Decomposition of $^{13}$C-labelled wheat root systems following growth at different CO$_2$ concentrations

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Abstract

We tested whether the amounts of carbon (C) mineralized from decomposing wheat (Triticum aestivum L. cv. Tonic) roots were related to the quantity (i.e. root dry weight per plant) or the chemical composition of material which had been grown at ambient or elevated CO$_2$ concentrations (350 or 700 $\mu$mol CO$_2$ mol$^{-1}$). Plants were grown in $^{13}$C-depleted CO$_2$ to distinguish root-derived C from soil-derived C. Over periods of up to ca. 400 d, root C, soil C and nitrogen (N) mineralization were measured from: (i) root systems left in situ in soil; (ii) soil after removal of visible roots; and (iii) equal amounts of roots added to fresh soil. Root systems in situ showed transiently faster C mineralization rates after growth at elevated [CO$_2$] compared with ambient [CO$_2$]. Ultimately, there were no [CO$_2$]-related di/C128erences in the amounts of C or N mineralized from root systems in situ. Specific rates of C loss from extracted roots were not significantly di/C128erent for roots from the two [CO$_2$] treatments. The potential accuracy of the $^{13}$C method was demonstrated and $^{13}$C/$^{12}$C fractionation during root decomposition was negligible. We conclude that when wheat is grown under elevated [CO$_2$], subsequent root decomposition will not necessarily be affected. If it does, it is likely to do so via an effect of [CO$_2$] on the amounts of root material produced per unit of soil rather than on the chemical quality of that material.

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1. Introduction

Plant biomass production usually increases when atmospheric carbon dioxide concentrations ([CO$_2$]) are elevated and its chemical composition may differ from that produced at current ambient [CO$_2$] (e.g. Conroy and Hocking, 1993; Owensby et al., 1993; Jackson et al., 1994). These effects depend on plant species and interactions with other environmental factors, e.g., water and nutrient availability, temperature. Likewise, effects of [CO$_2$] on litter decomposition and nutrient mineralization rates depend on changes in organic matter quantity and quality, soil biota and interactions with physicochemical conditions in the soil (O’Neill and Norby, 1996; Arp et al., 1997; Heal et al., 1997).

Inputs of plant organic matter to soils occur continually, but at variable rates, during the life of a plant, i.e. as above- and belowground plant parts, leachates and exudates. Large, discrete inputs occur when individual plants die. For annual crops, such inputs — particularly of roots — occur at harvest and may contribute nutrients to subsequent crops.

Only in a few studies (e.g. Swinnen et al., 1995) has in situ root decomposition of annual crops been investigated and none in relation to [CO$_2$]. Information is also lacking about how [CO$_2$]-induced changes in root quality (i.e. chemical composition) and quantity influ-
ence their subsequent decomposition. If elevated [CO$_2$] increases C-to-nutrient ratios of root debris, decomposition may be slower.

Information about the effects of elevated [CO$_2$] on root decomposition has been obtained on homogenized root subsamples, using grasses (Gorissen et al., 1995; Van Ginkel et al., 1996; Franck et al., 1997; Robinson et al., 1997), cotton (Torbert et al., 1995) and tree seedlings (Cotrufo and Ineson, 1995). In a study by Van Ginkel and Gorissen (1998), the in situ decomposition of $^{14}$C labelled perennial ryegrass ($\textit{Lolium perenne}$ L.) roots was measured. Often, studies of root decomposition have involved material sampled from young, non-senescent plants rather than naturally senescent material. This is a particularly important issue when considering C loss from the roots of annual species (e.g. cereals). These exhibit little root turnover pre-anthesis (Van Vuuren et al., 1997), most root mortality occurring synchronously at the end of the individual plant’s life. This pattern contrasts markedly with the continual root turnover seen in perennials (Fitter et al., 1996).

Here, we report experiments designed to test whether the amounts of C mineralized from decomposing wheat roots were related to the quantity (i.e. root dry weight per plant) or the chemical composition of root systems grown at ambient or elevated [CO$_2$] (350 or 700 μmol CO$_2$ mol$^{-1}$). We compared C mineralization from root systems in situ (in which the amount of root material in the soil was dependent on the previous growth of the plants under different [CO$_2$]) with that from equal weights of root material added to soil. We used $^{13}$C-labelling to distinguish root-derived C from soil-derived C, which also afforded an opportunity to test that technique’s accuracy in decomposition studies.

