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Original article

Root-derived respiration and non-structural carbon of rice seedlings

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ABSTRACT

Various methods have been suggested to separate root and microbial contributions to soil respiration. However, to date there is no ideal approach available to partition below-ground CO₂ fluxes in its components although the combination of traditional methods with approaches based on isotopes seems especially promising for the future improvement of estimates. Here we provide evidence for the applicability of a new approach based on the hypothesis that root-derived (rhizomicrobial) respiration, including root respiration and CO₂ derived from microbial activity in the immediate vicinity of the root, is proportional to non-structural carbon contents (sugars and organic acids) of plant tissues. We examined relationships between root-derived CO₂ and non-structural carbon of rice (*Oryza sativa*) seedlings using ¹⁴C pulse labelling techniques, which partitioned the ¹⁴C fixed by photosynthesis into root-derived ¹⁴CO₂, and ¹⁴C in sugars and organic acids of roots and shoots. After the ¹⁴C pulse ¹⁴C in both sugars and organic acids of plant tissues decreased steeply during the first 12 h, and then decreased at a lower rate during the remaining 60 h. Soil ¹⁴CO₂ efflux and soil CO₂ efflux strongly depended on ¹⁴C pools in non-structural carbon of the plant tissues. Based on the linear regression between root-derived respiration and total non-structural carbon (sugars and organic acids) of roots, non-rhizomicrobial respiration (SOM-derived) was estimated to be 0.25 mg C g⁻¹ root d.w. h⁻¹. Assuming the value was constant, root-derived respiration contributed 85–92% to bulk soil respiration.

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1. Introduction

Separation of root and microbial contributions to bulk soil respiration is an essential prerequisite for a better understanding of the carbon (C) and energy balance of plants, soils and

microorganisms, as well as of environmental factors controlling soil C cycling and C sequestration [30]. In addition, it is also essential for enhancing our capabilities to predict terrestrial ecosystem responses to climate change [3]. Hence, various attempts have been made to separate their

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contributions to the total CO₂ efflux, each of which has its advantages and disadvantages [3,16,26,30,41,45]. While there is no simple solution to partition soil CO₂ efflux, the combination of traditional methods with those based on isotopes seems especially promising for the future improvement of estimates [3,26]. Methodological advances are especially needed towards finding an approach to separate root and soil microbial contributions to soil CO₂ efflux, which will be widely applicable in the field [3,26,30,41].

Roots directly and indirectly contribute to bulk soil respiration through actual root respiration and rhizomicrobial respiration, which is based on the utilization of recently fixed C and does not have an effect on the long-term C balance in soils [26]. In this study we used the term “root-derived CO₂” for the sum of actual root respiration and CO₂ derived from microbial activity in the immediate vicinity of the root (rhizomicrobial respiration) and “SOM-derived CO₂” for microbial decomposition of soil organic matter (SOM) in root-free soil [26]. Root respiration is considered to be fuelled by non-structural carbon (NSC) rather than structural C fixed in cell walls of roots. Of NSC species (starch, organic acids and soluble sugars such as glucose, fructose and sucrose), starch is formed as a transitory reserve at times when the supply of soluble sugars exceeds the actual demand within the plant [16]. Soluble sugars and organic acids in plant tissues are important substrates for root respiration, which has been confirmed by a large number of studies. For instance, respiratory utilization of photosynthates such as sucrose in roots provides the essential energy for nutrient uptake, root growth and maintenance, as well as for symbiotic processes and defences [5,13,14,33,47]. A variety of organic acids are also involved in important respiratory pathways such as the Krebs cycle [37,39]. This implies that the concentration of NSC such as soluble sugars and organic acids might control root respiration, as suggested by previous studies [6,12]. Moreover, it has been shown that exogenously added sugars rapidly increase respiration rates of roots [42] and shoots [2,23].

