Rhizodeposition-induced decomposition increases N availability to wild and cultivated wheat genotypes under elevated CO2

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Abstract

Elevated CO2 may increase nutrient availability in the rhizosphere by stimulating N release from recalcitrant soil organic matter (SOM) pools through enhanced rhizodeposition. We aimed to elucidate how CO2-induced increases in rhizodeposition affect N release from recalcitrant SOM, and how wild versus cultivated genotypes of wheat mediated differential responses in soil N cycling under elevated CO2. To quantify root-derived soil carbon (C) input and release of N from stable SOM pools, plants were grown for 1 month in microcosms, exposed to ^13C labeling at ambient (392 ppm CO2) and elevated (792 ppm CO2) CO2 concentrations, in soil containing ^15N predominantly incorporated into recalcitrant SOM pools. Decomposition of stable soil C increased by 43%, root-derived soil C increased by 59%, and microbial-N was enhanced by 50% under elevated compared to ambient CO2. Concurrently, ^15N contents in the microbial biomass and mineral N pool decreased. Wild genotypes allocated more C to their roots, while cultivated genotypes allocated more C to their shoots under ambient and elevated CO2. This led to increased stable C decomposition, but not to increased N acquisition for the wild genotypes. Data suggest that increased rhizodeposition under elevated CO2 can stimulate mineralization of N from recalcitrant SOM pools and that contrasting C allocation patterns cannot fully explain plant mediated differential responses in soil N cycling to elevated CO2.

Keywords:
Elevated CO2
Rhizodeposition
N mineralization
^13C
^15N
Genotypes

1. Introduction

The rise in atmospheric CO2 concentrations stimulates photosynthesis in most plants, leading to an increase in plant production by approximately 20% (Ainsworth and Long, 2005; de Graaff et al., 2006). The stimulation of plant production should enhance soil C input, which in turn may increase soil C sequestration, thereby counterbalancing the rise in atmospheric CO2 (Gifford, 1994). However, the extent to which elevated CO2 stimulates soil C storage has proven hard to predict, because it is unclear whether the plant growth response to elevated CO2 can be sustained in the long-term (de Graaff et al., 2006; Reich et al., 2006; Van Groenigen et al., 2006).

Due to increased growth rates under elevated CO2, plant N demands increase (Luo et al., 2006). In addition, the greater soil C inputs associated with enhanced plant production lead to enhanced soil microbial N demands (de Graaff et al., 2006, 2007). Consequently, more N is retained in both plant tissues and soil pools under elevated CO2, which may result in progressive N limitation (PNL; Luo et al., 2006). The PNL concept posits that in unfertilized ecosystems, N availability progressively decreases under elevated CO2, because N retention in soil and vegetation is stimulated. This ultimately leads to a decline in plant growth and a concomitant decrease in soil C sequestration (Luo et al., 2004; de Graaff et al., 2006).

In a synthesis of results on plant growth and soil nutrient cycling under elevated CO2 in long-term field experiments, however, we showed that under low N availability elevated CO2 still stimulated plant production by ~10%, even though data suggested that PNL had developed in these ecosystems (de Graaff et al., 2006). In addition, plant production and soil C contents continue to increase under elevated CO2 in the Duke FACE experiment, despite there being no evidence of increased net N mineralization or nutrient-use efficiency (Finzi et al., 2001; Johnson, 2006; Finzi et al., 2006). This suggests that an unexplained internal source of N can alleviate PNL in unfertilized ecosystems exposed to long-term elevated CO2.

Hungate and Chapin (1995) postulated that if mineral nutrients are scarce in soils, microbes utilize rhizodeposits as a carbon-source, and decompose more SOM in order to acquire nutrients. More N is then moved into the active N pool in the soil where,
eventually, they may be made available to plants. This process is referred to as priming, which is defined as the stimulation of soil organic matter (SOM) decomposition caused by the addition of labile substrates (Jenkinson et al., 1985; Dalenberg and Jager, 1989). Since elevated CO₂ frequently stimulates rhizodeposition – an important contributor to labile soil C inputs – (Billes et al., 1993; Cotrufo and Gorissen, 1997) and increases decomposition of SOM (de Graaff et al., 2006; Carney et al., 2007), priming of more recalcitrant SOM may be the mechanism partially responsible for alleviating PNL under elevated CO₂ in low N environments. However, increased root-derived soil C input under elevated CO₂ and increased rates of N mineralization due to decomposition of recalcitrant SOM have never been directly linked.

Accumulation of SOM requires a positive imbalance between inputs to and outputs from SOM stocks (Jastrow et al., 2007). Thus, enhanced soil C sequestration under elevated CO₂ can only occur if the rate of soil C decomposition lags behind the CO₂-induced increase in soil C input (Raich and Schlesinger, 1992). Several authors have reported a decrease in soil C as a result of increased native SOM decomposition (i.e. priming) under elevated CO₂ (Carbon et al., 2001; Pendall et al., 2003; Hoosbeek et al., 2004). If rhizodeposition-induced decomposition of recalcitrant SOM actually is an important mechanism that increases N availability to plants under elevated CO₂, its concurrent effect on native C mineralization has to be measured to elucidate its potential impact on net soil C sequestration. Namely, the question ultimately is whether N mineralization induced by rhizodeposition promotes plant growth and a concomitant input of soil C sufficiently to counterbalance the increase in C decomposition.

