

Review

Sources of CO₂ efflux from soil and review of partitioning methods

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Abstract

Five main biogenic sources of CO₂ efflux from soils have been distinguished and described according to their turnover rates and the mean residence time of carbon. They are root respiration, rhizomicrobial respiration, decomposition of plant residues, the priming effect induced by root exudation or by addition of plant residues, and basal respiration by microbial decomposition of soil organic matter (SOM). These sources can be grouped in several combinations to summarize CO₂ efflux from the soil including: root-derived CO₂, plant-derived CO₂, SOM-derived CO₂, rhizosphere respiration, heterotrophic microbial respiration (respiration by heterotrophs), and respiration by autotrophs. These distinctions are important because without separation of SOM-derived CO₂ from plant-derived CO₂, measurements of total soil respiration have very limited value for evaluation of the soil as a source or sink of atmospheric CO₂ and for interpreting the sources of CO₂ and the fate of carbon within soils and ecosystems. Additionally, the processes linked to the five sources of CO₂ efflux from soil have various responses to environmental variables and consequently to global warming. This review describes the basic principles and assumptions of the following methods which allow SOM-derived and root-derived CO₂ efflux to be separated under laboratory and field conditions: root exclusion techniques, shading and clipping, tree girdling, regression, component integration, excised roots and *in situ* root respiration; continuous and pulse labeling, ¹³C natural abundance and FACE, and radiocarbon dating and bomb-¹⁴C. A short sections cover the separation of the respiration of autotrophs and that of heterotrophs, i.e. the separation of actual root respiration from microbial respiration, as well as methods allowing the amount of CO₂ evolved by decomposition of plant residues and by priming effects to be estimated. All these methods have been evaluated according to their inherent disturbance of the ecosystem and C fluxes, and their versatility under various conditions. The shortfalls of existing approaches and the need for further development and standardization of methods are highlighted.

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Keywords: CO₂ flux partitioning; Carbon sequestration; Rhizosphere and root respiration; Soil organic matter; Priming effect; Respiration by autotrophs and heterotrophs; ¹⁴C; ¹³C; ¹³C natural abundance; Mean residence time; MRT; Turnover rates; Separation methods

1. Introduction

After photosynthesis, CO₂ flux from soil remains the second largest carbon (C) flux in most ecosystems, and can account for 60–90% of total ecosystem respiration (Goulden et al., 1996; Longdoz et al., 2000). The realization that soils are a possible source of atmospheric CO₂, together with the continuous increase in atmospheric CO₂ concentration, has given rise to numerous methods to measure soil CO₂ efflux from ecosystems all over the world (Raich and Schlesinger, 1992). Despite the increasing popularity of the eddy covariance technique to assess ecosystem C exchange (Balocchi, 2003), classical chamber methods with measurement of CO₂ either by, infrared

gas analyzer or trapping in alkali, remain useful tools (Davidson et al., 2002). This is not only due to some limitations of the eddy covariance technique (Balocchi, 2003), and its comparatively high purchase and installation costs, but especially because chamber methods allow CO₂ fluxes to be measured directly from the soil. Micrometeorological techniques are only able to obtain the total CO₂ efflux and cannot partition total efflux into its individual sources (Buchmann, 2002).

Some studies have simultaneously used eddy covariance and chamber methods to separate net ecosystem CO₂ exchange from soil respiration (Lavigne et al., 1997; Dore et al., 2003), as well as to correct fluxes obtained by eddy correlations during night periods (Anthoni et al., 1999; Law et al., 1999; Dore et al., 2003). Most chamber methods and all micrometeorological techniques are only useful to obtain the total CO₂ efflux without differentiation of the CO₂ sources. In contrast to fluxes of other greenhouse gases (CH₄, N₂O), values for total CO₂ efflux from soil do not provide sufficient information to

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decide whether the soil is a net source or net sink for atmospheric CO₂. This is because not all of the CO₂ coming from the soil is soil-derived, i.e. it is not all produced by the decomposition of soil organic matter (SOM, see Section 2).

The majority of soils in the world are covered with vegetation, and the vegetation may contribute strongly to the total CO₂ efflux by root and rhizomicrobial respiration. However, this contribution of vegetation to total soil CO₂ flux has no effect on long-term C balance in soils. The earliest studies of soil CO₂ flux concluded that the contribution of the vegetation itself (Reiners, 1968; Anderson, 1973), as well as vegetation induced changes of soil organic matter turnover (Dormaer, 1990; Cheng and Kuzyakov, 2005) strongly limit the use of total CO₂ efflux to determine whether the soil is a net source or a net sink of CO₂ and to estimate C balance in various ecosystems. Nevertheless, a number of studies continue to interpret total CO₂ efflux from soil as a direct measure of SOM decomposition (references in Hanson et al., 2000).

Over the last few decades, various methods allowing the separation of several CO₂ sources and the calculation of their contribution to total CO₂ efflux from soil have been developed and used under both laboratory and field conditions. However, the basic assumptions and principles of these methods, and the results obtained in the original papers, all differ from one another. It remains unclear whether the variation in results is method-dependent or reflects varying experimental conditions, i.e. various soils, plants, equipment, and environmental conditions etc. In some cases, the same method has been applied in different experiments to separate differing sources of soil CO₂ efflux. Additionally, a number of various terms are used to refer to the same CO₂ source, which makes the comparison of results obtained by various research groups very difficult. Therefore, the aims of this review are:

- (1) to reveal the most important sources of CO₂
- (2) to clarify the terminology
- (3) to describe the basic principles and assumptions, and identify possible sources of error, in both field and laboratory methods used to estimate the contribution of various C pools to the total CO₂ efflux from soil.

Although the contribution of diverse sources to the total CO₂ efflux from soils of various ecosystems is very important for the construction of global, regional and local C balances, this topic is not included in this review. The contribution of CO₂ derived from the sum of root and rhizomicrobial respiration to the total CO₂ efflux from soil was recently reviewed by Hanson et al. (2000), who dealt mainly with forest ecosystems.

2. Sources of CO₂ efflux from soil

2.1. Background

A classification of sources of CO₂ efflux from soils applicable to a range of investigations should consider:

- C pools in the soil and on the soil surface,
- the agents contributing to CO₂ efflux: heterotrophic or autotrophic organisms,
- the location of CO₂ production: root-free or root-affected soil (rhizosphere), and the litter horizon,
- turnover rates and mean residence times of the C pools contributing to CO₂ efflux,
- the contribution of individual CO₂ sources in the soil to changes in atmospheric CO₂.

These last two points will be considered in the context of the soil CO₂ flux classifications.

2.1.1. C pools

The three C pools that can be recognized as sources of CO₂ efflux from soil include (1) the SOM (see below for turnover of several pools within SOM), (2) above and below ground dead plant residues, and (3) organic substances released by living roots such as exudates, secretions, and sloughed-off root cells. The last group is frequently described as rhizodeposits (reviewed by Nguyen, 2003). Despite large differences in chemical composition, turnover times in the soil, and contribution to the total CO₂ efflux, these three pools have no sharp boundaries. There are many dead plant residues in the soil that are partly humified, thus part of SOM, and there are many rhizodeposits that originated from dead roots or root parts (root turnover).

2.1.2. Agents of CO₂ production in the soil

There are two main groups of organisms: heterotrophic and autotrophic organisms. The most important heterotrophs in the soil can be subdivided into two broad groups: (1) soil microorganisms (bacteria, fungi, actinomycetes and protozoans) and (2) soil macrofauna (macroscopic invertebrates and small mammals). The contribution of soil macrofauna to total CO₂ efflux from soils is usually only a few percent (Andren and Schnurer, 1985; Konate et al., 2003; Ke et al., 2005). Most CO₂ evolved by heterotrophic soil organisms is respired by microorganisms such as bacteria, non-mycorrhizal and mycorrhizal fungi, and actinomycetes. This component of soil CO₂ flux will hereafter be referred to as ‘microbial respiration’. Although the direct contribution of soil macrofauna is small, they can greatly increase microbial respiration not only by fragmentation and comminution of plant residues (Couteaux et al., 1991; Bonkowski et al., 2000), but also by predation of some groups of microorganisms (Clarholm, 1985; Griffiths, 1994; reviewed by Bonkowski, 2004). This intensifies their turnover rate and results in increasing CO₂ efflux from the soil (Mikola and Setälä, 1998; Wardle et al., 1998; Lavelle and Gilot, 1994).

Plants are the most important autotrophs contributing to CO₂ efflux from soil by root respiration. Other autotrophs: algae and chemolithotrophs are of minor importance. Algae cover only the upper 2–3 mm of the soil surface and are active mainly on hydromorphous and flooded soils. Chemolithotrophs make only a marginal contribution to CO₂ efflux from well-aerated soils. Furthermore, in contrast to higher plants, algae

and chemolithotrophs are unicellular organisms and thus, the location of C fixation (assimilation) and respiration is the same. So, for algae and chemolithotrophs, there is no necessity to separate both fluxes because respiration is compensated for by assimilation at the same location.

2.1.3. Locations of CO₂ production

Within the scope of this review, only three locations of CO₂ production are of interest: root-free soil, root-affected soil, and the O horizon. This separation is based on many studies, which have shown that the processes contributing to CO₂ production are greatly changed in the presence of growing roots (see below). In addition, the O horizon, which contains dead above-ground plant residues (and frequently contains a large amount of living roots), is extremely important for CO₂ efflux from soil.

2.2. Sources of CO₂ efflux from soil

Within the broad groupings described above, I suggest that the following five sources are the main contributors to total soil CO₂ efflux (Fig. 1, from right to left):

- (1) microbial decomposition of SOM in root free soil without undecomposed plant remains, frequently referred to as ‘basal respiration’,
- (2) microbial decomposition of SOM in root affected or plant residue affected soil (I will use the term ‘rhizosphere priming effect’ or ‘priming effect’, respectively; see below),
- (3) microbial decomposition of dead plant remains,
- (4) microbial decomposition of rhizodeposits from living roots, frequently referred to as ‘rhizomicrobial respiration’, and
- (5) root respiration.

It is important to note that pedogenic or anthropogenic acidification of soils containing CaCO₃ can also contribute to the CO₂ efflux. Since globally soils in the upper 1 m contain a total of about 722 Pg C as CaCO₃ (Batjes, 1996), its dissolution during pedogenesis contributes to the CO₂ efflux into the

atmosphere on a geological time scale. However, on the sub-annual, annual and decadal time scales, which are usually used in the soil–CO₂–flux measurements, its contribution is marginal. The CaCO₃-derived CO₂ usually accounts for not more than 3–4 g C m⁻² y⁻¹ (calculated based on decalcification of 1 m depth of soil from loess initially containing 20% CaCO₃ over a period of 10,000 years). This abiotic CO₂ efflux (although driven by biotic processes of acidification) is of minor importance in all ecosystems compared with the biotic CO₂ fluxes which range from 60±6 for tundra to 1260±57 g C m⁻² y⁻¹ for tropical moist forests (Raich and Schlesinger, 1992). This small abiotic flux of CaCO₃-derived CO₂ can be accepted as source 6 (not presented on Fig. 1). In contrast to biotic CO₂ sources (Fig. 1), the CO₂ efflux from CaCO₃ is unidirectional: if dissolved CaCO₃ is not precipitated again by drying or is not re-crystallized with CO₂ from biogenic sources (Kuzyakov et al., 2006), Ca²⁺ or CaHCO₃⁻ will be removed from soil by leaching. After removal of Ca²⁺, no significant pools for inorganic binding of atmospheric CO₂ remain in the soil. The minor abiotic flux of CaCO₃-derived CO₂ can be easily predicted from acid production in soil and inputs in precipitation, is of minor importance and is not included in this review.

2.3. Considerations with regard to the classification terms

2.3.1. C pools

CO₂ from the first and second sources originates from SOM decomposition; thus their sum represents SOM-derived CO₂ (Fig. 1). CO₂ from the three other sources is plant-derived CO₂, originates from either living or recently-dead plants. Two of the plant-derived CO₂ sources, rhizomicrobial respiration and root respiration, can be combined to form a smaller category, root-derived CO₂.