Microbes in the rhizosphere rely on rhizodeposits from the root system for their maintenance and growth, where a fraction of this C is released through their respiration. Rhizodeposits are made up of root exudates, mucilage, dead root hairs and sloughed-off rhizodermal cells. Exudates are most directly linked to photosynthesis and are released by roots into the soil in the form of a great variety of organic compounds [34,46], comprising between 8 and 12% of the net primary production of terrestrial ecosystems [15,38]. Sugars are most abundant in root exudates and represent the most significant C input into the rhizosphere [35]. They are rapidly utilized by microbes in the rhizosphere, thereby contributing to rhizomicrobial respiration [29] and regulating rhizomicrobial activity [1,43]. In this context it has been shown that photosynthesis controls CO₂ efflux from the maize rhizosphere [28]. In addition, several studies showed indirectly that root-derived respiration is proportional to both, above- and below-ground plant biomass [9,19].

Although there is evidence that root respiration is also strongly regulated by the supply of adenosine diphosphate, this control is generally considered to operate at the biochemical level over short time periods (minutes to hours) [11]. On a whole plant basis, however, root respiration is

controlled by the supply of NSC substrates over longer time periods (several hours to days) [11]. In addition, recent findings demonstrated that sugars are involved in the regulation of respiratory gene expression [11]. This implies a strong potential to separate root-derived respiration from the total CO₂ efflux using the relation of soil CO₂ efflux to the NSC pool of plant tissues. However, so far no direct measurements of the relation between root-derived CO₂ and NSC pools of plant tissues are available, although a causal relationship between root carbohydrate content and respiration rate has been widely suggested [31,49].

The goal of this study therefore was to test the hypothesis that root-derived CO₂ is proportional to the NSC pool of plant tissues and depends on C recently-fixed by photosynthesis. Additionally, we partitioned bulk soil respiration into root-derived CO₂ and SOM-derived CO₂ based on the examined correlation. We applied a pulse ¹⁴C labelling technique since ¹⁴C allows us to sensitively trace C flows in the plant-soil system [34]. This would be particularly interesting since ¹⁴C-NSC would represent the active NSC pool and NSC could probably include a significant storage fraction. We aimed to provide evidence for the applicability of a regression-isotope approach.

2. Materials and methods

2.1. Soil description

Soil material was collected from the Ap horizon of a loamy Haplic Luvisol (long-term field experimental station Karlshof of Hohenheim University). The soil originated from loess, contained no CaCO₃, and exhibited the following characteristics: pH 6.0, organic C 1.2%, total N 0.13%, clay 23%, silt 73%, and sand 4.4%. The soil was air-dried and sieved through a 2-mm screen before the experiment.

2.2. Growing conditions

Thirty-five large-volume centrifuge tubes (10 cm in height, 5 cm in diameter) with lids were used to be planted with rice seedlings. A hole was bored in the centre of each lid using a drill and a small centrifuge tube (2 ml, lid and bottom removed by knife), was fixed and sealed in this hole. At the same time, two small holes were bored on both sides of the big hole and two silicone tubes (one close to the bottom of the centrifuge tube and the other above the soil surface) were inserted and sealed in these holes in order to trap CO₂ from the soil compartment after ¹⁴C labeling. Then each centrifuge tube was filled with 50 g of air-dried soil through the hole at the centre of the lid. Homogenously sized rice seedlings were chosen and planted into these small open centrifuge tubes attached to the lid 2 days after germination. The plants were grown for 14 h per day at a light density of 800 μmol m⁻² s⁻¹, and a temperature of 27 ± 1 °C and 22 ± 1 °C (day:night). Soil moisture was controlled by weight and maintained at 35% of the available field capacity. Two and four weeks after planting, NH₄NO₃ was applied at a rate of 10 mg N g⁻¹ soil to maintain N supply.