Plant species differ in mediating changes in soil N cycling in response to global change, but these differences have not been predictable from a knowledge of species’ biology (Hungate et al., 1996). To predict plant species’ impacts on soil nutrient cycling under elevated CO₂, they have been classified into broad groups relying on a broad suite of related plant traits that may generalize how they respond to environmental changes (Evine and Chapin, 2003). However, such classifications have yet provided limited understanding of how plant species affect plant and microbial N acquisition under elevated CO₂ (Hungate et al., 1996; Zak et al., 2000). Using plants with genetic similarity, but contrasting C-allocation patterns may provide another approach for elucidating why plants vary in mediating soil N availability under elevated CO₂. Namely, C allocation to roots is a key plant trait for explaining differential responses in N cycling as it affects both rhizodeposition and nutrient uptake, and genetic similarity minimizes the number of other plant traits that can influence a plants’ response to climate change.

With this study, we aimed at determining how elevated CO₂ affects rhizodeposition of C and how this feeds back to N release from recalcitrant SOM under both wild (Triticum turgidum subsp. dicocoides) and cultivated genotypes (Triticum turgidum subsp. durum) of wheat. The wild and cultivated genotypes of wheat are expected to have contrasting C allocation patterns, since modern agriculture developed cultivar that function well in favorable soil environments but lack traits necessary for growth in low-resource environments (Rengel and Marschner, 2005). Thus, selection for increased yield under high-input agricultural systems produced cultivars with smaller root systems (Chapin et al., 1989; Siddique et al., 1990; Jackson, 1995) and greater C partitioning to shoots (Gifford et al., 1984).

We hypothesized that elevated CO₂ increases the inputs of root-derived C thereby stimulating decomposition of recalcitrant SOM and concurrently increasing soil N availability and plant N uptake. In addition, we hypothesized that greater C allocation to the roots of the wild wheat genotypes results in greater rhizodeposition-induced N mineralization from stable SOM pools under elevated CO₂. To quantify root-derived soil carbon (C) input and release of N from stable SOM pools, plants were grown for 1 month in soil microcosms containing ¹⁵N predominantly present in recalcitrant SOM pools while being exposed to ¹³C labeling at ambient (392 μmol mol⁻¹) and elevated (792 μmol mol⁻¹) CO₂ concentrations.

2. Materials and methods

2.1. Pre-treatment of the soil

The soil used for the experiment was derived from the Swiss Free Air Carbon dioxide Experiment (FACE) in March of 2003. This soil had received ¹⁵N fertilization treatments for 10 consecutive years, which has lead to incorporation of a significant amount of the ¹⁵N into the more stable SOM pools (de Graaff et al., 2008). The samples were taken to a depth of 25 cm from both ambient and elevated CO₂ field plots under L. perenne that had received N fertilization (140 kg N ha⁻¹ y⁻¹), with an atom% ¹⁵N excess of 0.3841 in 1995 and 1.0602 from 1996 to 2003. No differences in total N, C or fertilizer derived N were observed between the ambient and elevated CO₂ treatments after 10 years (Van Kessel et al., 2006).

The soil of the ambient and elevated CO₂ treatments was composited, air dried and sieved to 2 mm. Next, sterile sand (30% to dry weight) was added, to increase its volume and to facilitate root extraction at termination of the experiment. Water-holding capacity of the soils was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100 °C). Water was added to obtain 60% of water-holding capacity. Subsequently, the soils were incubated at 30 °C for 145 days in ten plastic filters (Nalgene Filter model 7111; Becton Dickinson Labware, Lincoln Park, NJ, USA) at 35 °C (Kaye et al., 2002). A glass fiber filter (Whatman GF/A, Whatman Inc., Ann Arbor, MI, USA), and an “extra thick” glass fiber prefILTER (Gelman Sciences, Ann Arbor, MI, USA) were used to replace the filter originally in the filter unit.

To deplete the soil from labile N and ¹⁵N, the soils were leached at days: 1, 8, 25, 43, 58, 86, 100, 120 and 145, with a leaching solution containing all essential nutrients except for N (Stanford and Smith, 1972; Nadelhoffer, 1990; Kaye et al., 2002). At each leaching event, 1 L of the N-free leaching solution was added to the top of the filter, allowed to equilibrate with the soil for 45 min. and then drawn through the filter with a weak vacuum until all the leachate was collected (Kaye et al., 2002). Leaching was terminated when the rate of leable ¹⁵N efflux from the soil was near zero (6.8 × 10⁻⁵ μg⁻¹ g⁻¹ day⁻¹) for the last three leaching events. The soil was removed from the filters and composited, after which it was transferred to the containers. Three pots with 25 g of control soil were placed in the chambers in closed specimen cups in order to confirm that ¹⁵N mineralization was insignificant in the absence of plants.

