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Determination of microbial versus root-produced CO₂ in an agricultural ecosystem by means of $\delta^{13}\text{C}$ CO₂ measurements in soil air

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ABSTRACT

The amounts of microbial and root-respired CO₂ in a maize/winter wheat agricultural system in south western Germany were investigated by measurements of the CO₂ mixing ratio and the ¹³C/¹²C ratio in soil air. CO₂ fluxes at the soil surface for the period of investigation (1993–1995) were also determined. Root respired CO₂ shows a strong correlation with the plant mass above ground surface of the respective vegetation ($R^2 \geq 0.88$); the maximum CO₂ release from roots was in August for the maize ($2.0 \pm 0.5 \text{ mmol m}^{-2} \text{ h}^{-1}$) and in June for winter wheat ($1.5 \pm 0.5 \text{ mmol m}^{-2} \text{ h}^{-1}$). Maximum CO₂ production by roots correlate well with the maximum amount of plant root matter. Integrating the CO₂ production over the whole growing season and normalizing to the dry root matter yields, the CO₂ production per gram dry organic root matter (DORM) of maize was found to be $0.14 \pm 0.03 \text{ gC (g DORM)}^{-1}$. At the sites investigated, root-produced CO₂ contributed ($16 \pm 4\%$) for maize, and ($24 \pm 4\%$) for winter wheat, respectively, to the total annual CO₂ production in the soil ($450 \pm 50 \text{ gC m}^{-2}$ for maize, $210 \pm 30 \text{ gC m}^{-2}$ for winter wheat).

1. Introduction

To qualitatively understand and predict changes in atmospheric CO₂, a detailed understanding of the behavior of its major sources and sinks, including soil and the terrestrial biosphere, is necessary. One crucial question in this context is the balance of photosynthesis and respiration, or the deposition of organic matter and its decomposition in soils. For this budget, assimilated plant material, litterfall, and decomposition have to be determined

in different ecosystems. Of these, litterfall is the most easily measured. Accessing the quantity of living plant matter above and below ground is however difficult without disturbing the whole ecosystem. In contrast, the decomposition rate is more easily measured through soil respiration. To derive the microbial decomposition rate from CO₂ flux measurements, the respiration of living plant roots has to be separated. Unfortunately, root respiration cannot in general be distinguished from the microbial decomposition and therefore, in most cases, only approximate estimates exist.

Various authors consider root respiration to contribute 30–70% of soil respiration. Assuming steady state conditions, Trumbore et al. (1995) calculated root respiration from the balance of carbon inputs and losses. In seasonal dry forest

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areas of eastern Amazonia, they found a contribution of root respiration of 50–65% to the CO₂ flux from the soils. In a literature compilation, Raich and Schlesinger (1992) reported that live root respiration contributes 30–70% of the total soil respiration. Due to the fact, that, in general, it is not possible to determine whether measured CO₂ was produced by microbial decomposition or root respiration (Hendry et al., 1993), direct measurements of root-produced CO₂ in natural ecosystems are very rare. Dörr (1980) investigated root respiration in a maize field in southwest Germany. From only 2 measurements he calculated a contribution of root respiration of $(22 \pm 3)\%$.

Here, we present a method which allows the separation of the different sources of CO₂ in the soil in an agricultural system. The presented method is, however, only applicable to ecosystems with crop rotation from C3 and C4 plants or vice versa.

2. Separation of soil CO₂ sources by means of $\delta^{13}\text{C}$ measurements in soil air

The isotope ^{13}C in CO₂ offers, in some cases, the opportunity to separate CO₂ produced by decomposition and root respiration. Plants can be divided into 3 groups with different photosynthetic cycles (C3, C4, CAM = crassulacean acid metabolism). These different metabolic pathways discriminate atmospheric $^{13}\text{CO}_2$ relative to $^{12}\text{CO}_2$ in a different manner, leading to characteristic carbon isotopic compositions of the plant material. In the commonly used δ -notation for C3 plants, a mean value of $\delta^{13}\text{C} = (-26.6 \pm 2)\text{‰}$ PDB (Peedee Belemnite) was found, whereas the mean value for C4 plants is $\delta^{13}\text{C} = (-12.6 \pm 2)\text{‰}$ PDB (Vogel, 1980). CAM plants can make use of either C3 or C4 metabolism. All $\delta^{13}\text{C}$ data in this work, except the literature data of Vogel (1980) are expressed relative to VPDB (Hut, 1987) using the delta notation (Craig, 1957):

$$\delta_{\text{sample}} = \frac{R_{\text{sample}} - R_{\text{reference}}}{R_{\text{reference}}} 1000 [\text{‰}], \quad (1)$$

with the isotope ratio $R = [^{13}\text{C}]/[^{12}\text{C}]$.