2.3. Labelling procedure

A Plexiglas chamber (0.5 × 0.5 × 0.6 m) was used for the ^{14}C labelling following the method described in detail by Cheng et al. [8]. The pulse labelling was started 36 days after rice seedlings were planted. Two days before the labelling, the soil water content of each centrifuge tube was adjusted to about 60% of the available field capacity. The day before labelling, plant shoots were sealed at the shoot base (by sealing the hole around the shoot base) using silicon rubber NG 3170 (Fa. Thauer & Co., Dresden, Germany) to separate above- and below-ground compartments and placed in the labelling chamber. A 20 ml glass vial containing 2 ml of 1 mM $\text{Na}_2^{14}\text{CO}_3$ solution (330 kBq) was connected by tubing with the chamber. The chamber was then closed and 5 ml of 0.5% H_2SO_4 was added to the $\text{Na}_2^{14}\text{CO}_3$ solution in the vial through a pipe into the chamber. This allowed complete evolution of $^{14}\text{CO}_2$ into the chamber atmosphere. The labelling started at 10:00 h in the morning and plants were allowed to assimilate $^{14}\text{CO}_2$ for 5 h. Thereafter, the chamber air was pumped through 1 M NaOH solution to remove the remaining unassimilated $^{14}\text{CO}_2$. Then the plants were exposed to normal atmosphere for the chase period of 72 h, with 14 h per day at a light density of $800 \mu\text{mol m}^{-2} \text{s}^{-1}$, and a temperature of 27 °C and 22 °C (day:night).

2.4. Sampling

Each centrifuge tube was connected with two tubes: one was inserted into the soil to about 7 cm in depth, the other tube was situated above the soil surface. After labelling, the tube inserted into the soil was immediately connected to a glass bottle containing 100 ml of a 1 M NaOH solution in order to provide CO_2 -free air to the soil compartment. The other tube was connected to a test tube containing 20 ml of a 1 M NaOH solution and a membrane pump. The CO_2 evolved from the soil-root system was trapped by continuous pumping at a rate of $100 \text{ cm}^{-3} \text{ min}^{-1}$. The NaOH solutions were changed at 1 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h after the labelling. At the same time, plants were harvested through destructive sampling of five centrifuge tubes each and separated into roots and shoots. The roots were carefully removed from the soil and rinsed with distilled water. Then the roots and leaves were immediately killed in a microwave oven (600 W, 90 s). Subsequently, they were dried at 60 °C in a drying oven for 48 h, then weighed and ground to a fine powder using a ball mill (MM2, Fa Retsch) to measure ^{14}C activity in the soluble C fractions.

2.5. Analyses

Sugars and organic acids in plant materials were prepared following the method described by Wanek et al. [48]. Briefly, 40 mg of plant material were placed into 2 ml reaction vials and extracted with 1.5 ml methanol/chloroform/water (12:3:5, v:v:v) for 30 min at 70 °C. The samples were centrifuged at $10,000 \times g$ for 2 min and an aliquot of the supernatant (800 μl) was transferred into a 2 ml reaction vial and

mixed vigorously with 800 μl H_2O and 250 μl chloroform. After phase separation by centrifugation at $10,000 \times g$ for 2 min, 1.2 ml of the upper aqueous phase was transferred into a 2 ml reaction vial for further analysis. Sugars and organic acids were separated by ion-exchange chromatography. Cation-exchange resin (DOWEX 50WX8, 50–100 mesh, H^+ form) and anion-exchange resin (DOWEX 1X8, 50–100 mesh, formate form) were prepared as follows. Anion-exchange resin (4 ml) was filled into a solid-phase extraction cartridge (6 ml volume, inner diameter 13 mm) with a low-density polyethylene frit on the bottom and mounted on a solid-phase extraction manifold. Cation-exchange resin (2.7 ml) was filled in a second SPE cartridge which was mounted on top of the anion-exchange cartridge. An aliquot of the sample (1 ml) was added to the top of the ion-exchange assembly, which was then washed with 25 ml of deionized H_2O . The flow-through containing sugars was collected. Thereafter, the cation-exchange column was removed and 10 ml of 1 M HCl was applied to the anion-exchange column to elute the organic acid fraction.