2.2. Experimental design and growing conditions

Plants were exposed to two (392 and 792 μmol mol⁻¹) atmospheric CO₂ concentrations in two controlled environment chambers for 32 days. The chambers were located in the Greenhouse Facility at UC-Davis. They were constructed from clear Plexiglas, had a volume of 2000 L and were entirely sealed for the duration of the experiment, to allow for continuous labeling with ¹³CO₂-gas. Carbon dioxide concentrations were measured with an IRGA (model 820-LC, LI-COR, Lincoln, NE, USA) in a closed air circulating system. The IRGA was connected to an automatic burette, which was pulsed if the IRGA-reading fell below the threshold
concentration of 350 and 750 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), for the ambient and elevated chamber, respectively. In the event of a pulse the burette dispensed 10 mL of NaHCO\(_3\) solution containing 0.40 g \( ^{13}\text{C} \) L\(^{-1} \) and 3.98 g \( ^{12}\text{C} \) L\(^{-1} \) in a 250 mL side-arm flask placed on a laboratory stirrer (Fisher Scientific small vessel stirrer, http://www.fishersci.com) and containing a H\(_2\)SO\(_4\) solution (2 M). The H\(_2\)SO\(_4\) solution was changed regularly. The CO\(_2\) gas evolving from this reaction was then pumped back into the chamber, and led to an increase in the atmospheric CO\(_2\) concentration of 42 \( \mu \text{mol mol}^{-1} \). Using a data-logger controlling two 3-way solenoid valves (Dayton electric, model CAT66P-120-A, IL, USA), this procedure was repeated for each chamber every 15 min. In preliminary tests we found that the CO\(_2\) dispensed into the chamber was mixed with the chamber-air after 15 min. To facilitate air mixing in the chamber we used three fans that operated continuously. Labelling with \( ^{13}\text{CO}_2 \), started after 7 days of growth and lasted for 25 days. During that time, the ambient chamber received a total of 52 mg \( ^{13}\text{C} \) due to greater plant growth, and due to an initial spike with \( ^{13}\text{C} \) to get the CO\(_2\) concentration up to 792 \( \mu \text{mol mol}^{-1} \) (Table 1). Our experimental set-up did not allow us to control for higher CO\(_2\) concentrations at night resulting from plant and soil respiration. Consequently, in the mornings the positive deviation of the target CO\(_2\) concentrations in the ambient CO\(_2\) chamber reached a maximum of 21\% and in the elevated chamber of 14\%. As a result, this study compared plant and nitrogen cycling responses to two levels of atmospheric CO\(_2\) (X and 2X), rather than exactly current and twice current CO\(_2\) concentrations.

The chamber conditions used a photosynthetically active \( (400–700\text{ nm}) \) photon flux rate of 500 \( \mu \text{mol m}^{-2} \text{s}^{-1} \), a 16/8 h (day/night) cycle, and 30 \( ^\circ\text{C} / 25 \text{ ^\circ\text{C} (day/night) temperature. Humidity was kept at 60\% and controlled by a humidity controller (model EH-100, Ohmic Instruments, Easton, Md, USA) that was connected to a dehumidifier (Model FDD40J1, Frigidaire Company, Augusta GA, USA). Temperature was controlled by a thermostat (model 1609-101, White-Rodgers, http://www.white-rodgers.com) connected to a 3-way solenoid valve (Dayton electric, IL, USA) that opened to release cool water into the chambers via radiators situated on the floor of the chambers if the temperature in the chamber fell above the threshold temperatures.

Three wild and three cultivated genotypes of spring wheat were selected for the experiment. The genotypes were classified as follows:

- *Triticum turgidum* subsp. dicoccoides (accession number: PI 467015), wild material, collected in Northern Israel (1982).
- *Triticum turgidum* subsp. dicoccoides (accession number: PI 467008), wild material, collected in Northern Israel (1982).
- *Triticum turgidum* subsp. dicoccoides (accession number: PI 534280), cultivated material, collected in Arusi, Ethiopia (1988).
- *Triticum turgidum* subsp. durum (accession number: PI 532239), cultivated material, collected in Oman (1987).

Table 1

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>ANOVA</th>
<th>Biomass production (Fig. 1)</th>
<th>Old and new C dynamics (Fig. 2)</th>
<th>N content (Fig. 3a,b)</th>
<th>(^{15}\text{N} ) content (Fig. 3c,d)</th>
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<td></td>
<td>Shoot</td>
<td>Roots</td>
<td>R:S ratio</td>
<td>Stable SOM C</td>
<td>Root derived C</td>
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<td>Wild genotypes CO(_2)</td>
<td>( P &lt; 0.05 )</td>
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<td>Cultivated genotypes CO(_2)</td>
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<td>Genotypes</td>
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Seeds of the plants were vernalized at 6 \( ^\circ\text{C} \) and germinated for 5 days in plastic Petri dishes with water agar as the growth medium. Subsequently, the plants were transferred to containers (Deeppot cells, D25L, Stuewe & sons, Corvallis, OR, USA; 25 cm height and 4.25 cm diameter at top) containing 250 g (dry weight) of soil. The containers were placed in a Deeppot-cell tray (Deeppot cell tray, D16T, Stuewe & sons, Corvallis, OR, USA). Each pot contained one plant and three plants of each genotype were grown in the ambient and elevated chambers. Hence, in total 18 pots containing one plant of which nine were wild (i.e. accession numbers: PI 467008 \( n = 3 \), PI 414718 \( n = 3 \), PI 534280 \( n = 3 \) ) and nine were cultivated (i.e. accession numbers: PI 534280 \( n = 3 \), PI 532239 \( n = 3 \), PI 520125 \( n = 3 \) ) genotypes were randomly placed in the chamber. Water-holding capacity (WHC) was determined by calculating the difference in weight of soils at saturation point and oven-dry weight (100 \( ^\circ\text{C} \)). Soils were re-wetted to 60\% of WHC prior to seeding and on a daily basis during the experiment. This was achieved by slowly pumping the needed amount of water into the chamber through tubes that were attached to the pots in the chamber and with a Luer-lock to 30 mL syringes outside the chambers. The Luer-lock attached to the syringe was closed when not in use and in addition, some water was kept standing in the syringe to prevent \(^{13}\text{CO}_2\) from escaping.

