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REVIEW

Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review

E. Blagodatskaya · Y. Kuzyakov

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Abstract The number of studies on priming effects (PE) in soil has strongly increased during the last years. The information regarding real versus apparent PE as well as their mechanisms remains controversial. Based on a meta-analysis of studies published since 1980, we evaluated the intensity, direction, and the reality of PE in dependence on the amount and quality of added primers, the microbial biomass and community structure, enzyme activities, soil pH, and aggregate size. The meta-analysis allowed revealing quantitative relationships between the amounts of added substrates as related to microbial biomass C and induced PE. Additions of easily available organic C up to 15% of microbial biomass C induce a linear increase of extra CO_2. When the added amount of easily available organic C is higher than 50% of the microbial biomass C, an exponential decrease of the PE or even a switch to negative values is often observed. A new approach based on the assessment of changes in the production of extracellular enzymes is suggested to distinguish real and apparent PE. To distinguish real and apparent PE, we discuss approaches based on the C budget. The importance of fungi for long-term changes of SOM decomposition is underlined. Priming effects can be linked with microbial community structure only considering changes in functional diversity. We conclude that the PE involves not only one mechanism but a succession of processes partly connected with succession of microbial community and functions. An overview of the dynamics and intensity of these processes as related to microbial biomass changes and C and N availability is presented.

Keywords Priming effect · Soil microbial community · Isotopic methods · Soil respiration · Carbon sequestration · Carbon and nitrogen turnover · Soil organic matter · Triggering effect · Glucose

E. Blagodatskaya · Y. Kuzyakov
Department of Agroecosystem Research, University of Bayreuth, 95440 Bayreuth, Germany

E. Blagodatskaya (✉)
Institute of Physicochemical and Biological Problems in Soil Science, Russian Academy of Sciences, 142290 Pushchino, Russia
e-mail: sblag@mail.ru

Introduction

Abiotic factors such as temperature, soil moisture, and pH are main drivers of C turnover in soil and act indirectly—mainly by affecting microbial activity that drives the mineralization of soil organic matter (SOM) and plant residues. Beyond these abiotic factors, however, many biotic factors directly affect C mineralization in soil. The term priming effect (PE) was introduced to describe changes in the SOM decomposition effected by the addition of organic or mineral substances (Jenkinson et al. 1985; Kuzyakov et al. 2000). These changes are due to changes in the microbial activity as a response to altered amounts and availability of C. The increase in the number of investigations on priming effects during the last decade (Fig. 1) reflects the interest in biotic mechanisms of carbon (C) turnover in soil (Bell et al. 2003; Brant et al. 2006; Carreiro et al. 2000; Chander et al. 1997; Cheng and Kuzyakov 2005; Conde et al. 2005; Falchini et al. 2003; Fontaine et al. 2003, 2007; Perelo and Munch 2005; Zyakun and Dilly 2005).

There might be various mechanisms for the changes in microbial activity in soil after adding organic or mineral substances (De Nobili et al. 2001; Fontaine et al. 2003;

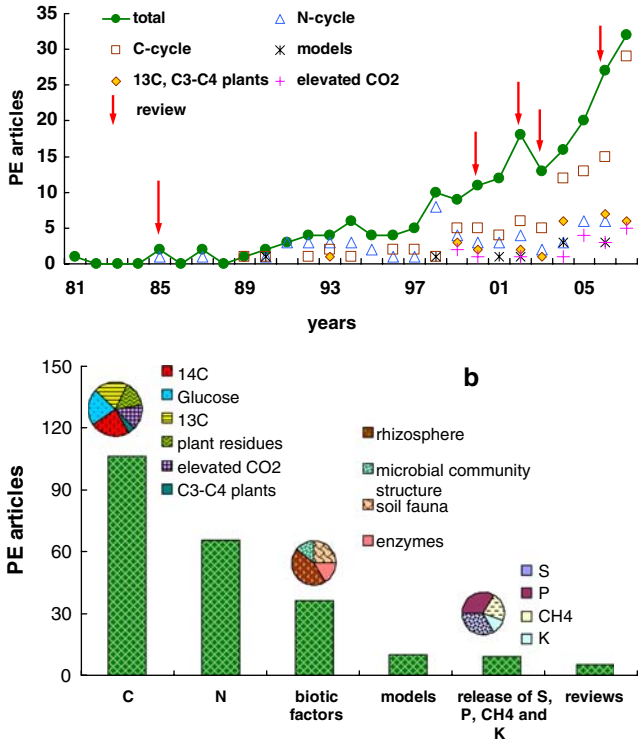

Fig. 1 Number of published papers related to the priming effects (PE) (**a**) and number of published papers dealing with C and N mineralization, effect of biotic factors on PE, models simulating PE, release of S, P, CH_4, and K, and review articles (**b**) from 1980 to late 2007. Web-of-Science citations with search options ("priming" AND "soil*") NOT "seed*" were evaluated

Jenkinson et al. 1985; Kuzyakov et al. 2000; Schimel and Weintraub 2003). However, most studies failed to prove the suggested mechanisms because the experiments were aimed at identifying priming effects (mainly by changes in CO_2 evolution) and not at identifying the mechanisms of these effects. This review will evaluate PE mechanisms based on CO_2 efflux data combined with other published data related to microbial biomass and activity. Therefore, we shall discuss the relationships linking activity, size, and composition of soil microbial communities and the magnitude of priming effects observed in studies over the last 30 years. We shall distinguish real (SOM decomposition) and apparent (changes in microbial biomass turnover without effects on SOM decomposition) PE by considering the extra released C as affected by the amount of added C and microbial biomass C content (Blagodatskaya et al. 2007; De Nobili et al. 2001; Hamer and Marschner 2005; Luna-Guido et al. 2001; Mondini et al. 2006). We shall also discuss the relationship between the phenomena of apparent or real priming effects and enzyme activity (Asmar et al. 1994; Marinari et al. 2000; Schimel and Weintraub 2003) and the role of soil properties, such as soil pH and aggregation, which were not considered earlier in affecting PE through their direct effect on biological activity.