In the case of 2 sources of CO₂ in the soil, each derived from a different metabolic pathway, e.g., C3 and C4, it is possible to distinguish root

respiration from microbial decomposition by their $\delta^{13}\text{C}$ signature. Unfortunately, in most natural systems both sources originate from the same plant species, and therefore the 2 source components remain indistinguishable. In ecosystems with a vegetation change from plants with C3 metabolism to plants with C4 metabolism (or vice versa), a distinction between both sources becomes possible, even if the decomposed material is a mixture of both kinds of plants. Examples for such ecosystems are the change from tropical forest to pasture (Trumbore et al., 1995) or seasonal changes in agricultural cultivation, e.g., from winter wheat (C3) to maize (C4).

However, even in the latter case an additional difficulty in determining root respiration exists. Here, the C4 derived part of the $\delta^{13}\text{CO}_2$ signal in the soil originates not only from active root respiration but also from decomposition of recent root exudations and recent plant root material (together = decomposed recent root material). Consequently, the CO₂ production by decomposed recent root material and the root respiration are summarized as root-produced CO₂. The part of recent root material which is not decomposed during the time of investigation does not need to be considered because it does not contribute to the CO₂ production in the soil on this time scale.

To compare the results of this work with published data of root respiration, we used the carbon balance calculations by Trumbore et al. (1995) and assume that 2/3 of the root-produced CO₂ originates from root respiration and the rest originates from decomposed root material.

If a change in vegetation takes place from plants with one photosynthetic cycle to plants with another, the isotopic composition of the soil organic carbon (SOC) differs from the isotopic composition of living plant matter. To determine the percentage of root-produced CO₂, a 2 component mixing calculation is applied:

$$\delta^{13}\text{CO}_{2\text{total}} = Y \delta^{13}\text{CO}_{2\text{root}} + (1 - Y) \delta^{13}\text{CO}_{2\text{decomp}} \quad (2)$$

$$Y = \frac{\delta^{13}\text{CO}_{2\text{total}} - \delta^{13}\text{CO}_{2\text{decomp}}}{\delta^{13}\text{CO}_{2\text{root}} - \delta^{13}\text{CO}_{2\text{decomp}}} \quad (3)$$

Y fraction of root-produced CO₂
(versus microbial decomposition)
 $\delta^{13}\text{CO}_{2\text{total}}$ mean isotope ratio of soil CO₂
in a depth profile (‰ VPDB)

$\delta^{13}\text{CO}_{2\text{decomp}}$	isotope ratio of CO ₂ produced by microbial decomposition (‰ VPDB)
$\delta^{13}\text{CO}_{2\text{root}}$	isotope ratio of root-produced CO ₂ (‰ VPDB)

Eq. (2) is an approximation which neglects the influence of different soil CO₂ concentrations in different soil depths. Calculated mean values of $\delta^{13}\text{CO}_{2\text{total}}$ weighted by soil CO₂ concentration profiles show maximum shifts of $\pm 1\text{‰ VPDB}$, usually $\pm 0.4\text{‰ VPDB}$, in the determined $\delta^{13}\text{CO}_{2\text{total}}$ values. Compared to the analytical uncertainty of the CO₂ concentration measurements, this is negligible (see Subsection 3.3).