2.3. Plant sampling

After 32 days of growth, the plants were harvested. The shoots were cut off at the soil surface, and dried at 70 \( ^\circ\text{C} \) for 3 days. The containers with the remaining soil were stored at 4 \( ^\circ\text{C} \) for one night prior to harvesting the roots. After washing the roots with water, the roots were dried at 70 \( ^\circ\text{C} \) for 3 days. Both the dry root and shoot material were ground in a ball mill and their C and N, and \(^{15}\text{N} \) and \(^{13}\text{C} \) isotopic composition was determined. Total C, N and the isotopic composition of the plant materials were analyzed by an automated N/C analyzer-isotope ratio mass spectrometer (ANCA-IRMS, Europa Scientific Integra, UK) at the UC Davis Stable Isotope Facility.

2.4. Soil sampling and analyses

The soil was mixed thoroughly after which a sub sample of 110 g was collected. Since the roots had occupied the entire volume in the containers, all the soil was considered rhizosphere soil. The roots were removed from the soil by hand picking. Twenty g of the soil was dried at 40 \( ^\circ\text{C} \) for 1 day and ground in a ball mill after which its total C and N and \(^{15}\text{N} \) isotopic composition was measured. Total C, N and the isotopic composition of the soil samples were analyzed.
A second sub sample (30 g) was used to determine mineral N in the soil, by extracting it with 120 mL 2 M KCl. The solution was shaken for 45 min, extracted and analyzed colorimetrically for NH₄⁺ and NO₃⁻ content (Forster, 1995). The amounts of ¹⁵N were derived by diffusing N according to Stark and Hart (1996), after which the ¹⁵N isotopic composition was measured. This method was also applied to the control soils.

The third and fourth sub samples (30 g each) were used to determine C in the active soil C pool and soil microbial biomass C and N by the fumigation-incubation method (Jenkinson and Powlson, 1976). Thirty g were stored at 4°C for 48 h, while the other 30 g were exposed to chloroform fumigation for 48 h. Subsequently, both the fumigated and non-fumigated soils were incubated for 10 days. Samples were transferred to 120 mL specimen containers and placed in ⅓ L Mason jars. Water was added to the samples to obtain 60% of WHC. In addition, 5 mL of water were added to the bottom of the jar to slow the drying of the soil.

A septum in the lid allowed gas samples to be removed with a 12 mL syringe. CO₂-samples were collected in 10 mL Vacutainers (Labco Unlimited, Buckinghamshire, UK). The concentration of CO₂ and its PDB ¹³C signature were determined using a continuous flow, isotope mass spectrometer (PDZ Europa TCII trace gas analyzer, Gero, 20–20 isotope ratio mass spectrometer, Cheshire, UK). Carbon mineralization data were expressed on the basis of oven-dry (40°C) weight of soil. Active soil C was defined as the CO₂-C efflux from the non-fumigated soil. Microbial C was defined as CO₂-C efflux from the fumigated soil minus soil CO₂-C efflux from the non-fumigated soil (Ec), using an efficiency coefficient, k, of 0.35 (Yance et al., 1987).

Both the non-fumigated and fumigated samples were extracted instantly after gas-sampling. Each sample was mixed with 120 mL of 2 M KCl and shaken for 45 min prior to filtration (Whatman #42). The extracts were analyzed colorimetrically for NH₄⁺ and NO₃⁻ content (Forster, 1995). Microbial biomass N was considered to be the total inorganic N flush in the fumigated samples compared to the non-fumigated samples. The ¹⁵N determination for both mineral ¹⁵N and microbial biomass ¹⁵N was performed by diffusing N from the extracts onto acidified disks sealed in Teflon tape (Stark and Hart, 1996). The disks were packed in tin capsules and analyzed for isotopic composition.

### 2.4.1. ¹⁵N and ¹³C recovery calculations

The amount of ¹⁵N recovered in the soil mineral N pool, the microbial biomass N pool and the plant tissues released by the stable SOM pool was calculated by multiplying the ¹⁵N atom% excess with the N contents in the concerning pool. The background atom% for ¹⁵N was 0.3663. Since the total amount of ¹⁵N present in the stable SOM pools after the leaching events and prior to plant growth was equal for each pot, we could directly compare the ¹⁵N recoveries in the plant and soil pools.

Recovery of ¹³C was calculated as: (1) recovery of ¹³C in the total plant biomass and soils was calculated as a percentage of total ¹³C added to the chambers; and (2) recovery of ¹³C in the individual plants and total soil C pool was calculated as a percentage of total ¹³C recovery per pot. The first calculation shows how much of the ¹³C applied to the chambers was taken up by plants and soils, how much remained in the chamber air and what the final loss was of ¹³C from the chamber, whereas the second calculation shows how much ¹³C was taken up per pot and the distribution of ¹³C in the plants and soils, which reflects the treatment effects.

The total CO₂-C efflux from the control soils of the fumigation-incubation at day 10 of the incubation represented both more active root-derived C and more stable SOM-derived C, enabling us to partition the measured CO₂ into root-derived C and C in the more stable SOM pools using the following equations:

\[ C_t = C_{SOM} + C_r \]

\[ C_t = C_t \times (\delta^{13}C_t - \delta^{13}C_0 / \delta^{13}C_r - \delta^{13}C_0) \]

\[ C_{SOM} = C_t - (C_t \times (\delta^{13}C_t - \delta^{13}C_0 / \delta^{13}C_r - \delta^{13}C_0)) \]

Where \( C_t \) is the total respiration of C from the soils at day 10 of the incubation, \( C_r \) is respiration of root-derived C at day 10 of the incubation, \( C_{SOM} \) is the amount of C respired from the more stable SOM pool at day 10 of the incubation, \( \delta^{13}C_t \) is the isotopic signature of the soil CO₂ efflux at day 10 of the incubation, \( \delta^{13}C_r \) is the isotopic signature of the roots, and \( \delta^{13}C_0 \) is the background \( \delta^{13}C \) value of the soil (–28%). We assumed that the \( \delta^{13}C \) value of rhizodeposition is equal to the \( \delta^{13}C \) value of root tissue.