Only the first source, microbial decomposition of SOM in the root free soil, contributes to the CO₂ efflux from all soils containing organic matter. The contribution of the four other sources depends on the presence of vegetation in the study year and/or in the previous few years. The presence of plants, especially the rhizodeposition of easily available C sources,

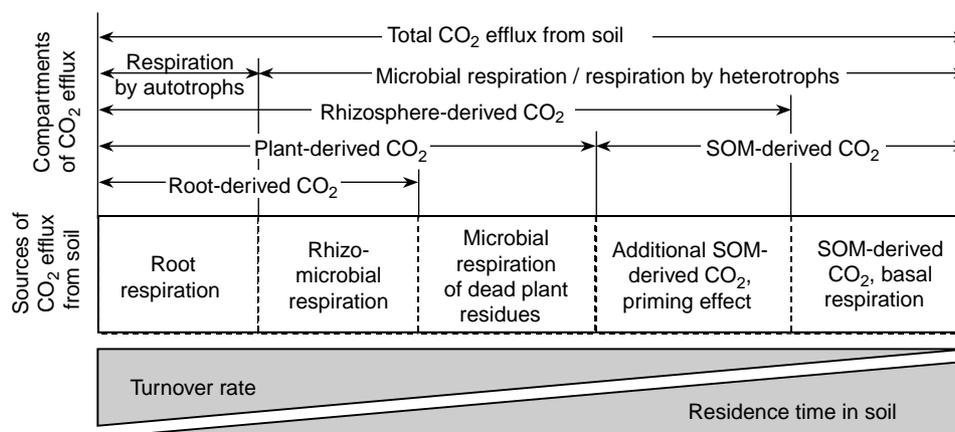


Fig. 1. Five main sources of biogenic CO₂ efflux from soil, ordered according the turnover rates and mean residence times of C in soil. The sources and compartments of the CO₂ efflux consider C pools with different turnover rates and MRT, the localization of C pools and the agents of CO₂ production.

can greatly change microbial activity in the rhizosphere. This increased microbial activity may change (increase or decrease) the intensity of SOM decomposition in the rhizosphere. Such a change is known as a ‘rhizosphere priming effect’ (Kuzyakov, 2002a), and it represents the interaction between growing roots and SOM decomposition (Dormaar, 1990; Paterson, 2003; Cheng and Kuzyakov, 2005). Similar changes in SOM decomposition have often been measured after addition of fresh plant residues to soil (Vanlauwe et al., 1994; Shen and Bartha, 1997; reviewed by Kuzyakov et al., 2000). Therefore, because of higher microbial activity, the CO₂ production by SOM decomposition at the interface between O and A (Ah) horizons may strongly differ from that of soils without an O horizon. CO₂ evolved as a consequence of a priming effect originates from SOM. Here, it is presented as a separate CO₂ source to highlight its importance.

2.3.2. Agents of CO₂ production in the soil

The first four CO₂ sources are produced by soil microorganisms, and therefore, will be termed microbial respiration or respiration by heterotrophs. Only the last CO₂ source, the actual root respiration is respiration by autotrophs. Because root respiration is the sole source of respiration by autotrophs, both terms are fully overlapping. It is especially important to note that the respiration of rhizosphere microorganisms decomposing rhizodeposits belongs to heterotrophic component of soil respiration and not to the autotrophic component, as it is often tacitly accepted to be.

It is important to highlight that the frequently used terms: ‘autotrophic respiration’ and ‘heterotrophic respiration’ are, in principal, incorrect, because the terms ‘autotrophic’ and ‘heterotrophic’ refer to the method of substrate and energy acquisition by an organism and are not connected with respiration themselves. Therefore, the terms ‘respiration by heterotrophs’ and ‘respiration by autotrophs’ will be used here. Other suitable and correct terms were suggested by Bond-Lamberty et al. (2004): heterotrophic and autotrophic components of soil respiration.

2.3.3. Locations of CO₂ production

It is important to note that the terms ‘rhizosphere CO₂’ or ‘rhizosphere respiration’, have frequently been used in the literature to refer to the sum of rhizomicrobial respiration and root respiration. However, strictly speaking, the term ‘rhizosphere CO₂’ or ‘rhizosphere respiration’ refers to the location of CO₂ production and not to the pool of C from which the CO₂ originates. If one is to use these terms to refer to the location of

CO₂ flux, then the CO₂ derived by microbial decomposition of soil organic matter in the rhizosphere must be included along with rhizomicrobial respiration and root respiration (Fig. 1). Therefore, I will use ‘rhizosphere CO₂’ or ‘rhizosphere respiration’ to describe the CO₂ evolved in the rhizosphere as the sum of root respiration, rhizomicrobial respiration, and SOM decomposition in the rhizosphere.

Despite the sharp boundaries between the CO₂ sources presented in Fig. 1, in reality there are no clear boundaries between the CO₂ sources or the C pools. The gradual boundary between SOM and dead plant residues becoming transformed into SOM, which can be best represented by a gradual distribution of SOM turnover times (Trumbore, 1997), and the boundary between plant residues and rhizodeposition were discussed above. Similarly, the boundary between root and rhizomicrobial respiration is not abrupt, because there are some bacteria and many fungi, especially ecto- and endomycorrhiza that penetrate root tissues (Rygielwicz and Andersen, 1994; Hodge et al., 2001; Jones et al., 2004) and produce CO₂ inside the roots. Though the boundaries are not sharp, the processes contributing to CO₂ production in each pool are different. Therefore, these CO₂ sources will be described separately.

2.4. Turnover rates and residence times of soil C pools contributing to the five CO₂ sources

A very important difference between the five sources of CO₂ efflux is the turnover rate (TR) of the C pools (Table 1). TR is the rate of cycling of C (or any other element or substance) in a pool or a system. If the pool is in steady state (input into the pool is equal to its decomposition), the value of the turnover rate is the ratio of the input amount per time unit to the total pool amount. Different turnover rates result in largely different mean residence times (MRT) of C in the SOM pools (Table 1). The MRT is inverse of TR (MRT=1/TR) and denotes the mean period of residence of C in the soil or in the SOM pool.

The MRT of C used for root respiration is the shortest one in the plant–soil system and lasts a few minutes to a few hours. In grasses, a maximum of 1–2 d are necessary for most (90–95%) of the C allocated to root respiration to return to the atmosphere as CO₂ (Gregory and Atwell, 1991; Cheng et al., 1993; Kuzyakov et al., 1999; 2001). Longer MRTs of about 4 days have been observed for tree roots (Ekblad and Höglberg, 2001). A portion of assimilated C can be stored in plants for longer time periods and utilized later for root respiration especially during periods of stress (e.g. cutting or grazing) or changing environmental conditions (drought, cold etc.). Such storage

Table 1
Turnover rates (TR) and mean residence times (MRT) of C in the pools of soil–plant system and their contribution to C sequestration

C pools	Process of CO ₂ efflux	TR (year ⁻¹)	MRT (1/TR)	C sequestration
Plant assimilates	Root respiration	$n^a \times 10^5 \dots 10^3$	Minutes–hours	No
Rhizodeposits	Rhizomicrobial respiration	$n \times 10^3 \dots 10^2$	Hours–days (weeks)	?
Plant residues	Microbial decomposition	$n \times 10 \dots 1$	Weeks–months	Very short-term
SOM in rhizosphere	Priming effect	$n \times 1 \dots 10^{-1}$	Months–years–decades	Short-term
SOM in root free soil	Basal respiration	$n \times 10^{-1} \dots 10^{-2}$	Decades–hundreds years	Long-term

^a n represents numbers from 1 to 9.

and reutilization occurs in the plant and not in the soil, so will not be considered here.

The C source that has the next shortest MRT is rhizodeposition. The decomposition rate of its fastest component, root exudates, is on the scale of few hours. Root exudates, consisting mainly of low molecular weight organic substances such as carbohydrates, carboxylic acids, and amino acids (Gransee and Wittenmayer, 2000; Jones et al., 2003; Nguyen, 2003; Kuzyakov et al., 2003), are utilized by microorganisms within a few hours and decomposed to CO₂ (Verburg et al., 1998; Kuzyakov and Demin, 1998; Jones and Hodge, 1999). High rates of photosynthesis during light periods tend to correspond with high rates of release of root exudates. The rapid decomposition of these exudates by rhizosphere microorganisms, often leads to diurnal dynamics of CO₂ efflux from soil (Baldocchi et al., 1986; Kim and Verma, 1992; Oberbauer et al., 1992; Kuzyakov and Cheng, 2001). Such diurnal variations in CO₂ flux from soil are more pronounced for grasses in comparison to trees because of the shorter distance that assimilates have to be transported after fixation. In most studies, diurnal CO₂ dynamics have been attributed to day–night variation of soil temperature, based on the assumption that higher soil temperature during the day leads to faster decomposition of organic compounds in the soil and thus higher CO₂ efflux. However, the high dependence of soil and air temperatures on solar irradiation hides the fact that larger amounts of easily decomposable substrates are released to the soil during daylight (Fitter et al., 1999). Because most decomposition processes in soil are substrate limited (Wardle, 1992; Ekblad and Nordgren, 2002), higher release of easily decomposable substrates leads to higher CO₂ production and results in diurnal CO₂ dynamics. Depending on the time lag between C fixation and the release of exudates to the soil, this close link between C assimilation and CO₂ efflux from soil can be shifted in time. This is especially pronounced in forest ecosystems (Ekblad and Höglberg, 2001). Because root exudates are quickly decomposed, their concentration in the rhizosphere remains at a very low level ($\mu\text{mol l}^{-1}$ soil solution) despite high rates of release ($\sim 150 \mu\text{g C mg}^{-1}$ DM of root growth, reviewed by Nguyen, 2003). Other components of rhizodeposition, such as sloughed off root border cells, root hairs, some high molecular weight secretions, need a few days or more for decomposition (Domanski et al., 2001; reviewed by Nguyen, 2003).

Plant residues remaining in soil after plant death usually require weeks to months to be completely decomposed (Cadisch and Giller, 1997; Hahn and Buchmann, 2004). Plant residues with high N and very low lignin content have the shortest decomposition time (weeks). Plant residues with high lignin and cellulose content (e.g. needles of conifers) are at the other end of the timescale. Decomposition of these residues can take to 2–4 years (Gaudinski et al., 2000). The much slower decomposition under cold (low microbial activity) or water saturated conditions (low oxygen supply), which are typical for peat, tundra and permafrost environment is not considered here (Dioumaeva et al., 2003).

The MRT of C of the last two SOM-derived C sources contributing to priming effect and basal respiration is much longer. Depending on climate and clay content, they can take

hundreds of years. By division of the total C stock in soils by the average annual CO₂ efflux (corrected for root-derived CO₂), Raich and Schlesinger (1992) estimated the average global turnover time for soil organic C as 32 years, varying between 10 and 520 years for various ecosystems. Typically, SOM is divided into at least two and frequently more pools: inert (or passive), recalcitrant, resistant, decomposable, available, active, etc. each with different TRs. There are no standard definitions for these SOM pools. Definitions vary depending on fractionation procedure, simulation model, and general concept (Six et al., 2002). It is generally accepted that the inert pool is complex and tightly bound to clay minerals. This SOM pool has a very slow decomposition rate; results of radiocarbon dating have shown that the MRTs of inert pools are thousands of years (Theng et al., 1992; Trumbore, 1997, 2000; Telles et al., 2003; Rethemeyer et al., 2004). Therefore, these inert pools make only minor contributions to annual CO₂ efflux from soil.

The other SOM pools (decomposable, available, active) can be decomposed by microorganisms either through basal respiration or by priming. Although the effects of priming last between a few days and a few weeks, most of the CO₂ released by the priming is from the comparatively available SOM pool, which has turnover times from years to decades (Jenkinson, 1971; Neff et al., 2002; Kuzyakov and Bol, 2006). If available, the same SOM pool is likely consumed during basal respiration as microorganisms use the C pools according to their availability (Kuzyakov and Bol, 2006).

2.5. Contribution of CO₂ sources to changing atmospheric CO₂ concentration

With regard to the CO₂-driven greenhouse effect, only SOM-derived CO₂ (the sum of two sources on the right in Fig. 1) contributes to changes in atmospheric CO₂ concentration. The long MRT of SOM (decades to hundreds of years) resulting in very slow turnover rates ($n \times 10^{-1} - n \times 10^{-2} \text{ y}^{-1}$, where $n = 1, \dots, 9$) relative to other less-recalcitrant respiratory substrates means that SOM is the only C pool that can be a real, long-term sink for C in soils. Despite long residence times in steady state, this very large reservoir of C in SOM, which exceeds the pools of C in aboveground biomass and CO₂ in the atmosphere (Eswaran et al., 1993), makes this pool a very large potential source of CO₂ if decomposition exceeds humification.

