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How to link soil C pools with CO_2 fluxes?

Y. Kuzyakov

Dept. of Agroecosystem Research, BayCEER, University of Bayreuth, Universitätsstr. 30, 95444 Bayreuth, Germany

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Correspondence to: Y. Kuzyakov (kuzyakov@uni-bayreuth.de)

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Abstract

Despite the importance of carbon (C) pools and CO_2 fluxes in terrestrial ecosystems and especially in soils, as well as many attempts to assign fluxes to specific pools, this challenge remains unsolved. Interestingly, scientists investigating pools are not closely linked with scientists studying fluxes. This mini-review therefore focused on experimental approaches enabling soil C pools to be linked with CO_2 flux from the soil. The background, advantages and shortcomings of uncoupled approaches (measuring only pools *or* fluxes) and of coupled approaches (measuring both pools *and* fluxes) were evaluated and their prerequisites – steady state of pools and isotopic steady state – described. The uncoupled approaches include: (i) monitoring the decrease of C pools in long-term fallow bare soil lacking C input over decades, (ii) analyzing components of CO_2 efflux dynamics by incubating soil without new C input over months or a few years, and (iii) analyzing turnover rates of C pools based on their ^{13}C and ^{14}C isotopic signature. The uncoupled approaches are applicable for non steady state conditions only and have limited explanatory power. The more advantageous coupled approaches partition simultaneously pools *and* fluxes and are based on one of three types of changes in the isotopic signature of input C compared to soil C: (i) abrupt permanent, (ii) gradual permanent, and (iii) abrupt temporary impacts. I show how the maximal sensitivity of the approaches depends on the differences in the isotopic signature of pools with fast and slow turnover rates. The promising coupled approaches include: (a) $\delta^{13}C$ of C pools and CO_2 efflux from soil after C_3/C_4 vegetation changes or in FACE experiments (both corresponding to continuous labeling), (b) addition of ^{13}C or ^{14}C labeled organics (corresponding to pulse labeling), and (c) bomb-^{14}C. I show that physical separation of soil C pools is not a prerequisite to estimate pool size or to link pools with fluxes. The future challenges include combining two or more promising approaches to elucidate more than two C sources for CO_2 fluxes, and linking scientific communities investigating the pools with those investigating the fluxes.

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1 Preamble

Two high-ranking international conferences motivated me to prepare this mini-review. At the first conference the results of various approaches to separate pools of soil organic matter (SOM), and thus carbon (C) pools in soil, were presented and discussed. These approaches are based on chemical and physical fractionations (extractability, particle and aggregate size, density, etc.) as well as their combinations (von Lutzow et al., 2007). Despite some progress to separate C pools of different age and thus of different turnover time, it was concluded that the pools obtained by any of the approaches are operationally defined – so they actually do not really exist (Bruun et al., 2010). Despite intensive testing, no approach was found to separate very old C pools (inert C), which are not involved in annual and decadal C cycles, from very recent C pools contributing to annual and interannual C cycles (Helfrich et al., 2007). The turnover time of the separated pools was estimated based on various isotopic approaches (Baisden et al., 2002; John et al., 2005). Based on the turnover time, possible contributions to the CO_2 fluxes from soil to the atmosphere were discussed, *but not measured*.

The second conference focused on CO_2 fluxes from soil and their partitioning. The goal was to evaluate possible sources of CO_2 and thus to gain insights into the C pools responsible for these fluxes. Again, the results of various approaches for CO_2 partitioning were mainly based on exclusion of some sources or on the C isotopic signature of CO_2 ($\delta^{13}C$, $\Delta^{14}C$) (Kuzyakov, 2006; Trumbore, 2006). The important part of the discussions and all outlooks of this conference were focused on the question: How we can find the C pools in soil that are responsible for these CO_2 fluxes?

Surprisingly, there was *no overlap of the colleagues participating in both conferences*!

2 Why it is crucial to link C pools with CO_2 fluxes?

Our thinking about ecosystem functioning is defined by pools and by fluxes. Therefore, nearly all ecosystem models (reflecting our thinking), including the models of soil C dynamics and CO_2 fluxes from soil, are based on pools linked together by fluxes within a system and with input and output. Accordingly, the accuracy and precision in predicting ecosystem functioning under a broad range of environmental conditions, but also under various disturbances, strongly depends on the correctness of the linkages between conceptual pools, estimation of their capacity, and rates and volume of the fluxes between the pools.

The *pools* reflect the *static components* of a system, and the *fluxes* are responsible for its *dynamics*. Thus, *pools* and *fluxes* are responsible for the *stability* and for *flexibility*, respectively, of any ecosystem. These static and dynamic ecosystem components have important consequences for the analysis of pools and fluxes. If we investigate the *pools* per se, which are the stable component – the analysis of *changes over the long term* is necessary. Over the short term the changes of pools are insignificant, especially considering high intrinsic variation of pools in all natural ecosystems. The changes of pools over the long period therefore provide a clear direction of the ecosystem alteration. In contrast to pools, *fluxes have a fast response* to changes of environmental conditions or of land use. So, the response of the fluxes is much faster than that of the pools. This is because most of the fluxes originate from *small pools, but having a (very) fast turnover*. Therefore, and in contrast to pools, the changes of fluxes over the long term may not clearly reflect the ecosystem changes, because the fluxes are highly variable depending on various biotic (Buchmann, 2000; Kuzyakov, 2010) and abiotic factors (Davidson et al., 2000; Kirschbaum, 2006). An important *consequence* of the mentioned contrasts *between pools and fluxes* is that the *mean residence time* (MRT) *of* C in *pools* is *much longer than the MRT of* C in *fluxes*. The common example for this fact is the discordance between $\delta^{13}C$ of microbial biomass and $\delta^{13}C$ of CO_2 efflux from soil after C_3-C_4 vegetation change (Werth et al., 2006). Due to this discordance between MRTs of pools and fluxes (Collins et al., 2000; Taneva et al., 2006), the calculation of the contribution of SOM pools to CO_2 fluxes based only on the MRT of C pools will underestimate the fluxes. Therefore, in this minireview, focused on the approaches linking C pools with CO_2 fluxes, I do not describe the approaches allowing estimation of MRT and turnover time of pools. This discordance between MRT of C in pools and in fluxes and its consequences, however, is a broad fascinating topic requiring for a separate review.