### 2.5. Statistical analyses

The procedure GLM in the SAS system for Windows™ V8 was conducted, with CO₂, and wild versus cultivated genotypes as treatments. We confirmed that there were no significant differences between the means of the three accessions within the wild and cultivated genotypes, after which we treated each genotype within the wild and cultivated types as a replicate (n = 3) to avoid pseudo replication. Means were compared by the Tukey test, if the analysis of variance was significant. The level of significance was P ≤ 0.01 and P ≤ 0.05.

### 3. Results

#### 3.1. Plant biomass production

Elevated CO₂ significantly stimulated aboveground biomass production of both the wild and cultivated genotypes, by 24.6% and 40.0%, respectively (Fig. 1a, Table 1). Root biomass production was significantly greater than the shoots and roots followed the results for biomass production (Table 4). Recovery calculated as ¹³C applied to the chambers was taken up by plants exposed to elevated CO₂ (Table 3). The allocation of ¹³C to the shoots and roots followed the results for biomass production (Table 4). Recovery calculated as ¹³C in plants and soil pools as a percentage of ¹³C recovery in a pot, showed that overall significantly more ¹³C was recovered in the shoots of the cultivated than the wild genotypes under both ambient and elevated CO₂ (+48.1%, on average). Elevated CO₂ did not alter R:S ratios for either one of the genotypes (Fig. 1b, Table 1).

#### 3.2. Carbon cycling

Total recovery of the ¹³C applied to the chamber in the total plant biomass, soil pools and residual CO₂ in the chamber atmosphere was 93.63% and 88.69% in the ambient and elevated chambers, respectively (Table 2), suggesting that the losses of ¹³C remained small. The \( \delta^{13}C \) enrichment of plant and soil pools in the elevated chamber was significantly greater than the \( \delta^{13}C \) enrichment of plant and soil pools in the ambient chamber due to: (1) the initial pulsing with ¹³C to raise the CO₂ concentration by 400 ppm; and (2) due to greater CO₂ assimilation by plants exposed to elevated CO₂ (Table 3).

The allocation of ¹³C to the shoots and roots followed the results for biomass production (Table 4). Recovery calculated as ¹³C in plants and soil pools as a percentage of ¹³C recovery in a pot, showed that overall significantly more ¹³C was recovered in the shoots of the cultivated than the wild genotypes under both ambient and elevated CO₂ (+11.9% on average). On the contrary, ¹³C recovery in the roots was greater in the wild compared to the cultivated genotypes under ambient and elevated CO₂ (+40.0% on average) (Table 4). Elevated CO₂ significantly increased ¹³C recovery in the shoots (+13.9%), but not in the roots of the cultivated...
genotypes. In contrast, $^{13}$C recovery was not enhanced in the roots and shoots of the wild genotypes under elevated CO$_2$ (Table 4).

Due to the high ratio of $^{12}$C inherent to the soil versus root-derived $^{13}$C, the incorporation of $^{13}$C in the total soil C pool was minor compared to the $^{12}$C background values in the ambient soils (Table 3), which made it difficult to accurately determine recovery of total soil $^{13}$C. However, $^{13}$C was clearly detectible in the active soil C pool in soils previously exposed to both ambient and elevated CO$_2$ (Table 3), hence we were able to partition soil C respiration in decomposition of more stable SOM C and root-derived C. Stable SOM-C decomposition was significantly greater under elevated CO$_2$ by 63.2% for the wild and 22.6% for the cultivated genotypes (Fig. 1), which made it difficult to accurately determine recovery of total soil $^{13}$C. However, $^{13}$C was clearly detectible in the active soil C pool in soils previously exposed to both ambient and elevated CO$_2$ (Table 3), hence we were able to partition soil C respiration in decomposition of more stable SOM C and root-derived C. Stable SOM-C decomposition was significantly greater under elevated CO$_2$ by 63.2% for the wild and 22.6% for the cultivated genotypes (Fig. 2a, Table 1). Root-derived soil C decomposition was significantly greater under elevated compared to ambient CO$_2$ levels by 65.2% for the wild and 54.2% for the cultivated genotypes, with no differences between genotypes. In addition, under elevated CO$_2$ significantly more of the root-derived $^{13}$C was incorporated into the microbial biomass compared to ambient CO$_2$, by 53.1% for the cultivated genotypes (Fig. 2c, Table 1).

### 3.3. Nitrogen cycling

The total release of $^{15}$N from stable SOM pools as measured in the mineral N pool and in the plants was significantly greater ($P < 0.01$) when plants had grown in the soils, compared to decomposition rates in soils without plants, by 7.8 and 8.4 times under ambient and elevated CO$_2$, respectively. In addition, the total release of total labile N from stable SOM pools as measured in the mineral N pool and in the plants was significantly greater ($P < 0.01$) when plants had grown in the soils, compared to decomposition rates in soils without plants, by 1.4 and 1.6 times under ambient and elevated CO$_2$, respectively. Initial soil N and $^{15}$N contents and soil N and $^{15}$N contents in the control soils at termination of the experiment are shown in Table 5. The total $^{15}$N recovery (sum of recovery in plants, microbial biomass and mineral N pool) as a percentage of the total amount of $^{15}$N incorporated in the stable SOM pools was 1.4% and 1.6% for the soils grown under ambient and elevated CO$_2$, respectively. Total $^{15}$N recovery at termination of the experiment was 93% on average for the planted soils, with no treatment differences.