Comparison of both subdivisions of SOM-derived CO₂ (Fig. 1) shows that the C pool involved in the rhizosphere priming effect should have a faster turnover rate than the C pool that contributes to basal soil respiration. Usually, it is only the available soil C pool, with turnover times of years to decades (Hahn and Buchmann, 2004) that contributes to processes on an annual or decadal time scale. It is possible to increase this pool of available soil C by several land management practices (higher manure or plant residue input, no tillage, perennial grasses etc. (Smith et al., 1997; Paustian et al., 1997; Falloon et al., 2002). However, after any increases, this pool will not all be converted to a stable C sink and will still remain a potential CO₂ source and sequestered C can be

Table 2
Summary of methods allowing partitioning of total CO₂ efflux from soil into root-derived CO₂ and SOM-derived CO₂

Method	Principle	Use ^a	Advantages	Disadvantages
Non-isotopic methods				
Planted vs. unplanted soil = Root exclusion (Section 3.1.1.1)	Difference of CO ₂ from planted and unplanted soil	F, L	Simple, cheap, various ecosystems	Different C, N and H ₂ O budget, different MO activity in planted vs. unplanted soil results in different SOM decomposition intensity
Trenching (Section 3.1.1.1)	Difference of CO ₂ from planted soil without and with trenched roots	F	Simple, cheap, mainly forest and shrubs	Dead root decomposition should be considered; different H ₂ O and nutrient budget, different MO activity in trenched vs. non-trenched soil → different SOM decomposition
Gap formation (Section 3.1.1.1)	Difference of CO ₂ from soil with vegetation and from the soil under the gap	F	Natural formation of gaps, mainly for forest	Mainly for forest vegetation; different C, N and H ₂ O budget, different MO activity in the soils under gap and trees → different SOM decomposition; differences in above litter decomposition
Shading (Section 3.1.1.2)	Difference of CO ₂ from soil with non-shaded and shaded plants	F, L	Simple, cheap, mainly grasslands and crops; mainly natural N uptake	Contribution of decomposition of previous rhizodeposits; RR and RMR are not completely eliminated; different H ₂ O budget, different MO activity in shaded vs non-shaded soil → different SOM decomposition
Clipping, clear felling (Section 3.1.1.2)	Difference of CO ₂ from soil with non-clipped and clipped plants	F, L	Simple, cheap, various ecosystems	Contribution of decomposition of previous rhizodeposits; dead root decomposition should be considered; RR and RMR are not completely eliminated; different H ₂ O budget, different MO activity under clipped vs. non-clipped plants → different SOM decomposition
Tree girdling (Section 3.1.1.3)	Interruption of C flows into roots by phloem girdling, difference of CO ₂ from soil under non-girdled and girdled trees	F, L	Suitable for forest ecosystems, natural H ₂ O regime and N uptake	Forest ecosystems only; dying of trees after girdling, contribution of dying roots to CO ₂ ; different C and N budget and MO activity under girdled and non-girdled trees → different SOM decomposition
Regression technique (Section 3.1.1.4)	Linear relation between root mass and root-derived CO ₂	F, L	Simple, cheap, various ecosystems	High number of replications is necessary; overestimation of root-derived CO ₂ when big and old roots are present; frequently poor correlation between root amount and total CO ₂ ; SOM-derived CO ₂ could be biased by far extrapolation of the regression line
Component integration (Section 3.1.1.5)	Manual separation of CO ₂ sources and measuring CO ₂ from each source	F → L	Separation of 3 (or 4) CO ₂ sources, cheap, various ecosystems	Strong CO ₂ flush after disturbance and root injuries; laborious; different CO ₂ decrease after the flush → dependence of the results on the CO ₂ trapping period
Excised roots (Section 3.1.1.6)	Measuring of respiration of excised roots separated from soil	F → L	Cheap, useful for roots of various plants	Strong CO ₂ flush after the disturbance and root injuries; different CO ₂ decrease after the flush → dependence of the results on the CO ₂ trapping period; extrapolation on field conditions is inexact; SOM-derived CO ₂ is estimated as difference to the total CO ₂ efflux
<i>In situ</i> root respiration (Section 3.1.1.7)	Respiration of living roots placed in a cuvette located in the soil	F, L	Respiration of living roots under field conditions	Extrapolation on field conditions is inexact; SOM-derived CO ₂ is estimated as difference to the total CO ₂ efflux; water and nutrient supply in the cuvette is necessary; mainly for trees and shrubs
Isotopic methods				
Continuous labeling of shoots in ¹⁴ CO ₂ or ¹³ CO ₂ atmosphere (Section 3.1.2.1)	Growing plants in ¹⁴ CO ₂ or ¹³ CO ₂ atmosphere and dilution of root-derived CO ₂ by unlabeled CO ₂ from SOM	L	The only one fully correct and exact method	Maintenance of a constant specific ¹⁴ C activity or ¹³ C: ¹² C ratio during long time period; temperature and air moisture control in the chamber; only laboratory application, special equipment is necessary; isolation of below-ground atmosphere is necessary
Pulse labeling of shoots in ¹⁴ CO ₂ or ¹³ CO ₂ atmosphere (Section 3.1.2.1)	Short-time labeling of shoots in ¹⁴ CO ₂ or ¹³ CO ₂ atmosphere + tracing of ¹⁴ CO ₂ or ¹³ CO ₂ from the soil	L, (F)	Easier than continuous labeling, recently assimilated C is mainly considered	Uneven distribution of labeled C between plant organs and C fluxes including CO ₂ ; calculations consider mainly the recently assimilated C; calculation of root-derived CO ₂ is based on many assumptions; isolation of below-ground atmosphere is necessary
¹³ C natural abundance (Section 3.1.2.2)	Growing C ₄ plants on a C ₃ soil or <i>vice versa</i> + tracing of ¹³ C in CO ₂ from the soil	F, L	Reasonable alternative for continuous labeling	Only for some soil-plant combinations applicable; not precise; high variation of ¹³ C in CO ₂ ; contamination with air CO ₂ should be considered

Free air carbon dioxide enrichment (FACE) (Section 3.1.2.2)	Application of additional CO ₂ with changed δ ¹³ C signature compared to air + tracing of δ ¹³ C of CO ₂ from the soil	F, L	Suitable for various ecosystems, wide range of plants	Expensive (additional CO ₂); correct only for the first year after FACE start; not precise; high variation of δ ¹³ C in CO ₂
Radiocarbon dating of soil CO ₂ (Section 3.1.2.3)	Different turnover rates of SOM and recent plant-derived C flows	F, L	Suitable for various ecosystems	Weighted age (Δ ¹⁴ C) of SOM-derived CO ₂ is necessary; can be biased by bomb- ¹⁴ C; very expensive (AMS analyses)
Bomb- ¹⁴ C (Section 3.1.2.3)	Different turnover rates of SOM estimated by 'pulse labeling' of SOM by bomb-derived ¹⁴ CO ₂ and of root-derived CO ₂	F, L	Suitable for various ecosystems	Weighted age (Δ ¹⁴ C) of SOM-derived CO ₂ is necessary, control of bomb- ¹⁴ C-affected SOM is necessary; very expensive (AMS analyses)
¹⁸ O of CO ₂ (Section 3.1.2.4)	Different δ ¹⁸ O of plant-derived CO ₂ and soil water CO ₂ and isotopic exchange between CO ₂ and H ₂ O	–	–	Not applicable until now, many uncertainties: see text

^a Application in 'F' field, 'L' laboratory, 'F→L' samples taken from field are analyzed in the laboratory (is also suitable for laboratory studies).

released as CO₂ again in the years following changes to land management.

Because of their fast turnover times, the three other CO₂ sources, microbial decomposition of plant residues and rhizodeposits, and root respiration, have no significant effect on C sequestration in the short or long-term. Since plant C sources frequently amount to more than half of the total soil CO₂ flux (Hanson et al., 2000; Larionova et al., 2003), the flux of plant-derived CO₂ masks the contribution of SOM-derived CO₂ when measuring CO₂ fluxes from planted soils. Furthermore, the contribution of vegetation to soil CO₂ flux is not constant and changes during the year. It is also strongly dependent on plant species (Fu et al., 2002), growth stage (Rochette et al., 1999b), available nutrients (Bradley and Fyles, 1996), and environmental factors such as intensity of photosynthetically active radiation (Ruimy et al., 1995; Craine et al., 1999; Kuzyakov and Cheng, 2001), soil moisture (Larionova and Rozanova, 1994; Flanagan et al., 2002), and temperature (Lambers et al., 1995; Buchmann, 2000). These complications make measurements of total soil CO₂ efflux unsuitable for direct estimations of the contribution of the soil to changes in atmospheric CO₂ concentration. Methods allowing separate estimation of individual CO₂ sources contributing to the total soil CO₂ efflux, particularly SOM-derived CO₂, are urgently needed for the evaluation of C sequestration.

3. Methods for separate estimation of components of CO₂ efflux from soil

3.1. Separation of SOM-derived and plant-derived CO₂

Very different prerequisites and assumptions are necessary for methods with and without the use of C isotopes (Table 2). For this reason, these methods are described separately.

3.1.1. Non-isotopic techniques

3.1.1.1. Comparison of planted and unplanted soil (root exclusion technique).

At first glance, it seems that the CO₂ efflux from unplanted soil under natural conditions should be a robust estimate of the soil's contribution to atmospheric CO₂, and the comparison of this flux with the CO₂ flux from planted soil should yield a fairly accurate estimation of root-derived CO₂. Several studies have used root-affected and root-free soil to estimate root-derived CO₂ as the difference between the two. This approach is frequently called the 'root exclusion technique'. In studies of CO₂ fluxes from forest sites, trenching of tree roots is frequently used as exclusion technique (Fisher and Gosz, 1986; Bowden et al., 1993; Buchmann, 2000; Ross et al., 2001; Lee et al., 2003). However, the root exclusion technique can give only a crude estimate of root-derived CO₂ and SOM-derived CO₂ from planted soil. This is due to the fact that the water regime and temperature balance in planted soil is very different from that in unplanted soil (Fisher and Gosz, 1986; Ross et al., 2001). The cycling of nutrients such as N, which affects the C cycle, also varies between vegetated and

unvegetated soil. The decomposition of SOM and other plant residues is dependent, not only on the physical effects (water regime and temperature balance) of vegetation on soil, but also on direct biological effects due to the presence of living roots (reviewed by Dormaar, 1990; Kuzyakov, 2002a,b; Paterson, 2003; Cheng and Kuzyakov, 2005). The CO₂ evolved as a consequence of the rhizosphere priming effect may be 2–3 times larger than that of basal respiration (Cheng et al., 2003). Because of the influence of the presence of plants on SOM decomposition, the CO₂ efflux from unplanted soil cannot be accepted as a suitable method for the estimation of SOM-derived CO₂ from vegetated soils. Some other variations of root exclusion techniques especially those used in forest ecosystems (root removal, trenching, gap formation) and their limitations were intensively discussed in the review of Hanson et al. (2000).

3.1.1.2. Shading and clipping. Shading or clipping of above-ground plant parts in grasslands (Craine et al., 1999) or clear felling in forests have been used as less-invasive alternatives to root exclusion, which avoid any influence of root-removal on soil conditions by partitioning of SOM-derived and root-derived CO₂. These methods are based on stopping leaf photosynthesis and thus excluding new assimilate transport to the roots. The advantage of these approaches is that shortly after the treatment (days), the water content and the nutrient turnover in the shaded or clipped treatment are nearly the same as in the untreated control. However, the disadvantage of these methods is that, although the flow of recent assimilate is interrupted, previously-fixed C in plant organic compounds can be used for root respiration and rhizodeposition. Thus, the contribution of root-derived CO₂ to the total soil CO₂ flux is not completely removed. The application of these techniques to many plants requires caution as plant C allocation is affected in two ways. Defoliation of grasses increases the sink strength for leaf growth and reduces below-ground C allocation (Detling et al., 1979; Holland and Detling, 1990; Mackie-Dawson, 1999). In addition, many plants store some of the C assimilated prior to defoliation in their roots, and utilize these C reserves for regrowth following cutting (Bokhary, 1977; Johansson, 1993). Increases in exudation and root and rhizosphere respiration after clipping have been reported (Paterson and Sim, 1999; Paterson et al., 2003; Fu and Cheng, 2004). These effects, especially changes of C allocation patterns have a significant effect on microbial community structure (Macdonald et al., 2004), and lead to differences in, but not elimination of root-derived CO₂ fluxes in clipped and un-clipped treatments.

