mixing ratios (11 ± 3 ppb) were found at similar levels as in the morning. At the vent of the Laiatico site H_2S gas mixing ratios were low in the morning from 05:00 to 09:00 h (H_2S: 2 ± 1 ppb) and the evening from 18:00 to 22:00 h (6 ± 2 ppb), but were high between 09:00 and 18:00 h (34 ± 15 ppb) (Fig. 2.4B).

The daily mean H_2S gas mixing ratio was 22 ± 18 ppb. SO_2 gas mixing ratios varied only in a small range at the vent of the Laiatico site ($SO_2 \leq 4$ ppb) with lower concentrations in the morning until 09:00 h (0.6 ± 0.2 ppb) and higher concentrations from 09:00 to 18:00 h (2.5 ± 0.8 ppb) (Fig. 2.5B). In contrast to the H_2S gas mixing ratio at this site, SO_2 gas mixing ratios increased again after a steep decline after 18:00 h. At the control area of both Bossoleto and Laiatico, neither H_2S nor SO_2 could be detected.

In contrast to the CO_2 gas mixing ratio a diurnal course of atmospheric H_2S and SO_2 was observed at the Solfatara site (Fig. 2.4C, 2.5C). The atmospheric gas mixing ratios of both sulphur gases were low in the early morning from 03:00 to 09:00 h (H_2S: 81 ± 59 ppb; SO_2: 3 ± 11 ppb) and in the evening from 18:00 to 20:30 h (H_2S: 51 ± 17 ppb; SO_2: ≤ 2 ppb). Atmospheric gas mixing ratio of H_2S and SO_2 increased at 09:00 h and fluctuated until 18:00 h around a mean of 245 ppb and 18 ppb, respectively. Considerable amounts of H_2S (H_2S mean: 12 ± 6 ppb; H_2S max.: 39 ppb) were measured even at the control area of the Solfatara site, whereas atmospheric gas

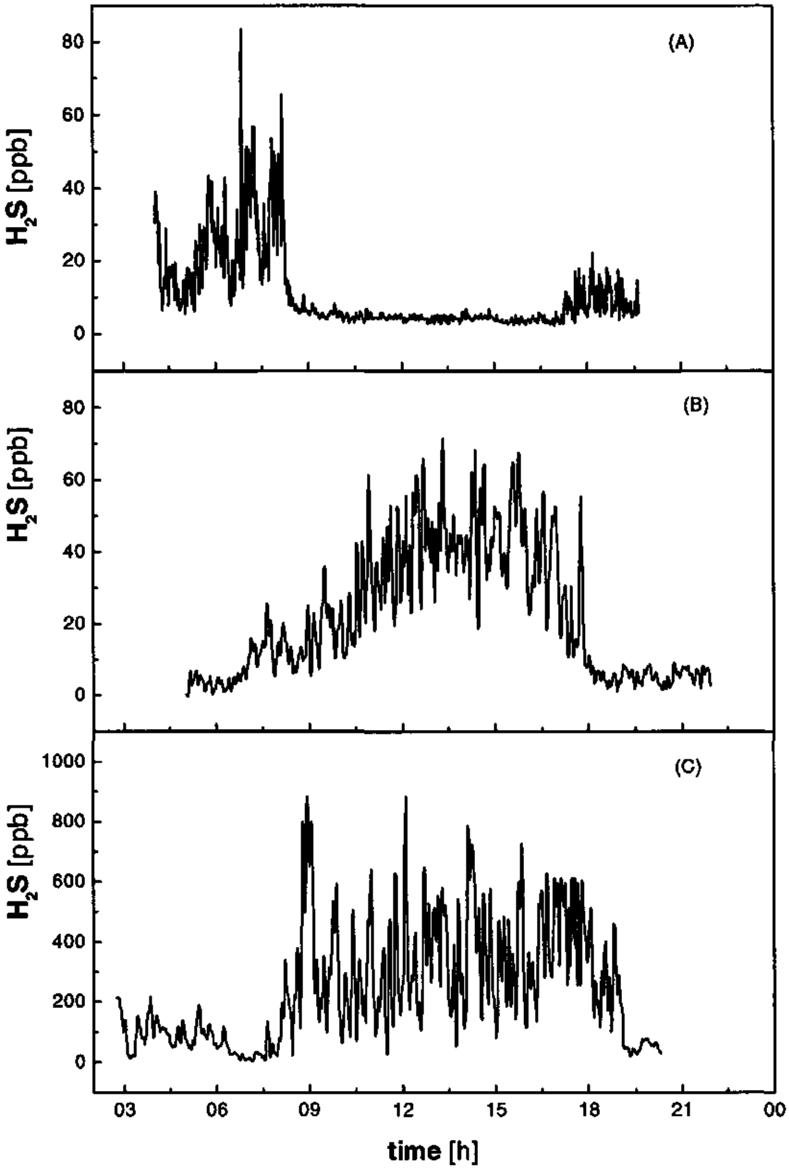

Figure 2.4. Atmospheric H_2S concentration in the vicinity of the CO_2 vent of three different sites in Central Italy: Bossoleto (A), Laiatico (B) and Solfatara (C). H_2S concentration was calculated by subtraction of the measured SO_2 concentration from the measured S_{total} concentration. S_{total} was measured with a flame photometric detection. The measurements were performed on a clear sunny day every minute continuously over ca 15 h per day using fully automated measuring systems. The data set was smoothed by calculation of 5 min means.

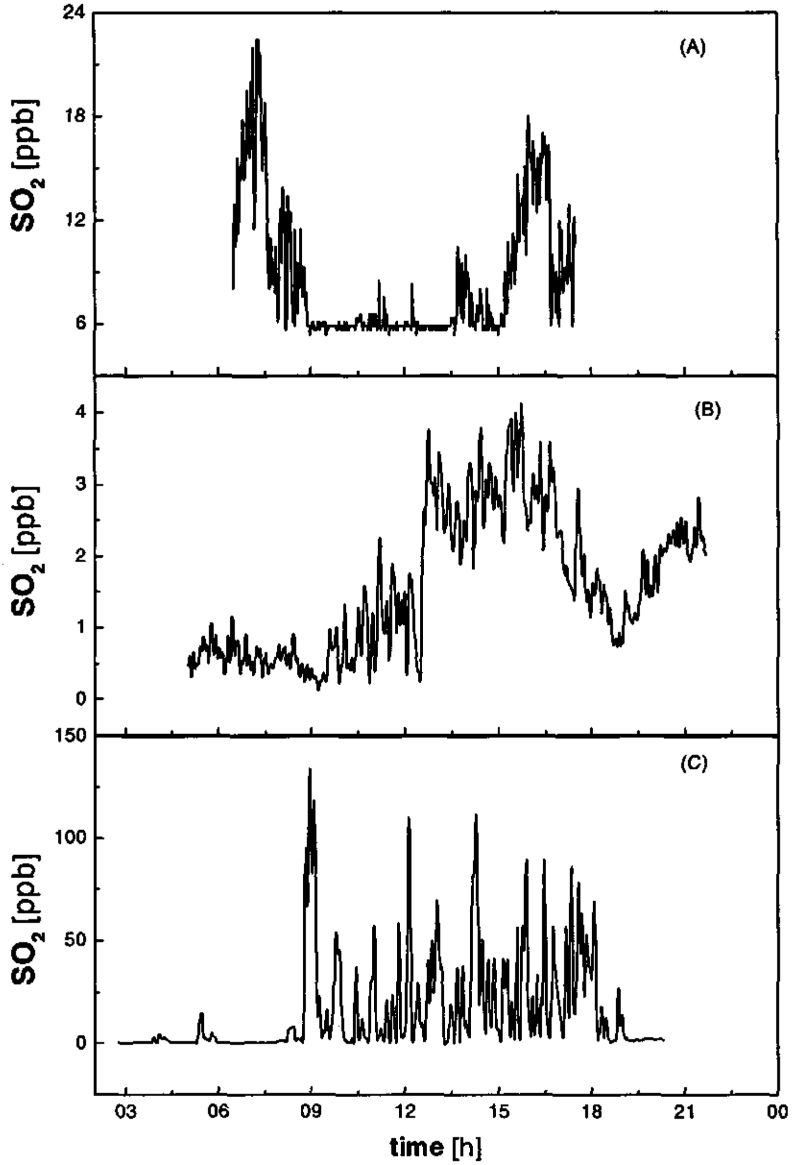

Figure 2.5. Atmospheric SO_2 gas mixing ratio in the vicinity of the CO_2 vent of three different sites in Central Italy: Bossoleto (A), Laiatico (B) and Solfatara (C). SO_2 was measured with UV fluorescence detection. The measurements were performed on a clear sunny day every minute continuously over c. 15 h a day using fully automated measuring systems. The data set was smoothed by calculation of 5 min means.

mixing ratios of SO_2 were negligible (SO_2 mean ≤ 2 ppb).

Atmospheric NO and NO_2 gas mixing ratios were similar at all three sites studied (5-10 ppb and 8-25 ppb, respectively) and did not change significantly with time and with increasing distance from the vents.

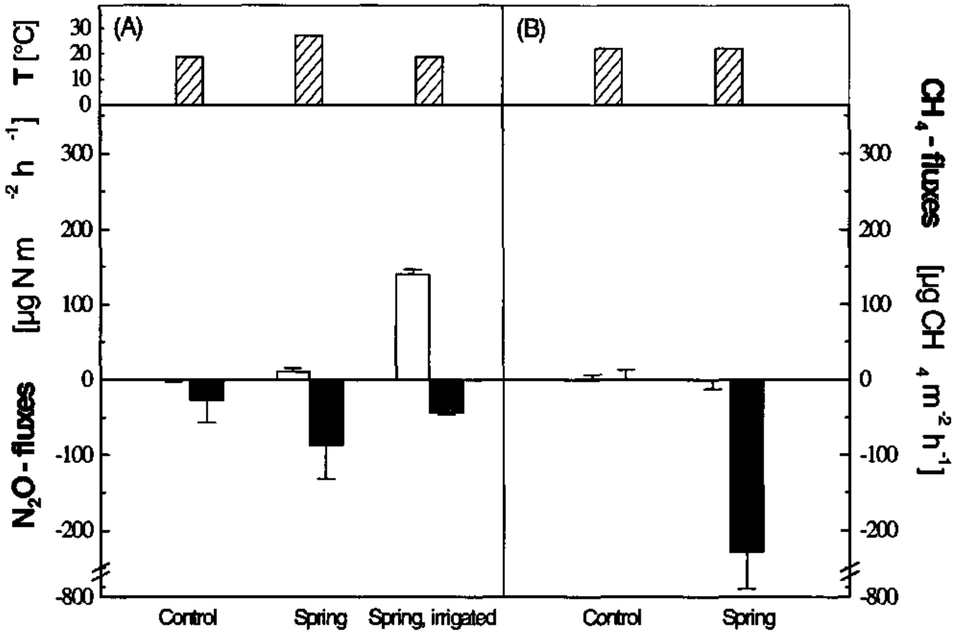

Figure 2.6. Comparison of mean N_2O (□) and CH_4 (■) flux rates from soil at the CO_2 vents and the control areas of the Bossoleto (A) and the Laiatico (B) site. At the vent of Bossoleto a rainfall event was simulated by irrigating soil with 8 mm water. N_2O/CH_4 concentrations were measured using the closed chamber technique (Schütz et al., 1989). Gas samples from the closed chambers were from the chamber's atmospheres taken every 40 minutes for 2 h several times per day using the vacutainer technique. Samples were analyzed for CH_4 and N_2O within 2 weeks by GC. Positive flux rates represent emission rates from the soil to the atmosphere, and negative flux rates represent deposition rates to the soil. The temperatures inside the chambers during the measurements are given at the top of the figure.

N_2O and CH_4 flux measurement

At the three sites, CH_4 and N_2O flux rates were determined from the soil of the Mediterranean oak forests grown either in the vicinity (elevated CO_2) or in some distance from the vent (ambient CO_2) (Fig. 2.6). A net flux of methane from the atmosphere to the soil was observed at the vents of all sites investigated. At the vent of the Bossoleto site deposition of atmospheric methane amounted to - 88 μg CH_4 m^{-2} h^{-1} which was 3 times higher than that at the control area. Also at the vent of Laiatico high amounts of CH_4 were deposited to the forest soil (-230 μgCH_4 m^{-2} h^{-1}), while at the control area only negligible CH_4 deposition was observed (- 2 μg CH_4 m^{-2} h^{-1}). Similar results were obtained for the vent of the Solfatara site (data not shown). Only minute N_2O fluxes were detected at the vents and control sites (Fig. 2.6). At the vent of the Bossoleto site, N_2O emissions of 11 μg N m^{-2} h^{-1} were detected; even lower N_2O emissions were found at the control area (0.6 μg N m^{-2} h^{-1}). Irrigation of the soil at the vent of the Bossoleto site increased N_2O emission by a factor of 13 compared to unirrigated soil, and slightly, but not significantly, reduced CH_4 deposition

Long-term CO_2 concentration estimates using a radioisotopic technique

^{13}C and ^{14}C contents

Table 2.1 shows the result of measurements of ^{14}C activity $(a^{14}C)_p$ and $\delta^{13}C$ of the twigs of *Q. ilex* performed at the Laiatico site. Equation (6) was used to calculate the local CO_2 concentration using the corrected activity $(A^{14}C)_p$ in Table 2.1. Delta ^{13}C ($\delta^{13}C$) in plant material ranged from -33 ‰ to -25 ‰ at the vent and from -30 ‰ to -26 ‰ at the control area. The average $\delta^{13}C$ of *Q. ilex* twigs was -28.1 ‰ for both the vent and control areas. This value is consistent with the assumption that the $\delta^{13}C$ from the vent was the same as that from the atmosphere. The ^{14}C activity $(A^{14}C)_p$ of *Q. ilex* twigs varied significantly between the two areas ($p=0.03$). It ranged from 78.2% to 129.2 % at the vent area and from 121.8% to 131.3 % at the control area with a mean of 105 % at the vent and 126 % at the control area. Variations in the ^{14}C activity at the vent area were about six times higher than variations at the control area (Table 2.1).

The highest ^{14}C activity in the twigs was 131.3 % was appreciably higher than the atmospheric value of 110.8 % in the sampling year 1995. We used the value of 131.3 % as the reference atmospheric ^{14}C activity $(A^{14}C)_a$ for ambient air at the Laiatico site during growth of the twigs. This value seems to be more representative since calculations with 110.8 % led to CO_2 concentration clearly lower than the present ambient CO_2

concentration. The value of 131.3% corresponds to the global average ^{14}C in the air for the period from 1968 to 1995 indicating that the twigs were 25-28 years old. The present-day atmospheric CO_2 concentration of 350 ppm was used as the normal ambient CO_2 concentration (p_a) at the sampling time (1995). Therefore we rewrote equation 6 as $p_s = 350 \times 131.3/(A^{14}C)_p$ ppm. At the control area (Fig. 2.7) the calculated CO_2 concentration varied from 350 ppm (see CS2, Fig. 2.7) to 381 ppm (see CS9, Fig. 2.7) with an average of 365 ppm (Table 2.1).

Table 2.1. $\delta^{13}C$ and ^{14}C activity of twigs of *Quercus ilex* growing at the Laiatico mineral CO_2 vent and estimated ambient CO_2 concentrations. Standard errors are in parenthesis.

Site	$\delta^{13}C$ (‰)	$(a^{14}C)_p$ (%)	$(A^{14}C)_p$ (%)	p_s ppm
Vent area				
S1	-27.4	121.8	122.4 (0.33)	376
S2	-29.2	83.1	83.80 (0.20)	549
S3	-29.0	90.7	91.50 (0.36)	503
S5	-29.4	77.7	78.40 (0.20)	587
S6	-33.1	76.9	78.20 (0.40)	588
S7	-28.4	124.4	125.3 (0.30)	367
S9	-25.8	129.0	129.2 (0.30)	356
S10	-28.4	102.0	102.7 (0.20)	448
S12	-25.1	115.0	115.1 (0.20)	400
S15	-26.1	123.2	123.5 (0.26)	373
S16	-25.8	111.5	111.7 (0.38)	412
S17	-30.6	97.7	98.90 (0.23)	465
Min.	-33	77	78	356
Max.	-25	129	129	588
Mean	-28	104	105	451
rSTD	-8	17	17	19
Control area				
CS1	-29.6	120.6	121.8 (0.20)	378
CS2	-27.8	130.6	131.3 (0.26)	350
CS3	-29.5	127.6	128.7 (0.26)	357
CS7	-25.9	126.9	127.1 (0.43)	362
CS9	-26.5	120.5	120.8 (0.30)	381
Min.	-30	121	121	350
Max.	-26	131	131	381
Mean	-28	125	126	365
rSTD	-5	3	3	3.6

‡, Percentage of 1950 activity (pre-bomb standard air); rSTD, relative standard deviation (%).

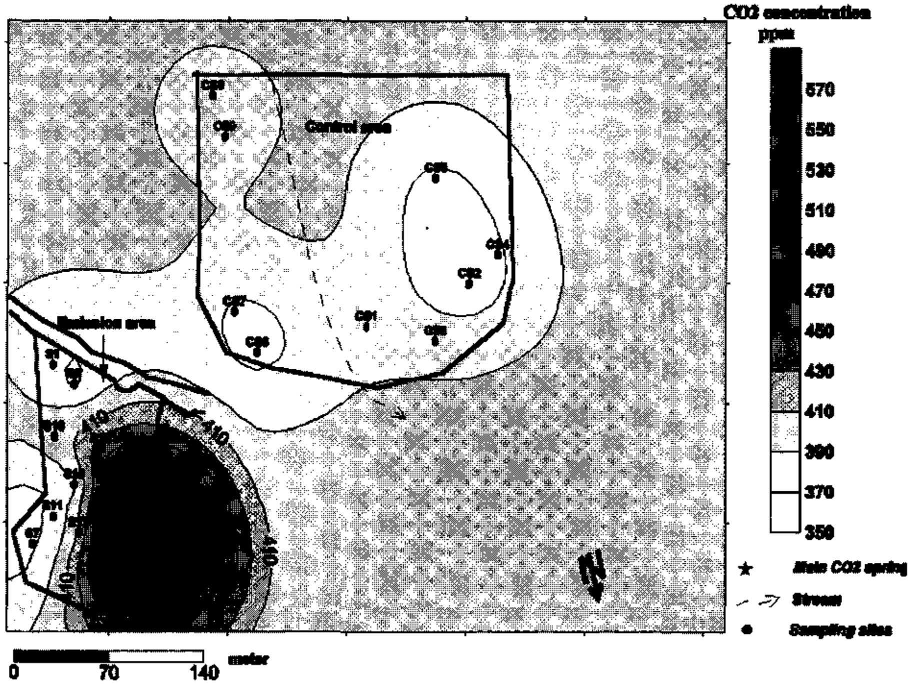

Figure 2.7. CO_2 concentration gradient at the Laiatico study area, estimated from ^{14}C activity in 25-28-year old *Quercus ilex* twigs, and known ^{14}C in ambient CO_2 and CO_2 from the vent. Sampling sites are marked by (•).

At the vent area the CO_2 concentration varied from 356 ppm to 588 ppm with an average of 461 ppm (Table 2.1). Variations in the calculated CO_2 concentration at the vent area were about five times higher than those at the control area. Two-tailed *t* test indicated a significant difference ($p=0.04$) in the local mean CO_2 concentration between the vent and control areas.

CO_2 spatial variability

Figure 2.8 shows a semivariogram of the CO_2 concentration. The sill value of 7333, the nugget effect of 963 and a range of 70 indicate a strong spatial dependency. The variogram was best described by the spherical function, which had an error of 5 %. Other models gave very similar results. Figure 2.8 shows the isopleths of the effective CO_2 gas mixing ratio constructed based on the calculated CO_2 concentration (Table 2.1) and the spherical semivariogram values. The CO_2 concentrations at the vent area are clearly elevated close to the vent, reaching ambient values within 50 to 100 m away from the vent. The mean CO_2 concentration emission directly above the vent was predicted to be 510 ppm.

The sum of the error in the estimate of p_s is about ± 5 %, slightly higher than that reported by van Gardingen et al. (1995). This is likely associated with the larger number of sites and the larger area we sampled. The CO_2 concentrations estimated with the ^{14}C method gave a very consistent pattern with values increasing downslope of the vent, and decreasing upslope. The maximum CO_2 concentration that we found is close to the predicted CO_2 concentration of 600 ppm for the year 2050.

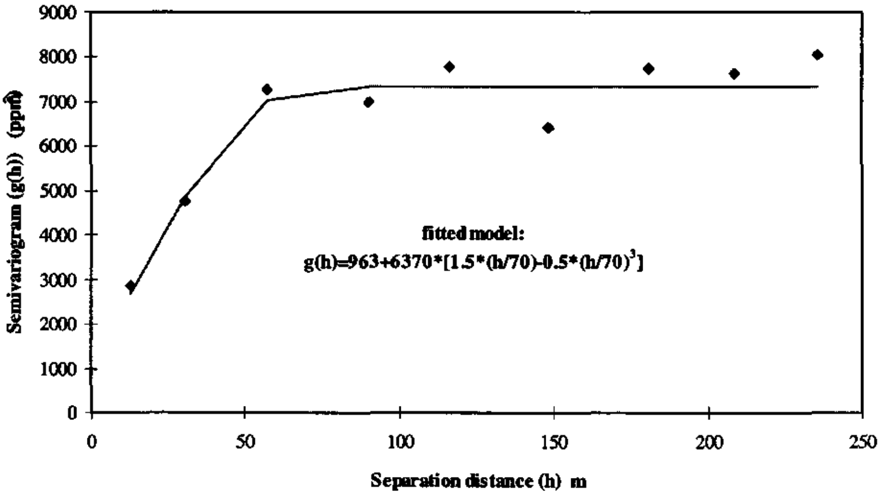

Figure 2.8. Semivariogram of ambient CO_2 concentrations around the Laiatico CO_2 vent and fitted spherical model.

DISCUSSION

The observed diurnal course for CO_2 (Fig. 2.3A) found in the present study at the Bossoleto site is in good agreement with earlier results at the same site by van Gardingen et al. (1995) and may be explained by the location of the CO_2 vent. At the bottom of the crater of the Bossoleto site dense CO_2-rich air accumulates during the night, and disperses as soon as sun radiation is incident on the bottom of the crater and temperature is rising (van Gardingen et al., 1995). In contrast to the Bossoleto site, the Laiatico site is located along a slope. During the day CO_2 is rising with increasing temperatures and spreading over the vegetation area of the vent. When temperatures decline during the night, the dense CO_2-rich air does not rise, and is flowing downhill close to the ground surface. It seems that at both sites, Bossoleto and Laiatico, the pattern of the CO_2 gas mixing ratio is mainly influenced by landscape characteristics and temperature differences during night and day. It remains unknown whether there is any change in CO_2-emission rates at all. In contrast to the Bossoleto and the Laiatico site, the Solfatara site is characterized by a flat area. There is neither a crater leading to an accumulation of CO_2-rich air nor a slope causing a downhill flow of CO_2. This difference in the topographic position may explain why the CO_2 gas mixing ratio - although highly fluctuating - does not exhibit diurnal course (Fig. 2.3C).

At all sites investigated CO_2 released from the vents was contaminated with atmospheric sulphur compounds (Fig. 2.4, 2.5). So far, H_2S was known to be the only phytotoxic gaseous component emitted by CO_2 vents in Italy (Duchi et al., 1985). The present study demonstrates that in addition to H_2S, SO_2 accompanies CO_2 as a trace gas. It has been suggested that negative effects on slow-growing plants can already be expected if long-term mean concentration of 30 ppb H_2S and 2-5 ppb SO_2 are exceeded (DeKok et al., 1983; Heber et al., 1987; Slovik et al., 1992). These concentrations are exceeded for both SO_2 and H_2S concentrations at the sites, but damage to the plants could not be observed (Miglietta et al., 1993c; Badiani et al., 1993). Therefore, the threshold values of phytotoxicity mentioned above are questionable for plants growing at natural CO_2 vents. Enhanced growth and biomass accumulation under elevated CO_2 (Seegmüller et al., 1996) requires enhanced reduced sulphur for protein synthesis of growing tissues. Therefore, it may be assumed that a rapid flux of SO_2 or H_2S into cellular sulphur pools may even support growth and development under these conditions. However, CO_2 as well as SO_2 and H_2S are taken up predominantly via the stomata (Garsed and Read, 1977; Norby and Kozlowski, 1982; Olszyk and Tingey, 1985; DeKok et al., 1983). As a consequence, stomatal aperture, which may respond to fluctuations in CO_2 on a scale of seconds to minutes (Raschke, 1972) strongly determines the sulphur gas flux into the plants in the course of a day or a vegetation period. Thus, the atmospheric concentrations measured in this study do not provide reliable information on the dose of atmospheric sulphur the plants were exposed to.

It is difficult to estimate long-term (annual average) CO_2 concentration distribution around the vent from direct measurements of highly fluctuating atmospheric CO_2 concentrations. In the present study the vegetation was used as an integrator using the known ratio of $^{14}CO_2$ activity of the vent to $^{14}CO_2$ activity of the ambient air. The CO_2 gas from the vent is isotopically depleted of ^{14}C, so all the ^{14}C in the plant tissues comes from the ^{14}C in the ambient air. The ^{14}C method was previously used by van Gardingen et al. (1995) and Bettarini et al. (1995) to estimate the effective average local CO_2 concentration experienced by plant material around natural CO_2 vents at the Bossoleto and the Solfatara site. The estimates of CO_2 concentrations calculated from ^{14}C abundance measurements of twigs from plants grown at the vent of the Laiatico site were in the same range as the mean CO_2 concentrations measured during the day using gas analysis. The tendency of the long-term CO_2 concentration to be lower than the short term daily mean CO_2 concentration can be explained by the different time-scale of the measurements (van Gardingen et al., 1995). While ^{14}C data are integrated over the lifetime of plant tissues, short-term CO_2 measurements reflect the CO_2 concentration over a period of hours. Both short-term measurements and ^{14}C contents clearly demonstrate the atmospheric CO_2 enrichment in the vicinity of the CO_2 vents.

Enhanced atmospheric CO_2 concentrations can cause enhanced rates of photosynthesis, but only small stimulation of above ground biomass production (Körner and Arnone, 1992; Wolfenden and Diggle, 1995). In contrast, root biomass production often tended to increase under elevated CO_2 by an increased C allocation to the roots (Seegmüller et al., 1996; Körner and Arnone, 1992; Norby et al., 1986). Increased C allocation to roots may explain enhanced consumption in the soils close to the CO_2 vent at Bossoleto and Laiatico (factor 3.2 and 115, respectively). Ecosystem processes mediated by soil biota are constrained by the input of energy-rich C-components into the soil, and it is likely that this input increases due to increased root production, root turnover or carbon exudation when CO_2 concentrations are rising (Curtis et al., 1994). Considering this context we can assume that methane consumption of several organisms of soil biota increased in the vicinity of the CO_2 vent, thereby causing enhanced methane deposition.

In contrast to the CH_4 flux rates, N_2O flux rates from the soil at the Bossoleto and the Laiatico site did not change significantly with enhanced atmospheric CO_2 gas mixing ratio [0.6 - 11 $\mu g\ N\ m^{-2}\ h^{-1}$ and 1.9 - (-2.9) $\mu g\ N\ m^{-2}\ h^{-1}$, respectively]. This finding is consistent with a study on N_2O emissions in a California annual grassland exposed to elevated CO_2 (Hungate et al., 1997).

However, when soil at the Bossoleto CO_2 vent was irrigated, N_2O emission increased dramatically by a factor of 13 compared to soil not irrigated. This increase in N_2O production confirms results obtained earlier in other studies on the effect of rainfall on N_2O production (Hungate et al., 1997; Davidson et al., 1991). Irrigation of dry soil may cause a flush of labile organic C and N from dead microorganisms (Bottner, 1985) or root material. This creates conditions to which bacterial and fungal biomass quickly responds, including changes in the rate of nitrification (Hungate et al., 1997) and denitrification (Rudaz et al., 1991). Therefore it can be assumed that elevated CO_2 not only alters soil microbial biomass but also soil emissions of N_2O during rainfall events. The magnitude of the contribution of N_2O from ecosystems with naturally CO_2 enrichment and rain events to the global budget can not be estimated from this study, since the data base available is too small.

In conclusion, the present study performed at three CO_2 vents in Central Italy demonstrates the heterogeneity of background environmental factors around a natural CO_2 vent and the high fluctuations of concomitant atmospheric trace gases. Atmospheric sulphur compounds complicate the extrapolation of CO_2 effects on plant metabolism. However, each of the three sites investigated offers a terrestrial ecosystem exposed continuously to naturally elevated CO_2 at varying sulphur trace gas exposure. Therefore, a comparison of the sites may be as a useful tool for estimating the impact of increasing global CO_2 concentration on natural ecosystems in predictive models.