Interestingly, although we are able to measure very precisely the input and output fluxes of a system, in most cases our experimental approaches fail to measure the exchange between ecosystem parts, and, thus, between individual pools within a system. This is particularly the case in systems as complex as soils. Frequently, we cannot even conclude whether some pools are linked together or not! For example, it still remains unclear whether SOM pools are under exchange, or whether plant and microbial litter C is directly incorporated into specific SOM pools and microbially decomposed thereafter without internal exchange. Thus, within a system, we cannot clearly separate the pools (even if they exist). This makes it difficult to link the pools with fluxes. Nonetheless, the correct linking of pools and fluxes is crucial for:

- understanding how the system works (what are the linkages between the pools)
- evaluating interactions within a system
- evaluating processes under steady state (see below) in a system
- quantifying biotic and abiotic drivers responsible for changes in individual pools and for overall changes in a system
- assessing the resilience and resistance and, closely connected, evaluating stability and flexibility of a system
- process-related prediction and mechanistic modeling of system behavior beyond the experimental conditions (in light of future global and climate changes, response to strong disturbances, etc.)

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This urgently calls for establishing links between pools and fluxes. This is especially important for soil, not only because it stores most of the terrestrial C, but also because in most global models soil still remains a "black box". Such a "black box" approach is surely insufficient to predict changes under new environmental conditions, as the processes (linkages between the pools) inside the box are not reflected. This "black box" approach underlines our weakness in linking pools with fluxes. This is because we are strongly limited by the appropriate experimental approaches. Therefore, this mini-review focuses on evaluating the known experimental approaches that can be used for this aim.

3 Steady state of pools and isotopic steady state

An important feature of soils (and many other systems) hampers process-oriented studies and the linking of pools with fluxes: many soils are in a *steady state* concerning the level of total C and C in the SOM pools (at least related to the duration of our experiments and funding). *Steady state is a state of an open system in which the input is equal to the output over a longer period*[1]. Steady state of an open system leads to steady state between the pools – the absence of pool changes over time. Thus, measuring the pool's size over time will not reveal any changes and we will not be able to investigate processes. Because of this hampering feature, *most studies on soils are still focused on the soil properties and properties of soil components, but not on processes*.

Only one methodological approach allows investigating *processes under steady state*: the *application of tracers*. The *tracer approach assumes identical behavior (including transformation) of the tracer with the substance (or pool) under investigation*. Because of nearly identical chemical and biochemical properties of isotopes of one

[1] Steady state in a *closed system* is termed *dynamic equilibrium* means identical rate of exchange between the pools, but without in- and output.

element, *isotopic tracers* are the most frequently used and the most powerful tracer application.

Most soils are not only under steady state of C pools, but also under *isotopic steady state*. This means that over a defined period, *there are no changes of isotopic composition of the input C and consequently of the C pools in soil and of the output C* (e.g. CO_2 efflux). Under such conditions – steady state of pools and isotopic steady state – there are no approaches that would enable investigating processes and no approaches that would enable linking soil C pools with CO_2 fluxes (Table 1).

Despite the absence of changes, the isotopic composition of individual pools under steady state may differ. This can be used (i) to evaluate ^{13}C isotopic fractionation in soil (Blagodatskaya et al., 2011a) and (ii) to estimate mean residence time of C in very slow pools by radiocarbon dating (not bomb ^{14}C) (Scharpenseel et al., 1989). In contrast, *disequilibrium in isotopic composition* can be used and *is a prerequisite for studying processes under steady state*. This means that the isotopic composition of the input C changes over time, and the isotopic composition of the SOM pools follow it with a *delay corresponding to the turnover time of individual pools*. Note here that the amount and quality of the C input should remain constant.

As shown below, some approaches linking soil C pools with CO_2 fluxes are suitable for non-steady state conditions, whereas other approaches using isotopic disequilibrium between C input and SOM pools can be applied for soils under steady state (Table 1).

4 Approaches to link pools and fluxes

The variety of approaches linking pools and fluxes is limited, and we can enumerate them on one hand (Table 2). Theoretically, linking pools and fluxes requires measuring both. Due to certain assumptions, however, some approaches allow to measure only pools *or* fluxes and to conclude about fluxes or pools, respectively. I will term these approaches *uncoupled approaches*. They usually deliver only relative results that are difficult to compare with other studies. The other group of approaches is based on the analysis of both pools *and* fluxes and will be termed *coupled approaches.* These coupled approaches allow more definite and precise conclusions. Therefore, I describe these groups of approaches separately.

4.1 Uncoupled approaches

The uncoupled approaches are based on measuring *changes* of pools *or* of fluxes during a time period in the absence of C input into the system (soil) (Table 2). This means that they can be used only under *non steady state* conditions. As the isotopic composition of pools and CO_2 is not analyzed, it is irrelevant whether the soil under isotopic steady state or not (Table 1).

4.1.1 Decrease of C pools in a bare soil (long-term bare fallow experiments)

This approach is based on repeated measurements of soil C pools physically separated by one of the fractionation methods mentioned above in long-term bare fallow (LTBF) experiments. Long-term absence of any C input (fallow soil) depletes the total C stock in soil (Fig. 1). This depletion differs for individual C pools. As the decomposition of each C pool in soil commonly adheres to first-order kinetics (Parton et al., 1987), a simple estimation of decomposition rates (k) of the physically[2] separated pools (P) can be done by parameter fitting of the equation:

$$P(t) = \sum_{n=1}^{i} P_i(0) \cdot \exp(-k_i \cdot t) \tag{1}$$

where $P_i(0)$ and $P(t)$ are the measured size of separated pools, i is the number of pools at time 0 and time t. The respective estimation (not measurement!) of the CO_2 flux

[2]Here and elsewhere: "physical separation" means separation of soil C pools by any fractionation method including chemical extractions; particle or aggregate size or density fractionation, or separation based on thermal stability or molecular mass fractionation.

during the whole period of the LTBF experiment, cumulative from all individual pools, corresponds to the decrease of the respective pools; at the time t, it can be assessed by:

$$CO_2^i(t) = P_i(0) \cdot (1 - \exp(-k_i \cdot t)) \quad (2)$$

The corresponding estimation or CO_2 efflux rates from individual pools (${}^{Rate}CO_2^i(t)$) at time t can be calculated as:

$${}^{Rate}CO_2^i(t) = P_i(0) \cdot k_i \cdot \exp(-k_i \cdot t) \quad (3)$$

The rate of the CO_2 efflux from all pools (${}^{Rate}CO_2^i(t)$) at time t will be

$${}^{Rate}CO_2(t) = \sum_{n=1}^{i} P_i(0) \cdot k_i \cdot \exp(-k_i \cdot t) \quad (4)$$

Because of the slow decomposition rates, the significant decrease of the C pools can be measured only after many decades (Jenkinson and Coleman, 1994). As there are only very few LTBF experiments (Askov, Bad Lauchstädt, Grignon, Kursk, Rothamsted, Ultuna, Versailles) without any inputs over decades (Barre et al., 2010), this approach can be applied only at these sites. To my knowledge this approach was used solely to calculate decomposition rates of SOM pools (Barre et al., 2010) and to verify SOM models (Foereid and Hogh-Jensen, 2004; Ludwig et al., 2007). These decomposition rates of pools, however, were fitted by one or two exponential approaches based on the decrease of total C content only (not on separated pools) and the results were not linked with CO_2 fluxes. Only once was this LTBF approach used to separate SOM pools and estimate their decomposition rates (Vasilyeva et al., 2011).