3.1.1.3. Tree girdling. With the exception of trenching to exclude roots, most methods for the partitioning of CO₂ sources are impractical to use in forest soils because of very large above and belowground tree biomass. Instead, tree girdling has been used to estimate CO₂ fluxes originating from SOM and root-derived C in forests (Keutgen and Huysamer, 1998; Högberg et al., 2001; Subke et al., 2004). The girdling of phloem interrupts the flow of assimilates from leaves to the roots. Shortly after girdling, the interruption in the flow of

assimilates leads to strong suppression of root and rhizomicrobial respiration. A comparison of CO₂ efflux from soils with and without girdled pine trees showed that about 56% of the CO₂ is derived from the rhizosphere and 44% is from the SOM (Högberg et al., 2001).

In comparison to trenching, the main advantage of girdling is that the moisture and temperature regime of the soil under girdled trees remains nearly the same as under non-girdled trees for at least for 2–3 months, and thus, it has no physical effect on the CO₂ efflux. In soil with trenched roots, which has a similar effect on C release into soil as girdling, the availability of C, as well as microbial C and N decreased, and dynamics of N_{min} concentrations and gross nitrification rates were altered (Lee et al., 2003; Fisher and Gosz, 1986). In the soil under girdled trees, microbial biomass C and DOC were 23–41 and 45% lower, respectively, than in control plots (Högberg and Högberg, 2002). However, one important problem remains unsolved by this method: if the rhizodeposition including root exudation decreases, the additional decomposition of soil organic matter in the rhizosphere (rhizosphere priming effect) also declines very quickly. In the experiment of Subke et al. (2004) combining girdling with decomposition of ¹³C labeled conifer needles, it was clearly shown that girdling of spruce led to decreased litter decomposition because of the absence of rhizosphere priming effect in the soil under girdled trees.

An additional shortcoming of tree girdling is that the microbial decomposition of roots that die after girdling contributes to the CO₂ flux from the soil. Bhupinderpal et al. (2003) observed that in the year after girdling, there was an apparent increase in the contribution of root-derived CO₂ in comparison to the first year. This result is also typically observed in the second year after root trenching (Fisher and Gosz, 1986). To actually calculate the contribution of root-derived CO₂ in the soil with girdled trees (or trenched roots), the decomposition of dead roots should be considered, for example in an incubation of soil samples with dead roots under the same experimental conditions (Fisher and Gosz, 1986; Ross et al., 2001). The contribution of dead root decomposition can amount to 20% of total CO₂ efflux (Nakane et al., 1996) and varies depending on the vegetation type and period after girdling or trenching.

In the original paper as well as in the subsequent studies, girdling was proposed as a method to separate autotrophic from heterotrophic respiration (Högberg et al., 2001; Bhupinderpal et al., 2003). However, this is misleading. The method does not allow for the separation of root respiration per se (the one and only significant source of respiration by autotrophs in soil) from rhizomicrobial respiration. As the assimilate transport into the roots is interrupted, both sources of root-derived CO₂ strongly decrease.

It can be concluded that all difference methods: root exclusion technique, trenching, clipping, girdling etc. have some value for estimating the SOM-derived and root-derived CO₂ if the rhizosphere priming effect (changes of SOM decomposition in the presence of plants) is assumed to be

small. However, all difference methods lead to changes in environmental conditions, especially an increase in soil moisture (Staples et al., 2001; Ross et al., 2001) and decreases in extractable C, microbial C and N (Ross et al., 2001).

3.1.1.4. Regression technique. The regression approach was firstly suggested by Kucera and Kirkham (1971) and is based on the assumed linear relationship between root biomass and the amount of CO₂ respired by roots and rhizosphere microorganisms. The amount of CO₂ derived from SOM decomposition corresponds to the intercept of the regression line between root biomass (independent variable) and total CO₂ evolved from the soil (dependent variable). In the original study, Kucera and Kirkham (1971) observed that about 60% of total CO₂ is SOM-derived. This method is comparatively simple and has been applied in some later studies (Gupta and Singh, 1981; Behera et al., 1990; Hill et al., 2004). One important uncertainty of the approach was highlighted in the original study: high variation of root biomass and CO₂ flux led to a relatively low R^2 (Kucera and Kirkham, 1971). This caveat is especially important if the regression line has to be extrapolated far outside the root biomass range to calculate the SOM-derived CO₂ efflux. This is also problematic in ecosystems in which horizontal distribution of roots is homogeneous, e.g. agricultural crops. The other shortcoming is that increasing root biomass is strongly correlated with the quantity of large, older roots, which respire and exude much less C than fine, young roots (Behera et al., 1990). Despite these shortcomings, which are actually minor compared to those of other methods (especially where soil is disturbed; see above and below), this approach has been used surprisingly rarely. Root biomass is very frequently measured concurrently with CO₂ efflux, so the regression approach can be applied without additional effort.

3.1.1.5. Component integration. The component integration method is based on the physical separation of C pools contributing to CO₂ fluxes and measuring the specific rates of CO₂ efflux from each component part under controlled conditions (reviewed by Hanson et al., 2000; Kuzyakov and Larionova, 2005). Usually, the following components are distinguished: litter (O horizon), roots separated from soil by shaking or washing, and root-free soil (Blagodatski et al., 1993; Larionova et al., 2003, 2005). In some studies, root-free and rhizosphere soil were also separated. Specific rates of CO₂ efflux from each component are multiplied by their respective mass and summed to yield an integrated total CO₂ efflux (Hanson et al., 2000). Ideally, the CO₂ efflux rates should be measured in parallel in an undisturbed soil and compared with the sum of CO₂ efflux from individual components.

The main disadvantage of this method is a strong effect of physical disturbance and root damage on the respiration rates (Hanson et al., 2000 and references therein). The sum of CO₂ efflux from each component can be two or more times that from untreated soil. Furthermore, the decrease in the rate of the CO₂ efflux from each C source over time (caused by depletion of respiratory substrates) is very different for root respiration

(Rakonczay et al., 1997), rhizomicrobial respiration and microbial SOM decomposition (Sapronov and Kuzyakov, 2004). Therefore, the time lapse between disturbance and measurement has a strong influence on the resulting contribution of each source and should be standardized for each one. The technique also relies on effective separation of the components, which is not trivial. For example, removal of all root and root hairs from soil is extremely time-consuming and invasive, if possible at all.

3.1.1.6. Respiration by excised roots. Excising roots is the ‘short version’ of component integration (Section 3.1.1.5). In the excised root approach, the respiration of living roots (few grams), incubated after separation from soil by washing or shaking, is measured by infrared gas analyser (IRGA), oxygen electrode or capture of CO₂ in alkali (Kelting et al., 1998; Craine et al., 1999; Cheng et al., 2005). The roots are incubated between a few minutes (Burton et al., 2002) and 24 h (Lipp and Andersen, 2003). Rakonczay et al. (1997) compared the separation of roots from soil by washing or shaking and found no differences between methods for three tree species. However, manual brushing or shaking can be preferable for roots collected from dry soil (Larionova, personal communication). Small amounts of mineral soil remaining on the roots do not significantly increase root respiration since the specific respiration rates of soil are 2–3 orders lower than those of roots (Larionova et al., *in press*). If the soil temperature differs from the incubation temperature, then corrections are made according to Q_{10} estimates.

The advantages of the method are that it is simple, it can be used for field studies and it allows estimations involving tree roots. The shortcomings are similar to those of the component integration approach: an increase of root respiration by 1.5–3 times after the separation of roots from the soil and subsequent decrease is typical. Investigating dynamics of root respiration by excised roots, Rakonczay et al. (1997) found that over 1 h following the excision a 2–3 fold decrease was typical. This decrease differed between tree species. The decrease is due to recovery from injuries and depletion of respiratory substrates in excised roots (Sprugel and Benecke, 1991). After about 1 h the CO₂ efflux rate remains almost constant for up to 10 h (Rakonczay et al., 1997). Therefore, alterations in the time between excision and measurement lead to different estimations of root respiration. In addition, high variability of roots makes the extrapolation of the root respiration obtained by incubation of a small amount root very uncertain on the field scale.

In contrast to component integration, the CO₂ efflux from other compartments (SOM, litter, rhizosphere soil) is not measured. The sum of all other compartments is accepted as SOM-derived CO₂ and is calculated as the difference between the root respiration and the total CO₂ efflux from soil. Therefore, the excised root approach does not allow a clear differentiation of SOM-derived from root-derived CO₂ efflux. As a consequence of this, the estimation of root respiration by excised roots approach is mainly used in root

ecophysiology studies and not in soil C balance or global change studies.

3.1.1.7. *In situ* measurements of root respiration. *In situ* measurement of root respiration has often been used to exclude changes in root respiratory CO₂ losses due to root excision, and to allow direct measurement under field conditions. In this technique, living roots are excavated, without severing, and placed in a cuvette. CO₂ efflux is then measured with an IRGA (Sisson, 1983; Cropper and Gholz, 1991; Cheng et al., 2005). The cuvette with roots (sometimes with soil adhering to roots) is placed back into the soil and CO₂ is measured after the roots have been allowed a little time to recover from injuries sustained during extraction from soil and cuvette installation. In comparison with the excised root method, the *in situ* method allows respiration measurement of living roots under field conditions. If rhizosphere soil adhering to roots is not removed from roots before they are placed in the cuvette, the root-derived CO₂ is estimated, rather than root respiration *per se*. Measurement of total CO₂ efflux from the soil surface in parallel to the *in situ* root respiration (with adhering soil) allows an approximate value for SOM-derived CO₂ to be calculated by difference. Measurement of *in situ* root respiration over a long period requires regular additions of water to the cuvette, but may underestimate root respiration due to its high dependence on nutrient uptake (Lambers, 1987).

Calculation of actual field CO₂ fluxes from the *in situ* method is subject to similar uncertainties as the excised roots approach (Cheng et al., 2005). The CO₂ efflux is usually obtained per root mass or volume, which is difficult to accurately extrapolate to soil surface or soil volume units because of the high variability in horizontal and vertical distribution of roots in soil.

3.1.2. Isotopic techniques

There are several ways to alter the ratio of carbon isotopes in plants from that of SOM or to use natural variation of carbon isotope ratios for the partitioning of CO₂ originating from several sources (Table 2).

3.1.2.1. Continuous and pulse labeling of plants in ¹⁴CO₂ or ¹³CO₂ atmosphere. Continuous labeling exposes plants to atmosphere with constant ratios of ¹⁴C- or ¹³C-CO₂ to total CO₂ over a long period, generally from leaf emergence to the end of the experiment. The share of root-derived and SOM-derived CO₂ is determined from the dilution of the applied ¹⁴CO₂ specific activity or ¹³C atom excess, originating from root-derived CO₂, by unlabeled CO₂ originating from microbial SOM decomposition (Johnen and Sauerbeck, 1977; Whipps, 1987). Because the total amount of C (¹⁴C or ¹³C) assimilated from the artificial atmosphere is known, continuous labeling is particularly appropriate for estimation of the total amount of C transferred by the plant into the soil and below-ground C pools during the labeling period (Meharg, 1994), and to determine whether the losses of soil organic C as CO₂ are compensated for by root C input. However, maintenance of constant isotope ratios over long periods of time and control of

temperature and moisture conditions inside the labeling chamber is fairly complicated and expensive. Separation of soil-derived and atmospheric air is especially difficult (Warembourg and Kummerow, 1991), making classical continuous labeling (other new approaches are detailed below) unsuitable for field conditions.

Pulse labeling (labeling of shoots for short periods in an atmosphere of ¹⁴CO₂ or ¹³CO₂) or repeated pulse labeling can be applied to evaluate the contribution of recently-assimilated C to the total CO₂ efflux from soil (Rygiewicz and Andersen, 1994; Kuzyakov et al., 2001). Calculation of the amount root-derived CO₂ after the pulse labeling is different from that following continuous labeling. It is based on the percentage of assimilated ¹⁴C (or ¹³C) evolved as root-derived CO₂ (trapped in alkali). Because the total ¹⁴C or ¹³C supplied to shoots is known, calculation of the proportion of evolved CO₂ which is root-derived is simple, but dependent on a number of assumptions. Continuous dilution of labeled root-derived CO₂ by unlabeled CO₂ assimilated after labeling also contributes to the root-derived CO₂ efflux. For the calculation, it is assumed that the distribution of labeled C is similar to that of unlabeled C for the whole growth period (Kuzyakov et al., 2001; Kuzyakov and Cheng, 2001) or for the chase period after the labeling (Augustin et al., 2003). Since, many studies have shown that the distribution of assimilated C between above- and below-ground pools varies depending on plant growth stage, the increment of plant mass of individual organs should be measured between the label application and the end of chase period.