2. Materials and methods

2.1. Soil and roots used in mineralization experiments

Soil and root samples were obtained from the experiment described by Van Vuuren et al. (1997); see Table 1. Briefly, spring wheat ($\textit{Triticum aestivum}$ L. cv. Tonic) was grown at 350 or 700 μmol CO$_2$ mol$^{-1}$ (ambient and elevated [CO$_2$] treatments, respectively) and with frequent or infrequent watering ('wet' and 'dry' treatments, respectively), in a factorial experiment. Plants were grown in long vertical containers ($120 \times 2.5 \times 5$ cm) with removable front covers, allowing access to the soil and the growing root system. Each container was filled with sandyloam soil (1.35 kg at 105°C; sieved (2 mm); 3% total C, 0.21% total N, 0.13% total P and 0.49% total K), of pH 6.1 and fertilized with N, P and K.

The CO$_2$ to which the plants were exposed was depleted in $^{13}$C (see Gordon et al., 1995), allowing C derived from the wheat to be distinguished from that present in the soil: see Table 1). In elevated [CO$_2$], plants were exposed to CO$_2$ more $^{13}$C-depleted than

### Table 1

<table>
<thead>
<tr>
<th>[CO$_2$] during plant growth (μmol CO$_2$ mol$^{-1}$)</th>
<th>350</th>
<th>700</th>
<th>350</th>
<th>700</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of plants at final harvest (116 d growth)</td>
<td>4</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Root video recording</td>
<td>n</td>
<td>n</td>
<td>y</td>
<td>y</td>
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<tr>
<td>Extraction of roots</td>
<td>y</td>
<td>y</td>
<td>n</td>
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<tr>
<td>Measurement of soil inorganic N at final harvest</td>
<td>y</td>
<td>y</td>
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<td>n</td>
</tr>
<tr>
<td>Series (i) Soil with root systems in situ</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Series (ii) Soil from which root systems were removed</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Series (iii) Soil to which equal amounts of roots were added</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Amounts and composition of extracted roots (mean values, S.E. in parenthesis)

| Root dry weight per plant (g) | 1.31 (0.05) | 1.42 (0.08) | n.d. | n.d. |
| Root C (mg g$^{-1}$ dry weight) | 418 (1) | 432 (3) | n.d. | n.d. |
| Root C per plant (mg) | 549 (23) | 611 (30) | n.d. | n.d. |
| Root $\delta^{13}$C (%) | $\approx$ 38.3 (0.1) | $\approx$ 41.1 (0.1) | n.d. | n.d. |
| Root N (mg g$^{-1}$ dry weight) | 24.1 (0.9) | 24.1 (2.7) | n.d. | n.d. |
| Root N per plant (mg) | 32 (1) | 34 (5) | n.d. | n.d. |
| Root C-to-N (mass ratio) | 17 | 18 | n.d. | n.d. |

*a One plant lost.*
were those grown at ambient [CO₂]. Plants were grown for 116 d, at which point they were in the ripening stage (Large, 1954), although not yet completely senesced.

Certain data in the decomposition experiments described below are quoted as ‘per plant’. This means that those amounts of C or N were calculated for that amount of soil which supported one wheat plant in the Van Vuuren et al. (1997) experiment. Throughout, all data are quoted as means ± S.E.

2.2. C and N mineralization experiments

Three series of experiments were performed.

2.2.1. Soil with root systems in situ, series (i)

This series was designed to measure the decomposition of root systems left in situ following the harvesting of shoots after 116 d growth (Table 1), simulating the fate of wheat roots in the field when soil is left unploughed at the end of the growing season. These root systems were present in containers which had been used for nondestructive video recordings of root growth in the Van Vuuren et al. (1997) experiment (Table 1).