Total content of CO_2 -C trapped in the NaOH solution was measured by titration of aliquots with 0.2 N HCl against phenolphthalein, after the addition of 0.5 N BaCl_2 solution [4]. The ^{14}C in CO_2 collected in the NaOH solution was measured by a liquid scintillation counter (Wallac 1411 at Hohenheim University, Germany). The ^{14}C in the sugars and organic acids of the plant materials was measured by a liquid scintillation analyzer (C1600 TRICARB at the University of Vienna, Austria).

2.6. Calculation and statistic analysis

The total CO_2 efflux and $^{14}\text{CO}_2$ efflux from soils were expressed as $\text{mg CO}_2\text{-C g}^{-1} \text{ root d.w. h}^{-1}$ and $\text{kDPM g}^{-1} \text{ root d.w. h}^{-1}$, respectively. ^{14}C activities of sugars and organic acids in both roots and shoots were expressed as $\text{kDPM g}^{-1} \text{ root d.w. h}^{-1}$ or $\text{kDPM g}^{-1} \text{ shoot d.w. h}^{-1}$. Similarly, ^{14}C activity of total soluble NSC (TNC, sugars and organic acids were pooled together) in roots or shoots ($^{14}\text{C}_{\text{NSC}}$, kDPM) were expressed as $\text{kDPM g}^{-1} \text{ root d.w. h}^{-1}$ or $\text{kDPM g}^{-1} \text{ shoot d.w. h}^{-1}$.

Since non-rhizomicrobial respiration mainly relies on soil organic matter, the residual soil respiration must be derived from the decomposition of SOM when there is no ^{14}C in the NSC pool left. This allows the estimation of SOM-derived CO_2 from the y-intercept of the linear regression between soil CO_2 efflux and ^{14}C in the NSC of roots, similar to the regression technique described by Kucera and Kirkham [25]. Assuming SOM-derived respiration was constant, we estimated the contribution of root-derived CO_2 to total soil respiration.

The significance of the differences in ^{14}C activity between sugars and organic acids in roots or shoots was examined using one-way analysis of variance (ANOVA). Critical LSD values for 5% error probability were calculated. The standard errors of means are presented in the figures as a variability parameter. Linear regressions were calculated between ^{14}C activities of TNCs in plant tissues and soil $^{14}\text{CO}_2$ efflux. This was performed using SigmaPlot 9.0 software package.

3. Results

3.1. ^{14}C activity of NSC and $^{14}\text{CO}_2$ efflux

Highest ^{14}C activities of sugars and organic acids in both shoots and roots were shown 1 h after the ^{14}C labelling (Fig. 1a,b). In shoots ^{14}C activities of organic acids were only one-tenth that of sugars' (Fig. 1a). By comparison, ^{14}C activities of sugars and organic acids in roots were within about the same range (Fig. 1b). All values decreased within the first 6 h steeply and then declined at a lower rate in the remaining 60 h. $^{14}\text{CO}_2$ was also highest 1 h after ^{14}C labelling

with a sharp decline until hour 6, but compared to the TNCs further decline was less distinct (Fig. 1c).

3.2. Relationships between $^{14}\text{CO}_2/\text{CO}_2$ efflux and ^{14}C of NSC

Soil CO_2 as well as $^{14}\text{CO}_2$ efflux correlated positively with ^{14}C activity of the NSC of the rice seedlings (Figs. 2–4). Soil $^{14}\text{CO}_2$ efflux continuously decreased with decreasing ^{14}C activity of sugars ($R^2 = 0.77$, $P < 0.0001$; Fig. 2a) and organic acids ($R^2 = 0.67$, $P < 0.0001$; Fig. 2b) in roots. Likewise, soil $^{14}\text{CO}_2$ efflux also showed a decreasing trend with lower ^{14}C activity