Definitions and terms

Priming effect is defined as a short-term change in the turnover of soil organic matter caused by the treatments (usually addition organic C) of the soil (Kuzyakov et al. 2000). Usually, since the SOM turnover is not directly measured, but is determined by changes in CO_2 efflux rates or N mineralization rates, the origin of extra CO_2–C (primed carbon) or N cannot be evaluated, and thus the real priming effect cannot be assessed. Other processes, such as accelerated microbial turnover may contribute to the changes in CO_2 efflux rates or N mineralization rates (Dalenberg and Jager 1981; De Nobili et al. 2001; Wu et al. 1993). Accelerated CO_2 evolution in response to the activation of microbial metabolism and higher microbial biomass turnover is not related to the SOM turnover, and in this case, we have an apparent priming effect (APE). Usually, the microbial succession initiated by the input of fresh organic matter is accompanied by activation of various, previously dormant microorganisms that respond specifically to the added substrate. Accelerated activity of such microorganisms may enhance the degradation of soil organic matter as a result of co-metabolism and higher enzyme production, i.e., "real" priming effect (RPE). Thus, both apparent and real priming actions are governed by microbial activity. Few studies, however, have evaluated PE as a function of microbial biomass C and community structure.

Jenkinson et al. (1985) suggested that RPE is an increase in the decomposition of recalcitrant SOM, whereas APE is an increase of microbial C turnover, which is not linked with changes of SOM decomposition. Usually, the term "apparent" has been used in relation to primed CO_2–C originating from soil microorganisms (Bell et al. 2003; De Neve et al. 2004; De Nobili et al. 2001; Fontaine et al. 2003; Gioacchini et al. 2002; Hamer and Marschner 2005; Hopkins et al. 2006; Mondini et al. 2006). However, the term "apparent" priming has been also used to interpret extra CO_2 evolution derived from experimental errors due to the use of non-uniformly labeled substrate, incomplete trapping of evolved CO_2, addition of high amount of substrate to soil (Brookes et al. 1990; Conde et al. 2005), or enhanced decomposition of organic matter in response to improved soil moisture (Niklaus and Falloon 2006). We suggest such PEs as artificial to distinguish them from microbially originated apparent PE.

According to Jenkinson et al. (1985), most changes in N mineralization after the addition of N to soil are to be considered as apparent effects caused by N displacement reactions (with fixed ammonium or with microbial N) or by pool substitution. They assumed that real acceleration of SOM mineralization requires an excess of fresh organic matter with a wide C/N ratio. We believe that this could be one cause, but not the sole one for real PE.

In recent years, new terms such as "signaling" and "triggering" (De Nobili et al. 2001; Mondini et al. 2006) have been proposed for some PEs, but they were not clearly defined. In this paper, we define the triggering effect as an acceleration of internal microbial metabolism with quick increase in the respiratory activity promoted by trace amounts of substrate. In many studies and under field conditions, the amount of C added to soil does not represent a significant source of energy but can promote changes whose energy demand can be much higher than the energy of the added substrate. This triggering effect can activate dormant soil microorganisms if promoted by the addition of available low molecular substrates such as glucose and amino acids to soil (De Nobili et al. 2001; Kuzyakov and Bol 2006). Triggering effect induced by the input of external substrate should be distinguished from quorum sensing, which includes a wide range of signal exchanges within soil microbial community producing multiple cell–cell signaling molecules (Lazazzera 2000; Burmolle et al. 2003; Wang and Leadbetter 2005) and inducing gene expression encoding a variety of both phenotypical and physiological responses upon reaching a critical threshold (Gray and Smith 2005). Since the underlying molecular mechanisms of triggering effect need to be investigated, we can hypothesize that permanent or occasional input of available low molecular weight substrates associated to plant residues, root exudates, excretes of soil animals, etc., can act as external inducer and can trigger the mechanism of microbial communication similar to quorum sensing that can regulate the behavior of a group of organisms (Raffa et al. 2005). Thus, triggering effect promoted by external compounds can represent the first step in a signaling pathways cascade, which alter the behavior on a population-wide scale by intra- and inter-species microbial interactions (Gray and Smith 2005; Waters and Bassler 2005; Little et al. 2008).

The amount of added substrate, the microbial biomass content, and priming effects

Adding easily available substances to soil provides C and energy sources to microorganisms. Depending on the amount of added C and energy, three types of changes can occur:

1. The amount of added substrate C is higher than the microbial biomass C content of soil, and both microbial growth and changes in the community structure can occur.
2. The amount of added substrate C is similar or less than the microbial biomass C content of soil, and changes in microbial activity and in turnover rate of the active microflora can occur; however, the added substrate is not sufficient to induce microbial growth.
3. The amount of added C is much less than the microbial biomass C content of soil, and the energy added is insufficient to directly accelerate microbial turnover. Either triggering or signaling effects can occur.

In this paper, we discuss the threshold values for the three levels of substrate addition.

As the content of microbial biomass can differ in various soils, the amount of added substrate C should be expressed as the percentage of microbial biomass C (Fig. 2). To find the threshold values for the three response possibilities mentioned above, we considered PE studies in which microbial biomass C was measured and a few studies (Conde et al. 2005; Falchini et al. 2003; Fontaine et al. 2004; Shen and Bartha 1997), in which we calculated microbial biomass C considering that it represents 2% of the organic C content (Paul and Clark 1989).