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(References are give on page 135)

Chapter 3

Long-term elevated CO_2 does not influence litter quality and decomposition in *Smilax aspera* L. growing around a natural CO_2 spring

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INTRODUCTION

Elevated atmospheric CO_2 has the potential to affect C and N cycling in terrestrial ecosystems in several ways. A possible effect of elevated CO_2 is an increase in plant growth rates via stimulation of photosynthesis, showing an increased C-uptake by terrestrial higher plants (Bazzaz, 1990; Amthor, 1995). The extra carbon taken up by terrestrial plants will then enter the soil through litterfall and root turnover, causing greater C pool sizes in the soil. Through increased sequestration of C in plants, the storage of atmospheric CO_2-C in the soil will ultimately provide a negative feedback to the rising atmospheric CO_2 concentration. Accumulation of C in the soil imposes a negative feedback on plant growth through N immobilization as well. This would therefore limit further plant growth at elevated CO_2. Quantitative impacts of the enhanced C assimilation on soil C pool sizes are difficult to assess, because the C pool in the soil is very large compared to the inputs (Hungate et al., 1996).

Elevated CO_2 may also affect soil C pools via effects on litter quality. Plant material grown at elevated atmospheric CO_2 may have a lower N content (Cotrufo et al., 1994; Cotrufo and Ineson, 1995, 1996; Coûteaux et al., 1991,1996; Lawler et al., 1997; Robinson et al., 1997) and higher lignin and polyphenolic compounds contents (Melillo, 1983; Lambers, 1993; Cotrufo et al., 1994; Coûteaux et al., 1996; Peñuelas et al., 1996,1997) than that grown at the present-day ambient concentration of 360 ppm. Lower N content, higher C/N ratio and content of lignin and polyphenol of leaf litter due to elevated CO_2 are often associated with a lower C and N mineralization rate (Coûteaux et al., 1991, 1996; Cotrufo et al., 1994; Cotrufo and Ineson, 1995, 1996). Reduced litter mineralization rates in turn could further increase sequestration of C in soils, and thus lessen the rate of increase of atmospheric CO_2. Reduced decomposability of litter would also result in a decrease in N mineralization, and hence N availability for plants. Lower N availability in turn would have a negative feedback to further C assimilation by plants at elevated CO_2. So far, some of the information available on effects of CO_2 on litter quality refers to green leaves and was obtained under artificially short-term greenhouse experiments at high CO_2 levels. Because nutrients are withdrawn before litter fall and re-translocated to stem and root (O'Neill, 1994), it is doubtful whether the chemistry of green leaves can be a good determinant of litter decomposability. Second, it is not known whether CO_2 effects on litter chemistry observed in short-term experiments are sustained over a longer period of exposure to elevated CO_2.

Effects of elevated CO_2 on the chemical nature of leaf litter may ultimately provide insight into possible changes in nutrient turnover, and subsequently in ecosystem dynamics at further elevated atmospheric CO_2 (van Breemen and van Dam, 1993). To predict changes in net ecosystem productivity (NEP), and to understand positive and (or) negative feedbacks between ecosystem's components, the chemical composition and decomposition of plant material grown at long-term elevated CO_2 is of great importance.

The possibility exists to test effects of elevated CO_2 on quality of senescent leaf litter and C and N cycling by studying the vegetation occurring near natural CO_2 springs. In this sense, CO_2 springs have been already considered useful tools to address long-term response of plants and ecosystems to elevated CO_2, since vegetation growing around these has been exposed to elevated CO_2 for generations (Miglietta et al., 1993a; Amthor, 1995). Another advantage of natural CO_2 springs is that we can study CO_2 effects on C and N cycling in a natural environment where belowground feedbacks are already under way. However, CO_2 springs suffer from a number of limitations such as the lack of true replicates and controls, high spatially and temporally variability in CO_2 concentration, the potential presence of pollutants and the lack of quantitative knowledge of the history of CO_2 concentrations (Miglietta and Raschi, 1993; Amthor, 1995). Large spatial variability in soil properties is also a drawback of natural CO_2 springs.

Our study specifically tested the hypothesis that litter quality of plants was decreased by long-term exposure to elevated CO_2, resulting in decreases in rates of C and N mineralization.

MATERIALS AND METHODS

Study location

The study site is near the village of Laiatico, approximately 35 km south-east of Pisa, Tuscany, Italy. The vegetation is coppiced-Mediterranean oak-ash woodland composed of *Quercus ilex, Quercus cerris, Fraxinus ornus, Quercus pubescens*, with shrubs such as *Erica arborea* and *Arbutus unedo*. The understory is dominated by the vine *Smilax aspera* L. The CO_2 spring is in a small gully. There is a gradient in CO_2 concentration from ambient levels (about 360 ppm), outside of the CO_2 enriched area, up to a few percent CO_2 very close to the main vent. Concentrations measured over the area by means of an

Infrared gas analyser may vary widely depending on the weather conditions and may rapidly fluctuate in response to short-term variation in atmospheric turbulence. Therefore, we estimated the distribution of mean CO_2 concentrations around the vent from ^{14}C activity in twigs of *Quercus ilex*, and known ^{14}C in ambient CO_2 and CO_2 from the vent as described in van Gardingen et al. (1995), Bettarini et al. (1995) and Schulte et al. (1998) Briefly, the use of radioactive carbon isotope to determine mean long-term levels of CO_2 exposure of vegetation depends on the fact that the CO_2 venting out at the spring is fully depleted in ^{14}C. The air and CO_2 mixture of the local atmosphere has, therefore, a well defined signal that is reflected by the ^{14}C content of the vegetation. The lower the ^{14}C content in the plant tissue, the higher the mean CO_2 exposure level of the vegetation.

Plant sampling of *S. aspera* was made from two areas: the first one was within about 5 m of the vent ("emission area"), and the second was somewhat uphill from the emission area about 150 m from the vent with similar soil and vegetation ("control area") (Fig. 3.1). Soils of the area are calcareous, brown silty clay loam, developed from Tertiary marl. To study effects of CO_2 on litter quality, we selected a species that is abundant throughout the area and that produces large quantities of leaf litter, the C_3 (AJ Munting, pers. comm.) evergreen vine *Smilax aspera*. Pooled samples of abscised leaves of *Smilax aspera* were collected at three sampling points in two plots in the emission area, one with a CO_2 level of about 540 ppm around the vent (high CO_2 plot) and, one with an annual mean CO_2 level of about 370 ppm at a distance of 80 m away from the vent (low-CO_2 plot). Since CO_2 concentrations may vary strongly within a short-term period of time (even from hour to hour), the low-CO_2 plot was included to take into account possible short-duration high CO_2 in the part of the emission area with low mean CO_2 concentrations. Similarly, three pooled samples were collected in a control area (150 m away from the vent) where the CO_2 concentration was not affected by the vent, and had the normal value of about 360 ppm.

Each plot was replicated three times, and all measured parameters are expressed on a plot basis. The mean local atmospheric CO_2 concentrations (n=3) were 363 ppm for control plot, 373 ppm for low-CO_2 plot and 543 ppm for high-CO_2 plot (Schulte et al., 1997).

The difference in local CO_2 concentrations between control and low-CO_2 plots was not statistically significant, but it was significant between high-CO_2 and low-CO_2 ($p=0.004$) and between high-CO_2 and control plots ($p=0.003$).

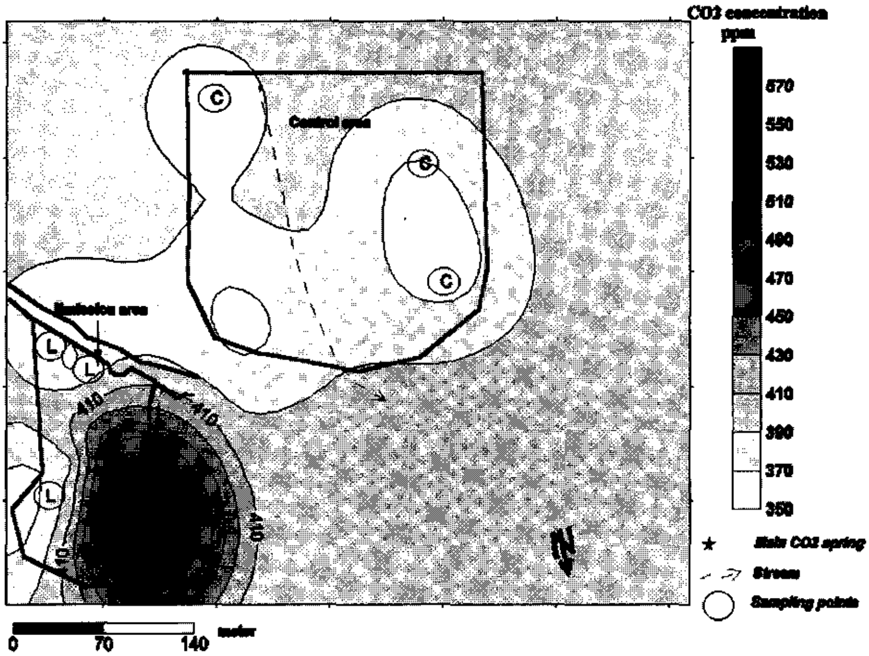

Figure 3.1. CO_2 concentration gradient in the Laiatico study area. *Smilax aspera* litter was collected in plots with an ambient(C), low (L) and high (H) atmospheric CO_2 concentrations. Sampling sites are marked by (O). For each plot, three field samples were selected and analyzed separately.

Litter chemical analysis

The leaf samples were washed with demineralized water to remove any soil particles, air-dried at room temperature, chopped and sieved over 1 mm sieve. Moisture content was determined from a subsample (1 mm size) after oven-drying at 105 °C overnight. One mm-mesh samples were reduced in size randomly to produce representative subsamples, and to promote sample homogeneity. A representative subsample was milled for total N and total C analysis. Five milled samples from each sampling point were analyzed (van Lagen, 1996) by dry combustion on a CHN element analyzer (Carlo Erba EA 1108, InterScience, Milan).

The concentration of total extractable polyphenols was determined using the Folins-Denis reagent (Anderson and Ingram, 1993). Three 1 mm-mesh samples from each sampling point were boiled in 50% methanol at 75 °C and filtered quantitatively over a S&S nr.589/3 filter. Next, 2.5 ml of the Folins-Denis was added to the extract, followed by 10 ml of 10% sodium carbonate. Tannic acid was used as standard and treated in the same way as leaf samples. The absorbance was read at 760 nm on a spectrophotometer (Perkin-Elmer 55E) on the day of extraction. Extracts that turned turbid after sodium carbonate addition, were left overnight for the precipitate to settle, and the analysis was repeated on the next day for the whole series. Total soluble polyphenols measured this way include hydrolyzable tannins, condensed tannin and non-tannic phenols. The values were expressed as tannic acid- equivalent polyphenols. The polyphenol contents were also expressed as % of total C (polyphenol-C) by using a tannic acid ($C_{76}H_{52}O_{46}$) with 54% carbon as a representative polyphenol. All concentrations were expressed on an oven-dry (105 °C) weight basis. A standard deviation of about 5% relative to mean values was accepted as measurement error in C, N and polyphenol analysis.

Incubation experiment

Potential C and N mineralization were studied in a laboratory incubation experiment at constant humidity and temperature for 48 days (C mineralization) or 60 days (N mineralization). A soil sample from the 0-10 cm mineral horizon of the Laiatico site was evenly mixed with non-calcareous beach sand (1 gram of Laiatico soil and 25 grams of sand). Properties of the materials used for incubation are reported in Table 3.1. One mm-mesh leaf litter was mixed with the mineral soil materials to obtain a homogeneous mixture. This procedure reduces variability related to the differences in the chemical composition of leaves and promotes direct contact between litter and soil particles (Randlett et al., 1996). Six litter samples of ninety five mg (oven-dry weight basis) from each sampling point (3 plots each with 3 sampling points) were mixed with twenty four g of the mixed soil (oven-dry weight basis) in a 250 ml incubation vessel. Litter-amended soils were moistened by adding 5 ml of demineralized water to a soil moisture content of about 65% of field capacity. All vessels were kept overnight in the dark at 20±0.1 °C prior to incubation. Three samples were analysed for initial inorganic N (NO_3-N, NO_2-N and NH_4-N). Initial mineral N was extracted with 1 *M* KCl and determined colorometrically on a Technicon AutoAnalyzer II (van Lagen 1996). The rest of vessels was placed in a soil respirometer to monitor CO_2 evolution. The cumulative C decomposition and respiration rates were determined by automated continuous measurement of CO_2 absorbed in 0.6 *M* KOH, by tracking the electrical conductance of the KOH. The CO_2 evolution was monitored hourly for 48 days at 20±0.1 °C, as described by Nordgren (1988). Sample

Table 3.1. Properties of the soil used for C and N incubation (n=4).

		Laiatico soil	Beach sand	Mixture
pH(H_2O)	(-)	7.9	-	-
pH(KCl)	(-)	7.6	-	-
C	(%)	3.6	0.009	0.20
N	(%)	0.30	0.003	0.013
C/N	(-)	12.0	3.0	15.4
Texture	(-)	silty clay loam	sand	-
Mineral N	(mg N. kg^{-1})			
	Initial	6.6	-	3.96
	Final	49.8	-	5.46
N mineralization rate ($month^{-1}$)		21.5	-	0.75

containers were opened for reaeration after 48 days and incubated again for 12 more days for final inorganic N content. Mineral N was extracted at the end of incubation (60 days). Soil moisture content was determined on day 60. The moisture loss was negligible (1.4% of the total moistened mixture). Twelve soil samples without added litter material were used as controls. Six controls were analyzed for initial N and six controls were analyzed for C mineralization and final inorganic N content. C and N mineralization data were expressed on an oven-dry (105 °C) weight basis. A relative standard deviation of about 8% for C mineralization and 15% for N mineralization was accepted as measurement error.

The cumulative C mineralization was calculated as the difference between CO_2 evolved from three soils containing litter and the mean values of CO_2 evolved from six soils without litter. C turnover was calculated as the ratio of cumulative C mineralization to total C content. Potential net N mineralization was calculated by subtracting initial mineral N from final mineral N for each litter sample. N turnover was expressed as % of total added N by dividing cumulative mineral N by total N content.

Statistical analysis

The significant differences between plots (CO_2 effect) was evaluated by one-way analysis of variance (ANOVA) using SigmaStat (Jandel Scientific, Germany). The data were tested for homogeneity of variance and normality before analysis. The Student Newman-Keuls test' multiple comparison was subsequently used for differences among means. Regression analysis was performed on linear relationship between litter quality parameters and C and N mineralization. Differences were considered statistically significant at $p \leq 0.05$, unless mentioned otherwise.

C mineralization data were fitted exponentially, using a two-compartment function:

$$C_t = C_1(1 - e^{-k_1 t}) + C_2(1 - e^{-k_2 t})$$

in which C_t is the cumulative CO_2-C (mgC kg^{-1} soil) at time t, C_1 is the labile pool size (mgC kg^{-1} soil), k_1 is the decay rate for the labile pool (day^{-1}), C_2 is the active pool size (mgC kg^{-1} soil), k_2 is the decay rate for the active pool (day^{-1}) and t is the time from the start of the incubation (day). The sum of C_1 and C_2 is the potentially decomposable C (C_o).

RESULTS AND DISCUSSION

N and polyphenol contents of leaf litter

We observed no significant difference in C, N and polyphenolic concentrations of *Smilax* litter between the three plots (Table 3.2). Elevated CO_2 also did not significantly influence polyphenol-C, and ratios of C/N, polyphenol/N and polyphenol-C/N (Table 3.2). Also the C and N contents and C/N ratio of green leaves of *Smilax* did not differ significantly between the three plots (data not shown).

Our results indicate elevated CO_2 did not affect litter chemistry. Another study showed that the N and polyphenol contents, and C/N ratio of *Quercus cerris* and *Quercus pubescens* litter growing at elevated CO_2 in the same study area were also unaffected by increased atmospheric CO_2 concentration (Raiesi et al., 1997a; Raiesi, 1998). This corresponds to results by Körner and Miglietta (1994), Miglietta et al. (1995), Bettarini et al. (1995) and Jones et al. (1995) who found no impact of elevated CO_2 on N content and C/N ratios of fresh leaves in a number of evergreen Mediterranean species growing around mineral-CO_2 springs in Central Italy.

Some other studies performed under controlled experiments also showed little or no impact of elevated CO_2 on chemical composition of plant residues (Curtis et al., 1989; Owensby et al., 1993, 1996; Kemp et al., 1994; Taylor and Ball, 1994; Randlett et al., 1996; O'Neill and Norby, 1996; Hirschel et al., 1997). Recently, Ball and Drake (1997) indicated that

***Table** 3.2.* Litter quality of *Smilax aspera* growing at control, low and high atmospheric CO_2 concentrations around the Laiatico mineral CO_2 spring, Italy. Each value represents means (n=3), standard deviations are included in parenthesis. Significantly different ($p<0.01$) means were only observed for the CO_2 concentration, as indicated by different letters.

		Plot		
Variables		Control	Low-CO_2	High-CO_2
CO_2	ppm	363 (15) b	373 (12) b	543 (80) a
C	(%)	55.9 (0.64)	54.6 (0.83)	55.7 (0.44)
N	(%)	1.01 (0.12)	1.03 (0.05)	1.07 (0.11)
C/N	-	56.1 (06.0)	53.1 (3.00)	52.6 (4.98)
Polyphenol	(%)	0.82 (0.09)	0.99 (0.13)	0.86 (0.11)
Polyphenol/N	-	0.81 (0.07)	0.97 (0.12)	0.81 (0.07)
Polyphenol-C	(%)	0.82 (0.07)	0.99 (0.14)	0.81 (0.07)
Polyphenol-C/N	-	0.83 (0.16)	0.97 (0.15)	0.77 (0.10)

the C/N ratio of the *Spartina patens* (a C_4 grass) litter (senescent material) grown at elevated CO_2 (ambient + 340 ppm) did not differ from that grown at ambient CO_2. In contrast, the authors observed a higher C/N ratio in *Scirpus olneyi* (a C_3 sedge) grown at elevated CO_2. Franck et al. (1997) reported that enhanced CO_2 had modest effects on C/N ratio of litter with increased C/N ratio of *Lolium* roots, but with decreased C/N ratio of *Avena* shoots.

Other studies based on short-term CO_2 enrichment experiments indicated a reduction in litter N and higher C/N ratio with CO_2 enrichment (see Bazzaz, 1990; Dìaz et al., 1993; Huluka et al., 1994; Cotrufo et al., 1994; Torbert et al., 1995; Coûteaux et al., 1996; Murray et al., 1996; Henning et al., 1996; Robinson et al., 1997; Lawler et al., 1997) and in a recent study, Robinson et al. (1997) reported that the total phenolic content in *Eucalyptus* foliage grown under elevated CO_2 increased as a consequence of increased carbon availability by elevated atmospheric CO_2 (793 ppm).

There are several possible explanations to reconcile these conflicting results. Differences in experimental conditions, such as application of nutrients, stage of development (seedlings vs. mature trees) and length of exposures, between controlled and "natural" exposure studies might be one of these. Also, plant species may differ in their responses to elevated CO_2 (Strain, 1987; Hunt et al., 1993; see Lee and Jarvis, 1995; Wullschleger et al., 1995;

Loehle, 1995). Some species like herbaceous plants may accumulate larger amounts of non-structural carbohydrates in the leaves even after long-term CO_2 exposure (Körner and Miglietta, 1994) and such an accumulation can cause changes in C/N ratio of the leaf litter. Others, like mature trees, may have the capacity to dissipate the extra-C fixed under elevated CO_2 towards a sink. This sink may be provided by enhanced root growth or by an enhanced production of secondary metabolites like volatile organic compounds (VOCs). Furthermore, long-term exposure of plants to elevated CO_2 may have caused acclimation or even adaptation of photosynthesis in this species (Miglietta and Raschi, 1993; Miglietta et al., 1993a) leading to a reduced capacity of CO_2 fixation and homeostatic adjustments of carbon uptake, especially in this N-limited ecosystems (Oechel and Vourlitis, 1996). Also Field et al. (1992) concluded that under nutrients-limited conditions, a characteristic of natural Mediterranean forests, natural ecosystems are less responsive to elevated CO_2 than managed ecosystems like agricultural crops.

C and N mineralization

Litter decomposition

Because atmospheric CO_2 did not markedly affect litter quality parameters such as C/N ratio and polyphenol content, we did not expect to see effects of CO_2 on mineralization rates. Initial (0-3 days) litter respiration rate was slightly ($p=0.06$) higher in the low-CO_2 plot (Fig. 3.2) than in the two other plots, but this effect disappeared with time (Fig. 3.3).

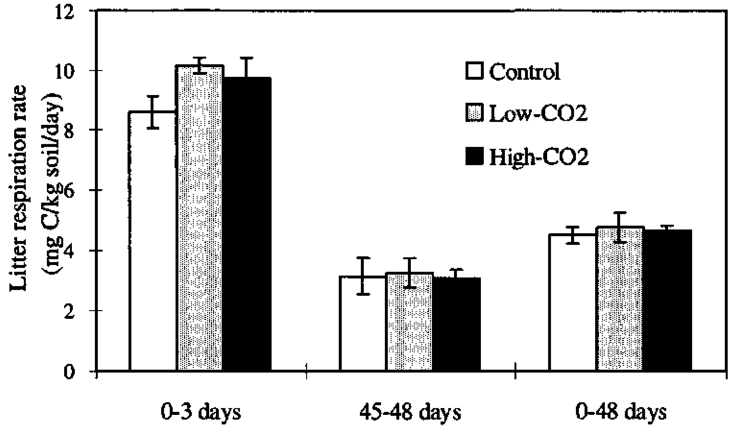

Figure 3.2. Initial (0-3 days), final (45-48 days) and mean (0-48 days) respiration rate in *Smilax aspera* litter produced at control, low and high-CO_2 concentrations. Each point represents means (n=3), bars indicate standard deviations.

The amount of C respired after 48 days of incubation did not differ significantly between the plots. Neither the relative nor the absolute cumulative C showed a significant difference between the three plots (Table 3.3).

We have fitted cumulative C mineralization data using a double-exponential model (Table 3.4) and a single-exponential model (data not shown). The goodness of fit (r^2) for the double-exponential model (0.999) was better than that for the single-exponential model (0.956). Litter from all plots had similar labile pool size, active pool size and decomposition rate constants for the labile and active fractions. A large proportion of C was allocated to the active pool with a turnover time of 183 days, e.g., the active pool size was 13 times as large as the labile pool size. The turnover time for the labile pool was about 6 days. We also observed no impact of elevated CO_2 on litter decomposability of *Quercus pubescens* and *Quercus cerris* grown under elevated CO_2 (Raiesi, 1998). The observed lack of impact of elevated CO_2 on litter decomposition corresponds with the results of Randlett et al. (1996), Henning et al. (1996) and Torbert et al. (1995), who reported that plant residues produced at elevated CO_2 had similar decomposability relative to plant residue produced at control CO_2.

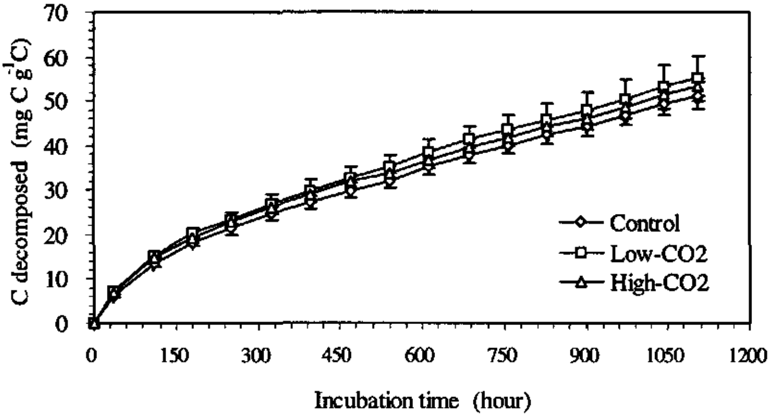

Figure 3.3. C decomposition in *Smilax aspera* litter produced at control, low and high- CO_2 concentrations during 48 days lab. incubation at 20 °C . Each point represents means (n=3), bars indicate standard deviations.

Kemp et al. (1994) observed a relatively minor CO_2 effect on the decay rate of naturally abscised litter in the tallgrass prairie ecosystems.

By contrast, Taylor and Ball (1994) observed that although the C/N ratio of residue was unaffected by elevated CO_2, soil respiration was significantly higher in soils amended with sorghum (C_4) residue from elevated CO_2 (700 ppm) than that measured in soils amended with residue from ambient conditions.

N mineralization

During the 60-day incubation, we observed a net N immobilization in all litter-amended soils (Table 3.3) indicating that N was incorporated into microbial biomass. The soil without plant material had a net N mineralization of 1.5 mg N kg^{-1} soil over the entire incubation (Table 3.1).

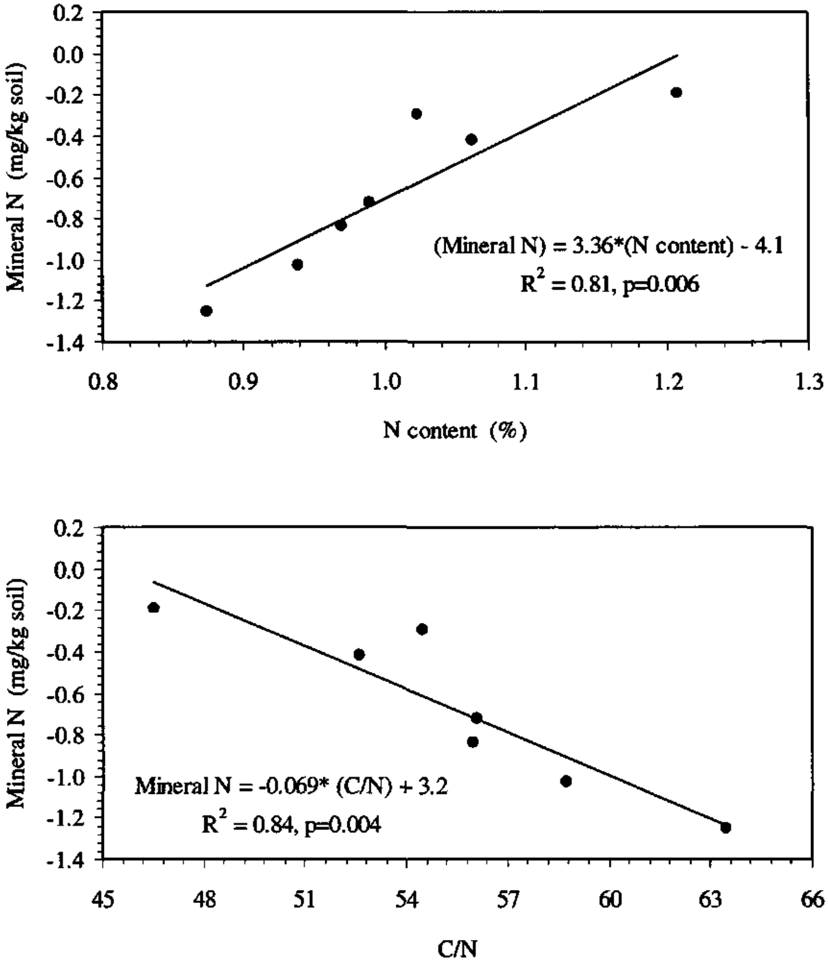

Figure 3.4. Linear regressions between initial N content (top panel) and C/N ratio (bottom panel), and N mineralization after 60 days of incubation in *Smilax aspera* litter. The regression equations and R^2 values are given.

N immobilization was higher in litter from the control plot and lower in litter from the high-CO_2 plot, but the difference was not significant (Table 3.3). N immobilization had a high standard deviation due to the low inorganic N content. Most of the variation in N immobilization between the plots would be explained by the litter N content (r^2=0.81, p=0.006), C/N ratio (r^2=0.84, p=0.004) and polyphenol-C/N ratio (r^2=0.45, p=0.10, Fig. 3.4).

In a previous study, we also found that N mineralization rate of litter from *Quercus pubescens* and *Quercus cerris* was not affected by elevated CO_2 (Raiesi, 1998) after 125 days of incubation. Our findings are similar to those of Henning et al., (1996), who observed no effect of enhanced CO_2 (705 ppm) on N mineralization of soybean and sorghum tissue after 70 days incubation and of Randlett et al., (1996) for net N mineralization in *populus* litter. Cotrufo et al. (1994) reported that N mineralization measured as cumulative NH_4^+ and NO_3^- over 155-day incubation was not affected by elevated CO_2 for ash, birch, sycamore and spruce litter. Our data, however, contrasted with other short-term CO_2 experiment, which observed a lower N mineralization with CO_2 enrichment (Torbert et al., 1995; Boerner and Rebbeck, 1995).

Table 3.3. C and N turnover rates in *Smilax aspera* litter derived from control, low and high atmospheric CO_2 concentrations around the Laiatico mineral CO_2 spring, Italy. C and N turnover rates were measured during a laboratory incubation at 20 °C for 48 days (C mineralization) or 60 days (N mineralization). Each value represents means (n=3), standard deviations are included in parenthesis. None of the mean were significantly different (p<0.05).