3. Sampling site and experimental methods

3.1. Sampling site

The Weiherbach area is located in the Kraichgau region in southwest Germany (30 km south east of Heidelberg, 200 m above sea level). The sampling sites are located in an area with loess/loam soils and agricultural land use. The crops at the 2 sites are rotated according to a regular scheme within a 2-year cycle. Maize (C4) is grown in the first year from April to September. Afterwards, winter wheat (C3) is sown in October and harvested in July of the following year. Thereafter, in September, mustard (C3) is grown until February of the third year and the field remains bare until April when again maize is sown. This cycle is shifted by one year for the second sampling site. These rotations have been performed at both sites for more than 5 years.

The winter wheat is harvested completely, i.e., only the below ground root matter remains in the soil. In contrast, after harvest of the maize, 45% of the above ground plant matter is mixed into the first 10–20 cm of the soil. Mustard is grown as green manure, its whole biomass is ploughed into the soil at the end of February.

3.2. Sampling methods

Soil air was collected through stainless steel probes (2–4 mm inner diameter) vertically installed in the fields at 7 different depths (10, 15, 20, 30, 50, 70, 100 cm). At the beginning of the

sampling, the probes were flushed with 100 ml of soil air by means of a 50-ml syringe. Then pre-evacuated glass flasks (300 ml) were connected and one set of air samples for CO₂ isotope measurements was sucked into the flasks by under pressure. For concentration analysis (URAS, see following paragraph), the syringe was used to pump a second set of soil air samples (~800 ml each) into 1-liter bags made of polyethylene-coated aluminum foil (TECOBAG, Tesseraux, Germany). This duplicate sampling was necessary because air samples stored in polyethylene bags cannot be used for mass spectrometric analysis of $\delta^{13}\text{CO}_2$ due to contamination of the mass spectrometer with ethylene from the coating.

For maize the total soil respiration rate was directly determined by the inverted cup method (Lundegardh, 1924). The cup had a diameter of 57 cm and a height of 25 cm. The incubation period varied from 30–60 min and the cup was placed interrow. CO₂ concentration analysis at the beginning and at the end of the incubation period were used to calculate the total soil respiration rate.

For winter wheat and mustard it was not possible to place the cup interrow, i.e., for all measurements it was placed over plants and, consequently, the CO₂ concentration at the end of the incubation period cannot be used to calculate the total soil respiration rate. Therefore, in this case, an alternative technique described by Dörr and Münnich (1990), using ²²²Rn exhalation measurements in combination with measurements of the ²²²Rn and CO₂ concentration profiles in the soil, was applied.

Sampling in the field was carried out weekly or bi-weekly for both, soil air and inverted cup measurements.

3.3. CO₂ and $\delta^{13}\text{CO}_2$ analysis techniques

CO₂ concentration was determined by non-dispersive infrared gas analysis (NDIR, URAS 1, Hartmann und Braun AG, Frankfurt, Germany) after diluting samples volumetrically under constant pressures with N₂ (Barth, 1980). The dilution of CO₂ with N₂ is part of the applied CO₂ concentration analysis technique. Cross-checks of CO₂ concentration measurements were made with a GC/FID system (Sichromat 1, Siemens, Karlsruhe, Germany) (Born et al., 1990). The

reproducibility (1σ) of the URAS CO_2 concentration measurements was typically $\pm 5\%$, for the GC measurement $\pm(0.5\text{--}10)\%$ (depending on the CO_2 concentration of the sample). Occasional comparison of measurements of the same sample with both systems showed variations below $\pm 5\%$ for a range of mixing ratios from 350 ppm to 60 000 ppm. Measurements of the CO_2 concentration of samples taken in glass flasks as well as in polyethylene bags also showed differences below $\pm 5\%$. $\delta^{13}\text{CO}_2$ was measured by isotope ratio mass spectrometry (Finnigan MAT 252 with Multiport Trapping Box C) with a precision of $\pm 0.03\text{‰}$ (1σ) (Neubert, 1998). To determine the $\delta^{13}\text{C}$ of dry plant material, the dry organic matter was first combusted and then the $\delta^{13}\text{CO}_2$ was determined by CO_2 by isotope ratio mass spectrometry.

4. Results and discussion

In order to apply the 2 component mixing calculation (eq. (2)), the isotope ratio of the different CO_2 sources in the soil, i.e., microbial decomposition of C_{org} and root-produced CO_2 , were determined.