Decomposition measurements began on the day that shoots were harvested (5 December 1994). Four replicate containers per [CO₂] treatment were watered to 0.22 g water g⁻¹ soil and their front covers replaced by fine nylon mesh. Each container was placed horizontally in a PVC pipe (1.3 m long, 64 mm dia) in an incubator room at 14°C (minimum 12°C, maximum 16°C) in darkness, after taking gas samples from the pipe via two gas sampling ports 34 cm from either end. The pipes were closed with rubber bungs, leaving a 1.25m inner pipe length and an estimated ‘head space’ of 2750 cm³ in each. At the final harvest (116 d: Table 1), concentrations of inorganic N with soil depth were measured by Van Vuuren et al. (1997); these were taken as the amounts of inorganic N present at the start of the in situ decomposition experiment.

The containers were incubated for 29 sequential periods, ranging from 3 d at the start of the experiment to 42 d at the end, over a total of 404 d. At the end of each period, duplicate gas samples were taken from each pipe to measure [CO₂]. For 17 of these periods, two other gas samples were taken from each pipe for determination of δ¹³C in CO₂. After sampling, each container was removed briefly from its pipe. Each pipe was flushed with outside air and one gas sample taken for background measurement of [CO₂]. If necessary, moisture losses from each container were replaced with distilled water (always < 10 g) and any condensation on the inside of the pipe removed.

After 404 d, each soil column was cut into 11 layers. Visible root fragments were collected manually from each and bulked for each plant. Roots were rinsed with water, oven-dried (60°C, 24 h) and weighed. Root [C], [N] and δ¹³C were determined as described in Section 2.3. Soil moisture contents and inorganic N concentrations were measured as described by Van Vuuren et al. (1997).

2.2.2. Soil from which root systems were removed, series (ii)

This series was designed to detect the presence of root-derived C in soil from which roots grown at different [CO₂] had been removed. Such C might be expected to be in the form of detached root fragments, soluble and insoluble rhizodeposits and in microbes and microbial metabolites.

After 116 d growth (Table 1), visible roots were extracted manually from soil and the soil retained for incubations. (Extracted roots were washed with water, dried (60°C, 24 h) and used in Series (iii) below; Table 1). The soil from each container was mixed, triplicate subsamples (75 g wet weight) weighed into separate 125 cm³ gas-tight glass jars and a sample dried at 105°C to measure its moisture content (9 December 1994). The soil-containing jars and three soil-free jars were closed with rubber bungs fitted with gas sampling ports. The average headspace volume of each jar was 63 cm³.

Jars were incubated for 20 sequential periods lasting from 6 to 33 d and 401 d in total. At the end of each period, two gas samples were taken from each jar for measurements of [CO₂] and δ¹³C, respectively. Headspace were flushed with outside air and [CO₂] measured in soil-free jars. At the end of the first period, soil moisture contents, which varied between 0.16 and 0.18, were adjusted to 0.22 g water g⁻¹ soil.

At the end of the experiment, 7 g subsamples of soil from each triplicate jar were combined and soil inorganic N concentrations determined in the composite sample. The soil remaining in each jar was dried to measure its moisture content.

The soil-only control was replicated six times. Each replicate comprised three subsamples of 75 g moist soil (0.22 g water g⁻¹ dry soil) in separate 100 cm³ jars. Three additional jars were soil-free. [CO₂] and δ¹³C of CO₂ were measured at the end of 14 sequential incubation periods, totalling 466 d. Incubation of controls began on 11 August 1994.

Incubations were initially at 15°C. However, technical problems caused the temperature to fall to ≈0°C for unknown periods of time. All jars were transferred to the 14°C incubator room mentioned above. By then, the incubation of soil-only controls had lasted for about 5 months. Thus the incubations had no reliable soil-only controls for C mineralization. Consequently, only time-averaged δ¹³C values of CO₂ will be pre-
sent. The incubation of soil from which visible roots were removed was affected only during the first month.

2.2.3. Soil to which equal amounts of roots were added, series (iii)

This series was designed to measure specific rates of C loss (i.e. C loss g⁻¹ initial root dry weight) from roots grown at different [CO₂] to test whether those treatments led to innate differences in root decomposability, reflecting possible differences in chemical quality.