There are a number of different C isotopes (¹⁴C, ¹³C and ¹¹C). Radioactive ¹⁴C has been used in most studies with continuous (and pulse) labeling so far. This preferential use of ¹⁴C is based on the high sensitivity, lower costs of purchase and analyses, and easier sample preparation as compared with ¹³C or ¹¹C. Because of safety restrictions with ¹⁴C and radioactive waste disposal, the application of ¹³C for continuous labeling experiments has increased in recent years, especially in connection with free air carbon dioxide enrichment (FACE) installations (see below). As ¹¹C has a very short half-life (20.4 min), only ¹⁴C and ¹³C are appropriate for continuous labeling.

Since continuous and pulse labeling approaches, their assumptions and possible errors have been discussed extensively in many other reviews (Warembourg and Kummerow, 1991; Meharg, 1994; Hanson et al., 2000; Kuzyakov and Domanski, 2002), these methods will not be discussed further here.

3.1.2.2. ¹³C natural abundance and FACE. The ¹³C natural abundance method is based on the differential discrimination of the heavier ¹³C isotope during CO₂ assimilation by plants with different types of photosynthesis. The enzyme Rubisco (C₃ plants) leads to a ¹³C depletion of about -19‰ compared to air CO₂, resulting in δ¹³C values of plant tissues of about -27‰ (-35‰ ≤ δ¹³C ≤ -20‰). Phosphoenol pyruvate carboxylase (the primary carboxylating enzyme in C₄ plants) results in smaller depletion, and therefore, the δ¹³C values of

C₄ plants are about -13‰ ($-15\text{‰} \leq \delta^{13}\text{C} \leq -7\text{‰}$). The $\delta^{13}\text{C}$ values of various plants are reviewed by Farquhar et al. (1989); Boutton et al. (1998), and Ehleringer and Cerling (2002). The effects of humification and other microbial processes during the incorporation of plant-derived C into SOM on $\delta^{13}\text{C}$ are usually less than 2‰ and thought to be negligible in most studies. Therefore, the soils developed under C₃ or C₄ vegetation contain SOM with $\delta^{13}\text{C}$ of -27 or -13‰ , respectively (Cheng, 1996). The ^{13}C natural abundance method is based on cultivation of C₄ plants on a C₃ soil, or *vice versa*, and estimation of the contribution of root-derived CO₂ according to the $\delta^{13}\text{C}$ value in the CO₂ evolved. This method can be considered as a variation of the continuous labeling, differing only in that the labeling of plants and soil occurs naturally.

The ^{13}C natural abundance method has important advantages over other techniques (Rochette et al., 1999b) since: (1) all C pools in the plant are labeled, (2) it is a non-invasive method, and (3) it does not involve handling of radioactive material.

The use of ^{13}C natural abundance techniques for the partitioning of CO₂ fluxes from soil into several fluxes began several years ago (Cheng, 1996). This method is used in many studies as standard procedure to separate root- (or plant-) derived from SOM-derived CO₂ (Qian et al., 1997; Rochette et al., 1999b; Flessa et al., 2000; Kuzyakov and Cheng, 2001; Fu et al., 2002; Fu and Cheng, 2002). This method can easily be used under field conditions (Rochette and Flanagan, 1997) because special equipment for plant labeling and isolation from the atmosphere is not necessary. This last feature, combined with developments in methods for measuring the $\delta^{13}\text{C}$ of CO₂: i.e. IR tunable diode laser absorption spectroscopy, which precision is now at 0.2‰ (McManus et al., 2002), FT-IR spectroscopy with precision of 0.1‰ (Esler et al., 2000; Bowling et al., 2003; Griffis et al., 2005), or NIR spectroscopy (Hörner et al., 2004), will promote the use of ^{13}C natural abundance approach in future investigations. These spectroscopic methods do not require special sample preparation or separation of gases (as in common mass spectrometry) and allowing quantitative on-line analysis in field or laboratory. They are also much cheaper to install and maintain compared to mass spectrometers.

Although the principle of the ^{13}C natural abundance method allows the calculation of the proportion of the root-derived and SOM-derived CO₂ in the total CO₂ efflux from soil, there are some important shortcomings of the ^{13}C natural abundance method that limit its application. Firstly, soil-plant pairs of C₃ plants growing in a C₄ soil or *vice versa* are unusual. Hence, the field application of this method is greatly restricted. All decomposable SOM must be derived from C₃ or C₄ vegetation. This is only possible after very long-term cultivation of a C₃ or C₄ plant species. Secondly, a maximal range of only $\sim 14\text{‰}$ is available for all variations of the $^{13}\text{C}:^{12}\text{C}$ ratio in CO₂ (it is calculated as the difference between the $\delta^{13}\text{C}$ value of cell tissue of C₃ and C₄ plants), whilst the variability of $\delta^{13}\text{C}$ in soil or plants is at least $\pm 1\text{--}2\text{‰}$ (Cheng, 1996) or more (Farquhar et al., 1989). Additionally, the ^{13}C discrimination by plants

depends on environmental conditions, such as temperature, water availability (Farquhar et al., 1989; Høgh-Jensen and Schjoerring, 1997; Lucero et al., 2000), air humidity (Ekblad and Högberg, 2001), N supply (Høgh-Jensen and Schjoerring, 1997), light intensity (Pate and Arthur, 1998), and plant characteristics such as specific root length (Poorter and Farquhar, 1994) or plant sex (Dawson and Ehleringer, 1993; Hill et al., 1996). Furthermore, CO₂ diffusion in soil leads to isotope fractionation (Cerling et al., 1991). These effects on plant tissue $\delta^{13}\text{C}$ values may shift the results of CO₂ efflux partitioning based on ^{13}C natural abundance.

In common with labeling methods, application of the ^{13}C natural abundance method requires gas-tight isolation of soil from the atmosphere, which has a $\delta^{13}\text{C}$ of about -7.5 to -8.5‰ . In order to overcome the practical problems associated with gas-tight isolation of soil from the atmosphere, so-called Keeling plots can be calculated (Keeling, 1958, 1961). Keeling-plots present the dependence of the $\delta^{13}\text{C}$ value on the reciprocal of the total CO₂ concentration in the mixture of soil and atmospheric CO₂. Plotted against the reciprocal of the CO₂ concentration, increasing the portion of soil CO₂ mixed with atmospheric CO₂ leads to linear decrease of $\delta^{13}\text{C}$ values of the mixture from two sources. So, the $\delta^{13}\text{C}$ value of the end-member-soil CO₂ can be calculated as the intercept with the Y-axis. This $\delta^{13}\text{C}$ of soil CO₂ is accepted as a weighted mean of all C pools in soil (living roots, root residues and SOM) contributing to the CO₂ efflux (Søe et al., 2004). The contribution of SOM and plant-derived CO₂ can be calculated from the $\delta^{13}\text{C}$ values of SOM and roots.

Another uncertainty of the ^{13}C natural abundance approach is the small, but progressive reduction of the ^{13}C content of the atmosphere (-0.02‰ y^{-1}) due to the release of ^{13}C depleted CO₂ from fossil fuel combustion (Fung et al., 1997). This subsequently changes the isotopic composition of SOM pools with high and intermediate turnover rates. This may also make a small difference to estimates of the proportions of root-derived and SOM-derived CO₂ in the total CO₂ efflux from soil. These limitations of the ^{13}C natural abundance method should be considered for an exact estimation of root-derived and SOM-derived CO₂.

Free air CO₂ enrichment (FACE) experiments with shifted C isotopic composition of added CO₂ also generate differences in $\delta^{13}\text{C}$ of CO₂ evolved from roots and SOM (Leavitt et al., 1996, 2001; Andrews et al., 1999; Van Kessel et al., 2000; Pendall et al., 2001; Søe et al., 2004). In most cases, the $\delta^{13}\text{C}$ signature of CO₂ used for FACE is about -45‰ . After dilution with atmospheric CO₂, the plants receive CO₂ with $\delta^{13}\text{C}$ between -20 and -25‰ depending on the $\delta^{13}\text{C}$ of the additional CO₂ source and the atmospheric air-to-additional CO₂ ratio. The isotopic discrimination of ^{13}C by C₃ photosynthesis of about -19‰ , results in plant tissues, rhizodeposits and root respiration with a $\delta^{13}\text{C}$ of about -40 to -45‰ . So, the difference of $\delta^{13}\text{C}$ values of plants and plant-derived CO₂ (-40 to -45‰) and that of unmodified SOM (-27 to -29‰) can be used to separate contributions to total CO₂ efflux from soil under FACE. Keeling-plots are also used in FACE experiments to consider the dilution of SOM and

root-derived CO₂ by atmospheric air and to estimate the original $\delta^{13}\text{C}$ value of organic substances contributing to CO₂ efflux from soil (Søe et al., 2004). It should, however, be noted that FACE experiments by definition can only provide this information for plants grown at CO₂ concentrations above those in the present-day atmosphere.

3.1.2.3. Radiocarbon dating of soil CO₂ and Bomb-¹⁴C. As mentioned above, the sources of CO₂ efflux from soil have different turnover rates leading to different radiocarbon age or $\Delta^{14}\text{C}$ values. All plant-derived CO₂ has modern age, meaning that its $\Delta^{14}\text{C}$ value corresponds closely to the $\Delta^{14}\text{C}$ value of the atmosphere. The radiocarbon age of SOM differs greatly from that of the atmosphere and usually consists of pools aged between a few tens and hundreds of years, reaching thousands for inert SOM pools (Scharpenseel and Neue, 1984; Trumbore, 2000; Rethemeyer et al., 2004). This difference in the TR, which leads to different $\Delta^{14}\text{C}$ values can be used in the isotopic mass balance approach to partition total CO₂ efflux from soil into SOM-derived and plant-derived CO₂. Some attempts have been made to do this (Dörr and Münnich, 1980; 1986; Trumbore, 2000; Gaudinski et al., 2000; Chambers et al., 2004).

The main problems of CO₂ efflux partitioning based on the radiocarbon age (or $\Delta^{14}\text{C}$ values) are connected with the differences in radiocarbon age of the SOM pools and the fact that $\Delta^{14}\text{C}$ values decrease strongly with soil depth (Gaudinski et al., 2000). These differences are much larger than the changes of $\delta^{13}\text{C}$ values with soil depth and lead to many uncertainties in $\Delta^{14}\text{C}$ values of SOM-derived CO₂. Consequently, the value of $\Delta^{14}\text{C}$ obtained for SOM-derived CO₂ is the average of CO₂ coming from diverse depths and pools, weighted for their decomposition rates (Trumbore, 1993) and shifted by isotopic discrimination during diffusion. It is very difficult and uncertain to predict the weighted $\Delta^{14}\text{C}$ value of CO₂ by SOM decomposition, although some modeling approaches based on measured CO₂ production in several horizons have aimed to do this (Gaudinski et al., 2000). Therefore, the bulk $\Delta^{14}\text{C}$ value of SOM, which does not consider the decomposability of SOM pools and their depth distribution, is not suitable for the partitioning of total CO₂ efflux from soil into plant- and SOM-derived CO₂ (Trumbore and Zheng, 1996). Simplifying assumptions (Wang et al., 1993; 1994) such as (1) constant total CO₂ production in the entire soil depth profile, (2) constant ¹⁴CO₂ content of root-derived CO₂ with depth, (3) a linear decrease in the ¹⁴CO₂ content of SOM-derived CO₂ with depth, have allowed the modeling of ¹⁴CO₂ content of soil air, but are not sufficient for the separation of plant- and SOM-derived CO₂ (Amundson et al., 1998).

Given these difficulties, at least two empirical approaches exist to use ¹⁴C for the partitioning of total CO₂ efflux into SOM-derived and root-derived CO₂ (modified from Amundson et al., 1998): (1) incubation of intact, plant-free, soil cores under controlled conditions with collection and measurement of ¹⁴C in respired CO₂, (2) collection and measurement of ¹⁴C in CO₂ respired in the field during periods with very low root activity.