Surprisingly, two opposite relationships between the amount of added substrate and induced PE were found with different levels of added substrate C as related to microbial biomass C (Fig. 2). When the amounts of added

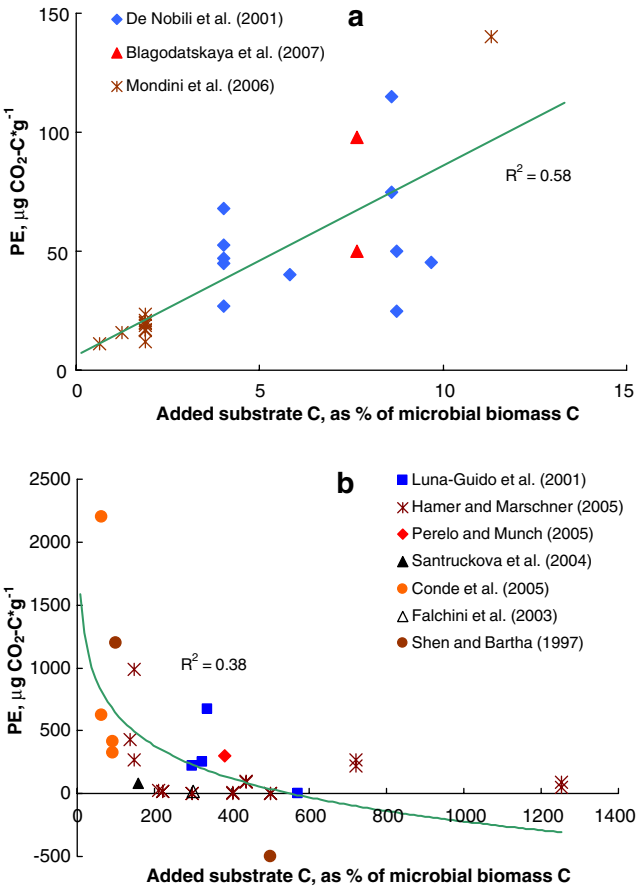

Fig. 2 Primed CO_2–C efflux as affected by the amount of easily available substrate C added and expressed as percent of microbial biomass C; added C is <15% of microbial C (**a**); added C is >50% of microbial C (**b**)

substrate C were lower than 15% of microbial biomass C, the magnitude of PE (here measured as extra CO_2) linearly increased with the amount of added C (Fig. 2a). Therefore, for low levels of the added substrate, the PE is substrate limited, and it confirms our hypothesis that priming effects are related to the energy input to the soil microorganisms by easily available substrates such as glucose and amino acids (Fig. 2). This means that microorganisms were able to utilize these substrates within a few hours or less, and thus, these inputs probably represent a pulse of available energy promoting the shift from dormant to active state.

When the amount of added substrates C exceeded 50% of the microbial biomass C value, the amount of primed CO_2–C decreased exponentially by increasing the added C (Fig. 2b). At the rates of substrate C exceeding 200–500% of microbial biomass C, the priming effect tended to be zero or even negative. Thus, the amount of added substrates switches the direction of PE. If easily available substrates were added in sufficient amounts, soil microorganisms start growing within 4–10 h (Blagodatskaya et al. 2007) and end within 1–3 days, depending on the used substrate and available nutrients. The 1- to 3-day period coincides with the maximal intensity of the observed priming effects. We suggest that such short-term priming effects induced at high levels of added substrates (comparable or higher than microbial biomass C) are mainly related with changes in microbial community structure and also involves their demand for other nutrients, such as N.

With a level of added substrate C being two to five times higher than microbial biomass C, priming effects tended to be close to zero or even negative. We assume that preferential microbial substrate utilization (Cheng and Kuzyakov 2005; Kuzyakov 2002) is the main process occurring at these levels of added substrates with the switch of substrate conditions for soil microflora from low available SOM to easily available and highly accessible added substrate. This can cause a lower SOM decomposition with a negative PE. It is important to underline that the magnitude of PE by high levels of the added substrate is about one order higher than the PE induced at levels of added organic C lower than 15% of microbial biomass C.

When plant residues were added to soil, the relationship between PE and the amount of added substrate was similar to that occurring when easily available substrates were added to soil (Fig. 3). The highest PE (expressed as percent of added substrate from study of Bell et al. 2003) was observed when the amount of plant residue C accounted for 4–9% of microbial biomass C. By increasing the amount of plant residues, an exponential decrease in PE was observed.

Therefore, studies comparing priming effects in different soils or in different horizons should consider the microbial biomass content, which can differ among different soils and among different soil horizons. By considering that

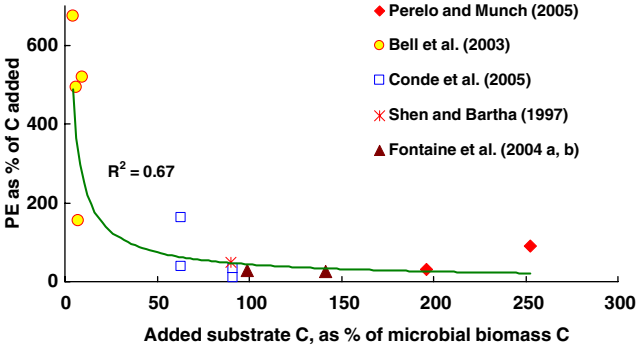

Fig. 3 Relationship between the input of low available substrate (plant residues) to soil expressed as percent of microbial biomass C and CO_2–C primed expressed as % of added C

microbial biomass C varied in range 0.2–1% of total organic C in the study of Hamer and Marschner (2005), the amount of organic C added to soil as 1.3% of the SOM C was by 1.4–12.5 times higher than microbial biomass C. This caused the absence of PE in some soil horizons when microorganisms were oversaturated by the added substrate. Regretfully, many reports on PE did consider neither organic C nor microbial biomass C content in soil.

In conclusion, when the added substrate C accounted for less than 15% of microbial C, a linear increase of extra CO_2 occurred by increasing amounts of added substrate C. When the amount of added substrate C was between 50% and 200% of microbial biomass C, an exponential decrease in primed CO_2–C was observed. When the amount of added substrate C accounted for more than 200% of microbial C, no PE or negative PE occurred. When the added substrate C was lower than 15% of microbial biomass C, the magnitude of PE was one order lower than PE induced by substrate C amounting to 50–200% of microbial biomass C.