Equal dry weights of roots obtained previously (see Series (ii) above; Table 1) were added to fresh soil. The roots had been dried at 60°C (24 h) and stored at room temperature before being clipped finely (<0.5 cm) and redried at 60°C. For each plant which had been grown under each [CO₂], triplicate 200 mg subsamples of root material were mixed with 75 g soil (0.22 g water g⁻¹ dry soil) in separate 0.5 dm³ gas-tight jars; one additional jar was soil-free (5 June 1995). Two replicate soil samples were used as soil-only controls. For each soil sample, triplicate 75 g subsamples of moist soil (0.22 g water g⁻¹ dry soil) were weighed in separate 0.5 dm³ jars. One additional jar was soil-free.

All jars were incubated for a total of 303 d in the 14°C incubator room mentioned above. Jars containing roots were incubated for 19 sequential periods, ranging from 3 to 37 d; [CO₂] and δ¹³C were measured at the end of 19 and 15 periods, respectively. Soil-only controls were incubated for eight sequential periods ranging from 22 to 54 d; [CO₂] was also measured within incubation periods. Before taking a gas sample, air in the jar was mixed by pumping it in and out of a 25 cm³ syringe. After flushing with outside air, [CO₂] was measured in the jar without soil.

At the end of the experiment, inorganic N concentrations were determined in one soil sample per jar, the remaining soil was dried to measure its moisture content.

2.3. Chemical and isotopic analyses

Gas samples for [CO₂] measurement were taken using 2 cm³ glass airtight syringes. The sample volume was reduced to 1 cm³ (at atmospheric pressure) just before injection into a Hewlett-Packard 5890A gas chromatograph. CO₂ gas standards (10 or 30 mmol mol⁻¹ = 1 or 3% v/v), sampled and injected in the same way, were used to calibrate the gas chromatograph for each sample run. [CO₂] was smaller than 30 mmol mol⁻¹ in about 90% of all gas samples analysed.

Before taking gas samples for δ¹³C analyses, [CO₂] data were used to calculate the volume of gas needed for sampling ca. 100 µl CO₂ (= 4.2 µmol CO₂ at 14°C and 101.3 kPa) This volume, up to a maximum of 12 cm³, was sampled from the head space using a 25 cm³ syringe and immediately transferred to a 12 cm³ evacuated glass vial (‘Exetainer’, PDZ Europa, Crewe, UK). Exetainers were stored at room temperature until δ¹³C analysis. δ¹³C was not determined in CO₂ from jars without soil. If CO₂ in the sample was <50 µl, δ¹³C data were discarded as being potentially unreliable.

Total C and N concentrations and δ¹³C in root samples were determined by continuous-flow isotope ratio mass spectrometry (CF-IRMS; Tracermass, PDZ Europa, Crewe, UK). δ¹³C in gas samples was determined using CF-IRMS with an automated gas-sampling and purification system (Roboprep-G Tracermass, PDZ Europa, Crewe, UK). δ¹³C values were expressed in parts per thousand (%), by reference to the international standard V-PDB:

\[ \delta^{13}C = \left( \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right) \times 10^3 \]  

where \( R = \frac{^{13}C}{^{12}C} \). Working standards calibrated to V-PDB were of 200 µl CO₂ (= 8.5 µmol CO₂ at 14°C and 101.3 kPa). In each mass spectrometer run, samples of 50, 100 and 200 µl CO₂ of two CO₂ standards were included (\( \delta^{13}C = -42, -33 \) or -25%). Over the range 50–200 µl CO₂, δ¹³C varied linearly with the volume of each CO₂ standard with a common slope, g. Sample δ¹³C values (\( \delta^{13}C_{\text{sample}} \)) were corrected for the effect of sample volume (\( v_{\text{sample}} \), µl) by calculating the δ¹³C value (\( \delta^{13}C_{200} \)) that a sample volume of 200 µl would have produced, according to:

\[ \delta^{13}C_{200} = \delta^{13}C_{\text{sample}} + (200 - v_{\text{sample}})g \]  

2.4. Calculations of C mineralization rates

The amount of CO₂ produced per jar or pipe (= net [CO₂]) and per incubation period was calculated by subtracting the mean [CO₂] of outside air from sample [CO₂] at the end of the period. Net [CO₂] was averaged for the three replicate jars per plant. Net [CO₂] data were converted to mg C mineralized per jar (or pipe) (= \( C_{\text{total}} \)), according to:

\[ C_{\text{total}} = 0.01 [CO₂]h_r m/v_m \]  

where \([CO₂] = \% CO₂ v/v\), \( h_r = \text{head space volume of jar or pipe (cm}^3\), \( v_m = \text{volume per mmol CO₂ at 14°C (23.6 cm}^3)\) and \( m = \text{mass of C per mmol C (i.e., 12 mg)}\).