This very simple approach has some hidden assumptions:

1. The main hidden assumption is that each C pool undergoes only decomposition and that there are no exchange between the pools (see above). This assumption

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cannot be tested because a homogeneous labeling (see below) of one soil C pool without labeling the others is impossible.

2. In order to correctly link the decrease of the C pools with the fluxes, it should be assumed that all losses of C from the respective pool are connected with mineralization of SOM to CO_2. This assumption is very probable: even on sites with high precipitation, DOC leaching is at least one and, in most ecosystems, two orders of magnitude lower than the CO_2 flux from the soil (Siemens, 2003; Kindler et al., 2010).

3. The calculation of decomposition rates (Eq. 1), and thus of the contribution to the CO_2 efflux from soil (Eq. 2), is based on first-order kinetics. Decomposition of a pool may be limited not only by the pool size, but may also involve other factors, e.g. microbial activity (Blagodatski et al., 2010), therefore decomposition does not necessarily decrease exponentially.

4. It is assumed that the measured depletion of pools in the bare soil (without C input) corresponds to decomposition rates of the pools with continuous C input or plant cropping. This implies the absence of priming effects (Kuzyakov, 2010).

An important advantage of the LTBF is that it is *the only approach allowing estimation of decomposition rates of slow pools*. Because fast pools are usually very small (see above), and will be depleted fast after the absence of C input, their changes are difficult to follow using the LTBF approach. In contrast, the gradual, continuous decrease of slow pools (e.g. black carbon, Vasilieva et al., 2011), can be estimated more precisely than by using other approaches.

Despite the very few sites and relatively narrow applicability, I would encourage using this comparatively simple approach on all LTBF experiments to estimate the decrease of C pools (especially those with slow turnover) and thus to indirectly estimate their long-term contribution to the CO_2 fluxes from soil.

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4.1.2 Kinetic approach in incubation studies

This approach (frequently termed biological approach or biological CO_2 fractionation) is based on the kinetics of CO_2 efflux from soil (without C input) and is typically used to evaluate the results of incubation (Kätterer et al., 1998; Pendall and King, 2007; Cabaneiro et al., 2008) or field studies (Taneva et al., 2006). The principle is also based on first-order kinetics (Parton et al., 1987), but of the CO_2 efflux from soil and not of the C pools as in the previous LTBF approach. The underlying assumption is that 1) the amount of C mineralized to CO_2 is proportional to the decomposition rates (k) and the pool size ($P(0)$), and 2) various pools (i is the number of pools) in soil contribute parallel (independently, i.e. without interactions; no priming effects) to the CO_2 efflux with different rates. Accordingly, the total C mineralized to CO_2 ($CO_2(t)$) until time t can be calculated as:

$$CO_2(t) = \sum_{n=1}^{i} P_i(0) \cdot (1 - \exp(-k_i \cdot t)) \tag{5}$$

If only one pool ($i = 1$) contributes to the CO_2 efflux, then the fitted parameters $P(0)$ and k correspond to the pool size and its decomposition rates. The size and the rate determine what this pool contributes to the total CO_2 efflux from soil. The same estimation can be based on CO_2 efflux rates (${}^{\mathrm{Rate}}CO_2(t)$):

$${}^{\mathrm{Rate}}CO_2(t) = \sum_{n=1}^{i} P_i(0) \cdot k_i \cdot (1 - \exp(-k_i \cdot t)) \tag{6}$$

Here the initial CO_2 efflux rates (${}^{\mathrm{Rate}}CO_2^i(0)$) from individual pools corresponds to:

$${}^{\mathrm{Rate}}CO_2^i(0) = P_i(0) \cdot k_i \tag{7}$$

Due to the relatively short duration (months to maximally a few years) of most incubation studies and thus the negligible contribution of slow pools to CO_2 flux, the fitted $P(0)$ pool size does not correspond to the total C content in the soil.

Because the total CO_2 efflux in the most incubation studies (especially long term) does not correspond to the exponential decay from one C pool (Magid et al., 2002), the parallel contribution of many C pools to the CO_2 efflux with different rates is assumed (Kätterer et al., 1998). Although in reality many C pools contribute to CO_2 efflux, most studies (e.g., Collins et al., 2000; Kalbitz et al., 2005) use only the sum of two exponents:

$$CO_2(t) = P_1(0)\cdot(1-\exp(-k_1\cdot t)) + P_2(0)\cdot(1-\exp(-k_2\cdot t)) \tag{8}$$

In some cases, three pool models were also applied (Taneva et al., 2006; Cohran et al., 2007). Due to the intercorrelation of the parameters by fitting, however, independent approaches to estimate the size or the rate of one of the pools are necessary (Paul et al., 2001). One recommendation is the successive subtracting of long-lived components – the approach frequently used in radiochemistry to determine independently decaying radionuclides (Taneva et al., 2006 and references therein).

Based on the common high variation of CO_2 efflux rates, the cumulative CO_2 efflux over a time period can be used. This allows a much more precise parameter estimation because variation of CO_2 efflux rates within a short period are smoothed over a long time. Accordingly, the integrative form of Eq. (3) should be used:

$$^{\text{cumulative}}CO_2(t) = \sum_{n=1}^{i}\left[\frac{P_i(0)}{k_i}\cdot(1-\exp(-k_i\cdot t))\right] \tag{9}$$

The fitting of parameters of Eq. (6) (or the respective two components in Eq. 8) results in 2 parameters for each of 2 pools (Paul et al., 2001): initial size of both pools ($P_1(0)$ and $P_2(0)$) and the respective decomposition rates (k_1 and k_2). These four parameters allow comparison of two pools, e.g. fast/active and slow pools with regard to pool size and decomposition rates (Collins et al., 2000; Kalbitz et al., 2003). Surprisingly, examining the studies that used this approach reveals that the sizes of the two pools differ by at least one order of magnitude ($P_{\text{fast}} \ll P_{\text{slow}}$), and the rates of the fast pool are at least one order of magnitude higher than that of the slow pool ($k_{\text{fast}} \gg k_{\text{slow}}$). This reflects one of the shortcomings of this approach: it is not possible to separate pools having similar decomposition rates. This is necessary because although two pools may have similar decomposition rates, they may differ considerably in pool size and functions. Therefore, the kinetic approach is unsuitable to consider the exhaustion of the one of the pools after some period, if any other pool has a similar decomposition rate.