Apparent and real priming effects

Until now, no clear-cut approach has been suggested to distinguish apparent from real priming effects, and it is not clear if the source of primed CO_2–C is SOM or the endogenous microbial metabolism (Bell et al. 2003). At least two different kinds of apparent PE can be caused by microbial activity when fresh available substrates are added to soil:

1. A triggering effect that is an acceleration of internal microbial metabolism by trace amounts of substrate with an immediate (several minutes to several hours) increase in the respiratory activity
2. A pool substitution with acceleration of microbial turnover revealed by a long-term (several days or weeks) increase in respiratory activity (Jenkinson et al. 1985)

The real PE depends on the composition of added substrate used by microorganisms. If some nutrients, mainly N, are lacking, this shortage can be supplemented by an accelerated SOM decomposition resulting in a real PE. When complex organic substrates are added, soil microorganisms can use the energy of most available compounds to synthesize enzymes hydrolyzing the low available compounds. Therefore, SOM can be co-metabolized by microorganisms. Thus, real PE can be due to

1. Mineralization of soil organic N to supply available N
2. Co-metabolism of SOM

The difficulties in distinguishing real and apparent PE also reflect the fact that both effects may occur simultaneously (Mondini et al. 2006).

We suggest to relate the response time with PE magnitude for a better interpretation of PE (Table 1). The following situations can occur:

1. *Short-term response: the amount of the primed carbon (C_{primed}) exceeds the rate of substrate additions (C_{added}), but it is lower than microbial biomass C (C_{BM}): $C_{added} < C_{primed} < C_{BM}$.* This type of PE is usually observed from hours to days after applying trace amounts (5–50 μg C g^{-1} soil) of substrate (Bell et al. 2003; Blagodatskaya et al. 2007; De Nobili et al. 2001; Mondini et al. 2006). It has been suggested that the quick short-term response with lower amount of primed CO_2–C versus microbial biomass C is a triggering effect, which can be due to accelerated metabolism of soil microorganisms rather

Table 1 Apparent and real priming effects (C_{primed}) as affected by the time period and the amount of added substrate (C_{added}) in relation to microbial biomass C (C_{BM})

Soil	Organic C, %	Total N, %	pH	Substrate	PE	Source
Short-term response: $C_{added} < C_{primed} < C_{BM}$						
Chromic Luvisol	0.94–2.22	0.1–0.2	6.5–7.1	Rhizosphere soil extract; root extract; glucose; amino acids	Apparent	De Nobili et al. (2001)
Agricultural soils under different management	0.38–0.52	0.02–0.03	6.3–6.6	^{12}C- and ^{14}C-plant residues	Apparent	Bell et al. (2003)
Eutric Cambisol	2.54	0.2	7.8	^{14}C-glucose; amino acids; protein hydrolysate	Apparent	Mondini et al. (2006)
loamy Luvic Chernozem	5	0.3	7.4	^{14}C-glucose	Apparent	Blagodatskaya et al. (2007)
Short-term response: ($C_{added} \approx C_{BM}$) > C_{primed}						
Artificial sandy-loam soil with composting ^{14}C-plant residues	3.25	0.03	7.35	^{12}C- and ^{14}C-glucose	Apparent	Dalenberg and Jager (1981)
Sandy loam field Cambisol	1.37	0.2	5.47	^{14}C-glucose	Apparent	Santruckova et al. (2004)
Typic Rendolls	3.9	0.3	8	^{35}S-($Na_2{}^{35}SO_4$), glucose	Apparent	Vong et al. (2003)
Long-term response: $C_{primed} > C_{BM}$						
Sandy clay loam	5.3	0.7	10	^{14}C-glucose; ^{14}C-maize	Apparent, partly real	Conde et al. (2005)
Alkaline saline soil	1.5–2.6	0.1–0.2	8.3–10.2	^{14}C-glucose	Apparent, partly real	Luna-Guido et al. (2001)
Dystric Cambisol	0.6–12.2		2.7–3.7	Fructose; alanine; oxalic acid	Apparent, partly real	Hamer and Marschner (2005)
Typic Udifluvent; Dystric Eutrochrept	1.7; 1.4	0.2; 0.2	5.9; 6.1	Glucose/nitrate solution	Real	Perelo and Munch (2005)
Combination of short and long-term responses						
Agricultural soils under different management	0.38–0.52	0.02–0.03	6.3–6.6	^{14}C-plant residues	Apparent, partly real	Bell et al. (2003)
Dystric Cambisol	0.6–12.2		2.7–3.7	Fructose; alanine	Apparent, partly real	Hamer and Marschner (2005)
Typic Udifluvent; Dystric Eutrochrept	1.7; 1.4	0.2; 0.2	5.9; 6.1	^{13}C- and ^{15}N-labelled white mustard	Real	Perelo and Munch (2005)

than to the decomposition of SOM. This hypothesis has been verified by adding unlabeled substrates to the ^{14}C-labeled microbial pool (Bell et al. 2003; Dalenberg and Jager 1981, 1989). The flush of microbial primed $^{14}CO_2$–C detected immediately after adding ^{12}C derived from the increased turnover of microbial C. Therefore, if the amount of primed CO_2–C is much less than microbial biomass C, and if the additional CO_2 (or N_{min}) is released very shortly after substrate addition, then an apparent PE is probably observed.

2. *Long-term response: the amount of the primed carbon (C_{primed}) is higher than microbial biomass C: $C_{primed} > C_{BM}$.* This PE usually occurs during long-term incubation ranging from weeks to months (Conde et al. 2005; Hamer and Marschner 2005; Perelo and Munch 2005; Shen and Bartha 1997) and can coincide with a significant increase in microbial biomass. The presence of the apparent PE cannot be excluded even when the amount of extra CO_2 exceeds the initial microbial biomass C value, since growing soil microorganisms can also contribute to the accelerated microbial turnover. Without applying isotopic-labeled compounds, the real PE can only be evaluated if the amount of the primed carbon is higher than both microbial biomass C (C_{BM}) and the added C ($C_{added} < C_{primed} > C_{BM}$).
3. *Short and long-term responses*. Added substrate can accelerate both microbial turnover and decomposition of recalcitrant SOM. Thus, the observed PE may be the combination of both real and apparent PE. The triggering effect immediately after substrate addition is replaced by an apparent PE caused by pool substitution and occurring during the intensive substrate decomposition (Hamer and Marschner 2005). Intensive mineralization results in a short-term increase in the active microbial biomass, which can also cause the real PE both during the intensive substrate mineralization and later at the beginning of microbial starvation. Thus, real PE can occur with apparent PE, as shown after the exhaustion of available ^{13}C-substrates added to soil (Perelo and Munch 2005). It was shown that real PE occurred as a significant increase in the incorporation of SOM-derived C in microbial biomass, and this preceded the extra CO_2 evolution. Perelo and Munch (2005) suggested that substrate input increased the immobilization of soil organic C by soil microorganisms, and then this microbial immobilized C was oxidized to CO_2 due to the accelerated microbial turnover.