\( C_{\text{total}} \) was partitioned into C mineralized from wheat roots (= \( C_{\text{roots}} \), mg) and C mineralized from native soil organic matter (SOM; = \( C_{\text{som}} \), mg) using the following equations (see Balesdent and Mariotti, 1996):

\[ \delta^{13}C_{\text{sample}} = \delta^{13}C_{200} + \left[ \frac{m}{v_m} \right] g \]
Croots \hat{=} C_{total} - C_{som} = \frac{c - a}{b - a} \tag{4}

where \( a = \delta^{13}C \) of CO\(_2\) mineralized from SOM, \( b = \delta^{13}C \) of roots extracted from soil and \( c = \delta^{13}C \) of CO\(_2\) mineralized from soil containing roots. \(^{13}C/^{12}C\) fractionation during the decomposition of root C was not taken into account and no correction was made for ambient air CO\(_2\) introduced during flushing of jars with outside air. For incubation periods without measurements of \( \delta^{13}C \), the parameter \( c \) was obtained by linear interpolation between \( \delta^{13}C \) measurements made during the previous and following incubation periods.

### 2.5. Statistical analyses

For the incubation experiments of soil plus or minus roots in jars (Series (ii) and (iii)), statistical analyses were done on mean values of the triplicate measurements per plant or per soil sample. Analysis of variance procedures in Genstat 5 (Genstat 5 Committee, 1993) were used for data that were not repeated over time (e.g. C mineralization per sampling day, total inorganic N per plant or per jar).

Amounts of C mineralized were repeated measurements for each of the original plants. The appropriate statistical analysis for such data was described in detail by Van Vuuren et al. (1997). Briefly, for each plant, data were summarized across time by fitting an orthogonal polynomial function of time using Genstat 5. The estimated parameters of this function were not repeated measurements and, for a given plant, the parameter estimates were uncorrelated. Effects of the \([\text{CO}_2]\) treatments on each parameter were analysed using analysis of variance as before.

### 3. Results

#### 3.1. Soil with root systems in situ (Series (i))

The amounts of C mineralized varied more among replicates of soil with roots grown at elevated \([\text{CO}_2]\) than among replicates with roots grown at ambient \([\text{CO}_2]\) (Fig. 1a,b). For both treatments, C mineralization rates decreased with time.

\( \delta^{13}C \) values of total C mineralized were initially more negative in the elevated \([\text{CO}_2]\) treatment (Fig. 1c,d) because plants in that treatment were more \(^{13}C\)-depleted (see Materials and methods; Table 1) and, initially, more root C was mineralized in that treatment.
Mean $\delta^{13}$C values of extracted roots were $-38.3$ and $-41.1$ for ambient and elevated $[\text{CO}_2]$, respectively (Table 1). As more of the root C decomposed, $\delta^{13}$C values of CO$_2$ became less negative and closer to that of CO$_2$ evolved from soil-only controls ($\delta^{13}$C = $-26.3\%$).

After 404 d, estimates of C mineralized from root systems grown at ambient and elevated $[\text{CO}_2]$ were $405 \pm 219$ and $480 \pm 253$ mg per plant, respectively; the corresponding amounts of C mineralized from SOM were $339 \pm 15$ and $392 \pm 21$ mg (Fig. 1.e,f; Table 2). Separate ANOVAs for each sampling day indicated that significantly ($P < 0.05$) more C was mineralized from roots grown at elevated $[\text{CO}_2]$ after 8, 11, 14 and 17 d ($P < 0.1$ up to 36 d). However, the accumulated amounts of C mineralized from roots grown at different $[\text{CO}_2]$ became more similar with time and the analysis of the complete data set indicated no statistically significant difference between $[\text{CO}_2]$ treatments. C mineralized from SOM also did not differ significantly between $[\text{CO}_2]$ treatments.