Plot	C turnover		N turnover	
	mg C kg^{-1} soil	mg C g^{-1} C	mg N kg^{-1} soil	mg N g^{-1} N
Control	215.3 (14)	51.3 (3.2)	-1.08 (0.25)	-6.40 (1.5)
Low-CO_2	228.7 (23)	55.1 (5.2)	-0.76 (0.36)	-4.47 (2.5)
High-CO_2	222.8 (07)	53.2 (1.9)	-0.54 (0.35)	-3.21 (2.2)

CONCLUSIONS

This experiment was conducted to study the impact of elevated atmospheric CO_2 on litter quality and C and N dynamics in *Smilax aspera* L. exposed to increased CO_2 concentrations in the vicinity of natural CO_2 springs for a very long period. The litter quality of *Smilax aspera* and its subsequent decomposability were not affected by elevated CO_2. We have evidence that the response of litter quality to long-term elevated CO_2 in two other dominant plant species in this ecosystem is similar to that of *Smilax aspera*. Therefore we speculate that effects of CO_2 on litter quality play no significant role in changing the carbon balance and SOM pools of Mediterranean ecosystems containing typical oak-ash woodlands under the future higher concentrations of atmospheric CO_2.

Table 3.4 Estimated mean values of model parameters using the doubled-pool exponential function fitted to the measured accumulated C decomposition during 48 days. None of the means (n=3) within each column appeared to be significantly different at $p \leq 0.05$, standard deviations are given in parentheses.

		Plot		
Variable		Control	Low-CO_2	High-CO_2
C_1	mgC kg^{-1}soil	49.2 (7.17)	54.8 (11.5)	56.5 (11.0)
C_2	mgC kg^{-1} soil	626 (148)	587 (113)	627 (069)
k_1	day^{-1}	0.1846 (0.03)	0.2015 (0.03)	0.1873 (0.03)
k_2	day^{-1}	0.0069 (0.00)	0.0075 (0.00)	0.0065 (0.00)
Co	mgC kg^{-1}soil	675 (155)	642 (114)	683 (073)
r^2	-	0.999	0.999	0.999
Litter turnover	day	160 (40.1)	141 (17.2)	162 (19.9)
Annual cumulative C^a	mgC kg^{-1}soil	605 (106)	599 (098)	620 (051)

^{a} predicted by model

This does not rule out the possibility that elevated CO_2 will stimulate soil organic carbon pool through higher litter input caused by increased net primary production. Evidence of such enhanced sequestration of SOM under elevated CO_2 is presented elsewhere (Raiesi et al., 1998b).

Litter N mineralization and consequently N availability is not affected by CO_2 in agreement with the lack of a CO_2 effect on litter quality, and therefore provides no negative or positive feedback on biomass production. Accordingly, plants may not suffer from a lower soil N availability, when they grow under elevated CO_2. In conclusion, our results do not support the general hypothesis that elevated CO_2 decreases litter quality and its C and N mineralization rates.

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(References are give on page 135)

Chapter 4

Impacts of elevated atmospheric CO_2 on litter quality, litter decomposability and nitrogen turnover rate of two oak species in a Mediterranean forest ecosystem

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Impacts of elevated atmospheric CO_2 on litter quality, litter decomposability and nitrogen turnover rate of two oak species in a Mediterranean forest ecosystem

ABSTRACT

Elevated CO_2 may affect litter quality of plants, and subsequently C and N cycling in terrestrial ecosystems, but changes in litter quality associated with elevated CO_2 are poorly known. Abscised leaf litter of two oak species (*Q. cerris* L. and *Q. pubescens* Willd.) exposed to long-term elevated CO_2 around a natural CO_2 spring in Tuscany (Italy) was used to study the impact of increasing concentration of atmospheric CO_2 on litter quality and C and N turnover rates in a Mediterranean-type ecosystem. Litter samples were collected in an area with elevated CO_2 (>500 ppm) and in an area with ambient CO_2 concentration (360 ppm). Leaf samples were analyzed for concentrations of total C, N, lignin, cellulose, acid detergent residue (ADR) and polyphenol. The decomposition rate of litter was studied using a litter bag experiment (12 months) and laboratory incubations (3 months). In the laboratory incubations, N mineralization in litter samples was measured as well (125 days). Litter quality was expressed in terms of chemical composition and element ratios. None of the litter quality parameters was affected by elevated CO_2 for the two *Quercus* species. Remaining mass in *Q. cerris* and *Q. pubescens* litter from elevated CO_2 was similar to that from ambient conditions. C mineralization in *Q. pubescens* litter from elevated CO_2 was lower than that from ambient CO_2, but the difference was insignificant. This effect was not observed for *Q. cerris*. N mineralization was higher from litter grown at elevated CO_2, but this difference disappeared at the end of the incubation. Litter of *Q. pubescens* had a higher quality than *Q. cerris*, and indeed mineralized more rapidly in the laboratory, but not under field conditions.

Key-words: litter quality, litter decomposability, N mineralization, mineral-CO_2 springs, Mediterranean ecosystems, *Quercus cerris*, *Quercus pubescens*

INTRODUCTION

The atmospheric CO_2 concentration is increasing and may reach twice its pre-industrial value of 280 ppm by the middle of the 21st century (Bolin, 1986; Keeling, 1993; IPCC, 1994; Schimel, 1995). Such elevated CO_2 influences plant physiology and growth, and increases net primary production (NPP) by 30-40% in C_3 plants (Kimball, 1983; Mooney et al., 1991; Poorter, 1993), which make up about 95% of the earth's vegetation. Increased NPP therefore needs to be considered in the global C cycle (Idso and Idso, 1994).

Increased standing biomass and higher carbon input to the soil via litter and root debris may lead to enhanced accumulation of carbon in the soil as soil organic matter (SOM), if other factors influencing decomposition, such as litter quality, temperature and soil moisture, remain unchanged. Increased litter input would provide a negative feedback to the atmospheric CO_2 level. However, if global air temperatures increase by 1.5-4.5 °C due to increasing atmospheric CO_2 (Mitchell et al., 1990), decomposition rates may increase (Jenkinson et al., 1991; Oechel et al., 1993) lowering the storage of SOM in the soil. If, however, increased mineralization would release more N to the soil, this could further help increased primary production and C sequestration (Schimel, 1995; Schimel et al., 1996). The net effect of these partly opposing processes is rather uncertain, and further investigations are required to increase understanding of these processes.

The situation is even more complex, because increasing atmospheric CO_2 may also influence the chemical composition of plants, litter quality, and subsequently litter decomposability. Many short-term experiments have indicated that plant material grown at elevated CO_2 tended to have higher contents of non-structural carbohydrates, like starch and sugar, of structural carbohydrate like lignin and cellulose, as well as of some secondary metabolites like polyphenols, and lower contents of nutrients, in particular nitrogen (Allen et al., 1988; Coûteaux et al., 1991, 996; Field et al., 1992; Lambers, 1993; Melillo, 1993; Körner and Miglietta, 1994; Cotrufo et al., 1994; Cotrufo and Ineson, 1995; Torbert et al., 1995; Boerner and Rebbeck, 1995; Peñuelas et al., 1997). Such changes in litter quality may decrease the turnover rates of carbon and nitrogen in the soil, at least in the short-run (Bazzaz, 1990; Coûteaux et al., 1991, 1996; Cotrufo et al., 1994). On the other hand, increased formation of easily decomposable non-structural compounds, like starch (Körner and Miglietta, 1994) would stimulate microbial activity, and would enhance N immobilization by soil microbes. This could then lead to a slower recycling of plant material through decreased soil N availability (Dìaz et al., 1993).

To provide insight in the role of soils as global sources and/or sinks of C, it is necessary to understand the effects of both changes in temperature and in litter quality on decomposition. Experiments to assess and quantify the hypothetical effects of increased CO_2 on terrestrial ecosystems rarely last longer than a few years. Because of downward regulation of photosynthesises at continued high CO_2 (Miglietta and Raschi, 1993; Miglietta et al., 1995; Oechel and Vourlitis, 1996), and because of the slow nature of decomposition of soil organic matter, longer-lasting experiments would be desirable. Such experiments are provided by nature in the vicinity of natural CO_2-emitting springs. These springs are recognized as potential experimental sites to address the long-term impact of global increased CO_2 concentration on terrestrial ecosystems (Miglietta and Raschi 1993; Miglietta et al., 1993a). I investigated the impacts of long-term CO_2 enrichment on i) litter quality, ii) subsequent litter decomposability and iii) N turnover rate around such a CO_2-emitting spring.

MATERIALS AND METHODS

Research location

The study area is near Laiatico (43°26'N, 10°42'E) in the Tuscany region of Italy, at an altitude of 190-240 m ASL. Annual rainfall is about 830 mm. The area (*ca.* 0.95 ha) has a general slope of about 40% facing north-east, and is covered by a species-rich coppice oak-ash forest ecosystem, typical for a Mediterranean climate. *Quercus cerris* L., *Fraxinus ornus* L. and *Quercus pubescens* Willd. are co-dominant in the tree layer. A dense layer of undergrowth vegetation includes *Smilax aspera* L. and scattered grass in open spaces. Grass is not found in places where the tree density is high and light is a limiting factor. Soils of the area 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(H_2O) 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 CO_2 vent is located in a narrow gully. Carbon dioxide is the main gas emitted by the vent, with local CO_2 concentrations ranging from 600 ppm near the main vent to 360 ppm within 50 m from the source. Shortly, the distribution of local CO_2 concentration at the site was determined by taking samples from 25-year-old twigs of *Q. ilex*, and determining the ^{14}C content. Because the carbon from the vent is isotopically depleted of ^{14}C, and the total ^{14}C in the twigs comes only from the air, we can estimate the effective CO_2 concentration using the known ratio of ^{14}C in the air to ^{14}C in the twigs (Schulte et al., 1998, see Fig. 2.7). The vent also emits H_2S at a concentration too low to affect plant growth (Schulte et al., 1998, Miglietta pers. comm.). For the sake of simplicity the site within 50 m of the vent is termed the "emission area" in this work. A control area with similar slope, parent material, morphology, vegetation and hydrology was selected at a distance of about 150 m south-east of the emission area. The CO_2 concentration in the control area is 360 ppm. The control area was selected in such a way that represents similar physical and chemical properties of the soil, such as pH, texture, etc., to the emission area. Total soil N and C/N ratio in the forest floor and mineral surface horizon (0-10 cm) were comparable in both the emission and control areas (Raiesi et al., 1998 b, c).

Litter sampling and treatments

I grouped the litter samples according to CO_2 level of the site by selecting plots as follows:

-High-CO_2 plot (P1-P2): CO_2 concentration in the emission area of 530-450 ppm.
-Low-CO_2 plot (P3-P4): CO_2 concentration in the emission area of 450-360 ppm.
-Control plot (P5-P6): ambient conditions, about 150 meters away from the emission area (around 360 ppm CO_2).

Freshly abscised leaves from *Quercus cerris* and *Quercus pubescens* in the emission area and in the control area were collected in December 1995 from two litter sampling sites (20m × 20m) in each plot (3.1). On both *Quercus* species, the senescent leaves remain on the tree for a few months in autumn, and shedding takes place around December. Duplicated litter samples (200 grams) of *Quercus cerris* and *Quercus pubescens* were picked manually from both the trees and the soil surface in each plot (3 plots × 2 replicated sites × 2 species). Litter samples were pooled for each sampling site to obtain large composite samples, representative for the selected sampling sites.

The samples were air-dried for one week at room temperature, cut into two pieces with scissors, and mixed carefully. Approximately forty grams of each leaf sample was used for litter bag experiments. Water content upon drying at 70 °C was determined. The rest was chopped to pass a 1-mm sieve for lignin and polyphenol determinations, and for C and N mineralization experiments. A representative subsample from 1 mm-sized material was taken and ball-milled for C and N analysis.

Quality analysis of litter

The moisture content of sieved material was determined by oven-drying at 105 °C overnight. Total C and N were analyzed on a CHN element analyzer (Carlo Erba 1108, Milan) (van Lagen, 1996). The lignin and cellulose contents were determined using the acid detergent fibre (ADF) method using the 1 mm material (Rowland and Roberts, 1994). The concentration of total soluble polyphenols including hydrolyzable and condensed tannins was determined using the Folin-Denis reagent in sieved material (Anderson and Ingram, 1993). All concentrations were expressed on oven-dried weight basis.

Litter bag experiment

Litter bags (n=240), each containing two grams of the air-dried leaves were prepared. The litter samples were placed into nylon net bags (20×18 cm; 1.5×1.5 mm mesh size). Twenty bags of each species were surface-buried in the F layer of the forest floor (at 2-4 cm depth) at the location where they were collected: at P1, P2, P3 and P4. In addition, twenty bags of each species from the emission area (P1) were buried at P3, and similarly, litter from P3 was buried at P1. Burial time was mid December 1995. Half of the litter bags were retrieved after 4 months (April 1996) and the remaining ones after 12 months (December 1996). Upon retrieval the litter bags were washed to remove any soil particles, oven-dried at 70 °C overnight and weighed for remaining mass.

N incubation experiment

200 mg-subsamples of air-dried, sieved litter were mixed thoroughly with 200 g of a fresh sandy forest soil (5-15 cm) from Wageningen. The chemical characteristics of the

soil are in Table 4.1. The mixture was put in 0.5 *l* plastic containers and 15 ml of distilled water was added. The experiment was carried out in triplicate. Five containers with soil, but without plant material were used as controls. The containers were closed tightly and kept in the dark in a temperature-controlled chamber at 20±1 °C. The samples were re-aerated weekly for adequate oxygen supply. 1-M KCl extractable (soil:extractant ratio of 1:5) inorganic N (NH_4^+ and NO_3^-) was analyzed colorimetrically after 0, 14, 28, 43, 70 and 125 days (van Lagen, 1996). The cumulative mineralized N was expressed as a fraction of total N initially present in the mixture.

Table 4.1. Characteristics of the Wageningen soil (5-15 cm depth) used for C and N incubation.

pH		C	N	C/N	Initial N (mg.kg^{-1})	ρ_b	Texture %		
(H_2O)	(KCl)	%	%		(NH_4-N+NO_3-N)	g.cm^{-3}	clay	silt	sand
3.8	3.6	1.3	0.06	20	22	1.31	4	12	84

C incubation experiment

500 mg-subsamples of 1 mm-sized, air-dried litter were mixed thoroughly with 15 g of a fresh sandy forest soil (5-15 cm depth) from Wageningen (see Table 4.1 for chemical characteristics). The mixture was put in 0.25 *l* plastic respirometer vessels, to which 5 ml of distilled water was added. The measurements were carried out in triplicate. Sixteen vessels with soil, but without plant litter were used as controls. The CO_2-C evolution rate was monitored automatically by hourly measurement of CO_2 absorbed in 0.6 M KOH through the electrical conductance of the KOH for about 3 months (Nordgren, 1988). The cumulative CO_2 was averaged for each period of 100 hours (4 days) and was expressed as a fraction of total C initially present in the mixture.

Statistical Analysis

The initial chemical composition of leaf litter and the C and N mineralization parameters were linearly regressed against the local CO_2 concentration. I analyzed differences in chemical composition and C and N mineralization data between CO_2 plots (3 plots) and *Q. pubescens* and *Q. cerris* litter using two-way analysis of variance (ANOVA). The original data were either arcsin or log transformed for equal variance and normal distribution before analysis, when needed. Differences were considered significant only when p values were lower than 0.05, unless stated otherwise. All statistical calculations were performed using SigmaStat (Jandel Scientific, Germany).

RESULTS

Litter quality

The chemical composition of *Q. cerris* and *Q. pubescens* litter is given in Tables 4.2 and 4.3. Chemical composition and elemental ratios were not significantly correlated with atmospheric CO_2 concentration (Table 4.4).

Table 4.2. C, N, acid detergent residue (ADR), lignin, cellulose and polyphenols concentrations in litter produced under high, low and control CO_2 conditions at the Laiatico mineral CO_2 spring. Values are means (n=2); standard deviations are in parenthesis.

Plot	C %	N %	ADR %	Lignin %	Cellulose %	Polyphenols %
Q. cerris						
High-CO_2	55.7 (0.4)	0.72 (0.0)	46.9 (1.6)	27.2 (1.6)	19.7 (0.1)	19.3 (0.7)
Low-CO_2	55.8 (0.6)	0.73 (0.0)	44.4 (1.9)	25.3 (2.0)	19.2 (0.1)	14.7 (4.8)
Control	56.2 (0.3)	0.76 (0.0)	47.3 (1.3)	26.8 (1.0)	20.6 (0.3)	20.5 (6.3)
Mean (n=6)	55.9 (0.5)	0.74 (0.0)	46.2 (2.1)	26.4 (1.8)	19.8 (0.6)	18.2 (5.3)
Q. pubescens						
High-CO_2	52.7 (0.2)	0.81 (0.0)	42.1 (3.8)	19.3 (2.7)	22.8 (1.0)	21.1 (0.4)
Low-CO_2	53.0 (0.1)	0.80 (0.0)	43.5 (2.6)	20.7 (1.7)	22.9 (0.9)	17.2 (5.1)
Control	52.3 (0.6)	0.84 (0.1)	39.9 (0.6)	18.5 (1.2)	21.4 (0.6)	19.7 (1.9)
Mean (n=6)	52.7 (0.5)	0.81 (0.1)	41.9 (3.1)	19.5 (2.2)	22.4 (1.1)	19.3 (3.5)
Source of variation		ANOVA	$p \geq F$			
CO_2	0.85	0.56	0.92	0.93	0.91	0.50
Species	0.00	0.05	0.05	0.00	0.00	0.73
CO_2 × Species	0.42	0.95	0.39	0.55	0.13	0.90

Also ANOVA revealed that the chemical composition of *Q. cerris* and *Q. pubescens* litter derived from high-CO_2 was similar to that of litter derived from ambient CO_2

plots. The interaction of CO_2 and species with regard to litter quality was not significant (Tables 4.2 and 4.3). Also lignin, cellulose and polyphenol expressed as percentage of total C were not significantly different between elevated and ambient CO_2 plots (data not shown). The results, however, showed that *Q. cerris* litter, on average, had a lower quality than *Q. pubescens* litter.

Table 4.3. C/N, lignin/N, cellulose/N, (lignin+cellulose)/N and lignin/cellulose ratios of litter produced under high, low and control CO_2 conditions at the Laiatico mineral CO_2 spring. Values are means (n=2); standard deviations are in parenthesis.

Plot	C/N	Lignin/N	Cellulose/N	(Lignin+cellulose /N	Lignin/cellulose
Q. cerris					
High-CO_2	77.7 (0.0)	38.0 (2.4)	27.4 (0.3)	65.5 (2.7)	1.4 (0.1)
Low-CO_2	76.8 (1.6)	34.8 (2.8)	26.4 (0.0)	61.2 (2.9)	1.3 (0.1)
Control	73.6 (1.7)	35.0 (1.8)	26.9 (0.9)	61.9 (2.7)	1.3 (0.0)
Mean (n=6)	76.0 (2.1)	35.9 (2.8)	26.9 (0.7)	62.9 (3.3)	1.3 (0.1)
Q. pubescens					
High-CO_2	65.2 (1.6)	23.9 (4.0)	28.3 (2.1)	52.2 (6.1)	0.8 (0.1)
Low-CO_2	66.6 (1.1)	26.0 (1.8)	28.7 (0.7)	54.7 (2.5)	0.9 (0.0)
Control	63.2 (8.0)	22.5 (4.1)	25.8 (2.2)	48.2 (6.3)	0.9 (0.1)
Mean (n=6)	65.0 (5.0)	24.1 (3.8)	27.6 (2.2)	51.7 (5.9)	0.9 (0.1)
Source of variation		ANOVA $p \geq F$			
CO_2	0.59	0.75	0.51	0.66	0.90
Species	0.01	0.00	0.55	0.02	0.00
CO_2 × Species	0.93	0.67	0.46	0.64	0.65

Litter decomposition (litterbag)

Litter decomposition was calculated as the fraction of the initial dry mass remaining (RM, %) after 4 and 12 months incubation under field conditions (Table 4.5). *Q. cerris* leaf litter produced at high-CO_2 and low-CO_2 had a similar remaining mass after 4

Table 4.4. Correlation coefficients (r) between litter quality parameters and ambient CO_2 concentration.

Variables		QP n=6	QC n=6	QP+QC n=12
C	%	0.19	-0.66	-0.04
N	%	-0.08	-0.70	-0.19
ADR	%	-0.15	0.09	0.00
Lignin	%	-0.26	0.21	0.01
Cellulose	%	0.11	-0.30	-0.05
Polyphenol	%	0.41	-0.01	0.15
C/N		0.05	0.51	0.13
Lignin/N		-0.17	0.46	0.08
Cellulose/N		0.10	0.47	0.15
(Lignin+Cellulose)/N		-0.07	0.48	0.11
Lignin/Cellulose		-0.41	0.37	0.04

QP= *Quercus pubescens*; QC= *Quercus cerris*

Table 4.5. Remaining mass (RM) for the leaf litter of two oak species produced at high and low CO_2 levels after 4 and 12 months incubated under field conditions. Values are means (n=2); standard deviations are in parentheses.

Plot	RM_4 %	RM_{12} %
Q. cerris		
High-CO_2	71.9 (1.6)	39.4 (0.2)
Low-CO_2	69.7 (3.2)	31.7 (3.3)
Mean (n=4)	70.8 (2.7)	35.5 (4.5)
Q. pubescens		
High-CO_2	66.2 (6.9)	30.6 (2.9)
Low-CO_2	62.9 (4.3)	30.0 (3.3)
Mean (n=4)	64.6 (6.0)	30.3 (3.1)
Source of Variation	ANOVA $p \geq F$	
CO_2	0.57	0.21
Species	0.23	0.13
$CO_2 \times$ Species	0.91	0.27

months incubation. After 12 months, *Q. cerris* leaf litter produced at high-CO_2 decomposed slower (-24%, but not significant) than the litter produced at low-CO_2. High variation (3.4%) in remaining mass after 12 months contributed to lack of statistically significant differences. The same lack of clear CO_2 effect was found for *Q. pubescens*. I observed no significant correlation between the field decomposition of litter and the local CO_2 concentration (Table 4.6) and no significant interaction between CO_2 and species with regard to litter decomposition under field conditions (Table 4.5). The two species showed small differences in litter decomposition pattern. After 4 and 12 months, *Q. pubescens* litter decomposed slightly faster than *Q. cerris* litter.

Q. cerris litter from the high-CO_2 plot decomposed significantly faster (14% after 4 months and 38% after 12 months) when buried in the low-CO_2 plot than *Q. cerris* litter from high-CO_2 buried in the high-CO_2 plot, and *Q. cerris* litter produced at low-CO_2 decomposed slightly slower (-10% after 4 months and -24% after 12 months) when incubated in the high-CO_2 plot than *Q. cerris* litter from low-CO_2 buried in the low-CO_2 plot (Table 4.7). For *Q. pubescens*, I found no differences.

Litter decomposition (laboratory incubation)

C mineralization

The cumulative C mineralization patterns of the incubated soil-litter mixture are represented in Figure 4.2. The contribution of the organic C in the soil used to incubate the litter (based on incubation of soil alone) to mineralized C was less than 3.5 % of that of the litter (data not shown).

Q. cerris litter from the three plots had similar decomposition patterns, with slightly higher decomposition at the end of the incubation for the litter from the control plot (Fig. 4.2A). The cumulative C mineralization during the entire incubation was higher for *Q. pubescens* litter from the control plot than that from the high and low-CO_2 plots in the emission area, but the differences between treatments were not significant. High variability in litter decomposition from the control plot (within plot variation) may have caused the lack of statistically significant differences. Decomposition of litter from the high and low-CO_2 plots in the emission area was similar (Fig. 4.2B).

Table 4.6. Correlation coefficients (r) between field decomposition, N mineralization and C mineralization vs. ambient CO_2 concentration. (See the text for symbols and abbreviations).

Variables	QP n=6	QC n=6	QP+QC n=12
Field study			
RM_4 (%)	-0.27	0.26	-0.08
RM_{12} (%)	-0.21	0.60	0.24
N mineralization			
N_{m125} ($g.kg^{-1}$ N)	0.56	0.79	0.29
C mineralization			
Cm_{90} ($g.kg^{-1}$ C)	-0.33	-0.51	-0.24

QP= *Quercus pubescens*; QC= *Quercus cerris*

Table 4.7. Remaining mass (RM) for the leaf litter of two oak species grown under high and low CO_2 levels during a cross-experiment at the Laiatico mineral CO_2 spring.

Litter from	Incubated in	RM_4 %	RM_{12} %
Q. cerris			
High-CO_2	High-CO_2	73.6	39.6
Low-CO_2	Low-CO_2	66.6	28.7
High-CO_2	Low-CO_2	64.9	28.6
Low-CO_2	High-CO_2	73.7	37.5
Q. pubescens			
High-CO_2	High-CO_2	59.3	27.6
Low-CO_2	Low-CO_2	58.6	26.8
High-CO_2	Low-CO_2	61.9	29.4
Low-CO_2	High-CO_2	57.9	27.4

There was no significant correlation between the cumulative C mineralization (C_{m90}) after 3 months and the local CO_2 concentration for the two species studied (Table 4.6). Also the CO_2 and species interaction was not significant for the cumulative C

Impacts of elevated atmospheric CO_2 on soil organic matter around naturally occurring mineral CO_2-springs

I. Elemental composition, pool sizes and ratios

ABSTRACT

Ecosystems that have been exposed to long-term enhanced CO_2 concentrations offer highly relevant information on feedback mechanisms between soil organic matter dynamics and the rising atmospheric CO_2 level. In this study the effect of enhanced atmospheric CO_2 on elemental composition, and C and N pools of soil organic matter formed around a mineral CO_2 spring was investigated in a Mediterranean woodland ecosystem. Samples from the forest floor (F and HA layers) and mineral soil (0-10 cm A horizon) were collected across the CO_2 gradient (360- *ca.* 600 ppm). We determined total C and N contents and calculated C and N pool sizes and C/N ratios. Total C contents in the F layer were higher at elevated CO_2, but not in the HA layer and 0-10 cm mineral soil. Total C pool sizes were higher in CO_2 enriched areas only in the forest floor (F+HA layers). For the three layers, total N contents and C/N ratios were not affected by elevated CO_2, while N pool sizes appeared to increase with CO_2 enrichment. Such increases in N and C pools resulted from a gradual build up of soil organic matter. It is concluded that elevated atmospheric CO_2 may lead to accumulation of organic carbon in the forest floor (F+HA layers) of Mediterranean-type forests through enhanced plant production, but not by changes in quality of input materials. Our findings also suggest that soil will act as a net sink of C in the long-run.

Key-words: elevated CO_2, Mediterranean ecosystems, natural CO_2 springs, soil C content and pool, soil N content and pool, soil C/N ratios

Abbreviations: NPP, Net Primary Production; SOM, Soil Organic Matter; TC, Total Organic Carbon; W&B-C, Walkley & Black Carbon

INTRODUCTION

The CO_2 concentration has been increasing globally from 280 ppm before the beginning of the industrial revolution to 350 ppm at present. The CO_2 level is expected to double by the middle of the 21st century from the industrial revolution CO_2 concentration of 280 ppm (Goldewijk et al., 1994; IPCC, 1995). The annual increase in the global atmospheric CO_2 concentration is caused by burning of fossil fuel (6 Gt carbon annually) and deforestation (1 Gt carbon annually), mainly in the tropics. The rising atmospheric CO_2 has raised much public concern about possible enhanced global warming due to an increased greenhouse effect.

Soil is the largest store of organic carbon in the biosphere, estimated at 1300-1600 Gt C, or 65-80 % of the total biospheric organic carbon (Schlesinger, 1993; Goudriaan, 1995; Schlesinger, 1995; Houghton, 1995). Through exchange of C between soil and the atmosphere, with heterotrophic decomposers and primary producers as intermediates, soil organic matter (SOM) plays an important role both in the global carbon cycle and in nutrient cycles in terrestrial ecosystems.

Warming plus increased atmospheric CO_2 have the potential to influence plant growth quantitatively (Mooney et al., 1991; Idso and Idso, 1994) as well as qualitatively (Drake, 1992; Körner and Miglietta, 1994). Elevated CO_2 may increase primary production (NPP) and, therefore storage of C in plants, both above and below-ground. Enhanced CO_2 also influences the chemical composition of plant material. Plant material grown at elevated CO_2 has been reported to have higher contents of non-structural carbohydrate (Allen et al., 1988; Körner and Miglietta, 1994), of lignin (Melillo, 1983; Field et al., 1992; Cotrufo et al., 1994; Cotrufo and Ineson, 1995, 1996) of secondary metabolites (polyphenolics, Lambers, 1993) and lower contents of nutrients, in particular nitrogen (Allen et al., 1988; Cotrufo et al., 1994; Cotrufo and Ineson, 1995; Körner and Miglietta, 1994; Boerner and Rebbeck, 1995; Torbert et al., 1995). Elemental ratios such as C/N ratio, and lignin/N ratio of plant tissues have been found to be higher at elevated atmospheric CO_2 (Bazzaz, 1990; Coûteaux et al., 1991,1996; Cotrufo and Ineson, 1995,1996).

