THE IMPACT OF ATMOSPHERIC CO₂ ENRICHMENT ON THE BIOGEOCHEMISTRY OF C AND N IN A MEDITERRANEAN FOREST ECOSYSTEM

Fayez Raiesi Gahrooei, Nico van Breemen and Peter Buurman

ABSTRACT - Elevated atmospheric CO₂ can affect the dynamics of soil organic matter (SOM), and consequently C and N cycling in terrestrial ecosystems. This study evaluates the effect of enhanced CO₂ on substrate quality and subsequent rates of its decomposition, and on soil organic matter by using a plant litter-SOM continuum around a mineral CO₂ spring in Central Italy. Elevated CO₂ did not change leaf litter quality or decomposition; neither did it affect chemical composition of SOM and C decomposition in the soil. Yet, total C and N pool sizes in the forest floor were doubled at elevated CO₂, probably as a result of increased plant production. This suggests that elevated CO₂ increases the soil-sink of atmospheric CO₂. Nitrogen immobilization in the forest floor was lower under elevated than ambient CO₂, whereas N mineralization in the A horizon remained unaffected.

This study casts doubt on the common idea that elevated CO₂ changes litter quality, and thereby slows down decomposability of litter and N release, but strongly suggests that elevated CO₂ increases SOM pools via higher litter quantity through increased net primary production.

Keywords: Climate Change, litter quality and decomposition, Mediterranean ecosystems, mineral CO₂ springs, soil organic matter

INTRODUCTION
Carbon dioxide is an essential substrate for living plants, which link two main C reservoirs: atmospheric C with soil C. It is also an important greenhouse gas, which contributes to global warming. Human consumption of fossil fuels and forest clearing in the tropics has caused approximately a 28% increase in the atmospheric CO₂ concentration since the beginning of the Industrialization Revolution in 1700. Increasing rates of consumption of fossil fuels and deforestation will result in a continuing rise in CO₂ concentrations in the coming decades (IPCC 1995; Schimel 1995). Burning of fossil fuel and deforestation has disturbed the global C cycle so that the imbalance between global C sources and known sinks implies the presence of an unknown C sink (the so-called "missing CO₂"). The missing CO₂ may be trapped in soils and vegetation via CO₂ fertilization, forest regrowth in the northern hemisphere and atmospheric N deposition (Gifford 1994; Schimel 1995).

The rise in CO₂ concentration, and related greenhouse effect, is expected to lead to an increase in global temperature of 1-3.5 °C by the end of the next century (IPCC 1995). Elevated atmospheric CO₂ concentrations and global temperature may result in an array of

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changes in terrestrial ecosystems, varying from short-term physiological changes (Eamus and Jarvis 1989; Poorter 1993; Luxmoore et al. 1993) to long-term modification in pools of soil organic matter (SOM) and nutrients (O’Neill and Norby 1996; Hungate et al. 1997). Global climate change may also have some direct or indirect consequences for the pedosphere. While global warming may accelerate soil carbon decomposition, elevated CO2 may change 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 through the allocation of more C to root systems. O’Neil and Norby (1996), McGuire et al. (1995), Ball (1997) and Cotrufo et al. (1998) have reviewed a great deal of the literature on possible changes in litter quality due to a doubling in the atmospheric CO2. On the other hand, enhanced CO2 may decrease stomatal conductance (Eamus and Jarvis 1989; Bettarini et al. 1998), and hence reduce transpiration while increasing water use efficiency (WUE). Reduced transpiration may increase soil water availability, which stimulates decomposition and mineralization in surface soils, in particular in water-limited ecosystems such as arid and semi-arid zones. Because soil plays a central role in plant growth and in global C and N cycles, it is essential to advance our knowledge and understanding of the effect of elevated CO2 on SOM. To investigate these effects, long-term experiments are necessary. Natural CO2 springs provide a convenient and useful environment to carry out such long-term studies on ecosystem functioning, especially with respect to effects on soil C.

The aim of this chapter is to discuss the long-term impacts of elevated CO2 on the dynamics of soil organic matter in a Mediterranean ecosystem which has been exposed to elevated CO2 for an extended period. In this chapter, we present results from natural CO2 springs where the effects of elevated CO2 on plant litter (quality and decomposability) and soil organic matter (pools and decay) continuum were studied. The important feedbacks that SOM may impose on plant growth and atmospheric CO2 are speculated upon. By obtaining soil data around CO2 springs, we also attempt to answer several fundamental questions, such as “Will elevated CO2 affect quality and decomposability of litter of mature plants growing in N-limited ecosystems exposed to long-term elevated CO2 for?” and “Are such CO2 effects on litter quality reflected in soil C dynamics?”