At the start of decomposition, C was significantly ($P = 0.005$) more concentrated in the dry matter of roots grown at elevated $[\text{CO}_2]$, although the total amounts of root C produced under different $[\text{CO}_2]$ were not different (Table 1; $P = 0.13$). After 404 d, only $96 \pm 20$ and $80 \pm 4$ mg root C per plant remained in roots recovered from the soil. These data indicate that, respectively, 83 and 87% of ambient and elevated $[\text{CO}_2]$ grown roots had decomposed during that time. The corresponding fractions estimated from measurements of evolved CO$_2$ and its $\delta^{13}$C were 74 and 79%.

The $\delta^{13}$C values of roots recovered at the end of the decomposition experiment were $-38.2 \pm 0.4$ and $-40.7 \pm 0.2$ for ambient and elevated $[\text{CO}_2]$, respectively, statistically indistinguishable ($P > 0.1$) from those at the start (Table 1).

After 404 d, soil which had supported plants grown at ambient and elevated $[\text{CO}_2]$ contained, respectively, $128 \pm 28$ and $121 \pm 24$ mg inorganic N per plant. Inorganic N was most concentrated in the upper soil layers (Fig. 2), corresponding to the greater root dry weights per unit soil volume at those depths (Van Vuuren et al., 1997). These amounts of soil inorganic N were...
substantially greater than those present at the start of decomposition, i.e., \(31 \pm 11\) and \(32 \pm 16\) mg per plant in ambient and elevated \([CO_2]\) treatments, respectively (Van Vuuren et al., 1997). Net soil N mineralization during root decomposition did not differ significantly \((P = 0.474)\) between \([CO_2]\) treatments, respectively. Soil-only controls contained \(3.4 \pm 0.2\) mg N at the end of incubation.

4. Discussion

4.1. Quality vs. quantity of decomposing wheat roots

Initially, more C was mineralized in situ from root systems grown at elevated \([CO_2]\) compared with those grown at ambient \([CO_2]\) (Fig. 1), but this effect was transient. It may have been caused by greater initial concentrations of easily decomposable, non-structural C compounds in roots grown at elevated \([CO_2]\) (e.g. Jongen et al., 1995).

\([CO_2]\) had only a small effect on the total amounts of C mineralized in situ, as it had on root dry weight in the frequently watered treatments of the Van Vuuren et al. (1997) experiment (which supplied the material used in this study: Table 1). When watering was infrequent, however, Van Vuuren et al. (1997) found a 27% increase in root dry weight per plant at elevated \([CO_2]\). Had root systems from the infrequently watered treatments been available for the decomposition studies, this large difference in their initial dry weights might have produced greater effects of \([CO_2]\) on total C mineralization than we observed. The effects of \([CO_2]\) on the percentage of roots and root-derived C mineralized in situ may be small. For example, Van Ginkel and Gorissen (1998) found that \(52 \pm 4\%\) of initial C was mineralized from ryegrass roots over 230 d irrespective of \([CO_2]\) or N treatment. But the initial C content of the \(14C\)-labelled soil used by Van Ginkel and Gorissen was 18 to 39% greater at elevated than at ambient \([CO_2]\), resulting in a 10 to 26% greater total C mineralization at elevated than at ambient \([CO_2]\).

There was little effect of \([CO_2]\) on the chemical composition of wheat roots in the Van Vuuren et al. (1997) experiment, whether the plants were well watered (Table 1) or not (M.M.I. van Vuuren, unpub data). That was reflected in the similarity of the specific rates of C loss measured on extracted roots irrespective of previous \([CO_2]\) treatment (Fig. 3). Some non-structural C compounds may have leached from the extracted roots during rinsing before the start of the decomposition experiment (cf. Cotrufo and Gorissen, 1997), perhaps explaining the absence of the initially rapid C mineralization from elevated \([CO_2]\) grown roots.