Total plant N concentrations were equal for all genotypes, and were significantly greater under elevated CO$_2$ than ambient CO$_2$ (Table 6). Consequently, the tissue C:N ratios of the roots (9.1%, on average) and shoots (27.1%, on average) were significantly enhanced under elevated CO$_2$ for both wild and cultivated genotypes similarly (data not shown). Total plant N contents were enhanced by elevated CO$_2$ in the shoots of the wild plants and in the roots of the cultivated plants only (Figs. 3a,b, Table 1). The $^{15}$N recovered in the total plant biomass was significantly enhanced by elevated CO$_2$ in the roots of the wild and in the shoots and roots of cultivated genotypes (+23% on average) (Figs. 3c,d, Table 1). In addition, the percent $^{15}$N recovery in the roots of wild genotypes was greater under both ambient and elevated CO$_2$ than in the cultivated genotypes (+33.9% on average) (Figs. 3c,d, Table 1).

Total soil N contents and the percentage total soil $^{15}$N recovery were not different between the soils exposed to ambient and elevated CO$_2$ and wild and cultivated genotypes. Total mineral soil N contents and percent mineral $^{15}$N recovery was significantly smaller under elevated compared to ambient CO$_2$ treatments for the wild genotypes (-30.8%), but were equivalent for the other treatments (Table 6). Percent microbial biomass $^{15}$N recovery and N contents were smaller under elevated compared to ambient CO$_2$ for the wild genotypes (+12%) (Table 6). There were no differences in microbial biomass N contents or percent $^{15}$N recovery between the wild and cultivated genotypes (Table 6).

### 4. Discussion

#### 4.1. Linking rhizodeposition to N released from recalcitrant SOM under elevated CO$_2$

In our microcosm study, elevated CO$_2$ significantly stimulated plant production and the amount of root-derived C in the active soil C pool and microbial biomass after 1 month of growth. These data...
suggest that elevated CO₂ increased rhizodeposition. A CO₂-induced increase in C rhizodeposition (Cotrufo and Gorissen, 1997; Van Ginkel et al., 2000) and root-derived C mineralization (Bazot et al., 2006) has been reported by several studies. It is still debated, however, whether elevated CO₂ increases the input of root-derived materials by stimulating the quantity of rhizodeposition per gram of root, or whether the increase in rhizodeposition is caused by a proportional increase in root growth (Darrah, 1996). In this study, the increase in root-derived soil C under elevated CO₂ was accompanied by a proportional increase in root biomass for each of the wheat cultivars (data not shown). This corroborates other studies showing that increased root production under elevated CO₂ was responsible for enhanced C rhizodeposition (Billes et al., 1993; Cotrufo and Gorissen, 1997). This suggests that elevated CO₂ is not likely to affect rhizosphere processes unless belowground biomass production is stimulated.

Nitrogen-15 release was approximately 12 times greater in soils with plants compared to soils without plants, suggesting plant-induced N mineralization had occurred. Similarly to the soils with plants, the soils without plants were kept at 60% of WHC in the growth chambers. Due to limited availability of ¹⁵N enriched soil, however, they were placed in specimen cups rather than plant-pots, which may have affected N mineralization. Nonetheless, a similar positive effect of plant growth on N mineralization was shown by Herman et al. (2006). More importantly, the recovery of ¹⁵N in the plants exposed to elevated CO₂ was significantly greater than ¹⁵N recovery in the plants exposed to ambient CO₂ (+7% total in shoots and roots), and specifically in the roots (+26%). The ¹⁵N release from stable SOM pools was preferentially stimulated by plants compared to total soil N release. No unlabeled N had been applied to the soils for 10 years, hence unlabeled N was incorporated in relatively more recalcitrant SOM pools than the ¹⁵N and therefore decomposed more slowly. The ¹⁵N recovery data suggest that increased root-derived soil C inputs under elevated CO₂ enhanced microbial turnover of recalcitrant SOM pools and promoted N availability to plants. The increase in decomposition of C from the more recalcitrant SOM pools as determined from the incubation with rhizosphere soil after the CO₂ experiment further

### Table 3

<table>
<thead>
<tr>
<th>CO₂-treatment</th>
<th>Domestication</th>
<th>Shoots</th>
<th>Roots</th>
<th>Total soil C</th>
<th>Active C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambient</td>
<td>Wild</td>
<td>935.7 ± 22.4</td>
<td>1021.0 ± 33.2</td>
<td>-20.7 ± 1.4</td>
<td>110.3 ± 12.5</td>
</tr>
<tr>
<td></td>
<td>Cultivated</td>
<td>935.9 ± 45.3</td>
<td>891.8 ± 76.7</td>
<td>-19.2 ± 2.2</td>
<td>110.6 ± 4.1</td>
</tr>
<tr>
<td>Elevated</td>
<td>Wild</td>
<td>3430.9 ± 52.0</td>
<td>3281.7 ± 99.1</td>
<td>-6.5 ± 8.6</td>
<td>485.2 ± 138.6</td>
</tr>
<tr>
<td></td>
<td>Cultivated</td>
<td>3388.5 ± 121.4</td>
<td>2974.2 ± 296.9</td>
<td>-3.7 ± 4.9</td>
<td>656.3 ± 68.4</td>
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</tbody>
</table>

Source of variation ANOVA

<table>
<thead>
<tr>
<th>Wild genotypes CO₂</th>
<th>Cultivated genotypes CO₂</th>
<th>Genotypes</th>
<th>CO₂ + Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 3).