4.1. Isotope ratio of root-produced CO_2

The isotope ratio of root-produced CO_2 is assumed to equal the isotopic composition of dry root organic matter. The carbon isotope composition of root matter is about 0.7‰ lower than the weighted average isotope composition of total plant material (Schnyder, 1992). Measurements of the isotope composition were made for above ground plant material consisting of leaves, peduncle and roots. We assume, that the obtained values $-\delta^{13}\text{C} = (-13.2 \pm 0.1)\text{‰}$ VPDB for maize ($n = 3$, uncertainty = 1σ), $\delta^{13}\text{C} = (-27.0 \pm 0.2)\text{‰}$ VPDB for winter wheat ($n = 2$) and $\delta^{13}\text{C} = (-28.8 \pm 0.2)\text{‰}$ VPDB for mustard ($n = 2$), represent the weighted average isotope composition of total plant material. Using these values the isotope composition of root plant material is calculated to be: $\delta^{13}\text{C} = (-13.9 \pm 0.1)\text{‰}$ VPDB for maize, $\delta^{13}\text{C} = (-27.7 \pm 0.2)\text{‰}$ VPDB for winter wheat and $\delta^{13}\text{C} = (-29.5 \pm 0.2)\text{‰}$ VPDB for mustard.

4.2. Isotope ratio of CO_2 originating from decomposed soil organic carbon

Direct measurements of the ^{13}C content of soil organic carbon (SOC) were not performed. Instead, the $\delta^{13}\text{CO}_2$ in soil air was determined prior to planting. Considering the diffusive enrichment of $^{13}\text{CO}_2$ in soil air (Dörr and Münnich, 1980) due to its smaller diffusion coefficient $D(^{13}\text{CO}_2)$ compared to $D(^{12}\text{CO}_2)$ ($D(^{12}\text{CO}_2)/D(^{13}\text{CO}_2) \equiv \alpha = 1.0044$), the ^{13}C content of the soil organic matter can be estimated, neglecting any fractionation during the decomposition process (Balesdent et al., 1987). The $\delta^{13}\text{C}$ of the soil organic matter varies from year to year because of the different isotope ratio of the deposited organic matter. In April 1993, just before the planting of maize, the mean ^{13}C content of the soil organic matter was found to be $\delta^{13}\text{C}_{\text{org}} = (-26.0 \pm 0.3)\text{‰}$ VPDB. For further calculations, it was also assumed, that the carbon isotopic composition of the soil organic matter only changed after the harvest, when plant residues were added to the soil, i.e., all changes in the isotopic composition of soil CO_2 are attributed to activities of the living plant roots or their fast consumable products.

4.3. Depth profiles of $\delta^{13}\text{CO}_2$ in soil air

Figs. 1a,b show depth profiles of $\delta^{13}\text{CO}_2$ in soil air at different times of the year corresponding to different vegetation phases. Note, that the maximum change of $\delta^{13}\text{CO}_2$ in soil air during the growth of maize is about $+(5\text{--}6)\text{‰}$, whereas for winter wheat, a change of only -2‰ occurs. This is due to the isotopic composition of the bulk soil organic matter ($\delta^{13}\text{C}_{\text{bulk}} = (-26 \text{ to } -25)\text{‰}$ VPDB) being closer to the isotope ratio of winter wheat. Calculated percentages of root-produced CO_2 , therefore, show a much larger uncertainty for winter wheat than for maize.

4.4. Seasonal pattern of the sources of CO_2 production in soil

Fig. 2 shows combined results from sites 1 and 2 of soil temperature, vegetation height and percentage of root-produced CO_2 for a crop rotation period of 2 years. The amount of root-produced CO_2 increases during the growing season up to 65% of the total CO_2 soil respiration rate and

Fig. 1. Typical $\delta^{13}\text{CO}_2$ profiles in soil air at Weiherbach sampling site 1 for maize (a) and winter wheat (b). The profiles at different times of the year correspond to different vegetation phases.

correlates with the vegetation height ($R^2 \geq 0.88$) except for the last month. This correlation, in Fig. 3 only shown for maize, is very similar for the 2 sampling sites and in different years.