The non-uniform distribution of bomb-¹⁴C (¹⁴C produced mainly in late 1950s and early 1960s after the testing of atomic bombs in the atmosphere) between SOM pools with different turnover rates is also problematic. Isotopic disequilibrium of bomb-¹⁴C (distribution of bomb-¹⁴C between the SOM pools is not proportional to their total C content and depends on the turnover rates of the SOM pools) makes relative contributions to total soil CO₂ efflux difficult to estimate. However, a modeling approach makes it possible to estimate the contribution of pools with turnover times of years and decades to total soil CO₂ losses from the bomb ¹⁴C signal (Trumbore, 1997; Gaudinski et al., 2000). In the first step, theoretical $\Delta^{14}\text{C}$ values for the SOM pools with different turnover rates are modeled (Fig. 5 in Gaudinski et al., 2000) based on the $\Delta^{14}\text{C}$ dynamics in the atmosphere (Burchuladze et al., 1989; Levin and Kromer, 1997). Then, $\Delta^{14}\text{C}$ values and total C are measured in SOM pools separated by a fractionation procedure (e.g. based on density) and turnover rates are estimated from the theoretical modeled $\Delta^{14}\text{C}$ values. In the third step, the contribution of each separated SOM pool to the total CO₂ efflux is calculated based on the C content of this pool and its turnover rate. Thus, the $\Delta^{14}\text{C}$ value of SOM-derived CO₂ efflux, weighted by the contribution of individual pools is calculated using a mass balance approach. In the last step, comparison of the calculated weighted $\Delta^{14}\text{C}$ value of the SOM-derived CO₂ efflux with the $\Delta^{14}\text{C}$ values of the total CO₂ efflux, allows the contribution of root-derived CO₂ (with a $\Delta^{14}\text{C}$ value of the current atmosphere) to be calculated by mass balance. The whole approach can be done for individual soil horizons (Gaudinski et al., 2000).

Despite the many assumptions involved in the modeling and subsequent calculations, the approach based on bomb-¹⁴C has two very important advantages: (1) The method does not disturb the soil, so interactions within the ecosystem are not disrupted, and (2) In addition to information on the separation of SOM-derived and root-derived CO₂ turnover rates and MRT of individual SOM pools are calculated. However, this technique is not widely used due to laborious sample preparation procedures and the extremely high cost of accelerator mass spectrometry (AMS), although some simplifications have been suggested recently (Hahn and Buchmann, 2004).

3.1.2.4. ¹⁸O of CO₂. Differences in the ¹⁸O isotopic composition of water in soil and plants, and ¹⁸O isotopic exchange between CO₂ and H₂O in leaves have been used to estimate the contribution of individual sources to fluxes of CO₂ and H₂O from ecosystems to the atmosphere (Riley et al., 2003). Despite some acceptable simulations, as yet the CO₂ efflux from soil has not been partitioned on the basis of the ¹⁸O content of CO₂. Factors preventing the successful application of this technique are (modified from Amundson et al., 1998): (1) ¹⁸O isotopic exchange between CO₂ and soil water (Yakir and Sternberg, 2000), (2) ¹⁸O isotopic discrimination due to dissolution of CO₂ in soil water, (3) isotopic discrimination of CO₂ during diffusion through the soil profile, (4) incomplete ¹⁸O equilibrium between CO₂ and H₂O in leaves that is especially pronounced in C₄ and C₃ grasses (Gillon and Yakir,

Table 3
Summary of methods allowing partitioning of root-derived CO₂ into root respiration and rhizomicrobial respiration (for details see Kuznyakov and Larionova, 2005)

Methods	Principle	Use ^a	Advantages	Disadvantages
Non-isotopic methods				
Component integration	Manual separation of sources and measuring CO ₂ from each source by incubation	F→L	Cheap, various ecosystems, separation of 3 (4) CO ₂ sources	Laborious; strong CO ₂ flush after disturbance and root injuries; different CO ₂ decrease after the flush → dependence of the results on the CO ₂ trapping period
Respiration by excised roots	Measuring of specific respiration rates of excised roots separated from soil	F→L	Cheap, useful for roots of various plants	Strong CO ₂ flush after disturbance; results depends on the duration of CO ₂ trapping
Substrate induced respiration	Different response of microbial respiration and root respiration on the addition of glucose	F, L	Cheap, various ecosystems, more exact than other methods; development of the method by C ₄ sugar applied for C ₃ soil is possible	Many treatments and measurements are necessary; laborious
Isotopic methods				
Comparison of root-derived ¹⁴ CO ₂ with rhizomicrobial ¹⁴ CO ₂	Comparison of root-derived ¹⁴ CO ₂ during continuous labeling with rhizomicrobial ¹⁴ CO ₂ by decomposition of uniformly ¹⁴ C labeled rhizodeposits from the same plants	L	Estimation of additional parameters as total C input by plants into the soil; separation of SOM-derived and root-derived CO ₂	Very long incubation is necessary; many treatments are necessary; uncertainties by estimation of the stabilization factor; only for grasses and crops
Isotope dilution	Dilution of ¹⁴ CO ₂ of rhizomicrobial respiration of labeled plants by CO ₂ from decomposition of added unlabeled glucose	L	No effect of roots on glucose addition; reasonable separation results	Assumed constant ratio between root respiration and rhizomicrobial CO ₂ after ¹⁴ CO ₂ labeling was not checked; laborious; many treatments
Model rhizodeposition technique	Comparison of ¹⁴ C in CO ₂ and remaining in soil in treatment with labeled plants or labeled model rhizodeposits	L	Comparatively simple	Strong underestimation of rhizomicrobial CO ₂ , overestimation of root respiration; laborious
Modeling of ¹⁴ CO ₂ efflux dynamics	Modeling of ¹⁴ CO ₂ dynamics from root respiration and from rhizomicrobial respiration after pulse labeling of shoots	L	Is based on dynamics of processes; reasonable separation results	The assumption of delayed rhizomicrobial ¹⁴ CO ₂ is not checked; many measurements are necessary; model parameterization is necessary; may be biased by diurnal CO ₂ dynamics
Exudate elution	Elution of root exudates labeled with ¹⁴ C before microorganisms decompose them and simultaneous trapping of ¹⁴ CO ₂ from root respiration	L	The only one methods allowing physical separation of CO ₂ and substances exuded by roots	Strong underestimation of rhizodeposition, overestimation of root respiration; laborious
δ ¹³ C values of CO ₂ and microbial biomass	Growing C ₄ plant on a C ₃ soil (or <i>vice versa</i> or growing plants under FACE) and calculation by δ ¹³ C values of: CO ₂ , microbial biomass, soil, roots	F, L	Absence of strong disturbance; many measurements in one canopy are possible; estimation of C sources for MB	Not tested; unconsidered isotopic effects may strongly shift the partitioning results; inactive microbial biomass dilute δ ¹³ C of active microbial biomass

^a Application in 'F' field, 'L' laboratory, 'F→L' samples taken from field are analyzed in the laboratory (is also suitable for laboratory studies).

2001), and (5) diurnal variations of the $\delta^{18}\text{O}$ of water pools (Riley et al., 2003).

In conclusion: the most accurate method to separate plant-derived from SOM-derived CO_2 is the continuous labeling of plants with $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ (Table 2). Where practical problems prevent the use of this technique, other techniques which avoid disturbance to the plant–soil system are preferable. Despite lower resolution, ^{13}C natural abundance method can be accepted as a reasonable alternative allowing application under field conditions. Bomb- ^{14}C having the fewer disturbances of natural CO_2 flows is an attractive, but very expensive approach. The difference methods, which rely on mechanical separation of components of the system and resulting in changed C, N, microbial biomass and SOM turnover should be avoided.

3.2. Separation of root and rhizomicrobial respiration: estimation of the contribution of autotrophic and heterotrophic organisms to root-derived CO_2

“Discriminating between CO_2 which is directly derived from root respiration and that which is derived from mineralization of the components of C-flow is exceptionally difficult and has presented one of the greatest challenges to quantifying rhizosphere C-flow” (Killham and Yeomans, 2001). Partitioning of root-derived CO_2 efflux from the soil into actual root respiration and microbial respiration of rhizodeposits is very important for determining the C and energy balance of soils and plants, C sources for rhizosphere microorganisms, rhizosphere priming effects, sources of soil organic matter, and linking net ecosystem C exchange with net ecosystem production.

In addition to the component integration method described above, some other approaches have been used to separate root and rhizomicrobial respiration in non-sterile soil (Table 3), thus allowing estimation of respiration by autotrophic and heterotrophic organisms. Detailed description of these approaches, their basics, advantages, shortcomings and limitations, which have been reviewed by Kuzyakov and Larionova (2005), is outside of the scope of this review. Only the background of these methods is described (Table 3):

- Respiration by excised roots is based on the estimation of specific respiration rates of roots washed or picked from the soil (see above).
- Substrate induced respiration (SIR) is based on the response of microbial respiration to the addition of glucose and the absence of a response of root respiration (Panikov et al., 1991). The differences in the response and the comparison of CO_2 efflux before and after glucose addition allow calculation of root respiration and microbial respiration. Ekblad and Höglberg (2000) greatly improved this method, and allowed it to be applied under field conditions, by applying C_4 sugar to soil with C_3 plants and measuring the $\delta^{13}\text{C}$ of respired CO_2 . The substrate induced respiration method for separation of root and microbial CO_2 should not be confused with the SIR method for microbial biomass estimation (Anderson and Domsch, 1978).
- Root-derived $^{14}\text{CO}_2$ (the sum of root respiration and rhizomicrobial respiration) during continuous labeling of plant shoots in $^{14}\text{CO}_2$ atmosphere is compared with rhizomicrobial $^{14}\text{CO}_2$ originating from the decomposition of uniformly ^{14}C labeled rhizodeposits from the same plants (Johansson, 1992). The estimation of the initial amount of rhizodeposits released by the plants is based on the stabilization of rhizodeposited-C in the soil after partial decomposition.
- The isotope dilution method is based on the dilution of $^{14}\text{CO}_2$ evolved from the rhizosphere of ^{14}C labeled plants by CO_2 from microbial decomposition of unlabeled glucose added to the soil (Cheng et al., 1993). The CO_2 evolved by decomposition of unlabeled glucose dilutes the $^{14}\text{CO}_2$ of rhizomicrobial respiration, and at the same time, $^{14}\text{CO}_2$ of root respiration remains undiluted. Addition of increasing amounts of unlabeled glucose allows root respiration to be calculated. Subtraction of root respiration from total root-derived $^{14}\text{CO}_2$ gives the rhizomicrobial respiration.
- The model rhizodeposition technique is based on the comparison of $^{14}\text{CO}_2$ efflux from the soil and ^{14}C remaining in the soil of two treatments (Swinnen, 1994): (1) plant shoots are labeled with $^{14}\text{CO}_2$; (2) ^{14}C labeled artificial rhizodeposits are added to the soil with unlabeled plants. The calculation of root and rhizomicrobial respiration by the model rhizodeposition method assumes a constant ratio of microbially respired ^{14}C to the ^{14}C of organic substances remaining in the soil with natural rhizodeposits (labeled plants) and with artificial rhizodeposits (Swinnen, 1994). In the first step, $^{14}\text{CO}_2$ evolved by rhizomicrobial respiration of natural rhizodeposits is calculated based on the decomposition of artificial rhizodeposits. In the second step, root respiration is calculated as the difference between root-derived $^{14}\text{CO}_2$ and $^{14}\text{CO}_2$ of rhizomicrobial respiration estimated in the first step.
- The modeling of $^{14}\text{CO}_2$ efflux dynamics (Kuzyakov et al., 1999; 2001; Kuzyakov and Domanski, 2002) is based on the $^{14}\text{CO}_2$ efflux dynamics from soil after pulse labeling shoots with ^{14}C and modeling C flow in the rhizosphere. The C flow simulation reveals a time delay in the release of CO_2 coming from microbial utilization of rhizodeposits vs. the CO_2 coming from root respiration. Tracing the $^{14}\text{CO}_2$ efflux from soil after ^{14}C pulse labeling of plants, fitting the model parameters on the measured $^{14}\text{CO}_2$ efflux, and subsequently modeling root respiration and the microbial decomposition of exudates allows the independent estimation of both flows.
- The exudate elution procedure is based on the elution of ^{14}C labeled exudates from the rhizosphere of plants labeled in $^{14}\text{CO}_2$ atmosphere before soil microorganisms take the exudates up and decompose them (Kuzyakov and Siniakina, 2001). The $^{14}\text{CO}_2$ evolved from root respiration is collected simultaneously. To achieve both aims, the soil is flushed with a continuous flow of air–water mixture. Here, it is important to mention that despite

underestimation of rhizodeposition and overestimation of root respiration, the exudate elution procedure is the only one method based on physical separation of both flows. All other methods (except component integration) are based on calculations that make assumptions, which are difficult to check.