Our data suggest that elevated \([CO_2]\) will not necessarily affect subsequent C cycling from arable crop residues in soil. When there is such an effect on wheat, it is more likely to arise from different amounts of root per unit of soil rather than from \([CO_2]\)-induced differences in the chemical quality of that material. How-
ever, even though we incubated root systems from senesced plants in situ, our experimental setup could not mimic field conditions precisely. There, more types of soil biota are present than in the sieved soil used in our experiments (Jones et al., 1998) and soil biota, nutrient availability, temperature and moisture conditions fluctuate in space and time. Moreover, during the decomposition experiments, our soils were without living vegetation exposed to ambient or elevated [CO₂]. The latter probably would have resulted in greater soil moisture contents at elevated than at ambient [CO₂] caused by smaller water use by plants growing at elevated [CO₂] (Jackson et al., 1994; Hungate et al., 1997a; Van Vuuren et al., 1997; Arnone and Bohlen, 1998; Nicklaus et al., 1998), thereby affecting soil biota and decomposition. Griffiths et al. (1998) could detect no effect of [CO₂] on the composition of the soil microbial community from an experiment involving the same wheat cultivar, soil type and growth conditions used by Van Vuuren et al. (1997). Our finding of no strong effects of [CO₂] on subsequent decomposition of wheat roots suggests little influence of [CO₂] on the functioning of that community in terms of C or N mineralization.

Previous experiments showed no difference (Cotrufo and Ineson, 1995; Torbert et al., 1995; Robinson et al., 1997), slower (Cotrufo and Ineson, 1995; Gorissen et al., 1995; Van Ginkel et al., 1996), or faster (Robinson et al., 1997) rates of decomposition of roots grown at elevated [CO₂] compared with those grown at ambient [CO₂]. In the experiments by Cotrufo and Ineson (1995) and Franck et al. (1997), the direction and magnitude of effects of elevated [CO₂] on root decomposition depended on species and, in the experiment by Robinson et al. (1997), on environments. Root quality and decomposition were measured on roots from senesced plants in the experiments of Torbert et al. (1995), Franck et al. (1997) and Robinson et al. (1997); in the others, root ‘litter’ was obtained by sampling roots from rather young plants.

Effects of [CO₂] on decomposition have been investigated more often on aboveground plant parts than on roots but, again, few experiments (e.g. Kemp et al., 1994; Akin et al., 1995; Franck et al., 1997) have used naturally senesced material. Though it is often assumed that elevated [CO₂] reduces nutrient concentrations and nutrient to carbon ratios in litter, evidence for this generalization is still inconclusive (Arp et al., 1997; c.f. Ball, 1997). Likewise, there is insufficient evidence to conclude that plants grown in elevated [CO₂] produce more recalcitrant litter which decomposes more slowly (O’Neill and Norby, 1996; Arp et al., 1997; Norby and Cotrufo, 1998).

Our work shows that small amounts of root C deposited in soil (relative to the amounts of organic C already present in the soil) can make a disproportio-

4.2. N mineralization

Substantial amounts of N were mineralized during the decomposition of wheat root systems in situ (Fig. 2). But, given the small effects of [CO₂] on C mineralization (see above), it is not surprising that we found no effect of [CO₂] on N mineralization. This contrasts with other studies in which significant effects of [CO₂] have been found on N cycling processes such as mineralization (Hungate et al., 1997b), immobilization (Torbert et al., 1995; Hungate et al., 1997b) and denitrification (Smart et al., 1997; Arnone and Bohlen, 1998; Robinson and Conroy, 1999). An obvious difference between those studies and ours is that the latter did not involve living plants. Living plants affect soil microbial processes by several mechanisms. C losses from living roots can increase the amounts of substrates available to support heterotrophic microbes (e.g. Smart et al., 1997). Differential water use by plants grown under elevated [CO₂] influences soil moisture and aeration, a particularly important influence on the balance between aerobic and anaerobic microbial processes (e.g. Hungate et al., 1997a; Arnone and Bohlen, 1998; Nicklaus et al., 1998; Robinson and Conroy, 1999). Living roots can also produce specific signalling molecules which influence microbial metabolism (e.g. Pierson and Pierson, 1996), although the effects of [CO₂] on such processes remain unknown.

[CO₂] does not always influence soil microbial processes even when living vegetation is present. For example Hungate et al. (1997b) and Nicklaus (1998) found that many N cycle processes in grasslands were affected by [CO₂] only if other nutrients were also available to plants and microbes. Franck et al. (1997) suggested that a large element of species specificity on the effect of [CO₂] on soil N processes is to be expected. We suggest that such specificity will apply especially when those processes are measured under living vegetation, but perhaps less so when living plants are absent, as they are in many decomposition studies. Then, the legacy of elevated [CO₂] effects on soil N processes may always be small, irrespective of the taxonomic provenance of the organic matter supplying that N.