Another shortcoming of this approach is the interdependence of the parameters obtained by fitting (Paul et al., 2001). Thus, slight changes of the CO_2 efflux curves (e.g. incubation period, sampling frequency and timing) may strongly bias all parameters. The results linking pools and fluxes obtained by this approach are therefore poorly comparable with other studies, because the fitted pool sizes and the rates strongly depend on incubation duration. Moreover, other experimental conditions (soil amount, incubation conditions, CO_2 sampling strategy, ...) strongly affect the obtained results. This complicates comparisons with other studies. The approach does enable comparing the results of incubations of various treatments of the same soil, e.g. soils from plots with contrasting fertilization over lengthier periods (Majumder et al., 2010). This makes it possible to evaluate whether the fast/active or the slow pools have increased and how the rates have changed. The results of pools and flux rates are therefore relative (Table 3).

The incubation approach may be coupled with preceding physical separation of individual pools, e.g. for particle size fractions (Ohm et al., 2007) or aggregate fractions with subsequent evaluation of active and slow pools. Similarly, this yields the relative pool sizes and decomposition rates, and comparisons with other studies are hardly possible.

4.1.3 Concluding remarks on uncoupled approaches

In conclusion, the uncoupled approaches allow comparatively simple calculation of fluxes based on the pools and vice versa. Therefore, the link between pools and fluxes is unidirectional and this link cannot be objectively proven. The long-term bare field

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approach is preferable to estimate linkages between slow pools and CO_2 fluxes. Physical separation of pools is necessary to better estimate pool decomposition rates by the LTBF approach. In contrast, the kinetic approach using incubation studies is quicker, requires no physical separation of pools, and is mainly suitable for estimating decomposition rates of fast pools. The results obtained on pool sizes and flux rates by the incubation approach cannot be easily compared with other studies. The main shortcoming of both approaches is that they are suitable *only for non-steady state conditions* – without substrate input.

Note that there are various other approaches allowing estimation of MRT of C in the pools based on changes of isotopic signature of the C input compared to that of the SOM (Table 2, see the description of some approaches below). These isotopic approaches allow estimation of MRT both under steady state and non-steady state conditions (Table 2). However, it is important that the discordance between MRT of C in pools and in fluxes may lead to underestimation of CO_2 flux based on MRT of pools.

4.2 Coupled approaches

All coupled approaches are based on simultaneous measurement of C pools *and* CO_2 fluxes (Table 2). As mentioned above, a clear physical separation of individual functional C pools in soil by existing fractionation methods is not possible now and probably will not be possible in the future. This calls for other approaches (Bruun et al., 2010). The prerequisite for linking pools and fluxes by coupled approaches is being able to partition the total C in soil at least for 2 pools and the total CO_2 flux from soil at least for 2 component fluxes. The only approaches allowing such partitioning without physical separation are based on the disequilibrium of C isotopes (^{13}C and/or ^{14}C) or, more precisely, on the changes in the C isotopic signature of the input and subsequently of SOM. Only the three options are available (Fig. 2 top):

- *Abrupt permanent*: fast change and remaining on the new level; this corresponds to *continuous labeling*.

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- *Gradual permanent*: slow continuous change to a new level.
- *Abrupt temporary*: fast change and return to the previous level; this corresponds to *pulse labeling*.

These changes in the isotopic signature of the input C will lead to contrasting changes in the isotopic signature of SOM (Fig. 2 bottom) that are described below.

Note that in further discussions of all these options that alter the isotopic signature of SOM, we assume a *steady state* of the input and of the decomposition and, consequently, of the SOM level and of individual pools. Further applications are certainly possible also for the *non steady state* conditions (Table 1), but this requires more complex calculations considering the changes of total C stocks.

4.2.1 Abrupt permanent impact = continuous labeling

Background: the abrupt permanent impact assumes a strong change in the isotopic signature of C input (the input remains nearly the same, steady state conditions) and that it remains on the new level. This corresponds to *continuous labeling* (Kuzyakov and Domanski, 2000). This will lead to asymptotic convergence in the isotopic signature of SOM, theoretically leading to a new constant level corresponding (isotopic fractionation should be considered, see Werth and Kuzyakov, 2010) to the isotopic signature of the C input (Fig. 2). Shortly after the change in the isotopic signature of the input and, consequently, of the fast pools, the C pools in soil can be well linked with CO_2 fluxes.

Applications: the well known and the most frequently used approach representing an abrupt permanent impact is a C_3-C_4 vegetation change (Balesdent et al., 1987). This provides a new isotopic signature for all soil components. Here, the amount (and quality) of C input remains nearly the same, but the δ^{13}C signature of the new input differs significantly from that of the previous vegetation. For the principles of C_3-C_4 vegetation change approaches for SOM studies, see Balesdent et al. (1987), Flessa et al. (2000)

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and Werth and Kuzyakov (2010). A similar application that provides a new isotopic signature is the Free Air Carbon dioxide Enrichment (FACE) studies, which add ^{13}C depleted CO_2 (Andrews et al., 1999; Van Kessel et al., 2000; Hoosbeek et al., 2004). In addition, the combination of C_3-C_4 vegetation change and FACE approaches was used to increase the differences in isotopic signature of the C input and SOM (Ineson et al., 1996). This, in turn, increases the sensitivity and resolution in the partitioning of pools and CO_2 fluxes. As an alternative to the C_3-C_4 vegetation change or FACE approaches, which provide a new isotopic signature at the level of natural abundance, continuous labeling with strongly enriched (Evdokimov et al., 2004) or depleted (Cheng and Dijkstra, 2007; Paterson et al., 2008; Gamnitzer et al., 2009) CO_2 may be used.

How we can use these approaches (C_3-C_4 vegetation change, FACE, or others) to link pools and fluxes? The basic prerequisite is that the isotopic signature (δ^{13}C) of C pools with different turnover rates will differ after the C_3-C_4 vegetation change (all further arguments are correct also for FACE). This means that the isotopic signature of SOM allows conclusions to be drawn about the minimal (C_3 signature) and maximal (C_4 signature) age of two C pools and of the CO_2 flux (Blagodatskaya et al., 2011a). This can be demonstrated by a following theoretical example (Table 3): The contribution of C_4-C to the total soil C ten years after C_3-C_4 vegetation changes is 25% (the original approach suggested by Balesdent et al. (1987) can be used to calculate contributions of old and new C based on δ^{13}C signature of the mixing pool and both endmembers). Accordingly, 25% of C in soil is younger and 75% of C is older than 10 yr (Table 3). The ratio of C_4-to-C_3 in the SOM is therefore 0.33. At the same time the contribution of the C_4-C to the total CO_2 efflux from soil is 50%, and the respective contribution of C_3-C is also 50%. Here, the ratio of C_4-to-C_3 in the CO_2 is 1.0. Considering the ratio of C_4-to-C_3 in the SOM and that in CO_2 efflux, the turnover of C that is younger than 10 yr (C_4-C) is 3.0 times faster that is of the C older than 10 yr (Table 3). This yields the relative turnover of the old (> 10 yr) and new (< 10 yr) C in SOM, estimating the contribution of the two SOM pools, with different age ranges, to the CO_2 efflux. Based on the δ^{13}C signature, two SOM pools were linked with two CO_2 fluxes. Despite its simple applicability, this approach was rarely used (Collins et al., 2000; Blagodatskaya et al., 2011a) to link pools in SOM and in microbial biomass with the CO_2 flux from soil. To evaluate the absolute contribution, the relative data (e.g., Table 3) should be multiplied by C stocks in SOM and by C fluxes as CO_2.