MATERIALS AND METHODS

Research site

The studied location is a Mediterranean forest ecosystem. These ecosystems are the subject of concern in IGBP-GCTE (the International Geosphere-Biosphere Programme - Global Change and Terrestrial Ecosystems) because of their vulnerability to global climate change (IGBP 1994). 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 sloping forested region with an irregular and rough surface. Soils in the area are calcareous, developed from Tertiary marl. The soil pH(H2O) is 6.9 in the topsoil and above 7.3 in the subsoil. The soil texture is silty clay loam in the topsoil and silty clay in the subsoil. The soil is covered by a litter layer (F+HA) of varying thickness. The study area is
a typical, semi-natural coppiced Mediterranean woodland which was last cut about 25 years ago. The vegetation comprises 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 CO2 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. This results in a CO2 gradient from ambient (350 ppm) to about 600 ppm at the vent (Raiesi, 1998). Because factors other than atmospheric CO2 concentration contribute to spatial differences in soil properties including SOM within the valley, a comparable transect (ambient CO2, similar parent material, slope-soil hydrological conditions, and vegetation) was selected for control area.

**Litter sampling and analysis**

Senescent leaf litter from oak (namely Quercus cerris, Quercus pubescens and Quercus ilex), ash (Fraxinus ornus) and one understory species (Smilax aspera), which contribute significantly to litter input to the soil, were collected from an area enriched long-term to CO2 concentrations of c.550 ppm, and from a control area with 360 ppm CO2. Litter samples were taken manually from both trees and soil surface. The samples were air-dried, mixed and ground to pass through a 1-mm sieve for quality analysis. Litter quality parameters such as N (all plant species), lignin and cellulose (only Q. cerris and Q. pubescens) and polyphenolic compounds (only Q. cerris, Q. pubescens and S. aspera) were determined in ground material (Anderson and Ingram 1993; Rowland and Roberts 1994; van Lagen 1996). The decomposition rate of litter was studied using a litter bag experiment (12 months) and laboratory incubations (3 months). In laboratory incubations, N mineralization in litter samples was measured (125 days). For S. aspera, incubation experiments lasted 48 (C incubation) and 60 days (N incubation).

**Soil sampling and analysis**

Soil samples were collected along a CO2 gradient of decreasing atmospheric CO2 levels from the source to ambient (360 ppm) between 50 to 100 m from the vent (for a map of CO2 concentrations and sample locations, see Raiesi et al., 1997; Raiesi, 1998). Topographically similar transects were selected and sampled at the control area. Undisturbed soil cores of 26 profiles (17 in the vet area, 9 in the control area) 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. Contents of C and N were determined to calculate the pools of C and N on the basis of measured soil bulk density. Additionally, the thickness of the forest floor (F and HA layers) as a function of atmospheric CO2 concentration was measured. C and N decay rates were studied by tracking CO2 evolution from, and by extracting N mineralized in, samples incubated at 20°C in the laboratory for 15 (C mineralization) or 30 (N mineralization) days.
Table 1. Leaf litter quality parameters of Mediterranean species growing under elevated and ambient CO₂ levels at the Laiatico mineral CO₂ spring. (from Raiesi et al. 1997a,b; Raiesi, 1998, 1999).