Figs. 4, 5 display monthly means of absolute values of soil respiration as well as root and microbially produced CO₂. Because similar patterns of the sources of CO₂ production in soil were found for both sampling sites, Figs. 4, 5 refer only to the site with the highest sampling frequency (site 1). To compare maize and winter wheat, calculated percentages were converted to total fluxes by multiplying by the total soil respiration rate, which at the maize field is twice as high as at the winter wheat field. This higher decomposition rate is due to the late start in the growing season for maize (May), when the soil temperature in the maize field soil is already higher than in the winter wheat field soil (Fig. 2). Winter wheat shows a strong rise in soil respiration rate from February to March 1994 also driven by a steep

increase of the soil temperature (Fig. 2). Root-produced CO₂ is negligible until April, then becomes comparable to the microbially produced CO₂ and is constant until June. The decrease in this component in July is caused by lower activity of the wheat plants towards the end of their growing season.

Up to March and for both vegetation types, only microbial decomposition contributes significantly to the soil respiration, because winter wheat plants were small and no maize was growing at this time (Fig. 2). Then, in April, winter wheat plants begin to grow significantly. Maize plants stay small until the end of June, when the Leaf Area Index (LAI) of winter wheat is about twice the LAI of maize. Higher values of the LAI cause lower direct solar insolation onto the soil surface and therefore, in May (and in June), the soil temperature is higher in the maize field soil than in the winter wheat field soil (by about 5°C at a depth of 10 cm). Assuming a Q_{10} value of 2 (Raich

Fig. 2. Combined results for the Weiherbach sampling sites 1 and 2 of (a) soil temperature (10 cm depth), (b) vegetation height and (c) the percentage of root-produced CO₂ for one 2-year crop rotation period. The vertical lines indicate the time of the harvest.

Fig. 3. Correlation between vegetation height and root-produced CO₂ for maize (linear regression: $R^2 \geq 0.88$). The same slope was observed for all 3 years. Values obtained at the end of the respective growing season are shown as open symbols. At this time, lower plant activity resulted in smaller amounts of root-produced CO₂.

Fig. 4. Monthly means of the different sources of soil produced CO₂ for winter wheat (Weiherbach sampling site 1, 1994).

Fig. 5. Monthly means of the different sources of soil produced CO₂ for maize (Weiherbach sampling site 1, 1993). After harvest, the $\delta^{13}\text{C}$ signal of C4-plants in the maize field was interpreted as microbial decomposition of plant residues and dead roots.

and Potter, 1995; Schüller, 1996), soil respiration in the maize field should be 40% higher compared to the winter wheat field. This difference was actually found in May (Figs. 4, 5).

At the maize field, a steep increase in total soil respiration rates was observed from April until the end of June due to rising soil temperatures caused by direct solar insolation onto the soil

surface (Fig. 5). The sharp decrease in July reflects the fact, that the now mature maize plants ($\text{LAI}_{\text{June}} \approx 2$, $\text{LAI}_{\text{July}} > 3$) shield the soil surface from direct radiation. The rising production of CO₂ by the maize roots at this time of the growing season was, however, too low to compensate for this effect. Similar to winter wheat, a decrease of root-produced CO₂ was observed towards the end

of the growing season. After harvest, the $\delta^{13}\text{CO}_2$ signal from maize can be explained by microbial decomposition of plant residues and dead roots.

4.5. Contribution of root-produced CO_2 to the total annual CO_2 production

Total CO_2 production is calculated from the monthly means of the directly measured CO_2 flux rates. Multiplying these values with the monthly mean of the percentages of root-produced CO_2 yields the total root-produced CO_2 for each month. From these values the amount of root-produced CO_2 during the growing season is determined (Table 1). The contribution of root-produced CO_2 to the total annual CO_2 production is determined by normalizing the amount of root-produced CO_2 during the growing season to the total annual CO_2 production. This is possible because of the special crop rotation scheme.

Our calculated values of root-produced CO_2 , $(16 \pm 4)\%$ for maize and $(24 \pm 4)\%$ for winter wheat of the annual soil respiration rate, are less than the lower end of the estimates found in the literature of 30–70% (Trumbore et al., 1995; Raich and Schlesinger, 1992). This is possibly due to shorter growing periods of maize and winter wheat, which is only 4–5 months, compared to natural vegetation (>6 months). Higher soil temperatures on cultivated land, compared to, e.g., forest ecosystems, may be another reason.