The photosynthesized CO_2-C ultimately reaches the soil through above and below-ground plant debris where it is largely decomposed to CO_2 and partly stored temporally as soil organic matter. Compared to ambient conditions, plant material grown under enhanced

CO_2 may differ in decomposition rate and this influences long-term soil organic carbon accumulation, but effects of elevated CO_2 on soil C dynamics are poorly-understood (van Breemen and van Dam, 1993). The net effect of elevated CO_2 may be increased accumulation of below-ground carbon in soil organic matter, thus providing a negative feedback to rising atmospheric CO_2 concentration, e.g. chemical changes in litter may decrease its decomposition rate (Coûteaux et al., 1991, 1996; Cotrufo and Ineson, 1995, 1996; Boerner and Rebbeck, 1995), but data on this subject is still inconclusive (O'Neill and Norby, 1996).

Changes in quantity and quality of SOM will also influence many environmentally relevant soil properties associated with SOM, such as the capacity of soil to supply sufficient nutrients and water. CO_2-induced changes in quality of litter input and SOM derived thereof slows down the mineralization rate and thus also the accessibility of major nutrients. On the other hand, enhanced CO_2 reduces the transpiration, decreasing the time period that decomposition and mineralization in surface soils are limited by water availability.

One problem with most published results is that they refer to short-term experiments. To test the hypothesis that natural forests soils under long-term elevated CO_2 will also have increased pools of SOM, decreased contents of N and decreased nutrient availability, we studied soil organic matter composition in a naturally enriched-CO_2 forest ecosystem in the vicinity of a CO_2 spring. Our objectives were to: 1) asses the impact of long-term elevated CO_2 on the elemental composition of forest litter and native SOM, 2) evaluate the distribution of organic carbon in the soil as a function of elevated CO_2 , 3) quantify the pool sizes of C and N, and 4) compare our results with those from short-term experiments.

MATERIALS AND METHODS

Research area

The study area is located in the Tuscany region, in the province of Pisa, Italy (43° 26'N, 10° 42'E), at 190-240 m above sea level (Fig. 1.2). The area (ca. 0.9 ha) is a rolling forested region with an irregular and rough surface, and with some incised streams and gullies, sloping down to the northwest. The presence of rock outcrops indicates the occurrence of erosion. The annual rainfall is 830 mm (UNESCO-FAO, 1963). The rainfall varies seasonally with a maximum in October and March and a minimum in July. The average yearly temperature is 15 °C and max. and min. monthly temperatures are 33 °C and -4 °C respectively. The CO_2 spring, consisting of one major vent and a number of smaller ones within a circle of 5 meters, is situated at the bottom of a gully. There is a CO_2 gradient from ambient (350 ppm) at some distance of the vent to about 600 ppm at the vent (Schulte et al., 1998). The CO_2 emission comes from thermal decomposition of carbonatic parent material under the influence of volcanic activity (Miglietta et al., 1993a).

The vegetation of the study location is representative of the major types of eu-Mediterranean and sub-Mediterranean ecosystems. The study area is a typical, semi-natural Mediterranean oak-ash coppice woodland which was last cut about 25 years ago. The main trees and shrubs species are listed in Table 5.1. Below the tree layer, which is composed of evergreen and deciduous trees of oak-ash stands, a shrub layer is formed mainly by a fast-growing, spiny vine (*Smilax aspera*) and with scattered grass locally. The tree distribution is not uniform, and the shrub layer is so dense that passage through the forest is locally difficult to impossible. The soil, derived from Tertiary marl, is covered by a litter layer of varying thickness. Table 5.2 presents some chemical and physical characteristics of the soil in the study area. We divided the forest floor into a F layer which contains partially decomposed plant materials with leaves still recognizable, and a HA layer which contains fully decomposed plant material with no plants material visible macroscopically. The soil texture is generally silty clay loam in the topsoil and silty clay in the subsoil. Except for the litter layer, the soil is rather homogenous throughout its depth.

Soil sampling was carried out in April 1995 to make an inventory of the distribution of SOM as a function of atmospheric CO_2 concentration and other potentially important (slope- and elevation related) soil forming factors. Because factors other than atmospheric CO_2 level may contribute to spatial variation of SOM, two control sites (ambient CO_2; similar soil parent material, slope, soil hydrological conditions, and vegetation) were selected. Soil samples were collected in the emission area along a CO_2 gradient of decreasing atmospheric CO_2 levels from the source to ambient (350 ppm) between 50 to 100 m from the vent (Fig. 5.1). Topographically similar transects were selected and sampled at the two control areas. The soil was sampled in duplicate at each sampling site. Sites were in rather flat, undisturbed locations within dense vegetation, so as to exclude

Table 5.1. The main forest species of Laiticao study area (Pisa, Italy).

Trees	**Abundance**	**Shrubs, Vines**	**Abundance**
Pistacia lentiscus	+	*Juniperus sp.*	+
Cupressus sp.	+	*Erica arborea*	+
Fraxinus ornus L†	++	*Smilax aspera*	+++
Quercus ilex L	+++	*Ligustrum vulgare*	+
Quercus robur L	+	*Myrtus communis*	++
Quercus pubescens Willd†	+	*Cytisus scoparius L*	+++
Quercus cerris L†	+++	*Cistus salvifolius*	++
Populus sp.	+	*Genista sp.*	++
		Arbutus unedo L	+

†: deciduous tree, +: less abundant, ++: abundant, +++: very abundant

Table 5.2. Soil properties of the Laiatico study area. †

Horizon	Depth cm	pH-(H_2O) -	pH-(KCl) -	TC %	W&B-C %	TN %	C/N -	Clay %	Silt %	Sand %	Texture -	$CaCO_3$ %
L	-12-(-7)	nd	nd	55	nd	0.68	81	-	-	-	-	0
F	-7-(-4)	6.3	5.9	39	31	1.70	23	-	-	-	-	0
HA	(-4)-0	6.4	6.1	33	25	1.60	21	-	-	-	-	0
A	0-10	6.9	6.4	4.6	3.9	0.30	15	36	62	2	si.cl.lo	5.8
B	10-20	7.3	6.8	2.8	2.2	0.20	14	39	59	2	si.clay	5.8
B	20-30	7.2	6.6	2.5	1.8	0.20	12	38	61	1	si.cl.lo	3.3
B	30-40	7.9	7.5	1.8	1.2	0.1	18	42	57	1	si.clay	11
B	40-50	7.9	7.5	2.3	0.8	0.1	23	40	60	0	si.clay	6.6
Bg	50-70	7.9	7.5	1.7	1.0	0.1	17	50	50	0	clay	9.1

†: Mean values (n=26 profiles) for the whole research area; TC: Total organic C; W&B-C: W&B C (wet oxidation); TN: Total N

effects of relocation of litter by erosion. Undisturbed soil cores of 26 profiles (17 in the emission, 9 in control areas) were sampled with a teethed auger (20 cm high and 8 cm ϕ).

The cores were sectioned vertically to obtain separate samples from the F and HA horizons in the forest floor and from 0-10 and 10-20 cm depths in the mineral soil. All samples were transported to Wageningen, The Netherlands, for analysis. All concentrations and measured values are expressed on an oven-dry (105 °C) weight basis.

Sample treatment and measurements

Core samples were weighed for bulk density. Moisture content was determined from a sub-sample after drying at 105 °C overnight. Next, samples were air-dried, crushed gently and sieved to obtain the fine earth fraction (<2 mm). Roots and stones were removed manually and weighed for bulk density correction. The stones were discarded, while the roots were stored for chemical analysis. Soil texture was determined by laser grain size analyzer (Buurman et al., 1997) on 23 randomly selected samples, after removing $CaCO_3$ by sodium acetate buffer solution (pH=5) and organic matter by H_2O_2 35%. Soil pH (pH-H_2O and pH-KCl), total C (TC) and N (TN) (element analyzer), and Walkley & Black C (W&B-C) using the Walkley & Black method were determined for all 225 samples (van Lagen, 1996). For the A horizon, those mineral samples with pH(H_2O) above 6.8 were subjected to 10 % HCl treatment to remove calcium carbonate, and analyzed for organic carbon. All the analyses were carried out in triplicate, unless mentioned otherwise. Methods of analysis are documented in van Lagen (1996).

Forest floor thickness

The thickness of the forest floor (F and HA layers) was measured for 55 randomly selected 1m×1m squares after removing the freshly fallen leaf layer (L), both in the emission and control areas. For each square, the thickness was measured for 5-3 points, and average thickness was taken for that particular square. Additionally, a metal ring of known diameter and height ($20\ cm^{-2} \times 5\ cm$) was inserted into the forest floor to determine the litter mass in relation to the litter thickness. The contents of the ring were oven-dried at 60 °C overnight and weighed.

The CO_2 gradient in the area

Because daily measurements of CO_2 concentration, based on a small numbers of days do not give a good representation of the mean annual atmospheric CO_2 concentration experienced during photosynthesis, we decided to use ^{14}C abundance of twigs of a specific age to determine the ambient CO_2 concentration (Schulte et al., 1998). We assumed that the CO_2 coming from the vent is isotopically (^{14}C) depleted, and collected twenty five-year-old fresh twigs of *Quercus ilex* to determine the mean ambient CO_2 concentration by analyzing the ^{14}C activity using the conventional ^{14}C age method. The ^{14}C analysis was done at the Center for Isotope Research, University of Groningen, The Netherlands. The CO_2 concentration for the rest of the sampling sites was interpolated by ordinary kriging. Figure 5.1 represents the isopleths of the CO_2 concentration for both the emission and control areas.

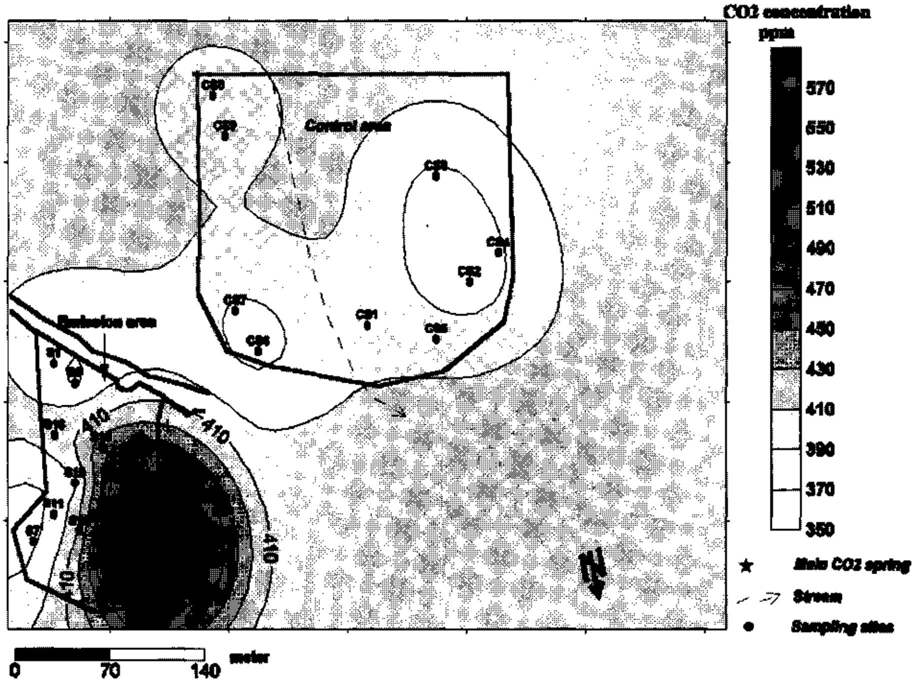

Figure 5.1. CO_2 concentration gradient in the study area, estimated from ^{14}C contents in 25-28-year old *Quercus ilex* twigs, and known ^{14}C in ambient CO_2 and CO_2 from the vent. Sampling sites are marked by (•).

Calculations and data analysis

We analyzed the data using classical statistics and an ANOVA procedure using the SigmaStat programme (Jandel Scientific, Germany). For each sampling site, concentrations for total C (TC), W&B C (W&B-C), total N (TN), total C and N pools and C/N ratios were calculated for F, HA and 0-10 cm horizons for the two areas. We carried out the statistical calculation for: 1) linear correlation between the elevation and the measured variables, 2) linear correlation between the CO_2 concentration and the measured variables and 3) multiple linear regression of the CO_2 concentration and the elevation against all variables. The emission area showed a wide range of the local CO_2 concentrations from the ambient level of 356 ppm up to elevated CO_2 level of 588 ppm. We therefore divided the emission area into two plots, e.g. low-CO_2 and high-CO_2. This resulted in 9 sites for the high-CO_2 plot, 8 for the low-CO_2 plot and 9 for the control plot for the F and HA layers, and 8 sites for each plot for the 0-10 cm mineral soil. We then used one way ANOVA to test differences in measured variables between the three plots. The data were either log- or reciprocally-transformed for equal variance and normal distribution prior to analysis, where necessary. We performed Student Newman-Keuls test's multiple-comparison to separate differences between plot means ($p<0.05$). In addition, a geostatistical procedure was used to map the litter thickness for the whole study area. The litter thickness was considered as a regionalized variable, and a semivariogram was calculated with the programme SPATANAL (Stein et al., 1994).

Table 5.3. C and N contents, C/N ratios and total C and N pool sizes of the F layer from the emission area (S1-S17) and the control area (CS1-CS9) at the Laiatico mineral CO_2 spring, Italy. (H: High-CO_2, L: Low- CO_2, C: Control-CO_2 plots).

			Concentrations (%)			Ratios		Pools ($g.m^{-2}$)		
Site	CO_2	Plot	TC	W&B-C	TN	C/N_{tc}	$C/N_{W\&B\text{-}C}$	TC	W&B-C	TN
S1	376	L	37.4	29.4	1.62	23.1	18.1	988	776	42.7
S2	549	H	50.3	34.5	1.79	28.1	19.3	1659	1138	59.0
S3	503	H	41.3	29.6	1.75	23.6	16.9	842	604	35.7
S4	430	L	35.5	28.8	1.69	20.9	17.0	596	484	28.5
S5	587	H	46.1	37.0	2.02	22.8	18.3	1119	898	49.1
S6	588	H	39.2	31.7	2.05	19.2	15.5	1224	988	63.8
S7	367	L	50.6	41.9	1.81	28.0	23.2	1350	1119	48.2
S8	513	H	42.5	33.8	1.85	23.0	18.3	1769	1406	76.8
S9	356	L	36.6	32.7	1.59	23.0	20.5	757	676	32.9
S10	448	H	43.2	32.6	1.63	26.5	20.0	855	645	32.2
S11	382	L	37.9	30.8	1.40	27.1	22.0	1070	869	39.4
S12	400	L	37.9	29.8	1.72	22.0	17.3	1058	833	48.1
S13	503	H	41.6	35.0	1.83	22.7	19.1	723	609	39.1
S14	494	H	42.9	34.4	1.70	25.2	20.2	1134	909	45.0
S15	373	L	44.3	36.0	1.67	26.5	21.5	1064	863	40.2
S16	412	L	38.4	30.6	1.54	24.9	19.8	656	522	26.3
S17	465	H	42.8	33.8	1.58	27.1	21.3	1620	1276	59.8
CS1	378	C	35.7	29.4	1.65	21.6	17.8	364	300	16.9
CS2	350	C	24.6	24.5	1.12	21.9	21.8	347	346	15.8
CS3	357	C	35.1	27.6	1.58	22.3	17.5	323	254	14.5
CS4	355	C	38.4	30.9	1.57	24.5	19.7	564	454	23.1
CS5	360	C	39.5	28.0	1.82	21.7	15.3	379	269	17.5
CS6	370	C	43.0	35.1	1.83	23.5	19.1	1471	1199	62.7
CS7	362	C	38.3	31.4	1.68	22.8	18.7	391	321	17.1
CS8	360	C	38.6	31.1	1.93	20.0	16.1	463	373	23.2
CS9	381	C	36.2	27.8	1.78	20.3	15.6	413	317	20.3

TC, total organic carbon (element analyzer); W&B-C, W&B C(wet oxidation)

RESULTS

C and N contents

The F layer: Table 5.3 represents the elemental composition of the F layer for both the emission (S1-S17) and control (CS1-CS9) areas. Total organic C contents (TC) had a significant positive correlation with the atmospheric CO_2 concentration ($r=0.47$, $p<0.05$, Table 5.6). The F layer in the CO_2-enriched area had significantly more TC ($p=0.015$) than in the control area (Table 5.7). The content of W&B carbon (W&B-C) was quantified to check whether or not easily decomposable C compounds (e.g. rhizodeposition material including root exudation), which may increase in plant roots growing at elevated CO_2, would increase in the soil.

Table 5.4. C and N contents, C/N ratios and total C and N pool sizes of the HA layer from the emission area (S1-S17) and the control area (CS1-CS9) at the Laiatico mineral CO_2 spring, Italy. (H: High-CO_2, L: Low- CO_2, C: Control- CO_2 plots).

			Concentrations (%)			Ratios		Pools ($g.m^{-2}$)		
Site	CO_2	Plot	TC	W&B-C	TN	C/N_{tc}	$C/N_{W\&B\text{-}C}$	TC	W&B-C	TN
S1	376	L	32.2	25.4	1.58	20.4	16.1	2126	1676	104
S2	549	H	47.3	37.0	1.83	25.8	20.2	3902	3051	151
S3	503	H	29.2	24.7	1.50	19.4	16.4	1491	1260	76.7
S4	430	L	32.5	25.1	1.65	19.7	15.2	1365	1056	69.3
S5	587	H	28.6	28.4	1.44	19.8	19.7	1736	1728	87.5
S6	588	H	28.1	22.5	1.52	18.5	14.8	2193	1753	118
S7	367	L	47.2	38.3	1.87	25.3	20.5	3152	2554	125
S8	513	H	31.1	25.7	1.48	21.0	17.3	3231	2672	154
S9	356	L	31.1	26.0	1.55	20.1	16.8	1608	1347	80.1
S10	448	H	34.2	26.6	1.42	24.0	18.7	1690	1319	70.5
S11	382	L	29.4	22.8	1.20	24.4	19.0	2073	1610	84.9
S12	400	L	32.6	26.2	1.58	20.7	16.6	2275	1831	110
S13	503	H	32.3	25.9	1.55	20.8	16.7	1404	1127	67.5
S14	494	H	36.3	29.7	1.57	23.1	18.9	2395	1961	104
S15	373	L	30.7	23.0	1.33	23.0	17.2	1844	1379	80.1
S16	412	L	30.4	23.8	1.39	21.8	17.1	1299	1018	59.6
S17	465	H	42.2	32.0	1.86	22.7	17.2	3989	3021	175
CS1	378	C	29.4	24.4	1.50	19.6	16.3	822	684	41.9
CS2	350	C	25.4	20.5	1.29	19.6	15.8	609	491	31.0
CS3	357	C	29.0	16.5	1.38	21.0	11.9	668	378	31.8
CS4	355	C	31.8	23.7	1.51	21.0	15.7	1048	780	49.8
CS5	360	C	34.8	28.2	1.89	18.5	15.0	836	678	45.2
CS6	370	C	38.6	29.4	1.80	21.5	16.4	3664	2792	171
CS7	362	C	33.8	27.9	1.65	20.5	16.9	862	710	42.1
CS8	360	C	25.7	19.8	1.46	17.6	13.6	771	595	43.8
CS9	381	C	32.5	23.6	1.65	19.8	14.3	927	672	46.9

TC, total organic carbon (element analyzer); W&B-C, W&B C(wet oxidation)

Although the W&B-C content was poorly correlated with the CO_2 concentration (Table 5.6), the mean W&B-C contents increased by 14% in the emission area relative to the control area. Parallel to the increased total C, the W&B-C content of the F layer of the area exposed to higher CO_2 level was significantly higher ($p=0.04$). Total N concentrations ranged from a low of 1.12% at 350 ppm (control area) to a high of 2.02% at 590 ppm (emission area). The N content showed a significant positive correlation with the CO_2 concentration ($r=0.53$, $p<0.01$). The mean N content was slightly, but insignificantly, higher ($p=0.14$, Table 5.7) in the emission area than in the control area.

Table 5.5. C and N contents, C/N ratios and total C and N pool sizes of the 0-10 cm mineral soil from the emission area (S1-S17) and the control area (CS1-CS9) at the Laiatico mineral CO_2 spring, Italy. (H: High-CO_2, L: Low- CO_2, C: Control- CO_2 plots).

			Concentrations (%)			Ratios		Pools ($t.ha^{-1}$)		
Site	CO_2	Plot	TC	W&B-C	TN	C/N_{tc}	$C/N_{W\&B\text{-}C}$	TC	W&B-C	TN
S1	376	L	9.01	5.98	0.50	17.9	12.0	99.1	65.8	5.5
S2	549	H	3.17	2.83	0.18	17.6	15.7	40.6	36.3	2.3
S3	503	H	3.59	3.84	0.27	13.2	14.0	40.6	43.4	3.1
S4	430	L	6.84	6.92	0.43	16.1	15.9	63.0	63.6	4.0
S5	587	H	4.91	4.36	0.36	13.6	12.1	63.3	56.3	4.7
S6	588	H	3.91	4.50	0.35	11.2	12.9	41.5	47.7	3.7
S7	367	L	3.60	3.92	0.32	11.3	12.3	40.7	44.3	3.6
S8	513	H	4.58	4.50	0.33	14.0	13.6	49.0	48.1	3.6
S9	356	L	3.60	2.74	0.23	15.8	12.1	42.9	32.6	2.7
S11	382	L	4.13	3.20	0.24	17.4	13.6	50.4	39.1	2.9
S12	400	L	2.64	3.69	0.26	10.3	14.2	31.7	44.3	3.1
S13	503	H	2.37	1.57	0.12	20.1	13.2	27.9	18.6	1.4
S14	494	H	6.70	5.13	0.37	18.1	13.8	77.1	59.0	4.3
S15	373	L	4.64	3.61	0.25	18.6	14.4	56.1	43.7	3.0
S16	412	L	3.25	3.20	0.25	13.2	12.9	40.3	39.7	3.1
S17	465	H	4.08	4.49	0.38	10.7	11.8	46.5	51.2	4.3
CS1	378	C	5.02	3.82	0.39	12.2	9.73	59.8	45.4	4.7
CS2	350	C	3.62	2.63	0.27	13.21	9.67	43.5	31.5	3.3
CS3	357	C	4.78	3.09	0.26	18.6	12.0	55.9	36.2	3.0
CS5	360	C	4.29	2.89	0.28	15.3	10.3	54.4	36.7	3.6
CS6	370	C	5.47	3.58	0.30	23.0	12.1	65.6	43.0	3.6
CS7	362	C	5.52	4.35	0.27	20.1	15.8	63.5	50.0	3.2
CS8	360	C	5.55	4.07	0.19	29.1	21.1	65.0	47.6	2.3
CS9	381	C	4.69	3.96	0.20	23.9	20.1	55.4	46.7	2.3

TC, total organic carbon (element analyzer); W&B-C, W&B C(wet oxidation)

The HA layer: Total organic C contents of the HA layer are given in Table 5.4. There was no significant correlation between the total C content and the CO_2 concentration for the HA layer (Table 5.6). On average, the total C content of the HA layer was slightly higher (10%) in the elevated CO_2 area, but this difference was not significant (Table 5.7). Elevated CO_2 had no effect on the W&B-C content of the HA layer. The correlation between the W&B-C and the CO_2 concentration was not statistically significant. Neither was the 18% increase of W&B-C content in the high-CO_2 plot ($p=0.12$). N contents were not correlated with the local CO_2 concentration for the HA layer (Tables 5.4 and 5.6). The mean N contents in the two areas were similar (1.57 %, Table 5.7). The W&B-C/TC ratio of the HA layer increased as the atmospheric CO_2 concentration increased ($r=0.46$, $p<0.05$), but the averaged W&B-C/TC ratios were similar for both areas ($p=0.15$).

0-10 cm mineral soil: Differences of TC, W&B-C and TN in deeper layers were rather small (Table 5.5). Total carbon content of the 0-10 cm layer did not show a large variation in the control area, whereas it did in the emission area (Table 5.7). The differences in TC and W&B-C contents between emission and control areas were not statistically significant. The total N contents were similar in both areas (0.3%). None of the variables we considered appeared to correlate with the CO_2 concentration of the site (Table 5.6). The ratio of W&B-C to the total organic C of the 0-10 cm mineral soil was significantly higher ($p=0.02$) in the emission area than in the control area (Table 5.8). The W&B-C/TC ratio of the 0-10 cm mineral soil had a positive, but marginally significant, correlation ($r=0.39$, $p=0.07$) with the local CO_2 concentration.

Total C and N Pool sizes

F layer: In agreement with its higher TC and W&B-C contents, the TC and W&B-C pools in the F layer of the high CO_2 area were significantly higher than those in the control area ($p<0.01$). The increased pool size was 133 % for TC and 121 % for W&B-C. Total C and W&B-C pool sizes were significantly and positively correlated with the CO_2 concentration (Table 5.6). Also, the averaged N pools differ significantly between the two areas (Table 5.8). N pool size in the high CO_2 area was twice as high as in the control area. Accordingly, the correlation between the CO_2 concentration and N pool size was positive and significant ($r=61$, $p<0.001$).

HA layer: Notwithstanding the large variation in C and N pools, there were significant correlations between CO_2 level and C and N pools. Both C and N pools increased as CO_2 concentration increased. The C and N pools in the F layer of areas exposed to elevated CO_2 were appreciably higher (more than 100%, $p<0.001$) than those in ambient CO_2 area (Table 5.7).

0-10 cm mineral soil: Neither total C nor total N pools showed significant correlation with CO_2 level or significant differences between emission and control plots.

Table 5.6. Correlation coefficients of simple and multiple regression of CO_2 concentration and elevation against chemical properties for all sampling sites (n=26).

Variable		Elevation	CO_2	Elevation+CO_2
F Layer				
TC	(%)	-0.32	0.47*	0.47*
W&B-C	(%)	-0.30	0.34	0.36
TN	(%)	-0.05	0.53**	0.63**
C/N$_{tc}$	(-)	-0.34	0.04	0.39
C/N$_{W\&B\text{-}C}$	(-)	-0.25	-0.18	0.49*
W&B-C/TC	(%)	0.07	-0.33	0.36
TC pool	g.m^{-2}	-0.51**	0.56**	0.60**
W&B-C pool	g.m^{-2}	-0.51**	0.53**	0.58**
TN pool	g.m^{-2}	-0.50**	0.61***	0.63**
HA layer				
TC	(%)	-0.23	0.09	0.23
W&B-C	(%)	-0.38	0.28	0.38
TN	(%)	-0.07	0.04	0.07
C/N$_{tc}$	(-)	-0.29	0.08	0.30
C/N$_{W\&B\text{-}C}$	(-)	-0.50**	0.37	0.51*
W&B-C/TC	(%)	-0.40*	0.46*	0.49*
TC pool	g.m^{-2}	-0.43*	0.47*	0.53*
W&B-C pool	g.m^{-2}	-0.47 *	0.52**	0.57*
TN pool	g.m^{-2}	-0.43*	0.53**	0.55*
0-10 cm mineral soil				
TC	(%)	0.33	-0.18	0.33
W&B-C	(%)	0.01	0.12	0.17
TN	(%)	-0.29	0.08	0.32
C/N$_{tc}$	-	0.80***	-0.29	0.85***
C/N$_{W\&B\text{-}C}$	-	0.60**	-0.26	0.79***
W&B-C/TC	(%)	-0.43	0.39	0.44*
TC pool	t.ha^{-1}	0.37	-0.21	0.37
W&B-C pool	t.ha^{-1}	0.04	0.13	0.21
TN pool	t.ha^{-1}	-0.31	0.06	0.35

TC, total organic carbon (element analyzer); W&B-C, W&B C(wet oxidation);* $p<0.05$, ** $p<0.01$, *** $p<0.001$

C/N Ratios

F layer: The C/N$_{tc}$ ratio of the F layer of areas exposed to elevated CO_2 was slightly, but insignificantly, higher (9.5%, p=0.07), than that in ambient CO_2 area (Table 5.7).

Similarly, the C/N$_{W\&B\text{-}C}$ was not significantly different (4.4%) between emission and control plots (Table 5.7). We did not observe a significant correlation between the C/N ratios and the CO_2 concentration (Table 5.6).

Table 5.7. Total C, W&B C and ratios of three soil layers from the emission area (high, low CO_2-plots) and the control area (control plot) at the Laiatico mineral spring, Italy. Each value represents means (n=9), standard deviations are in parenthesis. Similar letters indicate no significant difference among plots at $p<0.05$.