The described example reflects a single time window (here 10 yr) after the abrupt permanent impact and cannot be extrapolated to determine the changes of relative turnover of C pools that originated earlier or later. Thus, based on one such "screen shot", we can neither estimate the *function of the changing availability of C in SOM over time* (Bruun et al., 2010). Nonetheless, this would be precisely our main aim, if we want to link pools with fluxes! To calculate such a function of changing C availability would require analyzing the $\delta^{13}C$ signature of SOM and of the released CO_2 by the same approach over the longer period – at least several years. I was unable to find any studies with such an application and, therefore, tried to simulate them. The simulation was based on a simple model, taking the OM as a whole and assuming that the decomposition rate is a function decreasing exponentially with time (Fang et al., 2005). The model reflects such changes of the C_4-to-C_3 ratio in the SOM and that in CO_2 efflux (Fig. 3). This enables important conclusions to be drawn for linking pools and fluxes. Despite a slow asymptotic increase of C_4-C in the SOM, its portion in the CO_2 increases much faster. Thus, the C_4-to-C_3 ratio in the SOM increases and reach saturation, but C_4-to-C_3 ratio raises exponentially in CO_2 (Fig. 3, bottom). This means that after some period after the impact, despite the high portion of the remaining C_3-C in SOM, its contribution to the CO_2 efflux is negligible! For example, according to the modeling on Fig. 3, 100 yr after the vegetation changes, the C_3-C in SOM is still about 30% (i.e. high). At the same time, the C_3 contribution to the CO_2 efflux is only 1.0%. So, the relative turnover of old (> 100 yr) and new (0–100 yr) SOM is about 45 times (Fig. 3, bottom)! Similar results – a very low contribution of C_3-C to CO_2 despite its high portion in the SOM – have been frequently confirmed experimentally (Paul et al., 1997; Collins et al., 2000; Taneva et al., 2006). This approach therefore clearly shows the portion (and the amount) of the inert C pool, which contributes nothing or negligibly to

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the CO_2 efflux from the soil. The advantage is that no physical separation of the pools is necessary. Note here that the linkage between the C in the soil and the CO_2 efflux by the approach presented in Fig. 3 only one pool, but with turnover rates decreasing after the C entered the soil, was used. Different functions of the changes of SOM availability with its aging were suggested (Bosatta and Ågren, 1985; Manzoni et al., 2009; Bruun et al., 2010) but were never proven experimentally.

The approach described above is based on the asymptotic convergence of the isotopic signature of SOM to that of the C input (Fig. 2). A valuable alternative, but based on the same approach, was suggested by Taneva et al. (2006). They examined the disappearance of the old C under FACE. In contrast to the previous approach, it focused not on the increase of the new C, but on the decrease of old C. At first glance both approaches seem very similar: both use a similar exponential approach to estimate decomposition/turnover rates and would be expected to yield similar results on the contribution of C pools to CO_2 fluxes. However, based on the increase of new C, the contribution of faster SOM pools will be estimated (versus the approach based on the decrease of old C). This discordance in the contribution of old and new SOM pools, estimated based on disappearance of old and new C, is closely connected with the discordance of MRT of pools and fluxes mentioned above.

Sensitivity: the sensitivity of approaches to separate pools, and to link these pools with fluxes, is proportional to the maximal difference between the isotopic signatures of C pools with slow and fast turnover. This is schematically presented for each approach in Fig. 3 (bottom) by the slim lines “slow” and “fast”; the respective area between both lines shows the whole range of SOM pools with different turnover rates. *The larger the difference between the isotopic signature of “slow” and “fast” pools, the higher the sensitivity* of the approach. This sensitivity depends also on the period after the start of isotopic disequilibrium; and strongly decreases when the new isotopic steady state is approached.

The sensitivity of the abrupt permanent impact approach is maximal when the isotopic signature of fast pools is already close to the new steady state, but that of the

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slow pools is far from it. Considering turnover rates of SOM pools and depending on the pools being examined, the maximal sensitivity of this approach for linking pools with CO_2 fluxes will be reached after several years to few decades.

4.2.2 Gradual permanent impact

Background: the gradual permanent impact assumes slow, continuous changes in the isotopic signature and asymptotic convergence to a new isotopic steady state (Fig. 2). The gradual permanent impact is possible in two options: (i) gradual change of isotopic signature of the input, or (ii) gradual change of the isotopic signature of SOM.

Applications: the first possibility for gradual change in the isotopic signature of the input may occur e.g. by aridization of the climate, which slowly suppresses or replaces plants with C_3 photosynthesis with plants with C_4 photosynthesis. In contrast to the example described above (abrupt permanent), these changes occur very slowly and the rates of the changes are comparable with rates of SOM turnover. Similar, but reciprocal, changes can occur by climate humidization ($C_4 \rightarrow C_3$). Although such changes are well known in the past and are frequently used for regional reconstructions of paleovegetation and paleoclimate, they cannot be used for recent studies to link pools with fluxes. Firstly, the changed environmental conditions (aridization or humidization) lead to differences in SOM composition, structure and stabilization mechanisms. Secondly, in most cases, the C input amounts also change. Therefore, not only is a steady state of SOM absent (this can be considered in calculations), but the composition of SOM pools in the soil after the changes does not correspond to a soil with an unchanged environment.

The second possibility for gradual change in the isotopic signature of the input is the very small and long-term changes of δ^{13}C and Δ^{14}C of litter and, thus, of SOM caused by the Suess effect. The rates of δ^{13}C depletion of the atmospheric CO_2 are now about −0.02‰ per year (Swart et al., 2010). This is equivalent to about −2‰ per century.

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Therefore, the δ^{13}C changes of litter are very slow and contribute to the slow changes of δ^{13}C of SOM.