To distinguish apparent from real PE in long-term incubation experiments, the amount of primed CO_2–C should be related to changes in microbial biomass during the studied period. We suggest that the contribution of real and apparent PEs to the total PE can be estimated by distinguishing substrate- and SOM-originated pools in the newly formed microbial biomass (Schneckenberger et al. 2008). Further studies with different labeling of three most important pools (added substrate, microbial biomass, and recalcitrant soil organic matter) are required to distinguish apparent from real PE (Kuzyakov and Bol 2006) and to precisely estimate the real PE.

Priming effects and microbial diversity

Hamer and Marschner (2005), Mondini et al. (2006), and Chaves et al. (2006) suggested that differences in the observed PE may be related to soil microbial community structure—but they did not provide any experimental evidence. Only few studies have shown that PEs initiated by different substrates are accompanied by changes in composition of soil microbial community (Bell et al. 2003; Blagodatskaya et al. 2007; Falchini et al. 2003; Kramer and Gleixner 2006; Landi et al. 2006). Different microbial species could be activated by added substrate as dependent on the response on quality and quantity of the added substrate. Thus, the composition of the microbial community can change, and this can produce PE. However, microbial diversity generally does not alter the rate of processes such as C and N mineralization (Nannipieri et al. 2003) because these processes can be carried out by most of microbial species inhabiting soil. According to Johnsen et al. (2001), functional diversity is expressed as a number of functional guilds performing the different processes or carbon source utilization patterns taking place in a community. Therefore, it is important to underline that changes in microbial diversity should be linked to functional biodiversity with determination of the capacity of microbial species to decompose substrates with different composition and availability.

Microbial diversity as a factor controlling PE with different amounts and availability of substrate

Different changes in microbial community structure can be predicted during apparent and real PE depending on the amount of added substrate.

1. It is reasonable to suppose that no changes in microbial diversity can occur after adding low amounts ($\ll$ of microbial biomass C; Fig. 2) of easily available substrate such as glucose. Such inputs may only cause short-term activation of indigenous, fast-growing microbial species (r-strategy) with an apparent PE. In such cases, the denaturing gradient gel electrophoresis (DGGE) profiles revealed activation of species present in the control soil but no changes in diversity. Small

amounts of glucose did not change (Brant et al. 2006; Falchini et al. 2003) or caused minor changes in microbial community structure (Falchini et al. 2003; Landi et al. 2006), as revealed by bacterial DGGE profiles. We assume that minor community changes reflect the versatility of glucose as an available organic substrate. Since glucose is the monomer of most plant-originated organic polymers, most soil microorganisms are capable of metabolizing glucose (Anderson and Domsch 1978; Landi et al. 2006). Therefore, the PE after a low input of glucose is due to the activity of a non-specific microflora, physiologically active at the moment of substrate addition. Input of substrates other than glucose but of similar availability may activate substrate-specific microorganisms, which may also contribute to PE. Several glutamate-specific bacterial species were revealed during a PE after a low input of glutamic acid, whereas the same amount of glucose gave a positive PE but did not change the community structure (Falchini et al. 2003).

2. Low available substrates, such as oxalic acid, can be decomposed by specialized microorganisms, which are usually not active in soil. Input of such organic compounds can activate specific dormant microbial species, and these substrate-specific microbial species can grow and become dominant. Substrate degradation by these microorganisms can be accompanied by co-metabolism of SOM in the case of real PE. A significant shift in community structure was found by DGGE after a PE caused by oxalic acid (Falchini et al. 2003; Landi et al. 2006) and by ^{13}C-phospholipid fatty acid analysis after a PE caused by vanillin (Waldrop and Firestone 2004).
3. If the amount of applied complex substrate is sufficient to induce microbial growth, the resulting succession in microbial population can cause different kinds of PEs. As specific growth rates differ for various species, the abundance of various microbial groups may change during substrate utilization (Baudoin et al. 2003). The growth of the fast-growing species utilizing easily available compounds can occur as a quick response to substrate addition (Blagodatskaya et al. 2007). This may be followed by the activation of specific micro-organisms able to decompose low available substrates (Chaves et al. 2006; Landi et al. 2006) or by the activation of SOM-degrading slow-growing K-strategists (Fontaine et al. 2003), which are suggested to be responsible for the real PE, but this has not yet been proven experimentally.
4. Long-term substrate limitation may constrain microbial functioning in soil despite the high functional diversity. A significant increase in CO_2 production can occur after applying easily available substrates to soils that have not received fresh C for a long time, such as deep soil layers (Fontaine et al. 2007), soils with low respiratory activity (Hamer and Marschner 2005), or fallow soils (Panikov 1995). We assume that long-term absence of fresh C input into such soils favors the development of an oligotrophic microbial community characterized by a high diversity of metabolic pathways. New inputs of fresh C (root exudates, plant residues, and low molecular organic substances) can activate microbial groups that were dormant or inactive during the long absence of fresh C, with synthesis of a broad variety of enzymes and possible SOM decomposition. However, a significantly higher PE was observed in soil under winter wheat as compared with soils fallowed for 2 years (De Nobili et al. 2001). This may reflect apparent PE and not real PE, as activation of microbial metabolism was probably the source of primed CO_2–C. Indeed, a higher microbial biomass was present in soils under winter wheat than in the fallowed soil.