Plot	TC (%)	W&B-C (%)	N (%)	C/N_{tc} (-)	C/$N_{W\&B\text{-}C}$ (-)
F layer					
High-CO_2	43.3 (3.0) a	33.6 (2.0) a	1.80 (0.2) a	24.2 (2.6) a	18.8 (1.7) a
Low-CO_2	39.8 (4.8) ab	32.5 (4.2) ab	1.63 (0.1) a	24.5 (2.4) a	20.0 (2.1) a
Control	36.6 (4.8) b	29.5 (2.9) b	1.66 (0.2) a	22.1 (1.3) a	18.0 (2.0) a
HA layer					
High-CO_2	34.4 (6.2) a	28.1 (4.1) a	1.58 (0.2) a	21.7 (2.2) a	17.8 (1.6) a
Low-CO_2	33.3 (5.4) a	26.3 (4.7) a	1.52 (0.2) a	21.9 (2.0) a	17.3 (1.6) a
Control	31.2 (4.1) a	23.8 (4.1) a	1.57 (0.2) a	19.9 (1.2) a	15.1 (1.5) b
0-10 cm mineral soil					
High-CO_2	4.16 (1.2) a	3.90 (1.1) a	0.30 (0.1) a	14.8 (3.2) a	13.4 (1.1) a
Low-CO_2	4.72 (2.0) a	4.16 (1.4) a	0.31 (0.1) a	15.1 (2.9) a	13.4 (1.3) a
Control	4.87 (0.6) a	3.55 (0.6) a	0.27 (0.1) a	19.4 (5.4) a	13.8 (4.3) a

Table 5.8. The ratio of W&B C to total organic C, total C pool size and W&B C pool size, and total N pool size three soil layers from the emission area (high, low CO_2 plots) and the control area (control plot) at the Laiatico mineral spring, Italy. Each value represents means, standard deviations are in parenthesis. Similar letters indicate no significant difference among sites at $p<0.05$.

Plot	W&B-C/TC (-)	TC pool (t.ha^{-1})	W&B-C pool (t.ha^{-1})	TN pool (g.m^{-2})
F layer				
High-CO_2	77.7 (4.6) a	12.2 (3.6) a	9.41 (2.8) a	50.4 (15) a
Low-CO_2	81.6 (3.2) a	9.42 (2.4) a	7.68 (1.9) a	38.3 (08) a
Control	81.4 (7.2) a	5.24 (3.4) b	4.26 (2.8) b	23.4 (14) b
HA layer				
High-CO_2	82.3 (6.6) a	24.5 (9.6) a	19.9 (7.1) a	112 (38) a
Low-CO_2	79.0 (2.5) a	19.7 (5.6) a	15.6 (4.6) a	89.1 (21) a
Control	76.0 (7.7) a	11.4 (9.0) b	8.65 (6.9) b	55.9 (41) b
0-10 cm mineral soil				
High-CO_2	94 (15) a	48.3 (14) a	45 (12) a	341 (99) a
Low-CO_2	93 (22) a	53.3 (20) a	47 (11) a	349 (90) a
Control	73 (06) b	57.9 (07) a	42 (06) a	323 (70) a

HA layer: Both C/N_{tc} and $C/N_{W\&B\text{-}C}$ ratios differed significantly between the sampling sites (Table 5.4). The C/N_{tc} ratio was slightly higher ($p=0.08$) in the elevated CO_2 area, whereas the $C/N_{W\&B\text{-}C}$ ratio was significantly higher ($p=0.006$) in the elevated CO_2 area (Table 5.7). Neither C/N_{tc} nor $C/N_{W\&B\text{-}C}$ showed a significant correlation with CO_2 level (Table 5.6).

0-10 cm mineral soil: Mean C/N_{tc} and $C/N_{W\&B\text{-}C}$ values were similar for all plots. Neither C/N_{tc} nor $C/N_{W\&B\text{-}C}$ had a significant correlation with atmospheric CO_2 concentration (Tables 5.6 and 5.7).

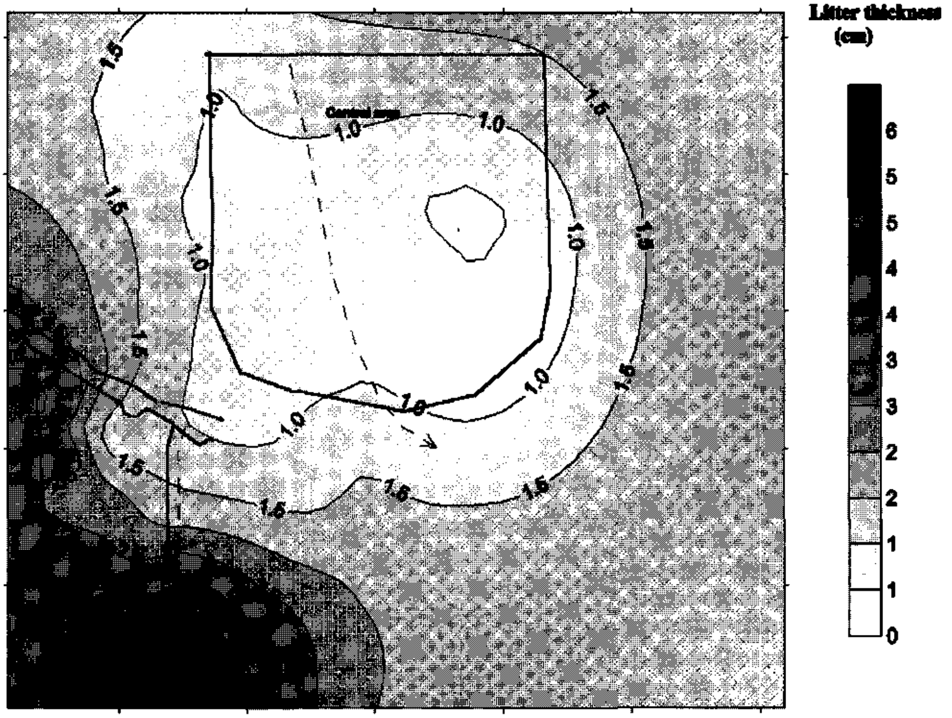

Figure 5.2. Isopleths of litter thickness in the study area. For more information see Fig. 5.1.

The thickness of the forest floor

The litter thickness was substantially higher in the emission area than in the control area (Fig. 5.2). The thickness ranged from a maximum value of 6.8 cm in the emission area to a minimum value of 0.4 cm in the control area. Although the relationship between the litter thickness and the CO_2 concentration showed a scattered pattern, the thickness increased

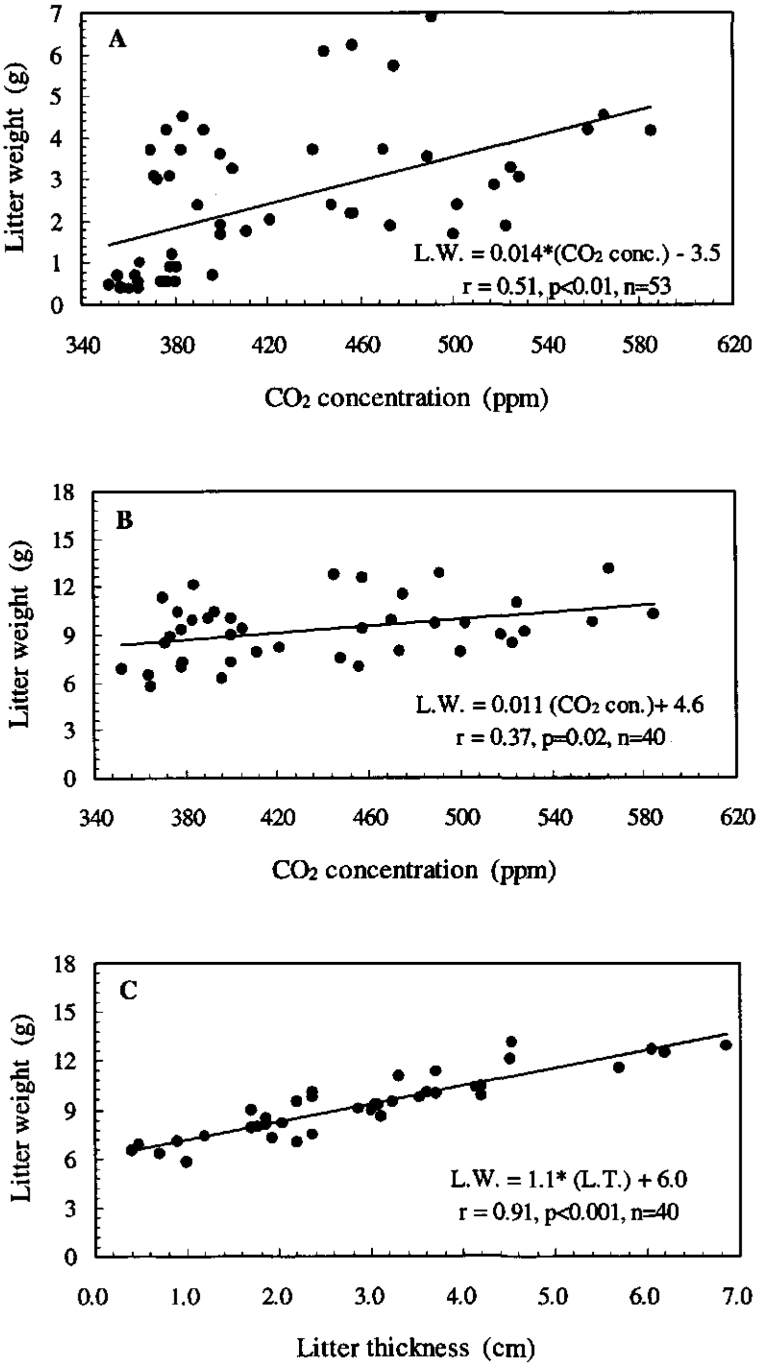

Figure 5.3. Litter thickness (L.T) is plotted against CO_2 concentration (CO_2 conc.) (A), litter weight (L.W) against CO_2 concentration (B) and litter weight against litter thickness (C) around the Laiatico CO_2 spring. (r values and significant levels are indicated).

significantly ($p<0.01$, $r=0.51$) with increasing CO_2 concentration (Fig. 5.3A). The correlation between the litter mass and the CO_2 concentration, however, was less significant with $p=0.02$ and $r=0.37$ (Fig. 5.3B). The litter thickness correlated strongly with the litter mass ($r=0.91$ at $p<0.001$, Fig. 5.3C).

Effect of elevation

In the emission area, the CO_2 concentration increased with decreasing elevation (data not shown). Slope position by itself may contribute to a difference in SOM composition and in C and N pool sizes, e.g. by downslope transport of surface litter. To differentiate between effects of CO_2 and slope on SOM, the influence of elevation on the SOM was tested for all variables. Table 5.6 gives the simple and multiple regressions of elevation and CO_2 with the measured variables. Some of the variables of the three layers were affected by the elevation of the site. Only the $C/N_{W\&B\text{-}C}$ of the HA layer and C/N ratios of the 0-10 cm layer, and pool size of C and N in the F and HA layer were significantly affected by site elevation. Generally, the correlation coefficients are positive with regard to CO_2 level, and negative with regard to elevation. The atmospheric CO_2 level explained most of variations in the variables better than elevation except for C/N ratios of the 0-10 cm mineral soil (Table 5.6).

DISCUSSION AND CONCLUSIONS

Soil C content and pool

The only significant correlation between atmospheric CO_2 and elemental composition was an increase in the total organic C content of the F layer at higher CO_2, whereas no such effects were seen in the total organic C content of the HA and 0-10 cm layers. Similarly, the W&B carbon content in the F layer of high CO_2 area was higher under elevated CO_2, but not in the HA and 0-10 cm mineral soil. It seems that elevated CO_2 had opposite impacts on total organic and W&B-C content (TC and W&B-C) of the forest floor and the mineral soil. Total organic C and W&B-C content were significantly increased in the F layer. In the HA layer and in the mineral soil, neither shows a significant difference.

Increased CO_2 caused a significant increase in TC and W&B-C pools of the F and HA horizons, but it did not affect C pool size of the 0-10 cm mineral soil. Higher C pools in the

F and HA layers may be caused by slower decomposition, higher litter production, or a combination of the two. The absence of CO_2 effects in the mineral soil suggests that litter production may have increased by elevated CO_2, because slower decomposition without increased litter production should have resulted in a larger pool in mineral horizons also. The increased carbon pools in the forest floor, therefore, may be due to increased net primary production (NPP) at elevated CO_2. The increased plant production will lead to an increase in input rate of C, which may influence the organic matter content in the early stages of decomposition. Henning et al. (1996) indicated that enhanced CO_2 could result in a net increase in C accumulation in soils under field crops only by increased C fixation. Unfortunately, we have no data about effects of elevated CO_2 on photosynthesis and biomass production for species at the Laiatico site. However, Jones et al. (1995) found that elevated CO_2 leads to higher growth rates for *Arbutus unedo* L. at the Rapolano CO_2 spring near Siena. This species also grows at our study site. Recently, Hättenschwiler et al. (1997) presented evidence that long-term elevated CO_2 caused a significant increase in radial stem increment of *Quercus ilex* L. growing around two natural CO_2 springs (Laiatico and Rapolano, Italy). Stem biomass production of trees increased especially during the early stages of exposure.

Previous studies carried out under short-term CO_2 exposure have reported no significant changes in total soil C content associated with CO_2 enrichment, corresponding with the lack of the CO_2 effect on C content in the HA and 0-10 cm layers (Zak et al., 1993; Rogers et al., 1992; Johnson et al., 1994; Rice et al., 1994; Ross et al., 1995; Arnone and Körner, 1995; Hungate et al., 1996). For example, Hungate et al. (1996) observed that surface soil C pools were not affected significantly by elevated CO_2 after four growing seasons of exposure of intact annual grasslands to ambient+350 ppm CO_2. Hungate et al. (1996) used ^{13}C isotope signature as a tracer to evaluate the input rate of newly-assimilated C in the soil, and found that the impact of elevated CO_2 on soil C pool was not detectable, because soil C pools are large and heterogeneous compared to annual plant input.

One problem with most experiments on effects of elevated CO_2 on litter quality and decomposability, and subsequently carbon storage (enhanced soil C pool) in the soil is that laboratory incubations mimic only part of the field conditions. For example, in most experiments the complexity of soil organisms (e.g. soil micro-, meso- and macro-fauna), which play an important role in the decomposition of soil organic matter (Tian, 1992; Coûteaux et al., 1991) is very low. Coûteaux et al. (1991) observed that the litter decomposition rate of two-year-old sweet chestnut grown under elevated CO_2 (C/N:80) for two years was lower than the litter decomposition rate of plants grown under ambient conditions (C/N:43) without the inoculation of decomposers. The pathway, however, changed where litter with higher C/N ratio from elevated CO_2 units decomposed faster (30%) than litter with low C/N ratio from ambient units with increasing diversity of soil organisms. Coûteaux et al. (1991) attributed the faster decomposition to a change in the composition, especially white-rot fungi, and the stimulation of microflora by the soil fauna present in their system. In our previous experiment (Raiesi, 1998), *Quercus pubescens* litter decomposed faster than *Quercus cerris* litter under controlled conditions (incubated at 20 °C), but this difference was not seen in an experiment with litter bags (field incubation), in spite of lower litter quality of *Quercus cerris*. The possible reason for that is the complexity and high diversity of soil organic matter decomposers in natural soils, whereas such a high diversity does not exist in controlled trails.

Assuming that a fraction of the W&B-C pool is accounted for by easily decomposable carbon compounds, such as rhizodeposits, exudates and soil enzymes, the increased W&B-C pool in the F and HA layers could partly be associated with increased exudation from the roots through increased C allocation to belowground components under elevated CO_2 levels (Norby et al., 1987; Rogers et al., 1994; Cardon, 1996; Darrah, 1996). Increased root rhizodeposits stimulate soil microbial activity, and therefore may accelerate the decomposition rate of soil organic matter (Körner and Arnone, 1992; Zak et al., 1993), especially in nutrient-limited ecosystems, such as Mediterranean forest ecosystems (Bottner et al., 1995). By contrast, Goudriaan and de Ruiter (1983), Lekkerkerk et al. (1990) and Kuikman et al. (1991) found that decomposition rates of native soil organic matter is slowed down due to preference of soil microbial communities for the production of easily decomposable compounds (rhizodeposition) induced by elevated CO_2.

Soil N content and pool

Long-term elevated CO_2 had no effect on N content in either the forest floor or mineral soil. Apparently, elevated CO_2 does not lower the N content of the soil in spite of a significant positive correlation of the N concentration with the CO_2 concentration, at least in the F layer. The total N content in the soil is influenced, among others factors, by the N content in leaf and root litter. The fact that we did not observe significant differences in N contents in the litter layer and mineral soil may indicate that the N content of the plant material reaching the soil via leaf litter (and probably root litter) was not decreased by elevated CO_2. As expected, the N contents in leaf litter of three oak species (*Quercus pubescens*, *Quercus cerris* and *Quercus ilex*), of one ash species (*Fraxinus ornus*) and of *Smilax aspera* produced under long-term elevated CO_2 were not actually reduced by higher CO_2 (Raiesi, 1998; Raiesi et al., 1998a). Another possibility is the stimulation of primary production by elevated CO_2.

Similarly, N content of the soil was not significantly affected by elevated CO_2 in some short-term CO_2-experiments. Niklaus and Körner (1996) reported that soil organic N in alpine grassland was not affected by elevated CO_2 after four seasons of CO_2 enrichment. Wood et al. (1994) found no change in soil N in three soil depths (1-5, 5-10 and 10-20 cm) after three years of cotton production under CO_2 enrichment (550 ppm). Also, soil total N in ryegrass/white clover turves of an Endoaquept soil (Ross et al., 1995) and in grass/clover turves of a Psammaquent (Ross et al., 1996) was not affected by elevated CO_2. A similar observation was reported for an artificial tropical ecosystem (Körner and Arnone, 1992). Zak et al. (1993) observed that total soil N was not affected by elevated CO_2, both in rhizosphere and bulk soil of *Populus grandidentata* Michx. grown in nutrient-poor soil at elevated atmospheric CO_2. Our results are also similar to those reported by Rice et al. (1994) who observed that elevated CO_2 did not affect soil N content in their unfertilized plots after four growing seasons in tallgrass prairie ecosystems (a mixture of C_3 and C_4 plants). Although it is difficult to compare our results with those from short-term experiments, all available data indicate that soil N concentration is usually not affected in soil-plant systems exposed to elevated CO_2.

Although the N contents in the forest floor remained unaffected, long-term elevated CO_2 led to a doubling of the N pool size. This is not surprising, as the C/N ratio of plant materials reaching the forest floor remained unchanged (Raiesi, 1998; Raiesi et al., 1998a) and elevated CO_2 stimulates plant production in the long-run (Jones et al., 1995; Hättenschwiler et al., 1997), the increased carbon input must be accompanied by an increased nitrogen input.

Similarly, Körner and Arnone (1992) observed that the N pool in litter was 33% higher under elevated than ambient CO_2 in the soil of an artificial tropical ecosystem, and that elevated CO_2 caused a 38 % increase in litter production suggesting that the litter C/N ratio is not changed by CO_2. However, the total N pools in the soil were similar for both elevated and ambient CO_2. The relative increase in N pool in the present study is still much higher than that reported for the experiment with humid tropical plant communities. Because the Laiatico forest has been exposed to elevated CO_2 for decades or centuries, the remarkable increase in N pools results from a progressive accumulation of organic N over a long period. After 2 years, N content, which can be translated to N pool size, at 5-20 cm depth of a soil under wheat grown at 550 ppm was significantly higher compared to ambient CO_2 (Prior et al., 1997), but such a CO_2 effect was not observed at 0-5 cm depth.

Elevated CO_2 and soil C/N ratios

Effects of CO_2 on soil C/N ratio are potentially important via relationship between C/N ratio and decomposition rate. The effect of increased CO_2 on C/N ratios was different for TC and W&B-C. The C/N_{tc} ratio was increased slightly in the F and HA layers and decreased slightly in the mineral soil under elevated CO_2, but these effects were insignificant. Only the $C/N_{W\&B\text{-}C}$ ratio of the HA layer of plots exposed to elevated CO_2 showed a significant increase, whereas no such effects were observed in the $C/N_{W\&B\text{-}C}$ of the F layer and 0-10 cm mineral soil. In spite of significant increases in the total organic and W&B-C content (and no changes in N content) in the F layer, the C/N_{tc} and $C/N_{W\&B\text{-}C}$ ratios were widened slightly by elevated CO_2. The small increases in C/N_{tc} and $C/N_{W\&B\text{-}C}$ can be linked to increased total and W&B-C contents at constant N contents. The $C/N_{W\&B\text{-}C}$ ratios of the F layer and 0-10 cm mineral soil were not affected by elevated CO_2, probably because of unchanged W&B-C and TN contents by CO_2 levels. It is likely that there was a downslope N movement that affected only deeper soil layers (HA and 0-10 cm). This N movement could account for slightly lower C/N_{tc} ratio of the 0-10 cm layer of the sites exposed to elevated CO_2. Generally, the current data suggest that the impact of the CO_2-enriched atmosphere on organic matter quality was more pronounced in the F layer than in the HA and mineral layers.

Our results that long-term exposure to CO_2 enrichment had little or no impact on soil C/N ratios is in line with similar results obtained with short-term experiments. Rice et al. (1994) did not find a significant CO_2 effect on soil C/N ratio of a tallgrass prairie ecosystem during a four year exposure to CO_2 enrichment. Niklaus and Körner (1996) found that, after four seasons of CO_2 enrichment in a late successional alpine grassland, soil C/N ratio was not affected by elevated CO_2 both in fertilized and unfertilized treatments. In a Mediterranean model ecosystem with *Bromus madritensis*, soil C/N ratios were similar in ambient and elevated CO_2 (700 ppm) levels (11.3 vs. 11.5) during the six month experiment (Dhillion et al., 1996).

The absence of a CO_2 effect on soil C/N ratios indicates that CO_2 enrichment would not affect the decomposition rate of soil C, if the ratio of C to N content is a good regulator of C decomposability in the ecosystem studied. It is, however, essential to know whether or not such a CO_2 effect on the quality of soil organic matter could be generalized before quantifying changes in the global C and N cycles, and carbon storage in terrestrial ecosystems at the future elevated CO_2.

Summarizing, we may conclude that:

(1) Under elevated CO_2 we found higher contents and pools of C in the litter layer. These results could not be ascribed to effects of elevated CO_2 on chemical composition of soil organic matter, and can probably be attributed to an effect of elevated CO_2 through increased plant production. Therefore, elevated CO_2 should lead to a larger C sequestration in the Laiatico ecosystem.

(2) Elevated CO_2 did not significantly affect N contents in litter or soil horizons, but it led to a remarkable increase in total N pool of the forest floor.

(3) Elevated CO_2 does not impact the C/N ratio of the three soil layers. The W&B-C to N ratio increases under elevated CO_2 only for the HA layer.

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(References are give on page 135)

Chapter 6

Impacts of elevated atmospheric CO_2 on soil organic matter around naturally occurring mineral CO_2-springs

II. C and N turnover rates

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Impacts of elevated atmospheric CO_2 on soil organic matter around naturally occurring mineral CO_2-springs

II. C and N turnover rates

ABSTRACT

Elevated atmospheric CO_2 may have the potential to alter C and N cycling in terrestrial ecosystems through its impact on C and N turnover rates. This work deals with the effect of enhanced atmospheric CO_2 on C and N mineralization in soil organic matter formed around a mineral CO_2 spring in a Mediterranean oak-ash ecosystem. Soil samples from the forest floor (F and HA layers) and mineral soil (0-10 cm A horizon) were taken at elevated and ambient CO_2 concentrations. C and N decay rates were studied by tracking CO_2 evolved from and by extracting N mineralized in samples incubated for 15 (C mineralization) or 30 (N mineralization) days under laboratory conditions. C mineralization rate of the three layers of areas exposed to elevated CO_2 did not differ from that of areas under ambient conditions. The rate of N immobilization in the F and HA layers at elevated CO_2 was lower than at ambient CO_2. N mineralization in the 0-10 cm A horizon was not affected by high CO_2. Furthermore, the mineralization of C and N showed significant correlations with the elemental composition of SOM. The latter was partially affected by the CO_2 concentration. Total C, N and C/N ratio were all correlated negatively with C mineralization, while they correlated positively with N mineralization. The content of W&B-C was most closely related to SOM decomposability. N mineralization showed a positive correlation with the C/N ratio. The results suggest that although C mineralization was not affected by elevated CO_2, N immobilization in soil samples was lower at elevated CO_2. Thus, decreased N immobilization in the forest floor with CO_2 enrichment would impose a positive feedback on plant growth, suggesting that CO_2 assimilation by plants growing at elevated CO_2 might increase. The net effect would be a decrease in rate of atmospheric CO_2 increase.

Key-words: C mineralization, C and N cycles, elevated CO_2, Mediterranean ecosystems, natural CO_2 springs, N mineralization

Abbreviations: NPP, Net Primary Production; SOM, Soil Organic Matter; TC, Total Organic Carbon; W&B-C, Walkley & Black Carbon; Cm, C mineralization ;Ni, N immobilization; Nm, N mineralization

INTRODUCTION

The atmospheric CO_2 concentration has increased by 30 percent compared with the pre-industrial level, and is expected to increase by at least another 50 percent by 2050 (Schimel, 1995; IPCC, 1995). The increased CO_2 concentration is expected to lead to an increase in global temperature of 1.5-4.5 °C (Mitchell et al., 1990). Increasing atmospheric CO_2 level and increasing global temperature may have many consequences on terrestrial ecosystem, ranging from short-term physiological impacts (Eamus and Jarvis, 1989; Poorter, 1993; Luxmoore et al., 1993) to long-term alteration in soil organic matter (SOM) pools (O'Neill and Norby, 1996). These changes may affect the functioning and structure of ecosystems. Elevated CO_2 stimulates photosynthesis of most plants, especially those with a C_3 photosynthetic pathway (Mooney et al., 1991; Poorter, 1993), which comprise 95% of terrestrial plants. Elevated CO_2 may increase net primary production by 30% if other growth factors such as soil N and water availability are not limiting (Brown and Higginbotham, 1986; Bazzaz, 1990; Tissue et al., 1993; McGuire et al., 1995). However, it is doubtful whether increased net primary production (NPP) is maintained under continued exposure to elevated atmospheric CO_2. This is largely because plants growing at continued elevated CO_2 may adapt to the steadily increasing CO_2 concentration (Miglietta and Raschi, 1993; Miglietta et al., 1995).

In addition to changing net primary production, enhanced atmospheric CO_2 may also alter chemical composition of plant material. Plant materials grown at elevated CO_2 tended to have a lower quality than those grown at ambient conditions (Gorissen et al., 1995; Peñuelas et al., 1996, 1997; Berntson and Bazzaz, 1997; Robinson et al., 1997; Hungate et al., 1997; Franck et al., 1997; Cotrufo et al., 1998). Changes in litter chemical composition may change its decomposability (Coûteaux et al., 1991, 1996; Cotrufo and Ineson, 1996; Boerner and Rebbeck, 1995; Gorissen, 1995, 1996; Franck et al., 1997). One possibility is that high-CO_2-grown plant material is likely to decompose slowly relative to that grown at the ambient CO_2 because of higher lignin and C/N ratio. A slower decomposition rate may provide a negative feedback to both plant growth and rising atmospheric CO_2 concentration. The extent to which elevated CO_2 will change litter decomposition in general, however, is largely unknown, partly because different plants respond differently to CO_2 enrichment, and partly because of the short duration of most experiments (Lee and Jarvis, 1995). Effects of elevated CO_2 on litter quality, and subsequent litter decomposability were mostly investigated in pot experiments (Coûteaux et al., 1991;

Gorissen, 1995; Boerner and Rebbeck, 1995). The extrapolation of results from pot experiment to field situations is full of pitfalls. In some experiments, effects of elevated CO_2 on litter quality are based on chemical analysis of green leaves. Because quantities of various chemical components differ between green and senesced litter (O'Neil and Norby, 1996), predictions based on green leaves will not be useful in studies of soil organic matter dynamics. Furthermore, results from pot studies seem to differ substantially from those obtained from field studies, while pot studies often showed decreased litter quality at elevated CO_2, field studies of temperate woody species, salt marsh, and tallgrass prairie have shown little change in chemical composition of plant material grown under elevated CO_2. (Owensby et al., 1996).

Elevated CO_2 may also stimulate microbial activity through increased accumulation of below-ground C in the rhizospheric zone. Accumulation of easily decomposable compounds in the rhizosphere may occur under elevated CO_2, which could lead to a faster decomposition of root material and lower decomposition of humified matter (Lekkerkerk et al., 1990). Changes in C and N mineralization rates would ultimately affect the availability nutrients, especially nitrogen. This effect may be especially important in nutrient-poor ecosystems (Arp and Berendse, 1993).

Numerous experiments have focused on effect of elevated CO_2 on plant growth and few on litter. Very few have addressed SOM itself. In an experiment around mineral CO_2 sites, soil C pool size in the surface soil layer was higher at elevated CO_2, while the soil N contents and C/N ratios were not affected by long-term CO_2 enrichment (Raiesi et al., 1998b). Increased C pool was attributed to enhanced plant production by CO_2 enrichment, since there was no CO_2 effect on the decomposability of source litter (Raiesi et al., 1997a,b; Raiesi, 1998; Raiesi et al., 1998a). Here, we present information on the impact of elevated atmospheric CO_2 on N and C turnover rates of SOM of a forest continuously exposed to elevated CO_2. We investigated the impact of elevated atmospheric CO_2 on the dynamics of soil organic matter in an enriched-CO_2 forest ecosystem in the proximity of a mineral CO_2 spring using laboratory incubation. Our aims were to: 1) quantify the effect of increased CO_2 on SOM decomposition rate; 2) test the impact of elevated CO_2 on N turnover rate; and 3) discuss the relationship between SOM quality and C and N mineralization rates.