<table>
<thead>
<tr>
<th>Species</th>
<th>Elevated CO₂ area</th>
<th>Ambient CO₂ area</th>
<th>Concentration (%)</th>
<th>Ratios (-)</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Lignin</td>
<td>Cellulose</td>
<td>Polyph.¹</td>
<td>C/N</td>
<td>Lignin/N</td>
<td>Cellulose/N</td>
</tr>
<tr>
<td><strong>Quercus cerris (n=2)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Elevated CO₂ area</td>
<td>0.72</td>
<td>27.2</td>
<td>19.7</td>
<td>19.3</td>
<td>77.7</td>
<td>38.0</td>
<td>27.4</td>
</tr>
<tr>
<td>Ambient CO₂ area</td>
<td>0.76</td>
<td>26.8</td>
<td>20.6</td>
<td>20.5</td>
<td>73.6</td>
<td>35.0</td>
<td>26.9</td>
</tr>
<tr>
<td><strong>Quercus pubescens (n=2)</strong></td>
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<td></td>
</tr>
<tr>
<td>Elevated CO₂ area</td>
<td>0.81</td>
<td>19.3</td>
<td>22.8</td>
<td>21.1</td>
<td>65.2</td>
<td>23.9</td>
<td>28.3</td>
</tr>
<tr>
<td>Ambient CO₂ area</td>
<td>0.84</td>
<td>18.5</td>
<td>21.4</td>
<td>19.7</td>
<td>63.2</td>
<td>22.9</td>
<td>25.8</td>
</tr>
<tr>
<td><strong>Smilax aspera (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO₂ area</td>
<td>1.07</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.86</td>
<td>52.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ambient CO₂ area</td>
<td>1.01</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.82</td>
<td>56.1</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Quercus ilex (n=3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO₂ area</td>
<td>0.75</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>72.8</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ambient CO₂ area</td>
<td>0.75</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>72.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td><strong>Fraxinus ornus (n=3)</strong></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO₂ area</td>
<td>0.75</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>69.8</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Ambient CO₂ area</td>
<td>0.76</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>69.2</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

¹ Polyphenols; n.d., not determined.
RESULTS AND DISCUSSION

Elevated CO₂ effect on litter chemistry and turnover

We studied changes in litter chemistry of plants following exposure to long-term elevated CO₂ and subsequent effects on C and N mineralization. The chemical composition (e.g. N, lignin, cellulose and polyphenols) and compounds-to-N ratios of *Quercus cerris* and *Q. pubescens* and *Smilax aspera* litter from high-CO₂ were not significantly different from those from ambient CO₂ (Tab. 1). Similar results were obtained with *Quercus ilex* L. and *Fraxinus ornus* L. leaf litter.

Carbon and nitrogen concentrations, and C/N ratios of non-senescent leaves of *S. aspera* were unaffected by CO₂ level (Tab. 2). Also the re-translocation of nitrogen during senescence (Table 2) appears unaffected by CO₂ level. This means that a decrease in efficiency of this translocation, as observed in other species (Arp and Berendse, 1993) is not found in *Smilax* exposed to long-term high CO₂ concentrations.

As expected from the litter quality data, rates of decomposition (measured with litter bags) in ambient and elevated CO₂ were similar in *Q. cerris* and *Q. pubescens*. Three months of incubation in the laboratory indicated that litter decomposition of neither species was affected by elevated CO₂ (Tab. 3). Similarly, decomposition under laboratory conditions in *S. aspera* litter was unaffected by the CO₂ level at which the plants were grown. 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 CO₂ than that at ambient CO₂ (Fig. 1), it was not affected by CO₂ in the long term (Tab. 3), again in accordance with the absence of a CO₂ impact on litter chemistry.

Results from long-term experiments around CO₂ springs provided evidence that increases in the atmospheric CO₂ concentration do not influence substrate quality, and therefore it is unlikely that rising CO₂ levels will affect C and N turnover rates of litter in terrestrial ecosystems. The lack of a CO₂-effect on litter decomposition, found in this type of system, corresponds with recent field observations in boreal forests (Verbürg 1998), lowland calcareous grasslands and lowland wet tropical forests (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 2. N content and C/N ratio of fresh green leaves, and N re-translocation before abscission in *Smilax aspera* growing at ambient and naturally enriched CO₂ concentrations around the Laiatico CO₂ spring.

<table>
<thead>
<tr>
<th>CO₂ level (ppm)</th>
<th>N (%)</th>
<th>C/N (-)</th>
<th>N re-translocation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>543</td>
<td>1.37</td>
<td>36.8</td>
<td>21.8</td>
</tr>
<tr>
<td>373</td>
<td>1.32</td>
<td>38.9</td>
<td>21.0</td>
</tr>
<tr>
<td>363</td>
<td>1.31</td>
<td>39.3</td>
<td>21.9</td>
</tr>
</tbody>
</table>

The absence of any CO₂ effect on litter quality could be due to either increased nutrient uptake by roots (Day *et al.* 1996) or the down-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 CO2. In N-limited systems, plants may take up inorganic N directly through absorption of amino acids (Chapin et al. 1993; Keilland 1994) or use atmospheric N deposition more efficiently when grown at elevated CO2. Decreased efficiency of N-withdrawal under CO2-enrichment (Aφ and Berendse 1993) may be another mechanism by which plants would counteract the N-dilution of increased C fixation rates under elevated CO2, but it seems that this mechanism can not explain unchanged litter quality with CO2 enrichment.