### Table 4

<table>
<thead>
<tr>
<th>CO₂-treatment</th>
<th>Domestication</th>
<th>Shoots</th>
<th>Roots</th>
<th>Total soil C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>Ambient</td>
<td>54.8 ± 1.6</td>
<td>27.6 ± 1.9</td>
<td>17.7 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>59.0 ± 2.1</td>
<td>30.0 ± 1.7</td>
<td>11.0 ± 3.5</td>
</tr>
<tr>
<td>Cultivated</td>
<td>Ambient</td>
<td>59.9 ± 2.8</td>
<td>17.3 ± 2.0</td>
<td>22.7 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>Elevated</td>
<td>69.7 ± 2.9</td>
<td>17.1 ± 2.3</td>
<td>13.2 ± 2.5</td>
</tr>
</tbody>
</table>

Source of variation ANOVA

<table>
<thead>
<tr>
<th>Wild genotypes CO₂</th>
<th>Cultivated genotypes CO₂</th>
<th>Genotypes</th>
<th>CO₂ + Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 3).

---

**Fig. 2.** Decomposition of more stable SOM-C (a), decomposition of root-derived C (b) and microbial biomass ¹³C contents (c) in the soils of the three wild and three cultivated wheat genotypes under ambient and elevated CO₂. Shading conventions as in Fig. 1. Values are means ± SE (n = 3). Significant differences between CO₂ treatments (P < 0.01, and P < 0.05) are indicated by ***, *, respectively. Additional statistics are presented in Table 1.
Cultivated genotypes CO2

Insignificance may have been caused because measured root-soil under elevated CO2 was prone to high turnover rates, which is found, most of the additional root-derived C entering the soil was incorporated in more stable SOM pools, the large background 12C values addition, our experimental set-up did not allow for measuring soil ment of differences in rhizodeposition between treatments. In though, can also take up organic N when faced with low N avail-

Increased efficiency of nutrient uptake could have also been responsible for the greater 15N recovery under elevated CO2. Yet, if SOM decomposition did not increase mineral N availability, increased fine root growth could not benefit N uptake. Many plants though, can also take up organic N when faced with low N avail-

The relatively large decomposition rates of 13C (+59.7%, on average), C from more stable SOM pools (+42.9%, on average) and the greater incorporation of 13C in the microbial biomass under elevated CO2 (+50%, on average), however, strongly indicate that increased rhizodeposition-induced microbial activity under elevated CO2 was responsible for the greater 15N uptake rates under elevated compared to ambient CO2. In addition, since the microbial N pool reflects the balance of N mineralization and immobilization (Hart et al., 1994), the reduction of microbial biomass 15N contents under elevated CO2, suggests that microbial 15N mineralization had increased. The smaller mineral 15N pool under elevated compared to ambient CO2 further indicates that the plants had taken up the mineral 15N made available from the stable SOM pools by microbes. Moreover, the stimulation of 15N uptake was significantly greater under elevated compared to ambient CO2 (+7%). The increase in C rhizodeposition rates, incorporation of root-derived C in the microbial biomass, and stable SOM-C decomposition along with the simultaneous decrease of 15N contents in the soil microbial biomass and mineral N pools and the concurrent increase of 15N uptake by the plants under elevated CO2 strongly support the hypothesis that CO2-induced increases in rhizodeposition can increase soil N availability.

If rhizodeposition-induced decomposition of recalcitrant SOM is an important mechanism contributing to N availability increases in the field as it does in our microcosms, it could explain sustained increases in plant production under long-term elevated CO2 in unfertilized ecosystems. Nonetheless, total plant N concentrations were still reduced by elevated CO2 in our experiment, which may suggest that the plant growth response to elevated CO2 in the long-term would still decrease. In addition, based on our experiment it seems unlikely that rhizodeposition-induced N mineralization

<table>
<thead>
<tr>
<th>Genotype</th>
<th>CO2 treatment</th>
<th>N concentration (mg g⁻¹)</th>
<th>15N concentration (µg g⁻¹)</th>
<th>Mineral N (µg g⁻¹)</th>
<th>Mineral 15N (µg g⁻¹)</th>
<th>Microbial N (µg g⁻¹)</th>
<th>Microbial 15N (µg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Shoot</td>
<td>Root</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild</td>
<td>Ambient CO2</td>
<td>15.93 ± 0.67</td>
<td>10.28 ± 0.29</td>
<td>11.60 ± 0.44</td>
<td>7.49 ± 0.24</td>
<td>1.29 ± 0.074</td>
<td>4.21 ± 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td>Elevated CO2</td>
<td>12.46 ± 0.93</td>
<td>8.64 ± 0.22</td>
<td>7.17 ± 0.97</td>
<td>6.02 ± 0.05</td>
<td>1.01 ± 0.049</td>
<td>2.53 ± 10⁻⁴</td>
</tr>
<tr>
<td>Cultivated</td>
<td>Ambient CO2</td>
<td>16.94 ± 0.42</td>
<td>10.80 ± 0.50</td>
<td>12.69 ± 0.60</td>
<td>8.05 ± 0.28</td>
<td>1.24 ± 0.079</td>
<td>3.87 ± 10⁻⁴</td>
</tr>
<tr>
<td></td>
<td>Elevated CO2</td>
<td>11.72 ± 0.25</td>
<td>9.56 ± 0.26</td>
<td>8.65 ± 0.09</td>
<td>7.04 ± 0.07</td>
<td>1.45 ± 0.15</td>
<td>3.27 ± 10⁻⁴</td>
</tr>
</tbody>
</table>