MATERIALS AND METHODS

Area description

The study was performed near Laiatico village in the region of Tuscany, Italy. The research area was described in detail by Raiesi et al. (1998b) and Raiesi (1998). The site (ca. 0.9 ha) is a sloping forested area, with a natural CO_2 spring situated in a small gully. There is a CO_2 gradient from ambient (350 ppm) to about 600 ppm close to the vent (Schulte et al., 1998). The vegetation of the area, an oak-ash coppice woodland last cut about 25 years ago, is typical for the Mediterranean climate. The vegetation is composed of evergreen and deciduous trees, comprising *Quercus ilex, Quercus pubescens, Quercus cerris, Fraxinus ornus* and *Quercus robur.* The shrub layer, which contains *Smilax aspera, Erica arborea, Arbutus unedo, Myrtus communis, Juniperus sp.* is locally very dense. Soils are calcareous, derived from Tertiary marl. The soil pH is 6.9 in the topsoil and above 7.3 in the subsoil. The soil texture is silty clayey loam in the topsoil and silty clay in the subsoil. The soil is covered by a litter layer (F+HA) varying from 0.4 cm to 7 cm. Some chemical and physical properties of the soil in the study area are given in Table 5.2.

Sampling, sample treatment and measurements

Details of soil sampling, treatments and measurements are reported by Raiesi et al (1998b). This paper deals with the quantification of the impact of long-term elevated CO_2 on C and N turnover rates. We measured C and N turnover rates as follows:

C and N mineralization

We weighed a representative sub-sample of 2 g of air-dried F or HA material or 10 g of air-dried mineral material into a 250-ml incubation vessel. Samples were rewetted by adding 4 ml of demineralized water to keep the moisture content at about 60% of the field capacity. The soil-water mixture was carefully homogenized. The samples were incubated in the dark at 20±1 °C. Water loss from the soil was negligible over the incubation period, and the amount of oxygen was enough for an aerobic C mineralization (P. Verburg, pers. comm.). Rates of CO_2 evolution were determined for all (52) samples of organic horizons and 120 samples of the 0-20 cm topsoils. The evolved CO_2 was absorbed in 0.6 M KOH, and the electrical conductivity of the KOH was automatically monitored (Nordgren, 1988).

The rate of CO_2 evolution and accumulated CO_2 were monitored hourly for 3 weeks. Because the C mineralization experiment lasted for 3 weeks, we therefore used the soil Walkley & Black C content to express C mineralization as a fraction of C available to microorganisms. The CO_2 production was expressed as mg C g^{-1} oven-dry soil. The cumulative CO_2 was corrected for the initial C flush due to dead microbial biomass by disregarding the data of the first 48 hours. All measurements were done in duplicate.

For the determination of soil N mineralization, a representative sub-sample of 30 g of air-dried F or HA material or 50 g of air-dried mineral soil was weighed into a 1/2 L incubation container and rewetted by adding 60 ml of demineralized water to organic samples or 20 ml to mineral samples. Containers were kept in the dark at 20±1 °C in a controlled-conditions chamber. Re-aeration took place after 10 and 20 days from the start of the incubation by reopening the lid. N mineralization was estimated from the increase in KCl-extractable inorganic N after incubating soil samples for 30 days. Initial inorganic N (NO_3-N and NH_4-N) was measured before incubation on an Autoanalyzer after extraction with 1 M KCl for 2 hr. using a soil:extractant ratio of 1:5 (van Lagen, 1996). To correct for the N flush, NO_3-N and NH_4-N were measured 4 days after incubation started. Final inorganic N (N-NO_3 and N-NH_4) contents were measured at the end of the incubation (after 30 days). We calculated N mineralization rate (either in mg N kg^{-1} soil $month^{-1}$ or in percentage of total soil N per month) as the difference between final (day 30) and initial (day 4) concentrations of inorganic N. All measurements were done in duplicate.

Statistical calculations

We analyzed the data using Pearson correlations 1) between the elevation and the C and N mineralization, 2) between the CO_2 concentration and C and N mineralization and 3) between elemental composition, C/N ratios, and C and N mineralization rates. We distinguished two plots in the emission area, e.g. high-CO_2 and low-CO_2 plots, because the local CO_2 concentrations were lower than 430 ppm for some sampling sites in the emission area. We then used one-way analysis of variance (ANOVA) to determine effects on C and N mineralization (high, low and control CO_2 as main treatments). Data were examined for homogeneity of variance and normality, and data were either arcsin or log-transformed prior to analysis to meet the assumption of normality. We analyzed some data using tests of the median because of heterogeneity of variance. We compared plot means using Student Newman-Keuls's multiple comparison test.

Table 6.1. C mineralization (Cm) and N immobilization (Ni)/mineralization (Nm) in three layers from 26 sites differing in atmospheric CO_2 concentration, measured during lab. incubation at 20 °C for 15 (C) or 31 (N) days, expressed either in mg per g or per kg soil, or as % of total organic C and N present. S1-17: emission area and CS1-9: control area. (H: high-CO_2, L: Low-CO_2, C: control-CO_2 plots).

			F layer				***HA layer***				***0-10 cm mineral soil***			
Site -	CO_2 ppm	Plot -	Cm ($mg.g^{-1}$)	Cm (%)	Ni ($mg.kg^{-1}$)	Ni (%)	Cm ($mg.g^{-1}$)	Cm (%)	Ni ($mg.kg^{-1}$)	Ni (%)	Cm ($mg.g^{-1}$)	Cm (%)	Nm ($mg.kg^{-1}$)	Nm (%)
S1	376	L	8.01	2.73	113	0.70	4.79	1.89	88	0.56	0.77	1.29	7.96	0.16
S2	549	H	7.08	2.05	91	0.51	5.88	1.59	94	0.51	0.63	2.24	14.16	0.79
S3	503	H	12.01	4.06	276	1.58	6.23	2.52	234	1.56	1.02	2.66	27.61	1.01
S4	430	L	7.58	2.63	84	0.50	5.61	2.23	306	1.86	1.07	1.55	87.14	2.00
S5	587	H	10.91	2.95	70	0.35	6.34	2.23	235	1.63	0.65	1.49	12.17	0.34
S6	588	H	8.28	2.61	235	1.15	5.39	2.40	203	1.34	0.71	1.57	32.50	0.93
S7	367	L	9.16	2.19	133	0.74	6.89	1.80	77	0.41	0.61	1.54	59.55	1.89
S8	513	H	10.71	3.17	121	0.65	6.69	2.60	138	0.93	0.52	1.16	21.28	0.64
S9	356	L	7.71	2.36	144	0.91	3.51	1.35	-62	-0.40	0.45	1.65	17.75	0.79
S10	448	H	11.12	3.42	208	1.28	6.62	2.48	47	0.33	nd	nd	nd	nd
S11	382	L	8.22	2.67	205	1.47	5.51	2.41	73	0.61	0.59	1.84	5.19	0.22
S12	400	L	7.66	2.57	108	0.63	4.70	1.79	238	1.51	0.63	1.72	12.57	0.49
S13	503	H	7.82	2.23	171	0.93	4.67	1.80	45	0.29	0.15	0.98	3.52	0.30
S14	494	H	6.22	1.81	93	0.54	4.51	1.52	128	0.81	0.61	1.19	5.83	0.16
S15	373	L	10.01	2.78	109	0.65	4.77	2.02	51	0.38	0.37	1.03	6.56	0.26
S16	412	L	8.68	2.84	184	1.20	6.77	2.84	-0.78	-0.01	0.65	2.04	1.56	0.06
S17	465	H	7.49	2.22	120	0.76	5.17	1.62	81	0.44	0.63	1.40	10.01	0.26
CS1	378	C	6.91	2.35	279	1.69	4.89	2.00	252	1.68	0.49	1.27	5.07	0.13
CS2	350	C	7.58	3.09	214	1.91	5.87	2.86	225	1.74	0.39	1.47	-2.70	-0.10
CS3	357	C	9.79	3.55	253	1.60	8.43	5.12	353	2.55	0.86	2.79	0.77	0.03
CS4	355	C	10.26	3.32	225	1.43	5.12	2.17	237	1.57	nd	nd	nd	nd
CS5	360	C	7.91	2.83	140	0.77	5.29	1.87	307	1.63	0.45	1.55	4.03	0.14
CS6	370	C	8.88	2.53	195	1.06	7.08	2.41	133	0.74	0.67	1.86	9.13	0.31
CS7	362	C	7.84	2.49	171	1.02	6.87	2.47	289	1.75	0.78	1.82	5.06	0.18
CS8	360	C	12.56	4.04	279	1.44	7.82	3.94	259	1.77	0.55	1.34	11.85	0.61
CS9	381	C	6.90	2.49	233	1.31	5.31	2.25	140	0.85	0.65	1.63	25.04	1.27

RESULTS

C mineralization

Table 6.1 gives the cumulative C mineralization in three soil layers. We did not observe a significant correlation between the cumulative mineralized C and the local CO_2 concentrations for the F layer (Table 6.2). ANOVA also showed that C mineralization in soil samples was not affected by elevated CO_2 for the F layer (Table 6.3).

Table 6.2. Correlation coefficients of simple and multiple regression of CO_2 concentration and elevation against cumulative CO_2-C production and net N mineralization for all sampling sites (n=26).

Variable		Elevation	CO_2	Elevation+CO_2
F layer				
Cm	($mg.g^{-1}$)	0.13	0.10	0.25
Cm^1	(%)	0.28	-0.08	0.29
Cm^2	(%)	0.31	-0.21	0.30
Ni	($mg.kg^{-1}$)	0.42*	0.27	0.42
Ni	(%)	0.35	0.41*	0.43
HA layer				
Cm	($mg.g^{-1}$)	0.32	-0.04	0.37
Cm^1	(%)	0.45*	0.20	0.45*
Cm^2	(%)	0.39	-0.05	0.44*
Ni	($mg.kg^{-1}$)	0.11	0.04	0.01
Ni	(%)	0.12	0.05	0.02
0-10 cm mineral soil				
Cm	($mg.g^{-1}$)	0.14	0.15	0.32
Cm^1	(%)	0.17	-0.01	0.21
Cm^2	(%)	0.12	0.11	0.26
Nm	($mg.kg^{-1}$)	-0.11	0.21	0.20
Nm	(%)	0.01	0.19	0.24

*$p<0.05$; [1] % mineralized C as % of total organic C (TC); [2] mineralized C as % of W&B C (W&B-C)

The total CO_2-C production of the HA layer is given in Table 6.1. Linear regression showed that C mineralization in the HA layer did not decrease with increasing CO_2 in the atmosphere (Table 6.2). One-way analysis of variance revealed that the rate of C mineralization in the HA layer was marginally significantly ($p=0.07$) higher in the control area than in the emission area (Table 6.3).

Table 6.3. C and N immobilization (F& HA)/mineralization (0-10 cm) in three soil layers from the emission area (high and low CO_2 plots) and the control area (control plot) at the Laiatico mineral spring, Italy. Values in percentage refer to mineralized C as % of W&B C or mineralized N as % of total N. Each point represents means (n=9); standard deviations are in parenthesis. Similar letters indicate no significant difference at $p<0.05$ within each column.

	Cumulative C mineralized		Cumulative N immobilized/mineralized	
Plot	($mg.g^{-1}$)	(%)	($mg.kg^{-1}$)	(%)
F layer				
High-CO_2	9.07 (2.0) a	2.72 (0.7) a	154 (68) a	0.86 (0.38) a
Low-CO_2	8.38 (0.8) a	2.60 (0.2) a	135 (39) a	0.85 (0.31) a
Control	8.74 (1.8) a	2.97 (0.6) a	221 (44) b	1.36 (0.34) b
HA layer				
High-CO_2	5.72 (0.8) a	2.08 (0.4) a	134 (71) a	0.87 (0.50) a
Low-CO_2	5.30 (1.1) a	2.04 (0.4) a	96.3 (112) a	0.61 (0.69) a
Control	6.30 (1.2) a	2.79 (1.0) a	244 (68) b	1.59 (0.50) b
0-10 cm mineral soil				
High-CO_2	0.62 (0.2) a	1.59 (0.5) a	15.9 (9.7) a	0.55 (0.31) a
Low-CO_2	0.64 (0.2) a	1.58 (0.3) a	24.8 (29) a	0.73 (0.73) a
Control	0.60 (0.2) a	1.72 (0.5) a	7.28 (7.3) a	0.35 (0.39) a

In the 0-10 cm layer, the accumulated CO_2-C did not correlate with the local CO_2 concentrations (Table 6.2). The averaged C mineralization rates were 0.62 (1.59%) for the high-CO_2 plot, 0.64 (1.58%) for the low-CO_2 plot and 0.60 mg g^{-1} soil (1.72) soil for the control plot. We did not observe significant differences in C mineralization among plots (Table 6.3).

Also when expressed as % of total organic carbon (TC), the C mineralization in the F, HA and 0-10 cm layers was essentially the same for all the plots (data not shown).

N mineralization/immobilization

Table 6.1 also presents the cumulative N mineralization in mg N kg^{-1} soil $month^{-1}$, corrected for the N flush during the first 4 days. Net N mineralization was negative (net immobilization) in all samples from the F layer and in most samples from the HA layer. All samples from the 0-10 cm layer displayed a positive net N mineralization after 30 days of incubation.

For the F layer, the amount of immobilized N was significantly lower in samples from the elevated CO_2 area than from the control area (Table 6.1). Net N immobilization showed a significant negative correlation ($r=-0.41$, $p<0.05$) with the atmospheric CO_2 concentration (Table 6.2). The mean N immobilization rates were 153 (0.86 % of total N) for the high CO_2 plot, 134 (0.85% of total N) for the low-CO_2 plot and 220 mg kg^{-1} soil $month^{-1}$ (1.36 % of total N) for the control plot. N immobilization in the F layer of high and low-CO_2 plots was significantly lower ($p<0.05$) when compared to the control plot (Table 6.3).

In the HA layer, two samples from the emission area showed a positive N mineralization from 0.78 to 62 mg N kg^{-1} soil $month^{-1}$ (Table 6.1). Net N immobilization for the low-CO_2 plot was significantly lower than that for high-CO_2 and control plots (Table 6.3). However, the standard deviation of N immobilization was quite high for the low-CO_2 plot. We found no significant relation between the net N immobilization rate and the CO_2 concentration of the site (Table 6.2).

Samples of the 0-10 cm layer showed net N mineralization. Generally, the net mineralization ranged from 0.77 mg N kg^{-1} soil $month^{-1}$ to 87.1 mg N kg^{-1} soil $month^{-1}$ (Table 6.1), while one sample showed N immobilization (2.70 mg kg^{-1}) in this layer. Variability in N mineralization was high and differences between plots were not significant ($p>0.10$, Table 6.3).

Relation between decomposition rate and elemental composition

The C and N contents of the three layers are reported in Table 5.7. We compared cumulative C evolution and N mineralization/immobilization rates with the elemental compositions by linear regression (Table 6.4). C mineralization (expressed in mg C g^{-1} soil) was not correlated with the initial soil N contents and C/N ratios for both F and HA layers.

We, however, found a significant positive correlation between C mineralization rate and initial total N content ($r=0.44$, $p<0.05$), W&B C content ($r=0.65$, $p<0.001$) for the 0-10 cm

Table 6.4 Linear regression of cumulative CO_2-C production (Cm) and N immobilization (F & HA) and mineralization (0-10 cm) against elemental composition and C/N ratios for all sampling sites (n=26).

Variable		Cm (mg.g^{-1})	Cm[1] (%)	Cm[2] (%)	Ni-m (mg.kg^{-1})	Ni-m (%)
F layer						
TC	(%)	0.22	-	-	-0.43*	-0.61***
TN	(%)	0.27	-0.21	-0.03	-0.14	-
C/N_{tc}	-	0.00	-0.34	-0.28	-0.36	-0.24
W&B-C	(%)	0.17	-	-	-0.45*	-0.57**
$C/N_{W\&B\text{-}C}$	-	-0.10	-0.15	-0.32	-0.27	-0.04
HA layer						
TC	(%)	-0.02	-	-	-0.31	-0.40*
TN	(%)	-0.06	-0.51**	-0.45*	0.03	-
C/N_{tc}	-	0.01	-0.40*	-0.28	-0.55**	-0.54**
W&B-C	(%)	-0.13	-	-	-0.33	-0.43*
$C/N_{W\&B\text{-}C}$	-	-0.18	-0.47*	-0.61***	-0.53**	-0.54**
0-10 cm mineral soil						
TC	(%)	0.41*	-	-	0.14	-0.01
TN	(%)	0.44*	-0.19	-0.23	0.31	-
C/N_{tc}	-	-0.08	-0.06	-0.04	-0.17	-0.05
W&B-C	(%)	0.65***	-	-	0.56**	0.39
$C/N_{W\&B\text{-}C}$	-	0.21	0.25	0.03	0.27	0.41*

*$p<0.05$, **$p<0.01$, ***$p<0.001$; [1] mineralized C as % of total organic C (TC); [2] mineralized C as % of W&B C (W&B-C)

layer. Correlation with quality parameters was improved, but still not significant, when C mineralization was expressed as % of W&B C (Table 6.4).

The net N immobilization (in mg kg^{-1} month^{-1}) in the F layer was correlated negatively with W&B C ($r=-45$, $p<0.05$). In the HA layer, the net N immobilization was negatively correlated with the C/N ratio ($r=-0.55$, $p<0.01$). The net N mineralization in the 0-10 cm was also significantly correlated only with W&B C ($r=0.56$, $p<0.01$). Expressing N immobilization-mineralization as a fraction of total initial N content improved the correlation coefficients for F, HA and 0-10 cm layers (Table 6.4).

Table 6.5. Correlations of net N immobilization (F & HA) and mineralization (0-10 cm) against C mineralization for all sampling sites (n=26).

Variable		Ni-m ($mg.kg^{-1}$)	Ni-m (%)
F layer			
Cm	($mg.g^{-1}$)	0.31	0.19
Cm^1	(%)	0.55**	0.60**
Cm^2	(%)	0.55**	0.51**
HA layer			
Cm	($mg.g^{-1}$)	0.37	0.41*
Cm^1	(%)	0.48*	0.57**
Cm^2	(%)	0.47*	0.56**
0-10 cm mineral soil			
Cm	($mg.g^{-1}$)	0.50*	0.41*
Cm^1	(%)	0.22	0.29
Cm^2	(%)	0.00	0.00

*$p<0.05$,**$p<0.01$; [1] mineralized C as % of total organic C (TC); [2] mineralized C as % of W&B C (W&B-C)

C mineralization against N immobilization/mineralization

Table 6.5 shows the correlations between C and N immobilization/mineralization rates. Regression analysis revealed a significant positive correlation ($p<0.01$) between relative C decomposition and N immobilization rates for both the F and HA layers. All significant levels and trends were rather similar for both F and HA layers (Table 6.5). In the 0-10 cm layer, the absolute C mineralization showed a significant positive correlation ($p<0.05$) with both absolute and relative N mineralization rates (r=0.50 for Nm in mg kg^{-1} soil $month^{-1}$ and r=0.41 for Nm in percent, Table 6.5).

DISCUSSION

Soil C turnover

Our data obtained from long-term CO_2 exposure raise objection to the hypothesis that the anticipated increase in the atmospheric CO_2 concentrations slows down soil C decomposition rate in terrestrial ecosystems, and thereby increases C sequestration in the biosphere. The results of our study show that elevated CO_2 did not affect the C mineralization rate of the F and HA layers of the forest floor and the 0-10 cm of the topsoil exposed to enhanced CO_2 level. The lack of a CO_2 effect on C mineralization is most likely related to the fact that elevated CO_2 did not significantly affect the C/N ratios of the F, HA and 0-10 cm layers (Table 5.7). The lack of CO_2 effects on C mineralization may indicate that substrate availability for soil microbes is the same under elevated and ambient CO_2, but we did not determine soil microbial biomass C in our study. In the studied system, the C balance of the ecosystem would not be affected via an effect of CO_2 on decomposition rate of SOM alone, and hence there would not be significant increases in soil C pools. As expected, the soil C pool sizes in the F and HA layers have been shown to be higher at elevated CO_2, but not in deeper soil layers (Raiesi et al., 1998b). The results from this study suggest that increased C pools in the forest floor layer are due to increased biomass production by CO_2 enrichment and unchanged decomposition rates.

Data from our previous experiments demonstrated that plant leaf litter grown at elevated CO_2 had similar decomposition rates to that grown at ambient CO_2 (Raiesi et al., 1997a,b; Raiesi, 1998; Raiesi et al., 1998a), indicating absence of CO_2 effects on decomposability of input litter was reflected in decomposing soil organic matter. Raiesi (1998) and Raiesi et al. (1998a) attributed unaltered litter decomposition rate to unchanged litter quality parameters under elevated CO_2.

Our data on soil C mineralization correspond well with results from earlier experiments indicating that long-term exposure to elevated CO_2 did not affect significantly soil respiration from tussock tundra in Alaska during a 2.5 growing seasons experiment (Oberbauer et al., 1986). Based on their results, Oberbauer et al. (1986) concluded that the conceptual hypothesis that elevated CO_2 depresses soil respiration is not supported, at least in tussock tundra. In contrast, some experiments have indicated increased soil respiration with CO_2 enrichment. For example, Vose et al. (1995) found an increase in soil respiration under elevated CO_2 in an experiment with ponderosa pine in open-top chamber. Elevated CO_2 enhanced soil respiration from ryegrass/white clover turves of an Endoaquept soil (Ross et al., 1995) and from grass/clover turves of a Psammaquept soil (Ross et al., 1996). Körner and Arnone (1992) observed a doubling of CO_2 evolution from the soil under elevated CO_2 in their model artificial tropical ecosystems. In woody systems (mature trees), soil respiration was either increased or not affected by elevated CO_2 (see Table 7.2

in Mooney et al., 1998), no matter what experimental conditions were and the ecosystem was.

Goudriaan and de Ruiter (1983) and Lekkerkerk et al. (1990) indicated that elevated CO_2 increases root-derived materials (such as root exudates, mucilages, dead and sloughed-off root cells) in the rhizosphere. These authors proposed that the easily-mineralizable root-derived compounds stimulate soil microbes, which depresses the decay of other organic compounds and causes increased storage of carbon in the soil. We observed slightly lower decomposition of SOM in the 0-10 cm mineral soil at elevated CO_2, but this effect was not reflected in C pool size of the 0-10 cm layer (Raiesi et al., 1998b). It is therefore essential to study the effect of elevated CO_2 on C allocation to below-ground elements and C fluxes in the rhizosphere using C isotopes at Laiatico before drawing a general conclusion about the potential impact of elevated CO_2 on SOM dynamics in this ecosystem.

In this study, we used SOM which was derived from plant materials exposed to elevated CO_2 continuously, and we considered the whole plant-soil system. By considering the whole plant-soil system, we took into account at least some plant-soil feedbacks such as impacts of elevated CO_2 on belowground C and processes and thereby on soil C via the plant. Most experiments, which have reported reduced C mineralization under elevated CO_2 are either short-term or used a soil-litter mixture that may not represent natural properties and processes as occur in the field. Soil fauna are excluded from some studies conducted under controlled conditions. Soil fauna undoubtedly play an important role in fragmentation of litter in the soil (Tian, 1992) so that in the most complex food webs, litter and/or organic matter with higher C/N ratio may decompose faster than litter and/or organic matter with lower C/N ratio, as indicated by Coûteaux et al. (1991, 1996). So, it seems that elevated CO_2 does not reduce soil respiration where the whole plant-soil system has been exposed to higher CO_2 concentrations regardless of being short- or long-term exposure (Obertbauer et al., 1986; Niklaus and Körner, 1996; Mooney et al., 1998). Our data may indicate that CO_2 impacts on SOM decomposability would not provide a negative feedback to the increasing atmospheric C pool. On the other side, the possibility exists that while CO_2 enrichment may not depress C turnover, more C is still released from the soil to the atmosphere through enhanced rates of C decomposition (Jenkinson et al., 1991; Oechel et al., 1993; Oechel and Vourlitis, 1994, 1996), if increases in soil temperature occurs with increasing CO_2 level in the atmosphere. The net effect would be a positive feedback to the rising atmospheric CO_2 level (Oechel et al., 1993). In this sketch, soils will act as a net source of atmospheric CO_2 rather than as a sink.

Soil N turnover

Long-term elevated CO_2 significantly affected N turnover rate. The net N immobilization in the F and HA horizons appeared to be lower for soil organic matter in elevated CO_2 environments despite unchanged C/N ratios and absence of changes in N concentration (Raiesi et al., 1998b). The N mineralization rate of the 0-10 cm layer was not significantly different for elevated CO_2 and ambient conditions.

In two short-term (<3 yrs) elevated CO_2 experiments with whole plant-soil systems, soil N mineralization rate increased significantly at high CO_2 (Körner and Arnone, 1992; Zak et al., 1993), providing a positive feedback on plant growth, and consequently a negative feedback to the atmospheric CO_2. These authors attributed increased N mineralization to stimulated activity of soil microbes through either increased root rhizodeposits, e.g. soil labile C (e.g. plant-derived C, Zak et al., 1993) or increased root turnover and root exudation (Körner and Arnone, 1992) at elevated CO_2. Both experiments were carried out on a nutrient-poor soil with small amounts of fertilizer application. It is not very surprising that processes similar to those reported by Körner and Arnone (1992) and Zak et al. (1993) may have taken place in our system, because Mediterranean ecosystems are often N limited (Bottner et al., 1995) so that soil microbes are forced to decompose SOM for energy and nitrogen uptake. However, in our system we did not detect an increase in C mineralization associated with elevated CO_2. Decreased N immobilization without increased C mineralization due to elevated CO_2 would result from N limitation rather than from C availability to microbes. This might explain why the effect was seen in the F and HA layers where the soil C/N ratio is higher than that of soil microbes and not in the 0-10 cm mineral soil where the C/N ratio is lower than that of microbes. One problem is that our C mineralization experiment lasted for 20 days, while N mineralization lasted for 30 days, so we can not exclude the possibility that the C mineralization was higher at elevated CO_2 than at ambient condition after 30 days.

Two further explanations may account for faster mineralization of N at elevated CO_2:

i) The total carbon pools in F and HA layers of the CO_2-enriched sites are substantially higher than those at sites with ambient CO_2 (Raiesi et al., 1998b) This could have increased the water holding capacity of the soil, stimulating microbial activity, and leading to faster N turnover. We should, however, bear in mind that such stimulating effects of soil moisture on N mineralization would take place only in the field, and not in the laboratory where N mineralization was studied at the same constant soil moisture content.

ii) Some workers have observed that plant material with high C/N ratio may decompose faster than that with low C/N ratio, at least at a high complexity of the decomposer foodweb (Coûteaux et al., 1991, 1996). The activity of white-rot fungi is stimulated by other decomposers, and therefore they can enzymatically degrade lignin, polyphenolic and polymerized constituents. In our study, we found a slight, but insignificant, increase in soil C/N ratio at elevated CO_2 (Table 5.7), but soil C/N ratio showed a significant negative relation with soil N immobilization rate in the HA layer, whereas we observed a positive relation between C/N ratio and N mineralization rate in the 0-10 cm mineral soil (Table 6.5).

Increased accumulation of carbon in the soil could be due to the increased litter production (i.e. aboveground production, and probably belowground C) by a combination of increased CO_2 from the air and increased N availability from the soil. That is why our previous results showed that the total C pool was increased significantly in the forest floor, but not in the 0-10 cm mineral soil (Raiesi et al., 1998b) without C mineralization rate being affected. Recently, Reich et al. (1997) studied aboveground net primary production (ANPP) as a function of annual net N mineralization rate in hardwood and conifer stands on diverse soils, and found a significant linear correlation between ANPP and annual N mineralization rate for all studied stands.

Correspondingly, in another study with two Mediterranean oak species and one undergrowth shrub from Laiatico, long-term enhanced CO_2 concentrations stimulated N mineralization from litter of *Quercus cerris* L. , *Quercus pubescens* Willd. and *Smilax aspera* L. in the initial stage of decomposition (Raiesi et al., 1997a; Raiesi, 1998; Raiesi et al., 1998a). This difference, however, disappeared with incubation time.

In general, it seems that the correlated initial increased N mineralization of litter (Raiesi et al., 1997b; Raiesi, 1998) with soil N mineralization rate at elevated CO_2 displays a positive feedback on plant growth, but whether such a positive feedback is sustained in the long-term can not be concluded from these short-term decomposition data. On the other hand, we believe that if increases in soil temperature takes place with increasing CO_2 level in the atmosphere there is also the likelihood of additional nutrients to come available via its stimulating effect on soil carbon decomposition (Jenkinson et al., 1991).