The second option: gradual change in the isotopic signature of SOM (at constant signature of the input) is ubiquitous for ^{14}C signatures, because of the radioactive decay of natural ^{14}C (Scharpenseel, 1971). As the decay constant of the ^{14}C isotope ($\lambda = 1.21 \times 10^{-4}$ a^{-1}; $T_{1/2} = 5730$ a) is comparable with the turnover rates of slow and very slow SOM pools (decades to millennia), the slow decrease of the ^{14}C content in SOM leads to changes of its ^{14}C/^{12}C ratio. This decrease in the ^{14}C/^{12}C ratio will be continuously compensated by the new ^{14}C with the fresh litter input. Both processes, radioactive ^{14}C decay and continuous input of new ^{14}C, stabilize the ^{14}C signature at a constant level (Cherkinsky and Brovkin, 1993) that corresponds to the turnover of the respective C pool. Linking C pools with CO_2 fluxes requires measuring the ^{14}C signature in SOM and in CO_2. Thereafter, the "age" (in practice the mean residence time of C in SOM; Cherkinsky and Brovkin, 1993) will be calculated and compared with the age of C in the CO_2 efflux from soil. So, in contrast to the "abrupt permanent impact" (described in detail above, using the C_3-C_4 approach example), the isotopic signature of the total SOM and its pools does not change over time, because they are in equilibrium with the input according to the turnover rates.

Sensitivity: an important shortcoming additionally limits the application of the gradual permanent impact approach to linking soil C pools with CO_2 fluxes: because of very slow changes in the isotopic signature of the input (e.g., Suess effect, ^{14}C radioactive decay), the isotopic signature of SOM also changes very slowly. Therefore, the isotopic signatures of pools with contrasting turnover rates are very close (Fig. 3, see the differences between the isotopic signature of fast and slow pools in the gradual permanent impact approach). Consequently, the separation of C pools and sources of CO_2 efflux with different turnover rates, based on isotopic composition, has a very low sensitivity. The slower the changes in the C input signature, the lower the sensitivity of the gradual permanent impact approach.

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Beside the low sensitivity, bomb-^{14}C (see below) strongly overprints the natural ^{14}C steady state between ^{14}C production in the stratosphere and radioactive decay in the soil. This is another reason why, beyond its low sensitivity, this approach, based on radioactive ^{14}C decay in soil, cannot be used in the future.

4.2.3 Abrupt temporary impact = pulse labeling

Background: an abrupt temporary impact on the isotopic signature of SOM is connected with a single strong change in the isotopic signature of the input (usually for less than one year and up to very few years) and the return to the previous level (Fig. 3). Subsequent changes in the SOM signature differ strongly in intensity and period, depending on the turnover time of the pools and pool connections (Manzoni et al., 2009). For fast pools, intensive, but short, changes of isotopic signature are common. In contrast, small and prolonged effects are typical for slow pools. These differences in isotopic signature of SOM pools enable linking them to CO_2 fluxes and evaluating the contribution of the pools.

Applications: the most common example for an abrupt temporary impact is the single (pulse) addition to soil of ^{13}C or ^{14}C labeled plant residues or individual organic substances (Sorensen, 1987; Verma et al., 1975; Kuzyakov, 1997). After microbial utilization of plant residues and their complete incorporation in SOM, various pools will have different ^{13}C or ^{14}C isotopic signatures. This difference can be used to evaluate the contribution of the C pools to CO_2 fluxes by the approach described for "abrupt permanent changes". The assumption, however, is that the isotopic signature of SOM is not changed during the CO_2 measurements. This is not entirely the case (in contrast to the "abrupt continuous changes" approach), because pulse labeling does not allow an isotopic steady state, i.e. between the isotopic signature of the input and that of the C pools. Nonetheless, the assumption is acceptable, if CO_2 is measured for a short period.

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A special and frequently used case of the abrupt temporary impact is the so-called "bomb-^{14}C". It is beyond the scope of this review to describe in detail the ^{14}C changes in the atmosphere and ecosystems after the atmospheric atom bomb tests in the 1950s and early 1960s, and I refer to original papers describing the bomb-^{14}C approach (Scharpenseel et al., 1989; Schuur and Trumbore, 2006). Bomb-^{14}C cannot be really accepted as a pulse labeling, because the ^{14}C content in the atmosphere increased for decades. This makes it comparable with the duration of SOM turnover, especially with the fast and intermediate pools. At the same time, bomb-^{14}C cannot be accepted as an abrupt permanent impact: the level of ^{14}C in the atmosphere is not constant and is continuously decreasing to the pre-bomb level (Burchuladze et al., 1989; Levin and Kromer, 1997). Despite the changing ^{14}C content in the atmosphere, the models simulating C fixation and subsequent incorporation of C into SOM enable accounting the $\Delta^{14}C$ signature to C pools with different turnover time. Subsequently, the $\Delta^{14}C$ signature of SOM and that of the released CO_2 can be used to link pools and fluxes (Gaudinski et al., 2000). This is done by an approach similar to that based on C_3/C_4 vegetation changes (see above), but considering the changing $\Delta^{14}C$ of the atmospheric CO_2 and thus of the C input into the soil.

Sensitivity: the sensitivity of the abrupt temporary impact approach is of special interest. In contrast to the two previous approaches, it has two sensitivity maxima (Fig. 3, bottom). The first maximum occurs shortly after the change of isotopic composition of the input: when the fast SOM pools have reached their maxima, but the slow pools remain nearly at the previous level. The second maximum is reached when the fast pools have returned back to the initial level prior to the labeling, but the slow pools have reached the maximum. These two maxima appear because the isotopic signature of the input actually changed twice: first by the labeling, and second after its absence. Note, however, that the sensitivity of the second peak to separate C pools with contrasting turnover rates, and thus to link them with CO_2 fluxes, is much lower than the sensitivity of the first one. This is because the isotopic signature of the slow pools after abrupt temporary impact (pulse labeling) is altered only little. The explanation is that

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most of the label is "utilized" by the fast pools, and also because of the strong dilution of the signature by the very large size of pools with slow turnover versus fast rates (K. Auerswald, personal communication, 2009).

4.2.4 Concluding remarks on coupled approaches

In conclusion, the coupled approaches are based on an analysis of the isotopic signature of SOC and CO_2 efflux from soil that allows to elucidate two C sources for CO_2. One important advantage of the coupled approaches is the direct linking of pools with fluxes. The second advantage is that they work under steady state conditions – with continuous input of new C. Depending on the change in the isotopic signature of the input C versus SOM-C, three cases are possible: (i) abrupt permanent, (ii) gradual permanent, and (iii) abrupt temporary impact. Nonetheless, only the abrupt permanent and abrupt temporary impacts, corresponding to continuous and pulse labeling, respectively, are useful because of their much higher sensitivity.