Contribution of bacteria and fungi to apparent and real PE

Most reports using molecular approaches in soil have studied bacterial community structure and have ignored changes in fungal community. However, fungal growth during microbial succession on substrates of various availabilities is also expected to contribute to the PE. The PE of a glucose-amended fallow Chernozem was initiated by intensive fungal growth, which was followed by the activity of a slow-growing bacterial population further contributing to the PE after fungal growth decreased (Panikov 1995). The priming effect induced by ^{14}C-labeled wheat straw in Ritzville silt loam soil was mainly due to fungal than bacterial activity (Bell et al. 2003). It was suggested that the initial phase of PE (fast response after substrate addition) was mainly due to the activation of the endogenous bacterial metabolism, whereas fungal activity was mostly responsible for the second and longer phase of PE. Therefore, changes in long-term PE dynamics can be explained by successional changes in the microbial community structure, with a gradual increase in the fungal/bacterial biomass ratio (Lundquist et al. 1999). This could be the case for the two- to fivefold increase in the extra-mineralized C between 100 and 300 h as compared to the first 100–200 h observed after single or multiple application of glucose or root extract to soils amended with cellulose (De Nobili et al. 2001). The stimulation of fungal activity was also suggested to be responsible for real PE with co-metabolism of SOM in long-term fallow soils (Panikov 1995). The input of fresh substrate to such soils may activate previously dormant microorganisms (spores and cysts), promote microbial succession, and increase microbial biomass turnover. The growth of filamentous fungi may enable fungal hyphae to penetrate previously inaccessible microzones. Available

SOM will be quickly consumed in such microzones, causing a real PE.

Despite the better determination of microbial diversity by the use of molecular techniques, the link between microbial and functional diversity in soil is still largely unclear (Nannipieri et al. 2003; Lynch et al. 2004). We expect an increasing application of molecular techniques in most of the upcoming PE studies. In this paper, we would like to underline that only few polymerase chain reaction (PCR)-based approaches for evaluating microbial diversity in soil have an appropriate resolution to detect functional diversity and distinguish inactive and active microbial cells (Nannipieri et al. 2003; Lynch et al. 2004). Underestimation of functional diversity is due to the low level of protein-coding genes, which is frequently less than threshold of PCR-based approaches (Krsek et al. 2006). In addition, most of these techniques do not allow determining the gene expression (Landi et al. 2006).

Characterization of both DNA and RNA failed to distinguish physiologically active and inactive microorganisms (Bastias et al. 2007) because of ribosomal DNA persisting in metabolically inactive cells. However, simultaneous direct extraction of DNA and RNA from soil (Pennanen et al. 2004), with calculation of their ratio, can estimate the contribution of active to total microbial biomass (Girvan et al. 2004). The combination of RNA and DNA DGGE/terminal restriction fragment length polymorphism profiles with estimates of rRNA-to-DNA ratios is very promising for monitoring the activity of definite groups of soil microorganisms during apparent and real priming. Among the few techniques linking genetic and functional diversity, the stable isotope probing cannot be successfully applied for priming studies because of the very high ^{13}C enrichments necessary for the density gradient separation.

Priming effects and enzyme activities

Due to the complex structure of soil organic matter, its decomposition can occur through several process initiated by extracellular hydrolysis (Marxsen and Witzel 1991) and completed intracellularly. Both extracellular and intracellular degradation of SOM involves enzyme activity. Thus, microbial enzyme responses to substrate addition may be important to clarify the mechanisms of priming effects.

Extracellular enzyme production as an indicator of real priming effects

The activity of extracellular enzymes, such as hydrolases (glycosidases and peptidases) and oxidoreductases (Table 2), has been supposed to play an important role in the SOM decomposition and thus in the real PE. The increased extracellular protease activity promoted by glucose caused a PE through the mineralization of soil organic N (Asmar et al. 1994). An increase in the extracellular activity of enzymes degrading cellulose and lignin was considered to be the cause of the real PE because these enzymes are also involved in the SOM decomposition (Fontaine and Barot 2005). Higher activity of cellulolytic enzymes such as glycosidases is predicted during initial SOM decomposition (Klose and Tabatabai 2002), whereas accelerated activity of ligninolytic enzymes (phenol oxidase and phenol peroxidase; Table 2) can be expected during later stages of SOM decomposition (Carreiro et al. 2000).

The increase in intracellular enzyme activities without any increase in the extracellular enzyme activities may be involved in the apparent PEs caused by the activation of the internal microbial metabolism. Thus, the type of enzymes activated after substrate addition and the accompanying extra CO_2 or N mineralization might be important for distinguishing apparent (by intracellular enzymes) from real (by extracellular enzymes) PE. However, the differentiation between intracellular and extracellular enzyme activities in soil remains an unsolved problem (Klose et al. 1999; Nannipieri et al. 2002; Nannipieri 2006).

The stimulation of microbial respiration during the apparent PE after repeated applications of trace amounts of peptides or amino acids was accompanied by a significant increase in β-glucosidase activity (Mondini et al. 2006). At the same time, urease, alkaline phosphatase, and acid phosphatase activity were not affected. Increased β-glucosidase activity could indicate enhanced metabolism of either extracellular substrate (e.g., soil carbohydrates) or endocellular energy reserves, or both (Mondini et al. 2006). Thus, although higher β-glycosidase activity might be a good indicator of priming action, it does not allow distinguishing real and apparent PE because the present assays do not differentiate intracellular and extracellular activities.

Application of fluorogenically labeled substrates (Freeman et al. 1995; Kandeler 2007; Sowerby et al. 2005) in combination with appropriate technique of microbial cells disruption may be promising for quick and precise distinguishing of intra- and extracellular enzyme activity and, thus, for assessing the mechanism of observed priming effects.