- Recently, the theoretical background to a new approach, based on $\delta^{13}\text{C}$ values of CO_2 and microbial biomass during growth of C_4 plants on a C_3 soil (or *vice versa* or growing plants in an atmosphere enriched or depleted in ^{13}C , e.g. FACE), has been suggested (Kuzyakov, 2004). However, this approach has not been tested experimentally.

Comparison of the results of separation of root respiration and rhizomicrobial respiration for grasses obtained by some of the isotopic approaches and by combination of some difference methods suggests that root respiration comprises on average 48% of root-derived CO_2 and rhizomicrobial respiration 52% (Kuzyakov and Larionova, 2005). However, there are still very few studies, which have attempted to partition root-derived CO_2 into root and rhizomicrobial respiration.

3.3. Separation of CO_2 efflux by microbial decomposition of plant residues

At the beginning of this section, it is important to note that the experimental estimation of the contribution of microbial decomposition of plant residues to total CO_2 efflux is much easier than the estimation of root-derived CO_2 . It is easier experimentally because assessment of the input of dead plant residues is much easier than the estimation of the total amount of rhizodeposits and intensity of root respiration. However,

whilst incorporation of dead shoot material into the soil can be estimated under field conditions, inputs of dead root are notoriously difficult to estimate with any certainty.

Methods similar to those used for separation of SOM- from plant-derived CO_2 can be used to distinguish between CO_2 coming from microbial decomposition of plant residues or organic fertilizers and CO_2 coming from SOM (Table 4). The common approaches are: (1) plant residue exclusion, (2) artificial ^{13}C or ^{14}C labeling, and (3) ^{13}C natural abundance.

The exclusion technique (Table 4) is based on the comparison of CO_2 efflux from soil of two treatments: (1) soil free from recent plant residues and (2) soil containing recent plant residues. The exclusion method does not differ greatly from those described above for the separation of root-derived from SOM-derived CO_2 . It has similar principles, advantages and shortcomings, and therefore, will not be described in detail here. The exclusion technique is used in practically all decomposition studies.

The labeling approach is based on the incorporation of plant residues, which have been uniformly labeled in an atmosphere of constant isotope ratio into unlabelled soil (Table 4). Liberated CO_2 is trapped in alkali solution. The contribution of plant residues (labeled) and SOM (unlabeled) to the evolved CO_2 is calculated according to the dilution of specific ^{14}C activity (Bottner and Austrui, 1998; Gale and Cambardella, 2000) or ^{13}C : ^{12}C isotope ratio (Lu et al., 2003) of CO_2 trapped in alkali. The results of these studies investigating decomposition of ^{14}C and ^{13}C labeled plant residues have been reviewed (Scharpenseel and Neue, 1984; Dormaar, 1990; Cadisch and Giller, 1997) and are beyond the scope of this review.

Recently, ^{13}C natural abundance (Rochette et al., 1999a; Liang et al., 1999) or litter labeled in FACE experiments (Subke et al., 2004) have been used to make the same kinds of

Table 4

Summary of methods allowing separation of SOM-derived CO_2 and litter CO_2 or estimation of CO_2 evolved by priming effect

	Methods	Principle	Use ^a	Advantages	Disadvantages
SOM-derived CO_2 from litter CO_2 (Section 3.3)	Plant residue exclusion	Comparison by difference of CO_2 from soil with and without plant residues	F, L	Simple, cheap, various ecosystems	Different C and N cycling in treated vs. untreated soil, different microbial activity → different SOM decomposition
	^{13}C or ^{14}C labeled plant residues	Addition of uniformly ^{14}C or ^{13}C labeled plant residues to soil and estimation of SOM-derived CO_2 according to the dilution of ^{14}C or ^{13}C in CO_2	F, L	Exact, the only one fully correct method, estimation of priming is possible	Uniformity of ^{14}C or ^{13}C distribution should be checked; laborious preparation of uniformly labeled plant residues; mainly for laboratory conditions
	^{13}C natural abundance or FACE	Addition of residues of a C_4 plant to C_3 soil or <i>vice versa</i> or addition of plant residues from FACE to any soil (preferably C_4 soil) + measuring the contribution according to the dilution of $\delta^{13}\text{C}$ of litter CO_2 by CO_2 from SOM	F, L	Simple, wide application is possible	Isotopic discrimination by decomposition should be considered, non-uniformity of distribution between individual substances in plant residues; not precise
Priming CO_2 (Section 3.4)	Difference of CO_2 evolved from SOM decomposition under different treatments	Comparison of SOM-derived CO_2 from planted soil (or soil with plant residues) vs. CO_2 from unplanted soil (or soil without plant residues). Any isotopic methods for estimation of SOM-derived CO_2 from planted soil (or soil with plant residues) can be used (see Table 2).	L (F)	No other methods available	Application of ^{14}C or ^{13}C is a prerequisite; very sensitive to all errors

^a Application in 'F' field, 'L' laboratory.

measurements. Despite low resolution of the ^{13}C natural abundance method, the absence of artificial labeling and nearly uniform distribution of ^{13}C make it a very useful tool to separate CO_2 coming from plant residues and CO_2 coming from microbial decomposition of SOM. By the combination of ^{13}C labeled needles (by FACE) with girdling of Norway spruce, Subke et al. (2004) estimated the contribution of three CO_2 sources: aboveground needle decomposition, root-derived CO_2 of spruce and SOM decomposition. This combined approach also allowed interactions between rhizosphere activity and needle decomposition to be evaluated. Interactions between plant residue and SOM decomposition remains one of the most important uncertainties in the use of difference methods for the evaluation of source contribution to total CO_2 efflux (see below).

Recent studies using compound specific $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}$ signature of individual substances, such as sugars, peptides, lignin etc.) instead of the ^{13}C of bulk plant residues have shown that $\delta^{13}\text{C}$ varies within plant tissues. The ^{13}C isotopic signature varies not only between plant organs and tissues, but also between individual substances such as lignin, cellulose, lipids, alkanes, and even between individual positions of C atoms in molecules (reviewed by Hobbie and Werner, 2004). In comparison to bulk plant material, lipids are depleted by 1.9–9‰, alkanes by 2.7–10.1‰, and fatty acids by 6.5–10.3‰ (Hobbie and Werner, 2004 and references therein). Thus, it may be expected that other compounds such as sugars, and amino acids should be ^{13}C enriched. Because sugars and amino acids are generally decomposed during the first few days following the incorporation of plant residues into the soil, the $\delta^{13}\text{C}$ value of evolved CO_2 should be ^{13}C enriched as compared with bulk material and lead to inaccuracies in estimates of the contribution of plant material to the total CO_2 flux. Later stages of decomposition may be similarly affected due to the ^{13}C depletion of more recalcitrant compounds such as lignin, lipids, alkanes and fatty acids. The interpretation of measurements is further complicated by the fact that ^{13}C fractionation between and within compounds (Rossmann et al., 1991) is different between C_3 and C_4 plants (Hobbie and Werner, 2004). Recently, the $\delta^{13}\text{C}$ of CO_2 evolved during decomposition of plant residues has been shown to vary by 3 to 5‰ over time (Hamer, 2004, pp. 131–150). Therefore, the results of separating SOM- and plant residue-derived CO_2 by ^{13}C natural abundance should be used with caution.

3.4. Estimation of CO_2 evolved due to the priming effect

The decomposition rates of SOM in soil with and without living plant roots or plant residues can differ (reviewed by Dormaar, 1990; Kuzyakov, 2002a; Paterson, 2003; Cheng and Kuzyakov, 2005). It seems that the decomposition intensity of SOM by the microbial community is changed if an easily available C source (e.g. rhizodeposition or plant residues) is present in the soil. Such effects, known as priming effects can be positive (increase of SOM decomposition) or negative (decrease of SOM decomposition, suppressing effect). Similar effects on N mineralization following additions of N

fertilizers have been accepted for many decades and have frequently been termed Added Nitrogen Interactions (ANI) (reviewed by Jenkinson et al., 1985; Kuzyakov et al., 2000).

To estimate the CO_2 evolved by the rhizosphere priming effect two prerequisites are necessary (Table 4). Firstly, a clear separation of SOM-derived from root-derived (or litter-derived) CO_2 is required. Only methods based on the use of C isotopes, such as continuous labeling of plants in $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ atmosphere (the best option) or ^{13}C natural abundance approach or FACE (worse option) allow such separation. Secondly, the SOM-derived CO_2 from soil with plants or plant residues should be compared with total CO_2 efflux from unplanted soil (equal to the SOM-derived CO_2). The unplanted soil should not contain any easily available organic substances such root or shoot residues or organic fertilizers, which are not the part of SOM.

The priming effect induced by the decomposition of fresh plant residues can be calculated in a similar manner to the rhizosphere priming effect. In this case, the use of ^{14}C or ^{13}C uniformly labeled plant residues for decomposition is necessary. Using a combination of uniformly labeled ^{13}C needles and spruce girdling, Subke et al. (2004) showed that rhizosphere priming of SOM decomposition was not significant, but decomposition of needles was increased in the presence of living roots.

It is important to emphasize that in both cases (rhizosphere priming and plant residue-induced priming) the calculation of priming effect is actually based on comparison of two different methods: (1) direct measurement of SOM-derived CO_2 from unplanted soil and (2) one of the isotopic methods separating SOM-derived CO_2 from planted soil. Therefore, all analytical and experimental errors associated with the separation of SOM-derived and root-derived CO_2 lead to errors in estimates of the priming effect (Table 4).

3.5. Approximation of C balance at the ecosystem scale

As detailed above, there have been many investigations of plant–soil system C balance. However, current concerns about increasing atmospheric CO_2 concentration and its effect on global climate mean that it is a matter of some urgency to generalize results for the evaluation whole ecosystem C dynamics. Hanson et al. (2000) reviewed results for forest ecosystems, but a recent review for grassland and agroecosystems is needed.

In order to generalize results for whole ecosystems, it is crucial to decide which approaches are adequate to do this. There are many differences between experiments, which make generalization difficult (Craine and Wedin, 2002). For instance, soils vary in C_{org} content and SOM pools, their decomposability, depth distribution, bulk density, and activity and composition of microbial biomass; site management practices vary in fertilization rate, and cutting or grazing frequency; vegetation varies in species composition, age, root depth distribution and biomass; and environmental conditions vary in moisture, temperature and light. Furthermore, interactions

between biotic and abiotic variables may not always be the same.

In my opinion, the amount of plant-derived CO₂ is easier to predict than the amount of SOM-derived CO₂. This is due to the fact that plant-derived CO₂, especially root-derived CO₂, is closely connected with physiological processes of plant and root growth, exudation and respiration. Indeed, Gifford (2003) showed that approaches used to assess plant respiration are adequate for most C balance applications. I, therefore, suggest a simple method for the approximation of the contribution of plant-derived CO₂ sources to the total CO₂ efflux from soils.

Models based on physiological and biochemical mechanisms of CO₂ exchange assume that plant respiration (R , g C–CO₂ d⁻¹) is proportional to the mass of plant dry matter (Gifford, 2003):

$$R = R_s(T)W$$

where $R_s(T)$ (g C–CO₂ g⁻¹ d⁻¹) is the temperature dependent specific respiration rate and $W(g)$ is the plant dry weight. For young plants grown in nutrient solution, specific respiration rates range from 12 to 100 mg C–CO₂ (g C)⁻¹ d⁻¹ (Gifford, 2003), but respiration rates of mature plants grown in soil are lower (Reich et al., 1998) and cannot be extrapolated from young plants grown in nutrient solution. Although the specific respiration rates of roots are similar to those of shoots (Reich et al., 1998), and root-derived respiration has been shown to be proportional to both above- and below-ground plant biomass (Craine et al., 2001; Hill et al., 2004), interspecies and other experimental variation leads me to suggest a refined method which is also based on plant dry weight.