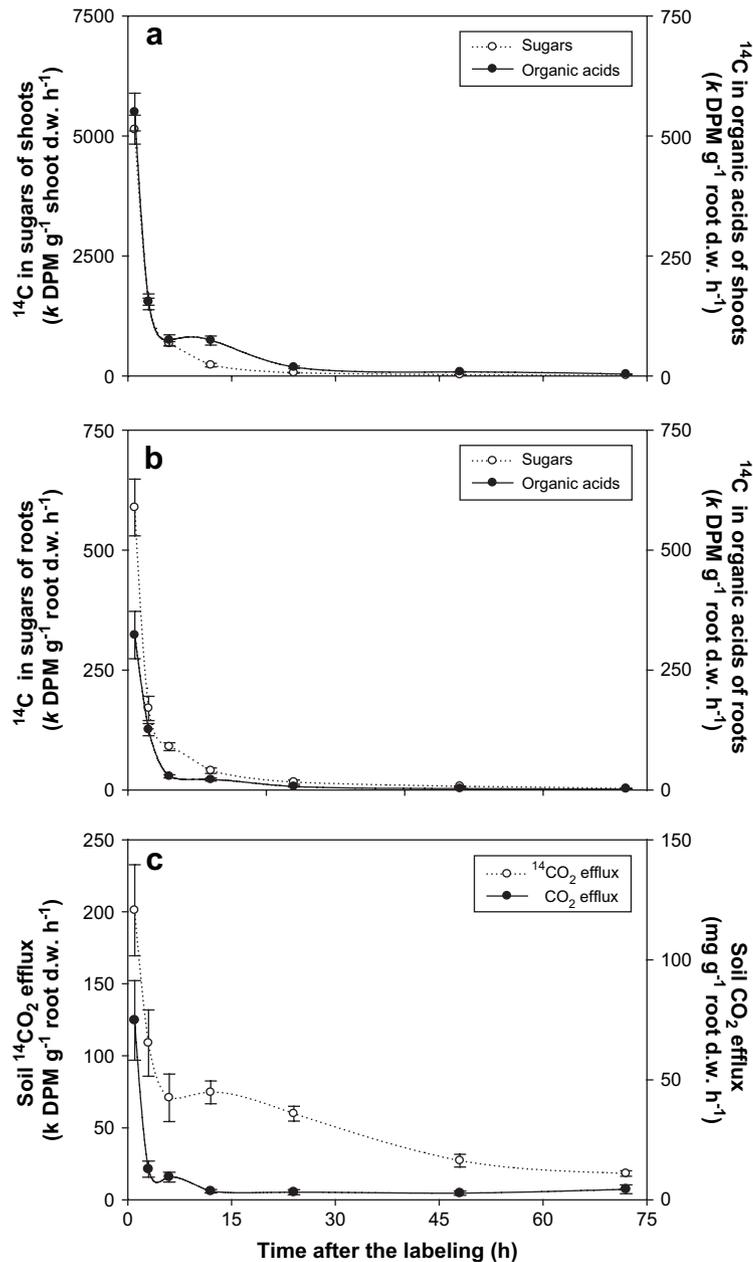


Fig. 1 – Dynamics of ^{14}C activity of the NSC, i.e. sugars and organic acids in shoots (a) and roots (b) of rice seedlings, as well as soil $^{14}\text{CO}_2$ efflux and soil CO_2 efflux (c) after exposing the plants to a $^{14}\text{CO}_2$ atmosphere. Values are means \pm SE of five replicates.