CONCLUSIONS

Mineralization of C and N were correlated significantly with the elemental composition of SOM. The latter was partially affected by the CO_2 concentration (Raiesi et al., 1998b). We found that the C and N contents and C/N ratios significantly influenced the C and N mineralization in the forest floor. Total C, N and C/N ratio were all negatively correlated with C mineralization, while they correlated positively with N mineralization. The content of W&B C was most closely related to SOM decomposability in our studied system. Surprisingly, N mineralization showed a positive correlation with the C/N ratio. This is contrary to the general concept of C/N being negatively correlated with N mineralization.

This study indicated that although C mineralization rate was not affected by high CO_2, N immobilization appeared to be smaller at high CO_2. Further experiments are needed to determine the interactions between elevated CO_2 and other relevant factors such as microbial activity, root activity and turnover, which regulate C and N turnover rates.

(References are give on page 135)

Chapter 7

General discussion and conclusions

General discussion and conclusions

Carbon dioxide is an essential substrate for living plants, which constitute an interface between the atmospheric C and the soil C. It is also an important greenhouse gas, which effectively contributes to the global warming. While global warming may accelerate soil carbon decomposition, elevated CO_2 may affect the composition and dynamics of soil organic matter (SOM) via (1) increased rates of addition of plant litter to the soil (2) alterations in litter quality and (3) enhanced rhizodeposition by more C allocation to roots. Because soil plays a central role in plant growth and in global C and N cycles, it is essential to improve our understanding of the effect of elevated CO_2 on SOM. To study these effects, long-lasting experiments are necessary. Natural CO_2 springs provide a useful environment to carry out long-term investigations on ecosystem functioning, especially with respect to effects on soil C. Previous chapters of this thesis gave detailed results from natural CO_2 springs where the effect of elevated CO_2 on a plant litter (quality and decomposability) and soil organic matter (pools and decay) were studied (chapters 3-6). This chapter presents a general discussion and synthesis. Finally, the important feedbacks that SOM *may* impose on plant growth and atmospheric CO_2 are speculated upon. Table 1 presents the summarized results from the previous chapters.

ATMOSPHERIC CO_2 AND GREENHOUES GASES

Short-term concentrations: Short-term measurements showed distinct diurnal courses of CO_2 gas mixing ratio, with high fluctuations at the site (chapter 2). The CO_2 gas (daily mean of 795 ppm) from the vent was accompanied by atmospheric sulphur compounds such as H_2S (daily mean of 22 ppb) and SO_2 (daily mean of 16 ppb), but NO and NO_2 were absent. Physiologically, negative effects of sulphur compounds on slow-growing vegetation can be expected when the mean concentrations exceed 30 ppb for H_2S and 2-5 ppb for SO_2 (DeKok et al., 1983; Heber et al., 1987, Slovik et al., 1992). The level of H_2S at the Laiatico spring is below, but that of SO_2 is above the threshold values of phytotoxicity. Effects of high levels of SO_2 on plants growing around mineral CO_2 springs were, however, not observed (Miglietta et al., 1993c; Badiani et al., 1993), suggesting that the threshold values for SO_2 toxicity are doubtful for plants growing around mineral CO_2 springs. While the emission of N_2O from the vent was negligible (11 μg N m^{-2} h^{-1}), irrigation of the soil exposed to elevated CO_2 increased the net flux of N_2O to the atmosphere by a factor of 13 at the Bossoleto spring (chapter 2). Higher N_2O emission upon irrigation is in line with results reported by Davidson et al. (1991) and Hungate et al. (1997), who found higher N_2O production with increasing rainfall. Increased N_2O emission from irrigated soil exposed to elevated CO_2 suggests that increased substrate availability to nitrifying and denitrifying bacteria could be a result of elevated CO_2. This is true especially when the labile C pool would increase under elevated CO_2 (Verburg, 1998). Another possibility is the decomposition of dead micro-organisms in a dry soil upon re-wetting (Bottner, 1985). The net flux of methane from the atmosphere to the soil was higher the vicinity of the vent (-230 µg CH_4 m^{-2} h^{-1}) than that in the control area (-2 µg CH_4 m^{-2} h^{-1}). High methane consumption with CO_2 enrichment might be due to a change in the activity of methantrophic bacteria. The short-term temporal variability of the atmospheric CO_2 and contaminant trace gases could be mainly due to variation in the air temperature, short-term variation in atmospheric turbulence and topographic position of the site.

Long-term concentrations: It is difficult to estimate the long-term (annual average) CO_2 concentration distribution around the vent from direct measurements of the atmospheric CO_2 concentration, which varies strongly in time. The ^{14}C concentration in vegetation around the vent was used as an integrator. The CO_2 gas from the vent is isotopically depleted of ^{14}C, so all the ^{14}C in the plant tissues comes from the ^{14}C in the air. Using the known ^{14}C concentration of the air in the control area, and correction of isotopic fractionation, the ^{14}C concentration in twigs of known age could be used to estimate the ambient CO2 concentration. *Quercus ilex* twigs were used to this purpose. The calculated CO_2 concentration varied from 350 ppm away from the vent to about 590 ppm around the vent. The CO_2 concentrations were clearly elevated close to the vent, reaching ambient values within 50 to 100 m away from the vent. The mean CO_2 concentration emission directly above the vent was predicted to be 510 ppm. The CO_2 concentrations estimated with the ^{14}C method gave a very consistent pattern with values increasing downslope of the vent, and decreasing upslope. The long-term CO_2 concentrations calculated by ^{14}C method were in the same range as the short-term daily CO_2 concentrations measured by infrared gas analyzer, but tended to be lower. The differences in the absolute CO_2 levels between the two methods can essentially be attributed to the time scale of measurements. The maximum CO_2 concentration that was found in this work is close to the predicted CO_2 concentration of 600 ppm for the year 2050.

ELEVATED CO_2 AND BIOMASS PRODUCTION AROUND CO_2 VENTS

The long-term elevated CO_2 levels may increase rates of C fixation by plants, and therefore stimulation of net primary production, even around mineral CO_2 springs (Jones et al., 1995; Hättenschwiler et al., 1997). Consequently, the C sequestration by soils could increase if N uptake by plants is high enough to support the continued plant growth under elevated CO_2. Plant growth in natural ecosystems is often N limited (Vitousek and Howarth, 1991), but extra N for plant uptake at elevated CO_2 could come from direct uptake of organic N (Keilland, 1994) or increased N uptake efficiency by roots, partly from atmospheric N deposition (Luxmoore et al., 1993; Gifford, 1994; Schimel, 1995; Day et al., 1996).

ELEVATED CO_2 EFFECT ON LITTER CHEMISTRY AND TURNOVER

Changes in litter chemistry of plants that were exposed to long-term elevated CO_2 and subsequent effects of such changes on C and N mineralization were studied (chapter 3 and 4). The chemical composition and elemental ratios of *Quercus cerris* and *Q. pubescens* (chapter 3) and *Smilax aspera* (chapter 4) litter at high-CO_2 were similar to those at ambient CO_2 (Table 1). Similar results, which are not reported in this thesis were obtained with *Quercus ilex* L. and *Fraxinus ornus* L. leaf litter. Also carbon and nitrogen concentrations, and C/N ratios of *green* leaves of *S. aspera* were not affected by CO_2 level. *Q. cerris* and *Q. pubescens* litter from elevated and ambient CO_2 levels decomposed at similar rates (remaining mass) under field conditions. Laboratory results indicated that litter decomposability of neither species was affected by elevated CO_2 over 3 months of incubation (Table 1). The unchanged litter decomposability is in agreement with the lack of a CO_2 effect on litter quality parameters. Also C mineralization under laboratory conditions in *S. aspera* litter was not affected by CO_2 level. N mineralization in leaf litter was monitored for 125 days (oaks) or 60 days (*S. aspera*). Although, the initial N mineralization in litter was higher at elevated CO_2 than that at ambient CO_2 (chapter 3 and 4), it was not affected by CO_2 in the long-run (Table 1), in accordance with the absence of a CO_2 impact on litter chemistry.

Results from long-term experiments around CO_2 springs provided evidence that the expected increase in the atmospheric CO_2 concentration does not have an influence on substrate quality, and therefore it is unlikely that rising CO_2 level affects C and N turnover rates of litter in terrestrial ecosystems. The lack of a CO_2 effect on litter decomposability reported in this thesis corresponds well with recent field observations from boreal forest ecosystems (Verburg, 1998), lowland calcareous grassland and lowland wet tropical forest ecosystems (Hirschel et al., 1997), tall grass prairie (Kemp et al., 1994; Owensby et al., 1993, 1996), temperate deciduous forest (O'Neill and Norby, 1996), and some agricultural crops (Henning et al., 1996; Taylor and Ball, 1994).

Table 1. The overall impact of elevated CO_2 on a leaf litter-soil organic matter continuum in a Mediterranean forest ecosystem, Italy.

	Ecosystem compartment		
Parameter	Plant	Forest floor	Mineral soil
		Concentrations	
C	=	+	=
N	=	=	=
Others†	=	n.a.	n.a.
		Ratios	
C/N	=	=	=
Others†	=	n.a.	n.a.
		Decomposition-Mineralization	
C	=	=	=
N	=	+	=
		Pool sizes	
C	(+)	+	=
N	(+)	+	=

= : no effect observed, +: increase observed, (+): increase estimated, † Others: lignin, cellulose, polyphenols and their ratios, n.a.: not applicable

The absence of any CO_2 effect on litter quality could be due to either increased nutrient uptake by roots (Day et al., 1996) or downward regulation of photosynthesis (Ryle et al., 1992; Grulke et al., 1993; El Kohen et al., 1993; Miglietta et al., 1995; Oechel and Vourlitis, 1996; Hättenschwiler and Körner , 1996) at elevated CO_2. The possibility also exists that in N limited systems, plants take up inorganic N directly through absorption of amino acids (Keilland, 1994) or use atmospheric N deposition more efficiently when grown at elevated CO_2 (Luxmoore et al., 1993; Gifford, 1994; Schimel, 1995).

Collectively, there is no convincing evidence that leaf litter quality of mature trees grown in N-limited Mediterranean forest soils and under continuous exposure to elevated CO_2 changes at the ecosystem level. These long-term CO_2 studies cast some doubt on the general concept that elevated CO_2 changes litter quality of plants, and thereby slows down decomposability of litter and N release. Although a number of authors draw the conclusion that although the CO_2 effect on litter quality is inconclusive, many field studies suggest that elevated CO_2 will not change litter quality, and therefore rates of C decomposition will not change (Norby et al., 1995; O'Neill and Norby, 1996; Koch and Mooney, 1996; Henning et al., 1996; Randlett et al., 1996; Hirschel et al., 1997; Verburg, 1998; Mooney et al., 1998).

So, it can be speculated that effects of CO_2 on quality of input materials play no significant role in changing the carbon balance and SOM pools of Mediterranean woodland ecosystems containing typical oak-ash trees. This does, however, not rule out the possibility that long-term elevated CO_2 will gradually increase the soil organic carbon pool through higher litter input caused by increased net primary production.

SPECIES COMPOSITION AND SOM (C AND N CYCLES)

Although elevated CO_2 had no significant impact on litter quality and decomposability, there were significant differences in litter quality and decomposition parameters between species (chapter 3 and 4). *S. aspera* had a higher litter quality than the two oak species, while *Q. pubescens* litter had a higher quality than *Q. cerris* litter. *Q. pubescens* litter decomposed faster than *Q. cerris* litter under controlled conditions, but such an effect was not seen in field decomposition (chapter 4). This suggests that that under field conditions plant leaf litter with a high quality does not necessarily decompose faster than plant leaf litter with a low quality. The fundamental reasons lie in specific micro-site characteristics (e.g. water, temperature), and high spatial micro-variability in some soil properties (e.g. soil microorganisms, root distribution).

This study suggests that differences in litter quality of different plant species are greater than impacts of elevated CO_2 on litter quality and C and N cycling so that changes in plant species composition may be more important for soil C sequestration than direct effects of elevated CO_2 on the litter quality of species. Because the response of individual plant species can not be used to predict the response of plant

2) The effect of high CO_2 on species composition needs to be investigated. Any impact of elevated CO_2 on plant species composition and succession would influence soil C dynamics and the N cycle, especially at community and population level.

3) The response of soil organisms (e.g. composition and activity) to elevated CO_2 needs to be further researched, because shifts in C allocation and in species decomposition due to high CO_2, and CO_2-induced changes in litter quality can have dramatic impacts on soil decomposer community, and therefore on ecosystem processes.

4) Biotic factors such as soil micro-organisms, and abiotic factors such as soil temperature and moisture content, which may be affected by global climate change could have more striking impacts on SOM dynamics than small changes in the chemical composition of plant materials. Because changes in CO_2 concentration, air temperature and rainfall patterns may be linked, we need to understand the combined effects of biotic and abiotic factors on decomposition processes with respect to global climate change on a broad scale. It is essential to pay more attention to the impact of changes in abiotic factors associated with elevated CO_2, such as soil moisture content and temperature, on C and N dynamics. Further, field studies are highly desirable to understand the interaction between abiotic and biotic factors, such as soil microbial activity and litter quality, and the resulting effect on organic matter decomposition in terrestrial ecosystems.

5) More studies about the effect of elevated CO_2 on the stage of developmental and succession in natural ecosystems are desirable to understand soil C dynamics, since mature trees cover a large area of the Earth's surface and because very little is known about CO_2 effects on litter quality of mature trees. Because of long-term dynamics of SOM, long-term CO_2 experiments at scales larger than a pot and a small field plot are required to project the consequence of elevated CO_2 on the structure and functioning of ecosystems.

(References are give on page 135)

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Summary

The atmospheric CO_2 concentration has dramatically increased from the pre-industrial revolution value of 280 ppm to the present value of 360 ppm, and further increases are foreseen within several decades. One effect of elevated CO_2 on terrestrial ecosystems is likely to be changes in C balances of vegetation and soils by increased plant production, by increased rhizodeposition via more C allocation to belowground and by alterations in the decomposition of soil organic matter (SOM). Previous studies have indicated that elevated CO_2 increases quantity and decreases quality of plant material. Such changes may slow down C and N turnover rates so that soil would act as a net sink of the increasing atmospheric CO_2. However, the long-term impact of elevated CO_2 on litter quality and subsequently decomposability, and thereafter on SOM dynamics are not fully known, especially in natural ecosystems which often suffer from nutrients limitations. The aim of this research was to test the hypotheses that enhanced CO_2 decreases substrate quality and subsequent rates of decomposition of leaf litter. Reduced litter quality and decomposability could increase residence times of surface litter and SOM or increase pools of surface litter and of SOM in the long-run. Increased accumulation of C in the soil eventually decreases nutrient availability. This research concerns effects of naturally increased CO_2 on a plant litter C (young C)- native soil C (old C) "continuum" by obtaining data from mineral CO_2 springs in Central Italy.

The study site is located near the village of Laiatico, approximately 35 km south-east of Pisa, Italy (43° 26'N, 10° 42'E, at 190-240 m above sea level, with annual rainfall of 830 mm; average yearly temperature of 15 °C). The area (ca. 0.9 ha) is a rolling forested region with an irregular and rough surface. Soils of the area are calcareous, developed from Tertiary marl. The soil $pH_{(H_2O)}$ is 6.9 in the topsoil and above 7.3 in the subsoil. The soil texture is silty clayey loam in the topsoil and silty clay in the subsoil. The soil is covered by a litter layer (F+HA) with varying thickness. The study area is a typical, semi-natural Mediterranean woodland which was last cut about 25 years ago. The vegetation is coppiced-Mediterranean oak-ash stands composed of *Quercus ilex* L., *Quercus cerris* L., *Fraxinus ornus* L., *Quercus pubescens* Willd., with shrubs such as *Erica arborea* L. and *Arbutus unedo* L.. The understory is dominated by the vine *Smilax aspera* L. The CO_2 spring, consisting of one major vent and a number of smaller ones within a circle of 5 meters, is situated at the bottom of a gully. There is a CO_2 gradient from ambient (350 ppm) at some distance of the vent to about 600 ppm at the vent. The CO_2 emission comes

from thermal decomposition of carbonatic parent material probably under the influence of volcanic activity. Because factors other than atmospheric CO_2 level will contribute to spatial differences in soil properties including SOM within the valley, topographically a similar transect (ambient CO_2, similar parent material, slope-soil hydrological conditions, and vegetation) was selected as the control area.

In an effort to determine the CO_2 concentration experienced by the vegetation around the CO_2 vent, short-term and long-term integrating measurements were performed. To characterize the atmospheric mixing ratios of concomitant trace gases such as SO_2, H_2S, NO and NO_2 with a high resolution in time, atmospheric gas measurements were performed by use of fully automated measuring apparatuses. The short-term measurements showed distinct diurnal courses of CO_2 gas mixing ratio with high fluctuations at the site (chapter 2). The CO_2 gas from the vent was accompanied by atmospheric sulphur compounds like H_2S and SO_2, but no NO and NO_2. While the emission of N_2O from the vent was negligible, irrigation increased the net flux of N_2O from the soil exposed to elevated CO_2 to the atmosphere upon irrigation of the soil (chapter 2). The net flux of methane from the atmosphere to the soil exposed to elevated CO_2 was substantially higher than the soil exposed to ambient CO_2. *Quercus ilex* twigs were used to determine the mean local ambient CO_2 concentration by analyzing the ^{14}C activity using the conventional ^{14}C age method. The calculated CO_2 concentration varied from 350 ppm away from the vent to about 590 ppm around the vent. The CO_2 concentrations were clearly elevated close to the vent, reaching ambient values within 50 to 100 m away from the vent. The mean CO_2 concentration emission directly above the vent was 510 ppm. The CO_2 concentrations estimated with the ^{14}C method gave a very consistent pattern with values increasing downslope of the vent, and decreasing upslope. The long-term CO_2 concentrations calculated by ^{14}C method were in the same range as the short-term CO_2 daily concentrations measured by infrared gas analyzer, but tended to be lower. The differences in absolute CO_2 levels between the two methods can be attributed to the time scale of measurements.

To evaluate changes in litter chemistry, duplicate senescent leaf litter samples from *Quercus cerris*, *Quercus pubescens* and *Smilax aspera* were taken at elevated (>500 ppm) and ambient (<360 ppm) CO_2 concentrations, analyzed for chemical composition (C, N, lignin, cellulose, polyphenols). The rates of C and N mineralization in plant leaf litter were determined using litterbag experiments under field conditions (12 months) and laboratory incubation methods under controlled conditions (3-4 months). Results from the vegetation demonstrate that elevated CO_2 did not affect litter quality. Consequently, C and N mineralization rates were remained unaffected by CO_2 level. However, there were significant differences in litter quality and decomposition-mineralization between species. *S. aspera* had a higher litter quality than the two *Quercus* species, while *Q. pubescens* litter had a higher quality than *Q. cerris* litter. *Q. pubescens* litter decomposed faster than *Q. cerris* litter under controlled conditions, but such an effect was not seen in the field decomposition. So, species composition had more influence on C and N cycles than elevated CO_2. This may be a potentially important feedback from CO_2 level to the soil than the CO_2 impact on litter quality.

To determine the effects of elevated CO_2 on soil organic matter itself (e.g. native and old C) and to find out whether or not CO_2 effects on plant litter C (new C) are reflected in soil C dynamics in a natural environment, soil sampling was carried out to make an inventory of the distribution of SOM as a function of atmospheric CO_2 concentration and other potentially important soil forming factors. Duplicate soil samples were collected in the elevated area along a CO_2 gradient of decreasing atmospheric CO_2 levels from the source to ambient (350 ppm) between 50 to 100 m from the vent. Topographically similar transects were sampled in a control area. Undisturbed soil cores of 26 profiles (17 in elevated and 9 in control area) were sampled and analyzed for total C and N contents. The rate of CO_2 evolution and accumulated CO_2 were monitored hourly for 3 weeks. The evolved CO_2 was absorbed in 0.6 M KOH, and the electrical conductivity of the KOH was automatically monitored. N mineralization was estimated from the increase in KCl-extractable inorganic N after incubating soil samples for 30 days. Results show that elevated CO_2 increased the C content and pool size of the forest floor (F +HA layers). Such effects were not observed in deeper mineral 0-10 cm of the soil (chapter 5). A significant positive relationship between the litter thickness and CO_2 concentration was observed, indicating a higher litter production may be expected with increasing atmospheric CO_2. The N content and the C/N ratios of the three soil layers, however, were not affected by elevated CO_2 (chapter 5). Although, the N contents in the forest floor remained unaffected by elevated CO_2, long-term elevated CO_2 led to a doubling of the N pool size. The rates of soil C mineralization were not influenced by elevated CO_2 (chapter 6), in accordance with the absence of a CO_2 effect on the N content and C/N ratios of soil organic matter (chapter 5). Therefore, the possible explanation for increased C pool size in the litter layer may be attributed to increased rates of litter input by enhanced Net Primary Production (NPP) at elevated CO_2. The rates of N immobilization in the forest floor (F plus HA layers) were lower at elevated than those at ambient CO_2, whereas the rates of N mineralization in mineral soil were unaffected (chapter 6).

Indeed the absence of a CO_2 effect on litter quality and consequently decomposability was generally reflected in the unchanged chemical composition and decomposition of soil organic matter. Even though the decomposition of soil organic matter in a natural environment is a complex process, especially in a multi-species ecosystem, and all factors that control SOM decomposition have not been examined, evidence that SOM decomposition is reduced under increased CO_2 was not found. In this study, soil carbon which was derived from plant materials exposed to elevated CO_2 continuously was used, and it considered the whole plant-soil system. By considering the whole plant-soil system, we took into account at least some plant-soil feedbacks such as impacts of elevated CO_2 on belowground C and processes and thereby on soil C via the plant. Most experiments, which have reported reduced C mineralization under elevated CO_2 are either short-term or used a soil-litter mixture that may not represent natural properties and processes as they occur in the field. Because elevated CO_2 has no impact on litter quality and decomposability we may expect that soil organic matter of Mediterranean forest ecosystems will increase only by elevated CO_2 if that causes an increase in biomass production, because elevated atmospheric CO_2 resulted in an increase in organic carbon in the forest floor.

Main conclusions

- both short- and long-term measurements displayed that the air in the forest canopy was clearly enriched with CO_2 in the proximity of the spring.
- the CO_2 concentration estimated with the ^{14}C technique had a very consistent pattern, increasing downstream, decreasing upstream.
- CO_2 springs emit variable amounts of H_2S and SO_2, but not nitrous oxides.
- elevated CO_2 increased the net flux of methane from the atmosphere to the soil.
- N_2O emission from the vent was negligible, and irrigation enhanced the flux of N_2O from the soil exposed to elevated CO_2 to the atmosphere.

- litter quality parameters of mature trees grown in N-limited soils and under continuous exposure to elevated CO_2 may not change at the ecosystem level.

- the long-term elevated CO_2 had no effect on litter decomposability, in agreement with no changes in litter quality of plants due to elevated CO_2.
- litter N mineralization, and consequently N availability, was not affected by CO_2 .
- differences in litter quality of different plant species were more significant than impacts of elevated CO_2 on litter quality
- plant leaf litter with a high quality does not necessarily decompose faster than plant leaf litter with a low quality under field conditions.

- contents of organic carbon in the forest floor were increased by CO_2 level, but not in deeper layers.
- contents of N, and C/N ratios of the soil profile remained unaffected by elevated CO_2.
- elevated atmospheric CO_2 increased the C and N pools in the forest floor of Mediterranean-type ecosystems, but not in the A horizon.
- while the decomposition of soil organic matter was not affected by elevated CO_2, N immobilization in the forest floor appeared to be lowered by CO_2 and N mineralization in the mineral soil remained unchanged.

During this experiment, more research directions were identified to be included in studies on the potential effect of global climate change on terrestrial ecosystems. We need to understand the impact of elevated CO_2 on belowground processes, plant species succession, microbial composition and activity, and on interactions between them. We also need to consider the time scale of measurements, especially in studies on soil organic matter because of the long-lived turnover of slow and passive pools, which are important for long-term C storage in the soil.

Samenvatting

De atmosferische CO_2 concentratie is dramatisch toegenomen, van een pre-industriële concentratie van 280 ppm tot de huidige waarde van 350 ppm, terwijl verdere toename gedurende de komende decaden verwacht wordt. Een verhoogde atmosferische CO_2 concentratie kan de koolstof balans van vegetatie en bodem veranderen door 1) een verhoogde primaire productie; 2) een verhoogde rhizodepositie door meer koolstof allocatie in ondergrondse delen; en 3) door veranderde omzetting van organische stof in de bodem (SOM). Voorgaande studies hebben aangegeven dat verhoogde CO_2 concentries kunnen leiden tot verhoogde hoeveelheid en verminderde kwaliteit van plantaardig materiaal. Zulke veranderingen kunnen leiden tot verlaging van de omloopssnelheden van koolstof en stikstof, zodat de totale opslag van koolstof in de bodem zou kunnen toenemen, waardoor de stijging van de atmosferische concentratie van CO_2 afgeremd zou kunnen worden. Evenwel, het effect op lange termijn van CO_2 op strooiselkwaliteit - en hieruit voortvloeiende strooiselafbreekbaarheid - , en zijn doorwerking op dynamiek van de SOM zijn onvolledig bekend, in het bijzonder in natuurlijke ecosystemen, waarin de groei vaak beperkt wordt door beschikbaarheid van nutriënten.

Het doel van het onderhavige onderzoek was om de hypothese te testen dat verhoogd atmosferisch CO_2 zou leiden tot een vermindering van strooiselkwaliteit en, als gevolg daarvan, tot een verlaging van de omzettingssnelheid van strooisel. Verlaging van deze omzettingssnelheid zou automatisch leiden tot een verhoging van de omloopstijd van strooisel *en* van SOM, en tot verhoging van de in strooisel en SOM opgeslagen hoeveelheden koolstof op lange termijn. Toename van de koolstofvoorraad in de bodem zou weer kunnen leiden tot verminderde beschikbaarheid van nutrienten. Het onderhavige onderzoek betreft effecten van natuurlijk verhoogde CO_2 op het natuurlijke continuum van strooisel (jonge C) en oorspronkelijke koolstof in de bodem (oude C), zoals gemeten in de nabijheid van een CO_2-bron in midden Italie.

De onderzoeklokatie ligt bij het dorp Laiatico, ongeveer 35 km ten zuidoosten van Pisa (43°26'N, 10°42'O), op 190-240 boven zeeniveau. De jaarlijkse neerslag is ongeveer 830 mm, en de gemiddelde jaartemperatuur is 15°C. Het gebied, ongeveer 0.9 ha groot, is bebost en glooiend, met een onregelmatig oppervlak. De bodems zijn ontwikkeld uit Tertiaire mergels en bevatten kalk. De bodem pH (water) is 6.9 in de bovengrond en boven 7.3 in de ondergrond. De textuur van de bovengrond is een siltige, kleiige leem, die van de ondergrond een siltige klei. De bodem heeft een strooisellaag (F+HA) van wisselende dikte. De vegetatie is een typisch, semi-natuurlijk mediterraan bos, dat 25 jaar geleden voor het laatst werd gekapt. Het hakhoutstruweel bestaat uit eiken en essen, met *Quercus ilex* L., *Quercus cerris* L., *Fraxinus ornus* L., *Quercus pubescens* Willd. als dominante species, en struiken zoals *Erica arborea* L., en *Arbutus unedo* L. De ondergroei wordt gedomineerd door de slingerplant *Smilax aspera* L. De CO_2 bron, die bestaat uit een hoofdkanaal en een aantal kleinere, binnen een cirkel van 5 meter, bevindt zich in de bodem van een erosiegeul. Er is een CO_2 gradient van atmosferische CO_2 concentratie (350 ppm) op enige afstand van de bron, tot ongeveer 600 ppm rond de bron. De CO_2 emissie komt voort uit thermische decompositie van carbonatische gesteenten, vermoedelijk onder de invloed van vulkanische activiteit. Omdat andere factoren dan het atmosferische CO_2 niveau bijdragen tot ruimtelijke verschillen in bodemeigenschappen, met inbegrip van koolstofgehalten, binnen het gebied, is een tweede transect, met atmosferische CO_2 concentratie en vergelijkbare helling, vegetatie, bodem, en hydrologie, gebruikt als referentiegebied.

Teneinde de gemiddelde CO_2 concentratie vast te stellen die door de vegetatie rondom de CO_2 bron wordt waargenomen, is een aantal experimenten van korte en lange duur uitgevoerd. Volautomatische atmosferische gasmetingen met een grote tijdsresolutie werden uitgevoerd om de mengratio's te bepalen van de begeleidende spore-gassen zoals SO_2, H_2S, NO en NO_2. Deze korte-termijn metingen duiden op een duidelijke dagelijkse amplitude van de CO_2 mengverhouding, met hoge fluctuaties (zie Hoofdstuk 2). Het CO_2 gas uit de bron wordt begeleid door atmosferische zwavelcomponenten zoals H_2S en SO_2, maar niet door NO en NO_2. Hoewel de N_2O uitstoot uit de bron verwaarloosbaar was, had irrigatie in het hoog- CO_2 gebied een duidelijke verhoging van de N_2O flux van de bodem naar de atmosfeer tot gevolg (Hoofdstuk 2). De netto flux van methaan van de atmosfeer naar de bodem was hoger bij hoge dan bij normale CO_2 concentratie.