In a review of about 30 studies, in which grasses or agricultural cereals were labeled in a ¹⁴CO₂ atmosphere to evaluate below-ground C translocation by plants, it was concluded that wheat (as representative of agriculture cereals) uses 9% of its total assimilated C and pasture grasses (as representative for natural grass vegetation) use 15% for the root-derived CO₂ (Kuzyakov and Domanski, 2000). By calculation of the ratio of shoot mass to total below ground C translocation (Table 5), it can be estimated that for an average yield of 6 Mg ha⁻¹ y⁻¹ grain yield or dry matter production (for pasture), 0.9 and 1.2 Mg C CO₂ ha⁻¹ y⁻¹ will be lost as root-derived CO₂ (Table 2). This means that we can roughly predict (for the common agricultural cereals and pasture of average intensity) the amount of root-derived CO₂ based on the below-ground allocation of C and utilization of C in the plants. These values are not fixed, and allow only a very rough approximation of the amount of root-derived CO₂, but taking account of the plant lignocellulose fraction by consideration of plant N content may help to improve estimates (Reich et al., 1998; Burton et al., 2002; Gifford, 2003).

This approach is suitable only to a rough estimate the contribution of roots of grasses and cereals to the total CO₂ flux during the 2–4 month growing season. It is not suitable for shorter-term predictions (days), because environmental factors, especially those affecting photosynthetic rate can strongly change the amount of root-derived CO₂.

Table 5

Approximation of total C input in the soil and sources of root-derived CO₂ efflux from the soil under wheat with 6 Mg ha⁻¹ grain yield and in a pasture of about 6 Mg ha⁻¹ dry matter production

	Percent of total assimilated		Mg C ha ⁻¹ ^a	
	Wheat	Pasture	Wheat	Pasture
Above-ground C	75	60	7.5	4.8
Shoot C	50	30	5.0	2.4
Shoot respiration (dark + photo)	25	30	2.5	2.4
Below-ground C	25	40	2.5	3.2
Root C	13	20	1.2	1.6
Soil + MO ^b	3	5	0.3	0.4
Root-derived CO ₂ ^c	9	15	0.9	1.2
Root respiration (48%)	4.3	7.2	0.43	0.58
Rhizomicrobial respiration (52%)	4.7	7.8	0.47	0.62
Total assimilated C	100	100	10	8.0

Combined from Kuzyakov and Domanski (2000); Kuzyakov and Larionova (2005). It is accepted that total above-ground plant mass is 2 times higher than the grain yield.

^a C content in dry mass of shoots and roots is accepted by 40%.

^b C remains in soil and microorganisms.

^c Root-derived CO₂: the sum of root and rhizomicrobial respiration (the contributions are taken from Kuzyakov and Larionova, 2005).

A comparison of methods for the separation of root and rhizomicrobial respiration estimates that root respiration accounts for about 48% and rhizomicrobial respiration about 52% of root-derived CO₂ (Kuzyakov and Larionova, 2005), although methods for partitioning of these fluxes are subject to a good deal of uncertainty. This further separation of root-derived CO₂ into root respiration and rhizomicrobial respiration (as shown in Table 5) provides better evaluation of the balance of below-ground translocated C and very rough estimation of the amount of rhizodeposits. However, values for root-derived CO₂, and root and rhizomicrobial respiration need further experimental verification especially with respect to different plant types. Respiration estimates for perennial and woody vegetation should be applied with caution, as data for C distribution and root-derived CO₂ fluxes (Table 5) are generally estimated from experiments lasting less than one growing season. Furthermore, the pattern of assimilate distribution in trees is very different from that of non-woody species. Högberg et al. (2002) calculated that trees use about 75% of below-ground translocated C for respiration and exudation and only 25% for root growth, which is far from the 1:1 ratio frequently accepted for grasses (Barber and Martin, 1976; Barber, 1979). Estimation of below-ground C partitioning for individual vegetation types is urgently required for ecosystem-level estimation of root-derived CO₂ fluxes from soil. When root-derived CO₂ fluxes from soil are estimated, the SOM-derived CO₂ efflux can be estimated by difference from measured values of total CO₂ efflux.

4. Comparison of methods

After reviewing the partitioning methods described above, the important question is: Which method is the best one? There

are no partitioning methods without significant shortcomings (Tables 2–4), so it is not possible to choose an ideal approach for the partitioning of below-ground CO₂ fluxes. However, the criteria for an ideal method can be suggested. The ideal approach for CO₂ partitioning should:

- (1) have no (or insignificant) disturbance of the ecosystem studied or CO₂ fluxes,
- (2) be suitable for the separation of CO₂ originating from all five sources mentioned in Fig. 1,
- (3) be universally applicable to a range of ecosystems: grassland, crops, shrubs and forest,
- (4) generate reproducible and reliable results independent of the personnel conducting experiments, and
- (5) be inexpensive and simple in terms of equipment, maintenance and analysis.

In this enumeration, I have tried to order the requirements according to their importance. One of the most important criteria for the results is the first one, as disturbance has unquantifiable effects on flux estimation. Most of the shortcomings of the reviewed methods are associated with disturbance. In a graphical representation of method suitability (Fig. 2), this first criterion can be used as one axis. Since none of the methods (including component integration) fulfill the second requirement, I have combined the last three requirements (3, 4 and 5) to form the second axis and named it ‘Universality’. The weighting of the three requirements comprising the axis ‘Universality’ is very subjective, since the importance of each criterion depends on the exact

information required, ecosystem studied, scale of investigation and available facilities. For comparison of the methods all approaches described in the review (see Tables 2–4) were placed in the field of both axes: ‘Disturbance’ and ‘Universality’ (Fig. 2). Methods suitable for separation of SOM and root-derived CO₂ cannot be directly compared with methods aimed on separation of root and rhizomicrobial respiration, so the latter have been presented with gray shading on the plot of ‘Disturbance’ and ‘Universality’ (Fig. 2). Methods allowing the estimation of CO₂ from the litter layer (O horizon) are mostly based on approaches similar to those for SOM and root-derived CO₂ partitioning (component integration, application of uniformly labeled plant residues, FACE or C₃/C₄ combination); so these methods are not shown separately on Fig. 2. The best method is located top left of the plot (highest universality and lowest disturbance) and the worst at the bottom right (lowest universality and highest disturbance).

Fig. 2 reveals that the method with the lowest disturbance and highest universality is the regression technique. This approach can be used in many ecosystems, is comparatively cheap, and hardly disturbs the system during CO₂ flux measurements. Although it has uncertainties associated with its reliance on correlation, it is surprising that this method has not been used more frequently. Despite large (probably the highest) disturbance, the most universal method remains component integration. This method is able to differentiate between three (root respiration, microbial decomposition of SOM, litter decomposition) or even four (+ rhizomicrobial respiration) sources of CO₂, but requires further development,

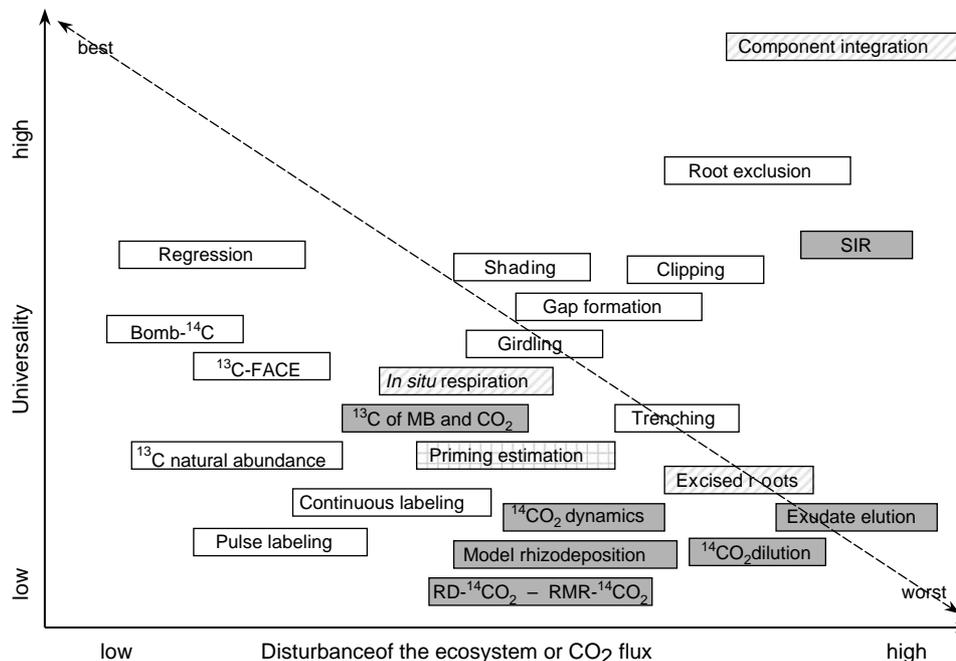


Fig. 2. Methods for CO₂ flux partitioning positioned according their disturbance effect on the ecosystem studied (X-axis) and the universality of the method based on its applicability to various ecosystems, reliability of results, and costs (Y-axis). An ideal method would be located on the top left and the worst on bottom right (see broken arrowed line). Methods for separation of SOM-derived and root-derived CO₂ are in white. Methods for the partitioning of root-derived CO₂ into root respiration and rhizomicrobial respiration are in gray. Methods suitable for total CO₂ partitioning and estimation of the actual root respiration are crosshatched in gray. An untested method is bordered with a broken line.

standardization and quality control before it can be used to provide reliable estimates. Isotopic methods, which have received a positive evaluation in this review, are located no higher than the bottom half of Fig. 2, because their application is limited, especially under field conditions.

All methods for the separation of root from rhizomicrobial respiration are located at the bottom right of Fig. 2, showing high disturbance and low universality. Therefore, most results obtained by these methods (reviewed Kuzyakov and Larionova, 2005), should be considered as preliminary and their further improvement is greatly needed. Although many approaches have been suggested and applied, many uncertainties remain due to their reliance on unproven assumptions.

Given the variety of methods already in existence (Fig. 2), it is not likely that many techniques based on wholly new approaches will be suggested in the near future. Further improvement to methods for partitioning of CO₂ fluxes may be based on the combination of more than one method for one study. The few studies, in which two or more approaches were combined have yielded much more information than the sum of the individual methods. Kelting et al. (1998) combined trenching with excised roots method to partition the total CO₂ efflux in forest with *Quercus rubra* and separated (1) root respiration, (2) rhizomicrobial respiration, and (3) SOM-derived CO₂. By combining root exclusion with component integration, root respiration, rhizomicrobial respiration and SOM-derived CO₂ have been separated for maize, barley and wheat, and integrated over the whole growing season (Larionova et al., (in press)). Combining shading, clipping and incubation of excised roots in grassland, Craine et al. (1999) estimated root respiration by two approaches. The combination of traditional methods with approaches based on isotopes seems especially promising for the future improvement of estimates (Gaudinski et al., 2000; Högberg et al., 2001; Kuzyakov and Cheng, 2001; Subke et al., 2004; Cheng et al., 2005; Kuzyakov and Bol, 2006).

5. Conclusions and future needs

Reviewing existing approaches shows that it is critical to estimate the contribution of individual sources to total soil CO₂ efflux, and no single, fully satisfactory partitioning method yet exists. To date, the most reliable methods for the separation of root-derived from SOM-derived CO₂ are based on continuous labeling of plants in ¹⁴CO₂ or ¹³CO₂ atmosphere (the best option, but only suitable for laboratory conditions), ¹³C natural abundance or the use of the δ¹³C of CO₂ in FACE experiments (satisfactory and applicable under field conditions). Bomb-¹⁴C is the approach which allows CO₂ sources to be separated with the least disturbance, but very high costs of analyses and some uncertainties limit its application. Because most isotopic methods are difficult to apply under field conditions they cannot be used for broad-range screening of various ecosystems on diverse scales. Therefore, the non-isotopic methods should be calibrated and standardized by comparison with isotopic approaches. In addition, simple calculation methods (e.g. based on plant physiology) should be developed

to approximate root-derived CO₂ for the extrapolation of results to whole ecosystems.

Future developments in the partitioning of below-ground CO₂ fluxes are likely to be based mainly on the combination of the existing approaches and their standardization.

The most challenging areas are the separation of root respiration from rhizomicrobial respiration, and the evaluation of the effects of living roots on the decomposition SOM (rhizosphere priming effects). Approaches allowing reliable quantification of these fluxes/effects, under both laboratory and field conditions are urgently needed.

One important group of approaches not mentioned in this review is the modeling of CO₂ fluxes from the soil. Experimental partitioning of CO₂ fluxes for various ecosystems and evaluation of the main environmental and plant factors controlling CO₂ fluxes from individual C pools will allow their modeling to scale up the results from point measurements to ecosystem, regional and global scales.

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