Changes in extracellular enzymes activity during microbial succession promoted by the input of fresh organic substrate were hypothesized as a possible explanation for observed PEs (Conde et al. 2005; Degens and Sparling 1996; Hamer and Marschner 2005; Luna-Guido et al. 2001; Xue et al. 2003). De Nobili et al. (2001) suggested that extracellular enzymes were stabilized in soil and were not involved in PE. However, free extracellular enzymes, which are short-lived unless they are adsorbed on surfaces or

Table 2 Changes in enzyme activities as related to the real and apparent priming effects

Enzyme	Nomenclature	Action	Producer	Enzyme activity during PE	Source
Oxidoreducatases EC 1.					
Dehydrogenase	EC 1.x.	Oxidizes a substrate by transferring one or more protons and a pair of electrons to an acceptor	Many living organisms	Increase	Kandeler (2007)
Phenol oxidase	EC 1.10.3.2, 1.14.18.1	Ligninolytic enzyme	Plants, animals and fungi	Increase but reduced by increased N availability	Carreiro et al. (2000)
Phenol peroxidase	EC 1.11.1.7.	Ligninolytic enzyme	White and rot fungi	Increase but reduced by increased N availability	Carreiro et al. (2000)
Hydrolases EC 3.					
Ester hydrolases	EC 3.1.	Acting on ester bonds			
Alkaline phosphatase	EC 3.1.3.1	Remove phosphate groups from many types of molecules	Bacteria, fungi	Real PE, increase; APE, no changes	Kandeler et al. (1999); Mondini et al. (2006)
Acid phosphatase	3.1.3.2	Free attached phosphate groups from other molecules during digestion	Plants, animals, bacteria, fungi	APE, no changes	Mondini et al. (2006)
Arylsulfatase	EC 3.1.6.1	Catalyze the hydrolysis of organic sulfate esters	Heterotrophic bacteria, rhizobacteria, plants	No changes, increase	Klose et al. (1999); Vong et al. (2003)
Glycosylases EC 3.2.; glycosidases EC 3.2.1.—hydrolysing *O*- and *S*-glycosyl compounds					
N-acetyl-b-D-glucosaminidase	EC 3.2.1.30	Hydrolyzes residues from the terminal non-reducing ends of chitooligosaccharides	Bacteria, fungi, plants, invertebrates, humans	Increase is expected during real PE	Parham and Deng (2000)
Xylanase	EC 3.2.1.8	Degrade the linear polysaccharide β-1,4-xylan into xylose, thus breaking down hemicellulose, which is a major component of the cell wall of plants	Herbivorous micro-organisms, fungi	Increase is expected during real PE	Kandeler et al. (1999)
β-1,4-Glucosidase	EC 3.2.1.21	Exocellulases that remove glucan units from the ends of the cellulose chains	Fungi, bacteria, termites	Increase; no changes	Carreiro et al. (2000); Mondini et al. (2006),
Cellobiohydrolase	EC 3.2.1.91	Hydrolysis of 1,4-β-D-glucosidic linkages in cellulose, releasing cellobiose from the non-reducing ends of the chains	Fungi, bacteria, and protozoans	Increase	Carreiro et al. (2000)
Peptidases EC 3.4. Acting on peptide bonds					
Protease	EC 3.4.	Hydrolysis of the peptide bonds, casein hydrolyzing	Occur naturally in all organisms	Increase	Asmar et al. (1994); Carreiro et al. (2000)
EC 3.5. Acting on Carbon-Nitrogen bonds, other than peptide bonds					
Urease	EC 3.5.1.5	Hydrolysis of urea into carbon dioxide and ammonia	Many bacteria, several species of yeast and a number of higher plants	APE, no changes	Mondini et al. (2006))
Deaminase	EC 3.5.4.4.	Involved in purine metabolism, breakdown of adenosine turnover of nucleic acids in tissues		Increase	Kandeler et al. (1994)

associated with humic molecules (Nannipieri et al. 2002; Renella et al. 2005), are continually released by active soil microorganisms. Free extracellular enzymes are immediately degraded if attached by proteases. Due to their limited lifetime, the content of free extracellular enzymes is not stable in soil, at least not during intensive mineralization processes (Six and Jastrow 2002). Both the content and activity of free extracellular enzymes may change considerably along with the succession of microorganisms involved in the decomposition of organic substrate (Table 2).

The different origin of enzymes may be used to distinguish real and apparent PE. Increases in enzyme activities associated to fungal activity such as xylanase, protease, and alkaline phosphatase activities (Kandeler et al. 1999) can occur during real PE caused by the development of fungi, since saprophytic fungi are the major litter decomposers. However, xylanase, protease, and alkaline phosphatase can be also synthesized by other soil microorganisms than fungi (Table 2). It has been suggested that the determination of β-glucosaminidase activity can be used as an indicator of fungal activity because this extracellular enzyme was highly correlated with fungal biomass (Miller et al. 1998; Parham and Deng 2000). Extracellular lignin-degrading oxidoreductases (phenoloxidases, peroxidases, and laccases) are generally synthesized by fungi, while enzymes degrading nonlignitic material glycosidases (cellulases) and esterases (lipases) can also be of bacterial origin (Carreiro et al. 2000; Six and Jastrow 2002). In addition, an increase in lignin-degrading oxidoreductase activity may indicate a real PE, since lignin and aromatic compounds of SOM are supposed to have a similar chemical structure (Schubert 1965; Stevenson and Cole 1999).