5 Challenges

This overview clearly demonstrates that only very few approaches enable linking pools and fluxes. Importantly, all the approaches (except the bare soil approach) allow elucidating two C pools and two fluxes only. Clearly, separation of two pools and two fluxes is insufficient to understand underlying mechanisms and to predict future changes. The first challenge, therefore, is to suggest approaches allowing partitioning of *more than two C sources and link them with respective components of CO_2 flux*.

Such partitioning may be based on a combination of two (or more) approaches, mainly isotope based. Thus, combining the C_3/C_4-vegetation-change approach with bomb-^{14}C or with partitioning of CO_2 efflux by incubation, would enable partitioning of 4 C sources of different age with 4 components of CO_2 fluxes. This would be a strong contribution in evaluating the availability of SOM pools (as suggested on

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Fig. 3) and their contribution to the CO_2 efflux. The combination of these approaches (C_3/C_4-vegetation-change and bomb-^{14}C) would also combine the abrupt permanent and abrupt temporary changes of isotopic signature.

A similar approach can be based on combining the C_3/C_4-vegetation-change approach with the addition of ^{14}C labeled substrates. Interestingly, this combination can be used for two aims: evaluation of (1) three or (2) four C sources in CO_2. Directly after adding ^{14}C labeled substrates, only three C sources can be evaluated: (i) old C_3-C, (ii) new C_4-C, and (iii) recently added ^{14}C labeled C (Blagodatskaya et al., 2011b). However, after complete utilization of the recently added ^{14}C labeled organics and ^{14}C incorporation in SOM with different turnover rates, four SOM pools can be elucidated as CO_2 sources: two based on ^{14}C and two based on $\delta^{13}C$ signature. To my knowledge, this approach has never been used before.

A combination of the C_3/C_4-vegetation-change approach with long-term incubation and chemical fractionation helped separate five pools and to estimate their absolute and relative turnover (Collins et al., 2000).

Another promising approach to evaluate the availability of SOM pools based on the partitioning of C pools and CO_2 fluxes for more than two components may be done *during* the changes in the isotopic composition of the SOM. As suggested above (see Gradual permanent impact), the C_3/C_4-vegetation-change approach can be done periodically on the same soil. The increasing period after the vegetation change will results in the increasing contribution of C_4 carbon to the less available SOM pools (Fig. 3). To my knowledge, such studies have never been done, even though they would clearly reflect the changes of SOM availability with time.

Further steps might include combining certain fractionation methods, especially fractionation by particle, density or aggregate size classes, with the analysis of CO_2 curves from soil incubations (Ohm et al., 2007). This approach would be especially valuable if the soil originated from studies with isotopic disequilibrium.

Last but not least, the challenge is to link two scientific communities: that investigating the pools with that investigating the fluxes!

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6 Conclusions

Furthering our understanding and prediction of C cycling in terrestrial ecosystems, and especially in soils, requires linking C pools and CO_2 fluxes. This overview underlines that only four approaches are available to enable this linkage: (1) decrease of C pools in bare soil, (2) partitioning of CO_2 efflux in incubation studies, (3) partitioning of SOC and CO_2 efflux after the C_3/C_4 vegetation changes or in FACE experiments, (4a) ^{14}C and ^{13}C labeling studies and (4b) bomb-^{14}C. Although the uncoupled approaches (1 and 2, measuring only C pools or CO_2 fluxes) have several shortcomings (e.g. not applicable under steady state), their easy application allows much broader use. The coupled approaches (measuring of both C pools and CO_2 fluxes) are more sophisticated, because they are based on simultaneous partitioning of C pools and CO_2 fluxes for two or more sources. They also provide more reliable data under steady state conditions and allow comparisons between studies.

Further elaboration of approaches for linking pools and fluxes is necessary. It remains a challenge to separate more than two pools and more than two CO_2 components in a single study. Such a separation is possible (i) by combining at least two described approaches or (ii) by using soil samples with different periods after the change in the isotopic signature of the input. Finally, the data from studies linking C pools in soil with CO_2 fluxes from soil should be organized into a data base, allowing broad conclusions to be drawn about the availability and turnover of soil C.

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Table 1. Possibility to study turnover processes and to link soil C pools with CO_2 fluxes depending on the presence of steady state of C pools and isotopic steady state.

Steady state of pools:	Isotopic steady state: No	Isotopic steady state: Yes
No	Coupled and uncoupled approaches	Uncoupled approaches
Yes	Coupled and uncoupled approaches	Impossible

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Table 2. Uncoupled and coupled approaches for linking soil C pools with CO_2 fluxes.

Approaches	
Uncoupled: analysis of C pools *or* CO_2 fluxes	Coupled: analysis of C pools *and* CO_2 fluxes
– Analysis of pools: • Decrease of C content in long-term bare fallow soil (LTBF) experiments • Mean residence time of C pools estimated by one of the isotopic approaches – C_3/C_4 vegetation change – Free Air CO_2 Experiments (FACE) – Input of ^{13}C or ^{14}C labeled organics – Bomb-^{14}C – Analysis of CO_2 fluxes • CO_2 flux dynamics by soil incubation	– Abrupt permanent impact: • C_3/C_4 vegetation change • Free Air CO_2 Experiments (FACE) – Gradual permanent impact (not used, see text) – Abrupt temporary impact: • Input of ^{13}C or ^{14}C labeled organics • Bomb-^{14}C

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Table 3. Example of approach to evaluate contribution of two SOM pools to CO_2 fluxes* based on *relative* turnover rates of old (C_3) and new (C_4) SOM pools after C_3-C_4 vegetation change. All values are presented as percentage of total C in SOM or in CO_2. (For experimental results based on this approach see Collins et al., 2000; Blagodatskaya et al., 2011a.)

	C_{total}	C_3 (old)	C_4 (new)	C_4/C_3
SOM	100	75	25	0.33
CO_2	100	50	50	1.0
Relative turnover rate: C_4 pools/C_3 pools (= relative contribution to CO_2)				3.0

* Note that on Fig. 3 the contribution of C_3 and C_4 to CO_2 efflux from soil is presented as percentage of C in SOM per year.