We found no effects of [CO₂] on N mineralized from wheat roots decomposing in the absence of living plants. Likewise, Randlett et al. (1996) found none when Populus leaves decomposed in the absence of Populus trees.
4.3. Using $^{13}$C to determine in situ mineralization of root C

We measured root C mineralization in situ in soil using $^{13}$C labelling of roots. The principle of this method is based on the difference in $^{13}$C/$^{12}$C ratio (expressed in $\delta^{13}$C values) between recently grown roots and native SOM. Similar methods have been used in studies of C dynamics in soils with a vegetation of C$_3$ plants ($\delta^{13}$C $\approx -32$ to $-20\%$o), being replaced by one of C$_4$ plants ($\delta^{13}$C $\approx -17$ to $-9\%$o), or vice versa, with measurements of shifts in $\delta^{13}$C of SOM or soil CO$_2$ (see Balesdent and Mariotti, 1996; Högb erg and Ekblad, 1996).

In previous experiments on effects of elevated atmospheric [CO$_2$], $^{13}$C labelling was used to quantify presumed [CO$_2$]-induced increases in soil C accumulation (Leavitt et al., 1994; Hungate et al., 1996; Leavitt et al., 1996). In these free air CO$_2$ enrichment experiments, only CO$_2$ in the elevated [CO$_2$] treatment was labelled with $^{13}$C. Ineson et al. (1996) and Nitschelm et al. (1997) grew C$_3$ plants on soils containing C derived substantially from C$_4$ vegetation to enable measurements of soil C input at ambient [CO$_3$] (and ambient $\delta^{13}$C) as well. In our experiments, CO$_2$ at both ambient and elevated [CO$_2$] had a $\delta^{13}$C more negative than that of ambient air. The $^{13}$C labelling was not strong enough to allow changes in $\delta^{13}$C of total soil organic C to be detected, but it greatly affected $\delta^{13}$C of CO$_2$ evolved from soil.

The potential accuracy of the $^{13}$C method was demonstrated by the experiment in which C mineralization was measured on soil only and on soil with known additions of roots (Series (iii)). We measured all the $\delta^{13}$C values required to calculate C mineralized from roots (i.e., $\delta^{13}$C of added roots, of CO$_2$ evolved from soil plus roots and of CO$_2$ evolved from soil only). Larger errors may be expected when $\delta^{13}$C of the root C source is less well known, as in our incubation of soil after removal of visible roots (Series (ii)). We are unsure why C mineralized from SOM per unit of soil was much smaller in that incubation compared with the incubation of soil with root systems in situ (Table 2). One reason is that the $\delta^{13}$C values of extracted roots may have been different from those of the actual root-derived microbial substrates, so influencing the calculations of C mineralization.

We did not take into account possible $^{13}$C/$^{12}$C fractionation during root decomposition, i.e., we used a constant $\delta^{13}$C value for the root C source in our calculations of root C mineralized. Two observations suggest that $^{13}$C/$^{12}$C fractionation was indeed negligible. First, the demonstrated accuracy of the method as it was used, as discussed above. Second, $\delta^{13}$C values of retrieved roots at the end of the decomposition experiment were statistically indistinguishable from those at the start (see Section 3.1). Högb erg and Ekblad (1996) measured no $^{13}$C/$^{12}$C fractionation during sucrose decomposition in soil; it seems that the same applies to the decomposition of more complex substrates.

5. Conclusions

1. C mineralization from root systems in situ was transiently faster after growth at elevated [CO$_2$] compared with ambient [CO$_2$]. Ultimately, however, there were no [CO$_2$]-related differences in the amounts of C or N mineralized from root systems in situ.

2. Rates of C loss from extracted roots per unit root dry weight were not significantly different for roots from the two [CO$_2$] treatments.

3. Root decomposition of wheat crops will not necessarily be affected if grown under elevated [CO$_2$]. Any [CO$_2$] effects are likely to arise from differences in the amounts of root material per unit of soil rather than on the chemical quality of that material.

4. $^{13}$C/$^{12}$C fractionation during root decomposition was negligible. The ‘near natural abundance’ $\delta^{13}$C method can be used to trace root-derived C in soil.

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