4.6. CO_2 production per gram dry organic maize root matter (DORM)

The portions of maize plant material above and below ground at the Weiherbach sampling site

Table 1. Total amounts of the CO_2 production in the soil for maize and winter wheat

CO_2 production in the soil ($\text{gC m}^{-2} \text{a}^{-1}$)	Maize	Winter wheat
total annual soil respiration	450 ± 50	210 ± 30
annual decomposition of SOC	380 ± 40	160 ± 20
total root-produced	70 ± 10	50 ± 10

Due to the special crop rotation scheme at the sampling site, the amount of root-produced CO_2 during the growing season equals the annual amount of root-produced CO_2 .

were determined by Dahmen (1994) for the year 1993. From these data the root/shoot ratios were calculated for the course of the growing season (Fig. 6). Region I corresponds to the period right after the germination, when maize plants are still very small (above ground plant matter $< 3 \text{ g m}^{-2}$, height $< 10 \text{ cm}$). At this time the plants mainly consist of roots. Therefore, the calculated root/shoot ratio is rather large (0.8 to 2). Region II displays the root/shoot ratio for the vegetative growth, after the plants were grown to a considerable size (above ground plant matter $10\text{--}500 \text{ g m}^{-2}$, height $< 20\text{--}120 \text{ cm}$). In this region the root/shoot ratio decreases to values of about 0.3 to 0.7, comparable to values found in the literature (0.5, Lieth and Whittaker, 1975). Towards the end of the growing season (Region III) when the maize plants are mature (above ground plant matter $> 1000 \text{ g m}^{-2}$, height $> 170 \text{ cm}$) the generative growth starts and root/shoot ratios of 0.2–0.3 are found.

Using this final root/shoot ratio, the amount of root matter at harvest is calculated from the above ground biomass at harvest, i.e., $2000 \pm 200 \text{ g m}^{-2}$ (Dahmen, 1994), to be $500 \pm 100 \text{ g m}^{-2}$. The ratio of total root-produced CO_2 and maize root material at the harvest yields the mean amount of root-produced CO_2 per g DORM for maize, namely, $0.14 \pm 0.13 \text{ gC (g DORM)}^{-1}$.

5. Conclusions

$\delta^{13}\text{CO}_2$ measurements in soil air can provide insight into below ground CO_2 production rates if a change from plants with one photosynthetic metabolism to another takes place. This method has the advantage of not disturbing the ecosystem under investigation.

Except for the end of the growing season, when plant activity is reduced, vegetation height and root-produced CO_2 are strongly correlated. Winter wheat and maize show similar patterns in time series of root-produced CO_2 . The patterns are shifted by approximately 2 months due to different growing seasons. The % of root-produced CO_2 differs between the 2 vegetation types, $(16 \pm 4)\%$ for maize and $(24 \pm 4)\%$ for winter wheat, due to different seasonality of the soil respiration rates most probably caused by different soil temperature regimes. The value of root-

Fig. 6. Calculated root/shoot ratios for maize at the Weiherbach sampling site for the course of the growing season 1993 (data taken from Dahmen (1994)).

produced CO₂ per g dry organic maize root matter was found to be $0.14 \pm 0.03 \text{ gC (g DORM)}^{-1}$.

Further investigations are not limited to ecosystems with crop rotation from C3 to C4 plants (or vice versa). Another possibility for applying $\delta^{13}\text{CO}_2$ measurements in soil air to determine root-produced CO₂ is to change the isotopic composition of the atmosphere surrounding the plant, which may be done with chambers. This method should work because plants discriminate against (constant) atmospheric $^{13}\text{CO}_2$ by a more or less constant value (Vogel, 1980). Consequently, a shift of atmospheric $\delta^{13}\text{CO}_2$ values by, e.g., 10‰ will cause a shift of 10‰ in $\delta^{13}\text{CO}_2$ values of root-produced CO₂. By this means the metabolic rate

of the species under investigation could also be assessed.

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