Our long-term CO2 studies cast doubt on the general concept that elevated CO2 changes the litter quality of plants, and thereby slows down decomposition of litter and N dynamics. Although a number of authors judge that the CO2 effect on litter quality is still undecided, many field studies indicate that elevated CO2 does not change litter quality, and that therefore rates of C decomposition are not expected to 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; Verbürg 1998; Mooney et al. 1998). The results of our study contribute to recent findings that there is no convincing evidence that leaf litter quality of mature trees grown in N-limited soils and under continuous exposure to elevated CO2, changes at the ecosystem level.

Table 3. Litter decomposition of leaf material from Mediterranean species originating from elevated and ambient CO2 levels at the Laiatico mineral CO2 spring. (from Raiesi et al. 1997a,b; Raiesi, 1999). Standard deviations between brackets.

<table>
<thead>
<tr>
<th></th>
<th>Annual remaining mass (%</th>
<th>Cumulative CO2-C respired (g. kg⁻¹ C)</th>
<th>Cumulative N mineralized (g. kg⁻¹ N)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quercus cerris</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO2 area</td>
<td>39.4 (0.2)</td>
<td>112 (3.0)</td>
<td>18.2 (0.5)</td>
</tr>
<tr>
<td>Ambient CO2 area</td>
<td>31.7 (3.3)</td>
<td>117 (1.0)</td>
<td>17.3 (0.6)</td>
</tr>
<tr>
<td><strong>Q. pubescens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO2 area</td>
<td>30.6 (2.9)</td>
<td>136 (7.0)</td>
<td>21.5 (1.1)</td>
</tr>
<tr>
<td>Ambient CO2 area</td>
<td>30.0 (3.3)</td>
<td>172 (28)</td>
<td>20.5 (1.0)</td>
</tr>
<tr>
<td><strong>S. aspera</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO2 area</td>
<td>n.d.</td>
<td>53.2 (1.9)</td>
<td>-0.54 (0.35)</td>
</tr>
<tr>
<td>Ambient CO2 area</td>
<td>n.d.</td>
<td>51.3 (3.2)</td>
<td>-1.08 (0.25)</td>
</tr>
</tbody>
</table>

1 the incubation time 12 months
2 the incubation time 90 days for Quercus species and 48 days for S. aspera
3 the incubation time 125 days for Quercus species and 60 days for S. aspera

Effects of CO2 enrichment on quality of litter apparently do not play a significant role in changing the carbon balance and SOM pools of Mediterranean woodland ecosystems containing typical oak-ash trees. This does not rule out the possibility that long-term elevated CO2 will gradually increase the soil organic carbon pool through higher litter input caused by increased net primary production.
Table 4. The characteristics of soil organic matter of three soil layers in a Mediterranean forest exposed to elevated and ambient CO$_2$ at the Laiatico mineral CO$_2$ spring. (from Raiesi et al. 1999b,c).

<table>
<thead>
<tr>
<th></th>
<th>C (%)</th>
<th>N (%)</th>
<th>C/N</th>
<th>C pool (t. ha$^{-1}$)</th>
<th>N pool (g. m$^{-2}$)</th>
<th>C decomposed$^1$ (mg. g$^{-1}$ soil)</th>
<th>N mineralized$^{2,3}$ (mg. kg$^{-1}$ soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>F layer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO$_2$</td>
<td>43.3</td>
<td>1.80</td>
<td>24.2</td>
<td>12.2 a</td>
<td>50.4 a</td>
<td>9.07</td>
<td>154 a</td>
</tr>
<tr>
<td>Ambient CO$_2$</td>
<td>36.6</td>
<td>1.66</td>
<td>22.1</td>
<td>5.24 b</td>
<td>23.4 b</td>
<td>8.74</td>
<td>221 b</td>
</tr>
<tr>
<td><strong>HA layer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO$_2$</td>
<td>34.4</td>
<td>1.58</td>
<td>21.7</td>
<td>24.4 a</td>
<td>112 a</td>
<td>5.72</td>
<td>134 a</td>
</tr>
<tr>
<td>Ambient CO$_2$</td>
<td>31.2</td>
<td>1.57</td>
<td>19.9</td>
<td>11.4 b</td>
<td>55.9 b</td>
<td>6.30</td>
<td>244 b</td>
</tr>
<tr>
<td><strong>0-10 cm mineral soil</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elevated CO$_2$</td>
<td>4.16</td>
<td>0.30</td>
<td>14.8</td>
<td>48.3</td>
<td>341</td>
<td>0.62</td>
<td>15.9</td>
</tr>
<tr>
<td>Ambient CO$_2$</td>
<td>4.87</td>
<td>0.27</td>
<td>19.4</td>
<td>57.9</td>
<td>323</td>
<td>0.60</td>
<td>7.28</td>
</tr>
</tbody>
</table>