Source of variation ANOVA

- Wild genotypes CO2  
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns

- Cultivated genotypes CO2  
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns

- Genotypes CO2 × Genotypes  
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns
  - P ≤ 0.05 ns

Values are means ± SE (n = 3).
would increase net soil C sequestration under elevated CO2, considering the significant increase in native soil C decomposition. Moreover, this mechanism does not contribute to a net gain of ecosystem N, but rather causes redistribution of available N. Redistribution of N under elevated CO2 is expected to have a diminishing effect on N availability and soil C sequestration in the future (i.e. decades to centuries) (Hungate et al., 2003).

The plants in this experiment were subject to a high degree of experimental manipulation. It has been demonstrated that the CO2 treatment effect in pot studies is enhanced compared to field studies (Norby et al., 2001; de Graaff et al., 2006). In our experiment the roots had occupied the entire soil volume in the pots after 32 days of growth and were left no choice but to take up N from a soil that was extremely limited for plant available forms of N. In a field situation the roots would probably have been exposed to greater nutrient heterogeneity and would have likely been able to access nutrient rich patches in the soil through foraging (Hodge, 2003). Hence, our experimental set-up may have exaggerated the impact of elevated CO2 on rhizodeposition-induced priming, and the relative importance of this process in the field for alleviating PNL remains to be determined. Nonetheless, it has been unclear what the source of the ‘extra N’ is that sustains increases in plant growth in some FACE studies (Finzi et al., 2001; Johnson, 2006; Finzi et al., 2006), and this study has provided a novel mechanistic understanding of the impact that increased root C inputs can have on soil N mineralization.

4.2. Genotypic variation in response to elevated CO2

In this study, we hypothesized that contrasting C allocation patterns between wild and cultivated genotypes of wheat may be an important parameter for explaining plant-mediated variations in soil N cycling under elevated CO2. Under ambient CO2 concentrations, more C was partitioned to the shoot compared to the root for the cultivated genotypes. On the contrary, the wild genotypes allocated more C to their roots. These data indicate that agronomic selection of wheat has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars (Gifford et al., 1984).

It has been argued that a high R:S ratio is a plant characteristic representing an “adaptive strategy” that enables plants to survive in stressful (e.g. low-resource) environments (Grime, 1977; Tilman 1988). On the contrary, the ability to allocate soil resources to stimulate shoot production is a characteristic of competitive species usually dominating fertile environments (Grime, 1979; Tillman, 1988; Ryser and Notz, 1996). Based on these ecological theories we hypothesized that cultivated genotypes are more likely to increase aboveground plant production in response to the CO2 fertilization effect, while wild genotypes are more likely to efficiently acquire nutrients in low-resource environments.

Elevated CO2 increased aboveground biomass production of both wild and cultivated genotypes, but the growth response of the cultivated genotypes was significantly greater than the growth response of the wild genotypes. By contrast, belowground biomass production was significantly greater for the wild compared to the cultivated genotypes under both ambient and elevated CO2. In addition, the wild genotypes stimulated stable C decomposition under elevated CO2 more than the cultivated genotypes. Nonetheless, they only acquired more 15N in their root biomass under elevated CO2 than the cultivated genotypes. There was significantly less 15N under elevated CO2 in the mineral N pool and the microbial biomass for the wild genotypes only, which may have been caused by greater N uptake. Since only the roots, but not the shoots acquired more 15N under elevated CO2, our data do not fully support the hypothesis that wild genotypes have an “adaptive life strategy” making them more likely to sustain nutrient acquisition when elevated CO2 reduces nutrient availability in the long-term. Therefore, more long-term research is warranted to elucidate whether agricultural systems that do not rely heavily on mineral nutrient fertilizer inputs might benefit from using plants with preferential C allocation to the roots under elevated CO2, and whether the ability of the wild genotypes to effectively compete...
with microbes for N makes them more likely to sustain growth and C sequestration in low N input systems.

5. Conclusions

Based on the increase in microbial activity due to enhanced rhizodeposition under elevated CO2, the concurrent stimulation of 15N availability by the microbial biomass from the stable SOM pools, and the subsequent increased mineral 15N uptake, we suggest that rhizodeposition-induced priming of recalcitrant soil N can be a mechanism that contributes to stimulating plant production under elevated CO2. The relative importance of this mechanism for preventing N limitation in ecosystems exposed to long-term elevated atmospheric CO2, however, should be tested under field conditions.

In addition, our data suggest that agronomic selection of wheat has resulted in a morphological tradeoff, where C allocation to organs associated with C assimilation compared to organs associated with nutrient uptake is favored in modern cultivars. Initially, this makes these cultivars more likely to increase biomass production under elevated CO2 than their wild relatives. However, the relatively large increase in belowground C-allocation in the wild genotypes did not unequivocally enhance their ability to effectively compete with microbes for N. Thus, contrasting C allocation patterns in this study could not fully explain plant mediated differential responses in soil N cycling to elevated CO2.

Acknowledgements

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References


Chapin III, F.S., Groves, R.H., Evans, L.T., 1989. Physiological determinants of growth at atmospheric CO2, however, should be tested under field conditions.