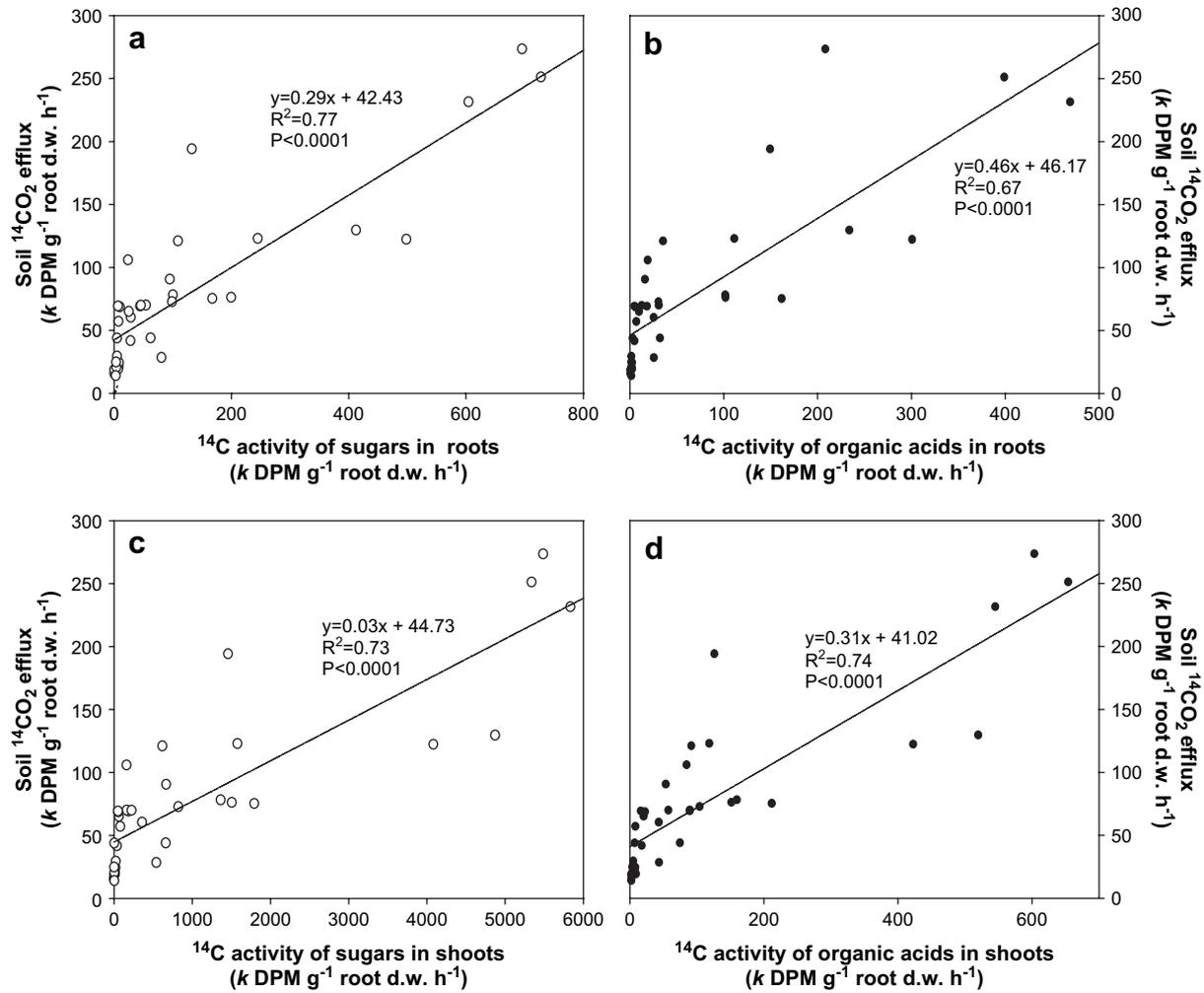


Fig. 2 – Relationship between soil $^{14}\text{CO}_2$ efflux and ^{14}C activity of the NSC fractions of different plant tissues. Correlation between soil $^{14}\text{CO}_2$ efflux and ^{14}C activity of (a) sugars in roots, (b) organic acids in roots, (c) sugars in shoots, and (d) organic acids in shoots.