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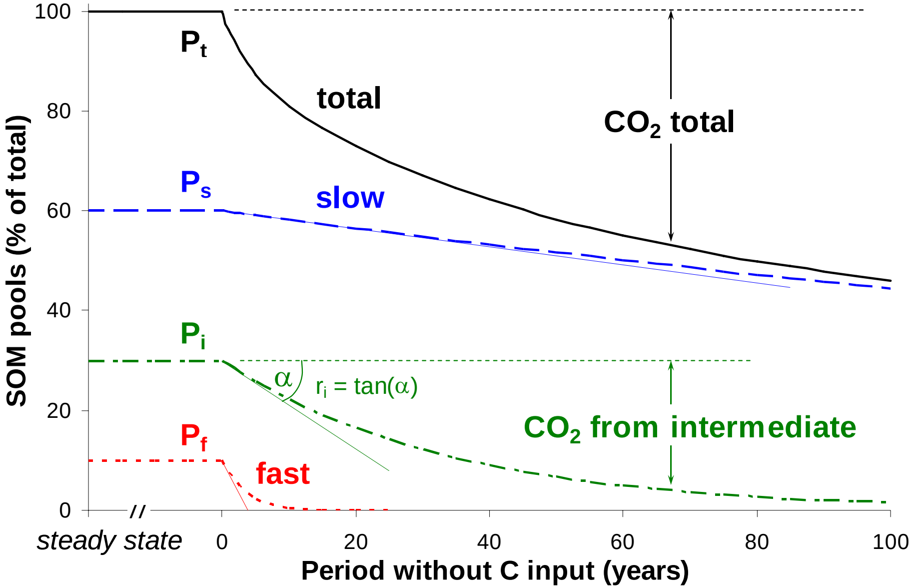

Fig. 1. Decrease of C pools in a soil by long-term bare fallow (LFTB) experiments. The decrease of three pools with fast (P_f), intermediate (P_i) and slow (P_s) decomposition rates and the respective amounts of CO_2 associated with these pools (for clarity it is shown only for intermediate pool and total C in soil) are presented. The decomposition is accepted by first order kinetics, and P corresponds to the pool size at steady state (before the start of long-term bare fallow), where the decomposition rates (r) are equal to the tangent of the angle α ($r = \tan(\alpha)$). Explanations in text.

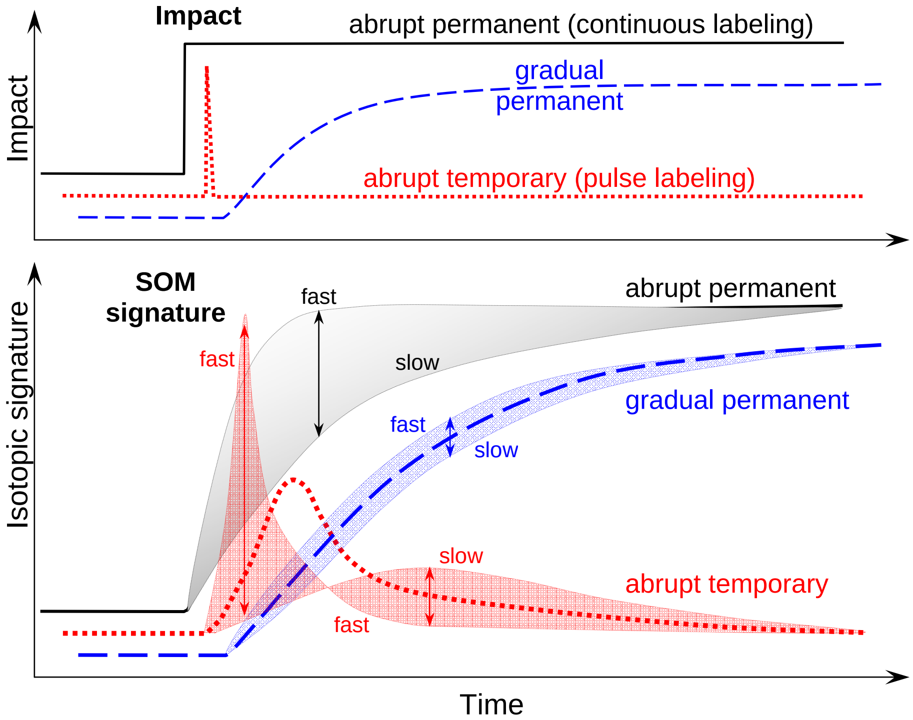

Fig. 2. Three types of impacts (above): abrupt permanent, abrupt temporary and gradual permanent; and their effect on changes of isotopic signature of soil organic matter (below). The changes of isotopic signature are presented for bulk SOM (fat curves), as well as for pools with slow and fast turnover. The hight of arrows and the shaded area showing the differences in isotopic signature between the slow and fast pools is proportional to the sensetivity of the approach for each period. Explanations in text.
Note that the three impacts are shifted in time and level to avoid overlapping of the curves. Isotopic fractionations are not considered here, and theredore the SOM pools have identical isotopic compositions before the impact and at new steady state.

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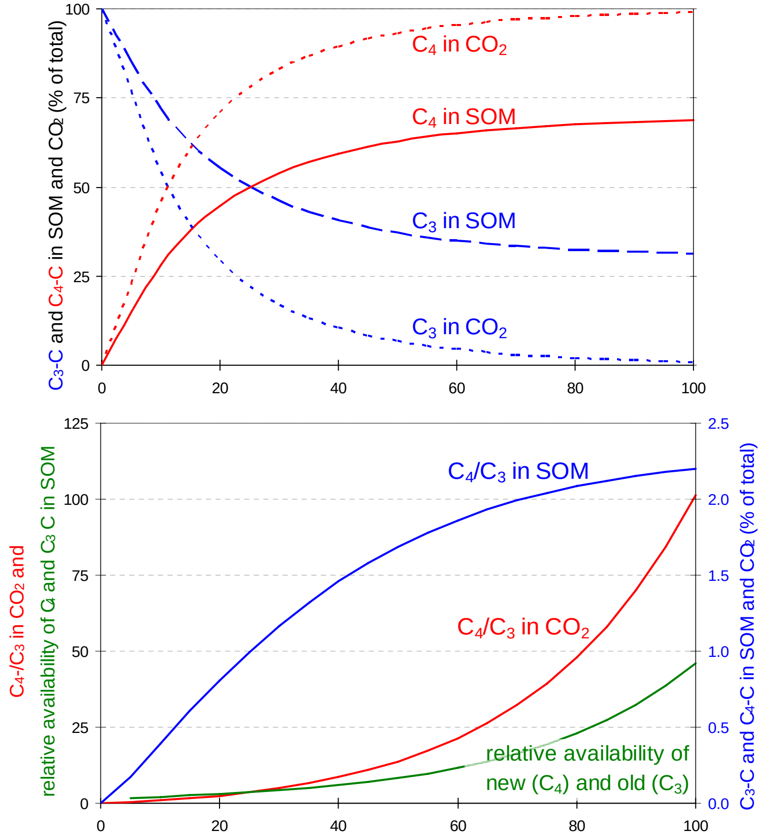

Fig. 3. Dynamics of C_3 and C_4 carbon in SOM and in CO_2 efflux (top) and the C_4/C_3 ratio in SOM and in CO_2 as well as relative availability of old (C_3) and new (C_4) carbon (bottom) after $C_3 \rightarrow C_4$ vegetation change (steady state of total SOM is assumed). Note very low contribution of C_3-C to CO_2 efflux after $\sim$50 yr despite it portion in SOM remains more than one third.