$^1$ the incubation time 2 weeks
$^2$ the incubation time 4 weeks
$^3$ Forest floor = N immobilization, 0-10 cm layer = N mineralization
Species composition and SOM (C and N cycles)
Although elevated CO₂ had no significant impact on litter quality and decomposition, there were significant differences in litter quality and decomposition parameters between species (Tab. 1 and 3). *S. aspera* exhibited a better litter quality than the two oak species, while *Q. pubescens* litter had a better quality than *Q. cerris* litter. *Q. pubescens* litter decomposed faster than *Q. cerris* litter under controlled conditions, but not in the field. Why under field conditions plant leaf litter with a high quality does not necessarily decompose faster than plant leaf litter with a low quality may be due to specific micro-site characteristics (e.g. water, temperature), and high spatial micro-variability in some soil properties (e.g. soil microorganisms, root distribution, etc.).

This study suggests that differences in litter quality between plant species are greater than impacts of elevated CO₂ on litter quality and C and N cycling within a species. This implies that changes in plant species composition may be more important for soil C sequestration than direct effects of elevated CO₂ on the litter quality of a given species. Because within one ecosystem, plant species composition may change in response to CO₂ enrichment, the response of individual plant species can not be used to predict the response of plant communities. Change of species, through different responses of individual species (Bazzaz 1990; Field *et al.* 1992) may have a important influences through effects on litter turnover (Kemp *et al.* 1994) and thereby on C and N cycling. Such long-term feedbacks may eventually affect the structure and functioning and of ecosystems (Bazzaz 1990; Canadell *et al.* 1996).

Effect of elevated CO₂ on composition and dynamics of SOM
Over the past decade, many experiments have addressed the effects of elevated CO₂ on plant growth, but few on litter. Very few studies have paid attention to changes in SOM itself. Changes in native SOM are potentially important, since effects on the global C cycle, due to climate change, should be reflected in the soil where carbon resides for decades to centuries. Amthor (1995) suggested that natural CO₂ springs are the best available sites to examine the effects of long-term elevated CO₂ on soil C pool sizes and dynamics. The impact of elevated atmospheric CO₂ on C and N pools, and C and N turnover rates of SOM were determined by analyzing pool sizes of C and N of native soil organic matter at different distances from a CO₂ spring, as a function of a long-term CO₂ gradient. Results demonstrate that elevated CO₂ increased the C content and pool size of the forest floor (F +HA layers, Tab. 4). Such effects were not observed in the upper 10 cm of mineral soil (Tab. 4). A positive relationship between litter thickness and CO₂ concentration was also observed (data not shown); indicating that higher litter production may be expected with increasing atmospheric CO₂. The N content and the C/N ratios of the three soil layers, however, were not affected by elevated CO₂. Although the N content of the forest floor remained unaffected by elevated CO₂, N pool sizes at long-term elevated CO₂ sites were double those at ambient sites. This is not surprising, as the C/N ratio of plant materials reaching the forest floor, and their decomposition rates, remained unchanged under elevated CO₂ (Raiesi , 1998, 1999; Raiesi *et al.* 1999a, Tab. 1 and 3), and elevated CO₂ stimulates plant production in the long term (Jones *et al.* 1995; Hättenschwiler *et al.* 1997). Therefore, increased rates of carbon input must be associated with increased rates of net nitrogen accumulation. Similarly, Prior *et al.* (1997) found after
2 years that N pool size at 5-20 cm depth of a soil under wheat grown at 550 CO2 ppm was significantly higher than that at ambient CO2, but such a CO2 effect was not observed at 0-5 cm of depth. However, the relative increase in N pools in the present study are much higher than those reported for experiments with wheat (Prior et al. 1997).