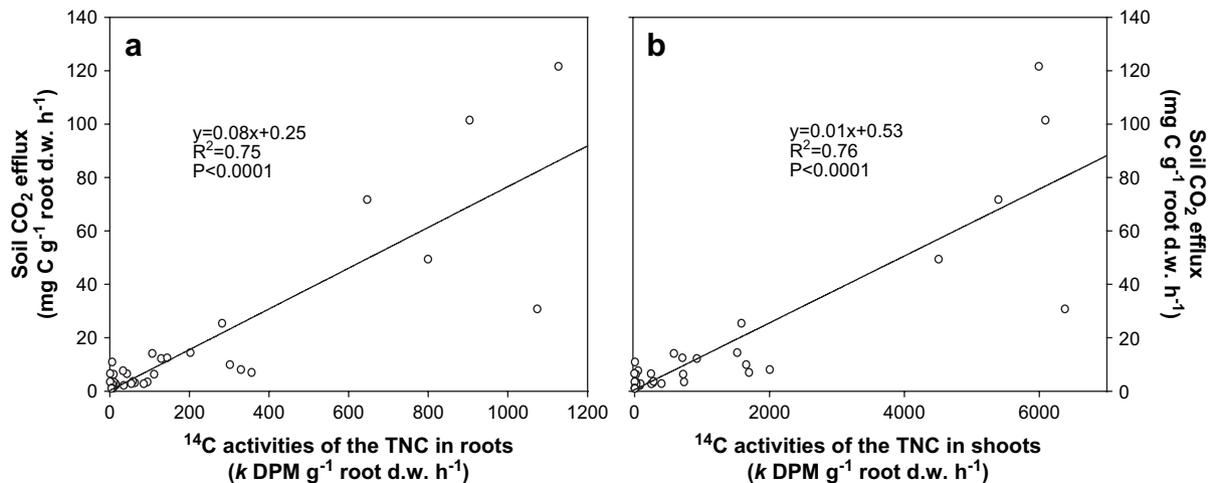


Fig. 3 – Relationship between soil CO_2 efflux and ^{14}C activity in the TNC of different plant tissues. Correlation between soil CO_2 efflux and ^{14}C activity of the TNC in (a) roots and (b) shoots.

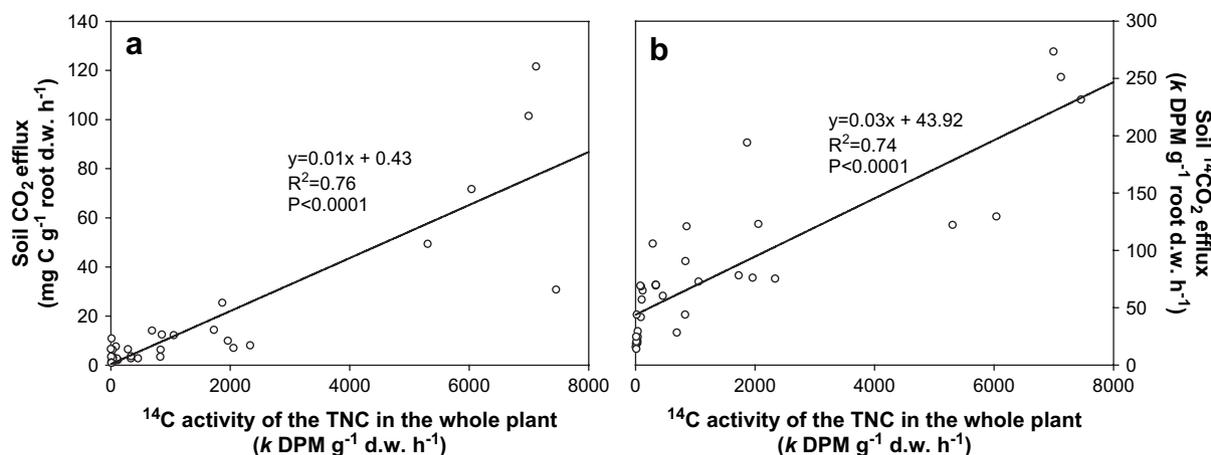


Fig. 4 – Relationship between soil respiration and ^{14}C activity in TNC of the whole plant. Correlation between (a) soil CO_2 efflux or (b) soil $^{14}\text{CO}_2$ efflux and ^{14}C activity of TNC of the whole plant.

of sugars ($R^2 = 0.73$, $P < 0.0001$; Fig. 2c) and organic acids ($R^2 = 0.74$, $P < 0.0001$; Fig. 2d) in shoots. A very similar correlation was also found between soil CO_2 efflux and ^{14}C activity of TNC in roots and shoots ($R^2 = 0.75$ vs. 0.76 ; Fig. 3). On a whole plant level, soil CO_2 efflux also showed very similar correlations with ^{14}C activities of the TNC (Fig. 4a). Soil $^{14}\text{CO}_2$ efflux also showed a significant correlation with ^{14}C activities of TNC of maize seedlings on a whole plant basis ($R^2 = 0.74$, $P < 0.0001$; Fig. 4b).