Het ^{14}C gehalte van *Quercus ilex* takjes werd gebruikt om de gemiddelde lokale CO_2 concentratie te meten. De berekende concentraties varieerden van 350 ppm op enige afstand van de bron tot 590 ppm rondom de bron. Atmosferische waarden werden gevonden op afstanden van 50-100 m van de bron. De gemiddelde CO_2 concentratie, gemeten direct boven de bron, was 510 ppm. De CO_2 gehalten die bepaald werden met behulp van ^{14}C metingen gaven een zeer betrouwbaar patroon, waarbij de waarden toenamen hellingafwaarts van de bron, en afnamen hellingopwaarts. De lange-termijn concentraties, zoals berekend uit de ^{14}C gehalten, kwamen goed overeen met de korte termijn metingen, maar waren gemiddeld iets lager. Verschillen tussen de twee methoden kunnen toegeschreven worden aan de verschillende tijdschalen.

Om veranderingen in chemische samenstelling van strooisel te meten, werden zuivere strooiselmonsters verkregen door duplo monsters te nemen van afgestorven blad van *Quercus cerris, Quercus pubescens,* en *Smilax aspera,* zowel bij hoge (>500 ppm) als bij lage (<360 ppm) CO_2 concentraties. In deze strooiselmonsters werden C, N, lignine, cellulose, en polyphenolen bepaald. De mineralisatiesnelheid van koolstof en stikstof in dit strooisel werd gemeten met behulp van strooiselzakken, die gedurende 12 maanden in het veld geïncubeerd werden, en door middel van gecontroleerde incubatie gedurende 3 tot 4 maanden in het laboratorium. De resultaten geven aan dat verhoogde CO_2 concentratie geen effect had op de strooiselkwaliteit. Als logisch gevolg hiervan, werd de afbraaksnelheid evenmin beinvloed door CO_2 concentratie. Desalniettemin waren er significante verschillen in strooiselkwaliteit en omzetting/mineralisatie tussen de soorten. *S. aspera* had een hogere strooiselkwaliteit dan de twee eikensoorten, en het strooisel van *Quercus pubescens* had een betere kwaliteit dan dat van *Quercus cerris.* Afbraak onder laboratoriumomstandigheden was sneller voor *Q. pubescens* strooisel, maar de langere incubatie onder veld-omstandigheden liet geen verschillen zien. De plantensoort lijkt meer invloed te hebben op koolstof en stikstofcycli dan het CO_2 gehalte van de atmosfeer.

Om na te gaan wat het effect is van verhoogd CO_2 gehalte op organische stof in de bodem ('oude C'), en of een CO_2 effect op strooisel ('nieuwe C') doorwerkt in de koolstofdynamiek van een natuurlijk systeem, werden bodemmonsters genomen. Duplicaat bodemmonsters werden verzameld langs een gradient van hoog CO_2 in de nabijheid van de bron, tot atmosferisch CO_2 op een afstand van 50 tot 100 meter. Topografisch vergelijkbare transecten werden bemonsterd in het referentiegebied. Ongestoorde boorkernen van 26 profielen (17 langs de CO_2 gradient en 9 in het refentiegebied) werden bemonsterd en geanalyseerd op C en N gehalte. In een incubator werd gedurende drie weken continu de CO_2 productie van de monsters gemeten. Vrijkomend CO_2 werd geabsorbeerd in 0.6M KOH en de geleidbaarheid van de KOH oplossing werd automatisch geregistreerd. Stikstofmineralisatie werd gemeten door bepaling van KCl-extraheerbaar anorganische stikstof vóór het experiment en na 30 dagen incubatie.

De resultaten laten zien dat verhoogd CO_2 leidde tot een vergroting van het C gehalte en van de C voorraad in de strooisellaag (F+HA lagen). Er werd geen verandering waargenomen in de bovenste 10 cm van de minerale grond (Hoofdstuk 5). Er was een significante positieve correlatie tussen dikte van de strooisellaag en CO_2 concentratie, die aangeeft dat strooiselproductie stijgt bij hogere CO_2 concentratie. Het stikstofgehalte en de C/N verhouding van de drie bodemlagen werden niet beinvloed door CO_2 gehalte (Hoofdstuk 5). Hoewel N gehalten van de strooisellaag niet werden beinvloed door lange-termijn blootstelling aan hoog CO_2 was er een verdubbeling van de totale N-voorraad in de grond.
In overeenstemming met het ontbreken van een CO_2 effect op strooiselkwaliteit, werd geen invloed van CO_2 op de mineralisatie van SOM gevonden (Hoofdstuk 5,6). De toename van de koolstofvoorraad moet daarom toegeschreven worden aan een toegenomen strooiseltoevoer als gevolg van verhoogde primaire productie bij verhoogde CO_2. Stikstofvastlegging in de strooisellaag was geringer bij hoge dan bij normale CO_2 concentratie, terwijl vastlegging in de minerale grond niet beinvloed werd door CO_2.

Het ontbreken van CO_2-effect op strooiselkwaliteit en -afbraak werd gereflecteerd in onveranderde chemische samenstelling en afbraaksnelheid van organische stof in de bodem. Hoewel afbraak van SOM in natuurlijke systemen een complex proces is, zeker in ecosystemen die vele species bevatten, en slechts een gering aantal factoren geevalueerd kon worden, is er geen bewijs dat afbraak van bodem-organische stof vertraagd wordt bij verhoogd CO_2.In het onderhavige onderzoek werd organisch materiaal gebruikt dat afkomstig was van planten die langdurig aan verhoogd CO_2 waren blootgesteld, en het gehele plant-bodem systeem werd geanalyseerd. Door deze geheel-systeem benadering zijn sommige van de plant-bodem terugkoppelingsmechanismen inbegrepen, zoals het effect van verhoogd CO_2 op koolstof verdeling boven- en ondergronds en, via dit effect, het totale effect op bodem-koolstof via de plant. De meeste experimenten die een vermindering van organische stof omzetting bij verhoogd CO_2 rapporteerden, waren óf van korte duur, óf gebruikten bodem-strooisel mengsels die niet-representatief waren voor een situatie in het veld.

Omdat verhoogd CO_2 geen effect blijkt te hebben op strooiselkwaliteit en -omzetbaarheid, kunnen we verwachten dat de koolstofvoorraad in Mediterrane bos-ecosystemen alleen zal toenemen door verhoogde productie van biomassa als gevolg van verhoogd CO_2. Dit laatste effect wordt gesuggereerd door de toename van de koolstofvoorrraad in de strooisellaag.

Belangrijkste conclusies

- zowel korte- als lange termijn metingen gaven aan dat de atmosfeer in het bos in de omgeving van de bron duidelijk verrijkt was aan CO_2.
- het CO_2 gehalte, gemeten met behulp van ^{14}C concentratie in twijgen, vertoonde een consistent patroon, met hoge gehalten hellingafwaarts en lagere gehalten hellingopwaarts van de bron.
- de uitstoot uit de bron van H_2S en SO_2 is variabel; er is geen uitstoot van NO_x.
- verhoogde CO_2 deed het netto transport van methaan van de atmosfeer naar de grond toenemen.
- irrigatie verhoogde het netto transport van N_2O van de grond naar de atmosfeer bij hoge CO_2 concentraties.

- in gronden met lage N-gehalten die blootgesteld zijn aan hoog CO_2, veranderen parameters die strooiselkwaliteit beschrijven niet op ecosysteem niveau
- in overeenstemming met afwezigheid van effect op strooiselkwaliteit, had verhoogd CO_2 geen effect op afbreekbaarheid van strooisel.
- stikstofmineralisatie in strooisel, en daarmee de beschikbaarheid van stikstof, werd niet beinvloed door het CO_2 niveau
- verschillen in strooiselkwaliteit tussen planten waren belangrijker dan het effect van verhoogde CO_2 op de strooiselkwaliteit.
- onder veld-condities wordt strooisel van hogere kwaliteit niet *per se* sneller afgebroken dan dat van lagere kwaliteit.

- koolstof gehalten in de strooisellaag werden verhoogd onder hoog CO_2 gehalte, maar de minerale grond vertoonde geen invloed.

- C en N gehalten, en C/N ratios van de bodem werden niet beinvloed door CO_2 gehalte van de atmosfeer.
- verhoogd CO_2 heeft, in een mediterraan bos, vergroting van de totale koolstofvoorraad in de strooisellaag tot gevolg, maar heeft geen invloed op de voorraad in de minerale grond.
- hoewel afbraak van organische stof in de bodem niet beinvloed werd door verhoogd CO_2 gehalte, lijkt stikstofvastlegging geremd te worden in de strooisellaag; in de minerale grond werd geen effect gemeten.

Gedurende het onderzoek werd een aantal additionele vragen geformuleerd, die beantwoord moeten worden om het effect van CO_2 op terrestrische ecosystemen te voorspellen. Het is nodig het effect te kennen van verhoogd CO_2 op ondergrondse processen, species samenstelling van het ecosysteem, samenstelling en activiteit van de microbiele biomassa, en interacties tussen deze factoren. Het is eveneens noodzakelijk de tijdschaal van waarneming in het onderzoek te betrekken, speciaal in studies van bodem-organische stof, omdat deze component een lange omlooptijd heeft met grote 'langzame' en 'passieve' voorraden, die van belang zijn voor lange-termijn vastlegging van koolstof in de bodem.

- پارامترهای کیفی در لاشبرگ درختان بالغی که تحت شرایط محدودیت ازت خاک بوده و بطور دائم در معرض غلظت بالای CO_2 رشد کنند، در مقیاس اکوسیستم تغییر نخواهد کرد.
- غلظت بالا و طولانی مدت CO_2 تأثیری بر تجزیه پذیری لاشبرگ نداشت که این یافته با عدم تغییر کیفیت لاشبرگ در اثر افزایش گاز کربنیک در توافق است.
- اختلافات مشاهده شده در کیفیت لاشبرگ بین گونه های مختلف گیاهی بمراتب مهمتراز تأثیرغلظت بالای CO_2 بر کیفیت لاشبرگ بود.
- تحت شرایط مزرعه لاشبرگ گیاهی با کیفیت بالا الزاماً سریعتر از لاشبرگ گیاهی با کیفیت پائین تجزیه نمی شود.

- افزایش CO_2 مقدار کربن آلی در کف جنگل را افزایش داد اما این اثر CO_2 در لایه های عمیق تر دیده نشد.
- افزایش CO_2 اثری بر مقدار ازت آلی و نسبت کربن به ازت در پرو فیل خاک نداشت.
- انبار کربن وازت در کف جنگل اکوسیستم مدیترانه ای تحت شرایط غلظت بالای CO_2 افزایش یافت ولی میزان آنها در افق A بدون تغییر باقی ماند.
- در حالیکه باافزایش غلظت گاز CO_2 سرعت تجزیه مواد آلی خاک تغییر نکرد، سرعت غیر متحرک شدن ازت در خاک کف جنگل کاهش یافت و سرعت معدنی شدن ازت خاک درافق معدنی خاک بدون تغییر باقی ماند.

در طی این آزمایش نیازهای تحقیقاتی بیشتری شناسائی شد که لازم است درمطالعه اثرات بالقوه تغییرات آب و هوائی برروی اکوسیستم های خاکی گنجانیده شوند. اطلاعات بیشتری در خصوص اثر غلظت بالای CO_2 بر فرآیندهای زیرزمینی، توالی گونه های گیاهی، ترکیب و فعالیت میکروبی در خاک و اثرات متقابل بین آنها نیاز است. در مطالعه پویائی مواد آلی خاک به دلیل اینکه بخشی از این مواد پایدار بوده و دارای سرعت تجزیه بسیار کندی می باشد، در نظر گرفتن مقیاس زمانی در اندازه گیری ها از اهمیت خاصی برخوردار است زیرا مواد آلی پایدار به عنوان ذخایر طولانی مدت کربن در خاک نقش مهمی بعهده دارند.

خاک کف جنگل (F+HA) در غلظت بالای CO_2 کمتر از این سرعت در غلظت های طبیعی CO_2 بود در حالیکه سرعت معدنی شدن ازت در افق های معدنی خاک تحت تأثیرافزایش CO_2 قرار نگرفت (فصل ٦).

بطور کلی عدم تأثیر CO_2 بر روی لاشبرگ گیاهی و در نتیجه تجزیه پذیری آن در ترکیب شیمیائی و تجزیه مواد آلی خاک منعکس گردید. هر چند تجزیه مواد آلی خاک در محیط طبیعی و بویژه در اکوسیستمهای چند گونه ای بسیار پیچیده است، وبا وجودیکه در این مطالعه کلیه عواملی که تجزیه مواد آلی خاک را کنترل می کنند بررسی نشد، شواهدی مبنی بر کاهش سرعت تجزیه مواد آلی خاک در غلظت بالای CO_2 بدست نیا مد. در این مطالعه منشأ کربن خاک مواد گیاهی بود که بطور دائم در معرض غلظت بالای CO_2 قرار داشت و سیستم کامل خاک-گیاه مورد بررسی قرار گرفت. با در نظر گرفتن این سیستم بعضی از پسخور های موثر بر سیستم خاک-گیاه از قبیل اثر CO_2 بر روی کربن و فرایندهای زیر زمینی و در نتیجه بر روی کربن خاک از طریق گیاه مورد توجه قرار گرفت. اغلب آزمایشاتی که کاهش سرعت معدنی شدن مواد آلی در نتیجه افزایش CO_2 را گزارش کرده اند یا کوتاه مدت بوده و یا از مخلوط خاک-لاشبرگ استفاده کرده اند که نمایانگر خواص و فرایندهای طبیعی به نحوی که در شرایط مزرعه رخ میدهند، نمی باشد. انتظار می رود که مواد آلی درخاک اکوسیستمهای جنگلی مدیترانه ای تنها در شرایطی افزایش یابد که غلظت بالای CO_2 موجب تولید بیشتر بیوماس گیاهی شود زیرا افزایش مواد آلی خاک در کف جنگل ناشی ازافزایش غلظت CO_2 بوده است و اینکه CO_2 بالا اثری بر روی کیفیت و تجزیه پذیری لاشبرگ گیاه نداشت.

جمع بندی نهائی

- اندازه گیریهای کوتاه و بلند مدت بوضح نشان دادند که هوای داخل کنوپی (canopy) گیاه در مجاورت چشمه های معدنی غنی از CO_2 است.
- غلظت CO_2 تخمین زده شده به روش کربن ١٤ روند با ثباتی ، افزایش در جهت شیب و کاهش در جهت مخالف شیب، داشت.
- چشمه های CO_2 مقادیر متغیری از H_2S و SO_2 تصاعد می کنند اما میزان اکسیدهای ازت متصاعد شده از آنها نا چیز است.
- CO_2 بالا شدت جریان گاز متان از اتمسفر به خاک را افزایش می دهد.
- تصاعد N_2O از چشمه ناچیز بود ولی آبیاری خاکی که در معرض گاز کربنیک بالا قرارداشت تصاعد N_2O از خاک به اتمسفر را افزایش داد.

گیاه نشان داد که افزایش CO_2 تأثیری بر روی کیفیت لاشبرگ ندارد و در نتیجه سرعت تجزیه کربن وازت تحت شرایط گاز کربنیک بالا بدون تغییر ماند. با وجود این اختلا فات بین گونه ای معنی داری در کیفیت لاشبرگ و سرعت تجزیه آنها وجود داشت. کیفیت لاشبرگ در *S. aspera* بالا تر از دو گونه *Quercus* بود، در حالیکه لاشبرگ *Q. pubescens* در مقایسه با *Q. cerris* کیفیت بالا تری داشت. تحت شرایط آزمایشگاهی لاشبرگ *Q. pubescens* سریعتر از لاشبرگ *Q. cerris* تجزیه گردید اما تحت شرایط مزرعه ای چنین اختلافی مشاهده نشد. بنا بر این تأ ثیر ترکیب گونه ای بر روی چرخش کربن و ازت بیش ازتأ ثیر گاز کربنیک بالا می باشد. به نظر می رسد که پسخور (feedback) اختلافات درترکیب گونه ای بر روی خاک در مقایسه با افزایش CO_2 اتمسفر به مرا تب مهمتر وقویتر می باشد.

برای مشخص کردن اثرات گاز CO_2 بر روی مواد آلی خاک (کربن قدیمی) و جهت پی بردن به این موضوع که آیا اثرات CO_2 بر روی لاشبرگ گیاهی (کربن جدید) تحت شرایط طبیعی در پویائی مواد آلی خاک منعکس خواهد شد، مطالعات صحرائی بعمل آمد. برای این منظور نمونه برداری خاک جهت بررسی اجمالی توزیع مواد آلی خاک بعنوان تابعی ازغلظت CO_2 اتمسفری و سایر عوامل موثر(از قبیل شیب، پوشش گیاهی) انجام شد. نمونه های خاک با دو تکرار در نا حیه ای با CO_2 بالا در امتداد شیب غلظت CO_2 از منبع تا ناحیه ای با غلظت طبیعی در فاصله ۵۰ تا ۱۰۰ متری از چشمه جمع آوری گردید. از نظر توپوگرافی ترانسکت های مشابهی نیز در ناحیه شاهد با غلظت طبیعی CO_2 نمونه برداری شد. خاک دست نخورده از ۲۶ پروفیل (۱۷ پروفیل در ناحیه CO_2 بالا و ۹ پروفیل در ناحیه شاهد) نمونه برداری و برای تعیین مقادیر کل کربن و ازت تجزیه گردید. سرعت تصاعد CO_2 با فواصل یکساعت و بمدت ۳ هفته اندازه گیری شد. معدنی شدن ازت پس از افزایش ازت معدنی قابل عصاره گیری با محلول KCl بدنبال خواباندن نمونه های خاک بمدت ۳۰ روز بر آورد گردید.

نتایج نشان می دهند که افزایش CO_2 میزان وانبار (pool) کربن در خاک کف جنگل (F+HA) را افزایش داد. این اثر گاز CO_2 در عمق ۱۰-۰ سانتی متری دیده نشد (فصل ۵). رابطه مثبت و معنی داری بین ضخامت لایه کف جنگل وغلظت CO_2 بدست آمد که نشانگر تولید بیشترلا شبرگ با افزایش غلظت گاز CO_2 در اتمسفر است. با وجوداین مقدار ازت و نسبت کربن به ازت (C/N) دراین سه لایه خاک تحت تأثیر غلظت CO_2 قرار نگرفت(فصل ۵). اگرچه مقدار ازت در خاک کف جنگل بدون تغییر باقی ماند ولی افزایش طولانی مدت CO_2 سبب گردید که انبار ازت دو برابر شود. سرعت تجزیه کربن آلی خاک تحت تأثیرافزایش CO_2 قرار نگرفت (فصل ۵) که این یافته با عدم تأثیر CO_2 بر مقدار ازت و نسبت کربن به ازت در مواد آلی خاک مطا بقت دارد. بنا بر این افزایش مخزن کربن در خاک کف جنگل ممکن است به افزایش سرعت ورود لاشبرگ بواسطه افزایش تولید خالص اولیه (net primary production) تحت شرایط CO_2 بالا نسبت داده شود. سرعت غیر متحرک شدن ازت در

آبراهه واقع شده است و شیبی در غلظت CO_2 از مقدار طبیعی (۳۶۰ ppm) در فاصله کوتاهی از چشمه تا حدود ۶۰۰ ppm در مجاورت چشمه وجود دارد. تصاعد CO_2 ناشی از تجزیه حرارتی مواد مادری آهکی است که احتمالاً تحت تأثیر فعالیت آتشفشانی در گذشته قرار دارد. به جهت آنکه عواملی به غیر از غلظت CO_2 بر اختلافات مکانی در خواص خاک (از جمله مواد آلی خاک) دخالت دارند، ترانسکت (transect) مشابهی (CO_2 طبیعی، مواد مادری، شیب، شرایط هیدرولوژیکی و گیاهان مشابه) به عنوان ناحیه کنترل انتخاب شد.

به منظور تعیین غلظت گاز کربنیک که رشد گیاه در معرض آن صورت گرفته است در اطراف چشمه اندازه گیریهای کوتاه و بلند مدت انجام شد. جهت تشخیص نسبت گازهای نادر همراه با CO_2 از قبیل NO_2, NO, H_2S, SO_2 و CH_4 با دقت زیاد زمانی، اندازه گیری گازهای اتمسفری به وسیله تجهیزات کاملاً خودکار صورت گرفت. اندازه گیری کوتاه مدت نشان داد که تغییرات روزانه مشخصی درغلظت CO_2 وجود دارد. با این حال در مقیاس زمانی کوتاه تر نوسانات زیادی مشاهده گردید(فصل ۲). گازمتصاعد شده از چشمه حاوی ترکیبات گوگردی از جمله H_2S و SO_2 بوده لکن اثری از گازهای NO و NO_2 دیده نشد. با وجود یکه تصاعد N_2O ازچشمه ناچیز بود، آبیاری موجب شد که شدت جریان N_2O به اتمسفراز خاکی که در معرض غلظت بالای CO_2 بود افزایش یابد. جریان متان از اتمسفر به خاکی که در معرض CO_2 قرار داشت بطور قابل توجهی بالا بود. برای تعیین متوسط غلظت CO_2 از طریق اندازه گیری غلظت کربن ۱۴ با روش رایج، از شاخه های کوچک بلوط (*Quercus ilex* L.) نمونه برداری شد. غلظت تخمین زده شده CO_2 از ۳۶۰ ppm دور از چشمه تا ۵۹۰ ppm در اطراف چشمه متفاوت بود. غلظت CO_2 در نزدیکی چشمه بطور مشخصی بالا بود و در فاصله ۵۰-۱۰۰ متری از چشمه به میزان طبیعی رسید. میزان تصاعد CO_2 دقیقاً بالای چشمه ۵۱۰ ppm بود. غلظت محاسبه شده CO_2 با کمک روش کربن ۱۴ الگوی ثابتی نشان داد بدین صورت که غلظت با افزایش شیب کاهش و با کاهش شیب افزایش یافت. علیرغم غلظت کمتر CO_2 در اندازه گیریهای طولانی مدت (روش کربن ۱۴) دامنه آن با غلظت کوتاه مدت CO_2 (روش infrared gas analyzer) مشابه بود. اختلاف در مقادیر CO_2 بین دو روش اندازه گیری را می توان به مقیاس زمانی اندازه گیری نسبت داد.

به منظورارزیابی تغییرات ناشی از گاز کربنیک زیاد در شیمی برگها، لاشبرگ گونه های *Q. pubescens*، *Q. cerris* و *Smilax aspera* در شرایط گاز کربنیک بالا (> ۵۰۰ ppm) و طبیعی (۳۶۰> ppm) نمونه برداری و برای تعیین ترکیب شیمیائی (کربن، ازت، لیگنین، سلولز و پلی فنل ها) تجزیه شدند. سرعت معدنی شدن کربن وازت در لاشبرگ گیاهی با استفاده از روش کیسه لاشبرگ (litterbag) تحت شرایط مزرعه ای (به مدت ۱۲ ماه) و تحت شرایط آزمایشگاهی به روش خواباندن (incubation) تحت شرایط کنترل شده (به مدت ۳ تا ۴ ماه) اندازه گیری شد. نتایج حاصل ازتجزیه

خلاصه

غلظت گاز کربنیک دراتمسفر بطور ناگهانی از ٢٨٠ ppm قبل از انقلاب صنعتی به ٣٦٠ ppm در زمان حاضر افزایش پیدا کرده است و ظرف دهه های آینده افزایش بیشتری نیز پیش بینی میشود. یکی از اثرات احتمالی افزایش گاز CO_2 بر روی اکوسیستمهای خاکی تغییر در توازون کربن گیاهان و خاک از طریق افزایش تولید گیاه، افزایش تر شحات ریشه ای با تخصیص بیشتر کربن به قسمت زیر زمینی و تغییراتی در تجزیه مواد آلی خاک می باشد. نتایج گذشته نشان داده اند که افزایش گاز CO_2 موجب افزایش کمیت و کاهش کیفیت اندا مهای گیاهی می شود. این تغییر و تحولات ممکن است سرعت تجزیه کربن و ازت را کاهش داده بطوریکه خاک به عنوان مخزنی (sink) برای گاز کربنیک در حال افزایش اتمسفر عمل کند. بااین وجود، اثرات طولا نی مدت گاز CO_2 بر روی کیفیت لاشبرگ گیاه و متعا قباً تجزیه پذیری آن و پس از آن بر روی پویائی (dynamics) مواد آلی خاک، بویژه در اکوسیستمهای طبیعی که از محدودیت عناصرغذائی رنج می برند، ناشناخته است. هدف از این تحقیق آزمون این فرضیه است که افزایش گاز CO_2 کیفیت مواد آلی و در نتیجه سرعت تجزیه لاشبرگ گیاهی را کاهش می هد. هر نوع کاهش در کیفیت و تجزیه مواد آلی ممکن است در طولانی مدت باعث افزایش متوسط زمان ماندگاری (mean residence time) لاشبرگ سطحی و مواد آلی خاک و یا موجب افزایش منابع (pools) لاشبرگ سطحی و مواد آلی خاک گردد. افزایش تجمع کربن در خاک نهایتاً قابلیت استفاده از عناصر را کاهش می دهد. این تحقیق اثرات افزایش طبیعی گاز کربنیک حاصل از چشمعه های معدنی را بر روی continuum کربن لاشبرگ گیاهی (کربن جدید)-کربن خاک (کربن قدیمی) در قسمت مرکزی ایتالیا بررسی می کند.

ناحیه مورد مطالعه نزدیک روستای Laiatico تقریباً در ٣٥ کیلومتری جنوب شرقی شهر پیزا در کشور ایتالیا (٤٣°٣٦" N, ١٠°٤٢" E، ١٩٠-٢٤٠ متر بالای سطح دریا، بارندگی سالیانه ٨٣٠ میلیمتر و متوسط درجه حرارت سالیانه ١٥ درجه سانتیگراد) واقع است. محل اجرای آزمایش (تقریباً به مساحت یک هکتار) ناحیه ای پوشیده ازجنگل بوده و دارای سطح نامنظم و ناهمواری می باشد. خاک منطقه آهکی بوده و بر روی سنگ مادر مارل از دوران ترشیاری (Tertiary marl) تکامل یافته است. pH خاک سطحی ٦/٩ و درخاک تحتانی بالا تراز ٧/٢ است. بافت خاک لوم رسی سیلتی درقسمت سطحی و رس سیلتی درقسمت تحتانی می با شد. سطح خاک دارای یک لایه مواد آلی است که خود شامل سه افق متمایز (L, F, HA) با ضخامتهای مختلف می باشد. ناحیه مطالعه جنگل شاخص و نیمه طبیعی مدیترانه ای است که حدود ٢٥ سال پیش قطع شد. بطور کلی ناحیه از درختان بلوط - زبان گنجشک متشکل از *Quercus ilex* L., *Fraxinus ornus* L., *Quercus pubescens* Willd., و *Quercus cerris* L. همراه با درختچه هائی از جمله *Erica arborea* L. و *Arbutus unedo* L. پوشیده شده است. دراشکوب تحتانی *Smilax aspera* L. گونه غالب است. چشمه متصاعد کننده گاز CO_2 در کف یک

CURRICULUM VITAE

Fayez Raiesi Gahrooee was born on June 2, 1967 in Gahroo, a village near the city of Shahr-Kord, Iran. He bears a second family name "Gahrooee" which is the name of his birth place. He began his study at primary school for five years followed by a three years middle school training. He entered the high school of Shahr-Kord in the field of Experimental Sciences in September 1983. After four years full time education at the high school level, he took part in a National Examination to get access to undergraduate studies. He was admitted as a soil science candidate to pursue his B.Sc. at the School of Agriculture of Tabriz University in Tabriz for four years. Shortly after his graduation, he participated in a National Specialized Aptitude Examination by which he was awarded a scholarship by the Ministry of Culture and Higher Education (MCHE) of Iran to continue his postgraduate studies towards the M.Sc. degree abroad. He was accepted to the M.Sc. Soil and Water programme of the Wageningen Agricultural University to obtain his M.Sc. in 1992. After 19 months he finished his M.Sc. curriculum in March 1994. Dr. Ir. P. Buurman supervised his M.S.c thesis with the title " Re-partitioning of trace elements in andesitic volcanic ash derived soils." Shortly after graduation, he was accepted as a Ph.D. candidate under supervision of Prof. Dr. Ir. N. van Breemen and Dr. Ir. P. Buurman at the Department of Soil Science and Geology at the same university. Back in Iran, he will work at a university and/or research center to fulfill his commitments to the MCHE. He spent six years of his life in Wageningen, the Netherlands. He married Tahereh in 1991. Their son "Armine" was born in Wageningen, the Netherlands, in October 1994. He returns home with his two hands full, one with M.Sc. and Ph.D. degrees, and the other one with his son.