Figure 1. - C mineralization in *Q. cerris* (A) and *S. aspera* (B) litter produced under elevated and ambient CO2 concentration, determined during a laboratory incubation at 20 °C (from Raiesi, 1999; Raiesi et al. 1999a).
Figure 2. - N mineralization in *Q. cerris* (A) and *Q. pubescens* (B) litter produced under elevated and ambient CO$_2$ concentration, determined during a laboratory incubation at 20 °C for 125 days. Soil blank is the control soil without the addition of leaf litter (from Raiesi, 1998).

The Laiatico forest has been continuously exposed to elevated CO$_2$ for more than decades or centuries so that the remarkable increased N pools at elevated CO$_2$ is the result of a progressive accumulation of organic N over a long period of exposure. In contrast, Körner and Arnone (1992) observed that total N pools in the soil of an artificial tropical ecosystem were similar at both ambient and elevated CO$_2$, despite of an 38 % increase in litter...
production. Notwithstanding, the N pool in plant litter was 33% higher under elevated than ambient CO₂, suggesting the C/N ratio remains constant for both treatments.

Rates of soil C mineralization, estimated over two weeks under laboratory conditions, were not influenced by elevated CO₂, in accordance with the absence of a CO₂ effect on the N content and C/N ratios of soil organic matter. Therefore, the possible explanation for increased C pool size in the forest floor must be attributed to increased rates of litter input through enhanced Net Primary Production (NPP) at the elevated CO₂. Evidence to corroborate this has been reported by Jones et al. (1995) and Hättenschwiler et al. (1997), who observed stimulations in forest growth and production following exposure to long-term elevated CO₂. The rate of N immobilization in the forest floor (F plus HA layers), measured during 30-days incubation under controlled conditions, was lower at elevated than that at ambient CO₂, whereas the rate of N mineralization in mineral soil was unaffected. The decreased rate of N immobilization in the forest floor at elevated CO₂ is surprising because the chemical composition of soil organic matter and its decomposition rate remained unchanged as CO₂ increased. N mineralization in leaf plant litter grown at elevated CO₂ was higher at the initial stage of incubation, but this difference disappeared with time.

The absence of a CO₂ effect on litter quality, and consequently on litter decomposition, was generally reflected in the unchanged chemical composition and decomposition of soil organic matter. Although the decomposition of soil organic matter in a natural multi-species ecosystem is a complex process, and only a few factors that control SOM decomposition have been examined, we found no evidence for reduced SOM decomposition under increased CO₂.

In this study, we used SOM that was derived from plant materials continuously exposed to elevated CO₂, and considered the whole plant-soil system. The experiment included implicitly some plant-soil feed-backs, such as impacts of elevated CO₂ on below-ground C and processes, and thereby on soil C via the plant. Most experiments, which have reported reduced C mineralization under elevated CO₂ are either short-term or have used a soil-litter mixture that may not represent natural properties and processes as they occur in the field. Soil fauna are excluded from some studies conducted under controlled conditions, while it undoubtedly plays an important role in fragmentation of litter in the soil (Tian 1992; Couteaux et al. 1991). We found that the rate of litter decomposition for the same litter type was not the same under laboratory and field conditions (Raiesi, 1998).

We deduce that the increase in the organic carbon pool of the forest floor, in the absence of an effect of elevated CO₂ on litter quality and decomposition, is explained by increased biomass production under elevated CO₂. Under elevated CO₂, soil N pools may also increase, but the rate of N immobilization in forest floor is lower than that at ambient CO₂. One reason to believe that sustained increases in plant growth under elevated CO₂ may be possible is the fact that N mineralization rates are stimulated by sustained CO₂ enrichment.

**CONCLUSIONS**

It is likely that changes in litter quality, litter decomposition and litter N mineralization rates, and consequently N availability, in response to higher levels of atmospheric CO₂
will not result in changes in the function of soil as a sink and (or) source of atmospheric CO₂. Evidence from a natural ecosystem, which has experienced long-term CO₂ enrichment, indicates a lack of positive or negative feedbacks from plant litter decomposition through atmospheric CO₂. At the same time, an increase in N pool-size and in soil N mineralization suggest that plant growth at elevated CO₂ may not be limited by low N availability in the soil. Higher C assimilation may lead to enhanced C accumulation in the soil, which may impose a negative feedback to the level of CO₂, in the atmosphere.

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