SOM-derived CO_2 was estimated to be about $0.25 \text{ mg C g}^{-1} \text{ root d.w. h}^{-1}$ (Fig. 3a) using the regression between soil CO_2 efflux and ^{14}C activities of the TNC pool in roots, based on the hypothesis that root-derived respiration is fuelled by root NSC. Considering the role of adenosine diphosphate in controlling root respiration over short time periods (minutes to hours), we estimated the contribution of root-derived CO_2 to soil CO_2 efflux only using CO_2 efflux data $>6 \text{ h}$ after the ^{14}C pulse. The contribution was estimated to be in the range of 85 and 92%.

4. Discussion

This study demonstrates a direct relationship between root-derived $^{14}\text{CO}_2$ and the NSC content (^{14}C activity) of plant tissues using a pulse ^{14}C labelling technique. In this study we partitioned the ^{14}C fixed by photosynthesis into three fractions: root-derived CO_2 , sugars and organic acids of plant tissues. We showed that root-derived $^{14}\text{CO}_2$ is strongly dependent on changes in the ^{14}C activity of the NSC pools of plant tissues (Fig. 2). This provides additional evidence that root-derived respiration is highly dependent on the supply of photosynthates to roots [10,18,21,22,28,36,40]. Actually, several approaches to disentangle root-derived (rhizomicrobial) and non-rhizomicrobial contributions to soil respiration also rely on the relationship between root respiration and the NSC in plants, e.g. tree girdling, the regression technique, shading and clipping, as well as clear felling [26]. Among them, tree girdling has successfully been applied to partition

autotrophic (rhizomicrobial) respiration and heterotrophic respiration in forests [20,24,44], based on the fact that shortly after girdling the interruption in the flow of assimilates from leaves to roots leads to strong suppression of root and rhizomicrobial respiration. Similarly, shading and clipping as well as clear felling also led to a reduction in photosynthate flow from leaves to roots [26]. By comparison, the regression technique is based on an assumed linear relationship between root biomass and the amount of CO_2 produced by roots and rhizosphere microbes [26].

In the current study the contribution of root-derived respiration to soil CO_2 efflux was quantified using a regression-isotope approach. The contribution was high due to the large fraction of rhizosphere soil, ranging from 85% to 92% (average of 88%), but still fell into the range of published contributions of root-derived respiration to soil CO_2 efflux [17,44]. Similar results were also reported for a number of crop and grass species [7,27,28,32].

We did not measure the NSC content of plant tissues in this study. This to some extent affects the reliability of our estimation, but our results still provide significant support for the combined regression-isotope approach, as suggested recently by Baggs [3], that the combination of non-invasive regression analysis with stable isotope approaches would be the way forward to partition soil CO_2 efflux. Theoretically, the refined regression approach, based on the NSC content of roots and root biomass, almost certainly provides a better estimate of root respiration than bulk root biomass alone, since the structural C fixed in cell walls, comprising the major root C pool, cannot be utilized for respiration by roots and is only slowly utilized by rhizospheric microbes. Actually, the observation by Kuzyakov and Cheng [28] that photosynthesis controls CO_2 efflux from the maize rhizosphere also provides strong support in this regard. Nevertheless, this approach needs to be further improved by examining the relationship between soil CO_2 efflux and the actual NSC content of plant tissue in future research. Using $^{13}\text{CO}_2$ instead of $^{14}\text{CO}_2$, this approach will be more widely applicable under field conditions.

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