Mechanisms of real priming effects and extracellular enzyme production

The causal relationship between enzyme activity and soil organic matter decomposition remains unclear (Sowerby et al. 2005). This can be due to potential rather than real activities given by the present enzyme assays used to measure enzyme activity in soil. Indeed, the present enzyme assays are carried out at saturating substrate concentrations, at optimal pH and temperature values, and usually in soil slurries (Nannipieri et al. 2003). Expression and repression of extracellular enzymatic activity can be important to understand the relation between extracellular enzyme activity, priming intensity, and direction of PE. The production of extracellular enzymes by soil microorganisms may be either nutrient or energy limited, and the ceasing of either nutrient or energy limitation may cause real PE. Two alternative mechanisms were, therefore, suggested to explain the role of extracellular enzymes in the priming effect (Fig. 4). The first mechanism supposed that the input

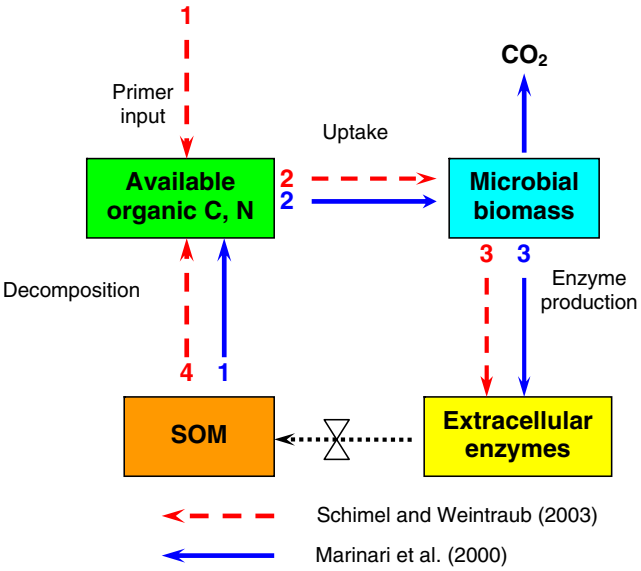

Fig. 4 Carbon and N fluxes during PE according to the hypotheses of Schimel and Weintraub (2003) and Marinari et al. (2000)

of a substance inducing PE can activate the microbial synthesis of intracellular and extracellular enzymes, such as dehydrogenase, protease, and acid phosphatase (Marinari et al. 2000). The second mechanism (Fig. 4) suggests that the fresh available substrate inducing PE can serve as an energy source for the production of extracellular enzymes with the subsequent increase in the decomposition of SOM, resulting in PE (Schimel and Weintraub 2003). Thus, enzyme activities are suggested to be the cause (Schimel and Weintraub 2003) or the consequence (Marinari et al. 2000) of the PE. To prove the validity of the first hypotheses, Shackle et al. (2006) added some enzymes to wetland soil and observed accelerated biodegradation of organic substances. Considering that any protein added to soil is quickly degraded by soil microorganisms (Nannipieri et al. 2002), Shackle et al. (2006) believed, however, that in their study, the supplemented enzymes were quickly immobilized on the soil matrix and retained their activity. Increased enzyme activity, which enhanced existing biodegradation processes after the addition of novel enzymes to soil can be assigned to the PE, since the amount of added enzymes (25–50 μg g^{-1}) was enough to serve as substrate promoting PE. Higher protease, deaminase, urease, and phosphatase activities were observed in soils after adding different organic substrates (Bell et al. 2003; Bolton et al. 1985; Kandeler et al. 1994). Probably, some of the increased enzyme activity can decompose SOM with production of microbially available substrates (Fig. 4). We suggest that if interpretation of PE mechanisms is based on increase in enzymes activity, the changes in microbial biomass and respiration should also be considered. Thus, increase in enzyme activity preceding the increase in microbial biomass

as a response to input of easily degradable organic compounds (Nannipieri et al. 1978, 1983) can be suggested as a feature of apparent PE. Whereas increase in microbial biomass coincided with or accompanied by the increase in enzymes activity (Landi et al. 2006) can be a feature of real PE.

Priming effects as related to substrate and soil properties

Substrate properties

As already mentioned, the availability, composition, and amount of substrate determine the magnitude and the type (real or apparent) of PE. The decomposition of easily available C sources (glucose, fructose, and alanine) led to a greater PE than the addition of low available substrates, such as catechol, oxalic acid, plant residues, manure, or slurry to soil (Conde et al. 2005; Hamer and Marschner 2005). Among the easily available substrates, glucose generally caused lower PE than L-glutamic acid (Mondini et al. 2006), a complex substrate mixtures (root extract, rhizosphere soil extract; De Nobili et al. 2001) or an amino acid mixtures (Mondini et al. 2006).

The amount of primed carbon is also dependent on the amount of available N in soil. Decrease in PE was observed when available N was applied to soil with organic C (Blagodatskaya et al. 2007; Cardon 1996; Liljeroth et al. 1994; Martin-Olmedo et al. 2002; vanGinkel et al. 1997). This confirms the preferential substrate utilization (the added versus the SOM) if the main nutrients, such as N, are present. In the case of an input of C-rich substrates without N, soil microorganisms are activated to decompose SOM to acquire N with production of a real PE. However, it has been observed that N addition with glucose and plant residues can also produce a PE (Conde et al. 2005; Hamer and Marschner 2005). Probably, this depended on still high C-to-N ratio despite the N addition. The synthesis and the activity of various enzymes involved in C and N cycling are, respectively, controlled by N and C availability (Carreiro et al. 2000; Chander et al. 1997). Thus, N addition stimulated the activity of cellulases, while the activity of phenoloxidase, an important ligninolytic enzyme, was greatly reduced by the increased N availability (Carreiro et al. 2000). Similarly, the activity of amidohydrolases (Deng and Tabatabai 1996) and other enzymes involved in N cycling (Skujins 1976) was influenced by the organic C content of soils.

In conclusion, the addition of trace amounts of easily available substrates induces a short-term triggering effect, whereas the input of available substrate without nutrients in amounts sufficient for microbial growth leads to activation of SOM decomposition. If nutrients are present, no PE occurs or there is a preferential substrate utilization leading to lower SOM decomposition.

Soil properties

Since the effect of chemical (such as nutrient status and the C-to-N ratio of the active SOM pool) and physical soil properties on the PE were discussed in detail in relation to priming effects in previous reviews (Kuzyakov 2002; Kuzyakov et al. 2000), we will focus in this paper on the effect of soil pH and aggregation on PE, as it is mediated by biological activity.

Soil acidity (pH)

The higher values of PE occur in neutral soils—in the pH range between 6 and 8—when both easily decomposable substances and plant residues are added to soil (Fig. 5). The changes of microbial activity and community structure as well as the enzyme synthesis are higher in soils with pH ranging from 5 to 8 than in acidic soils (Blagodatskaya and Anderson 1998).

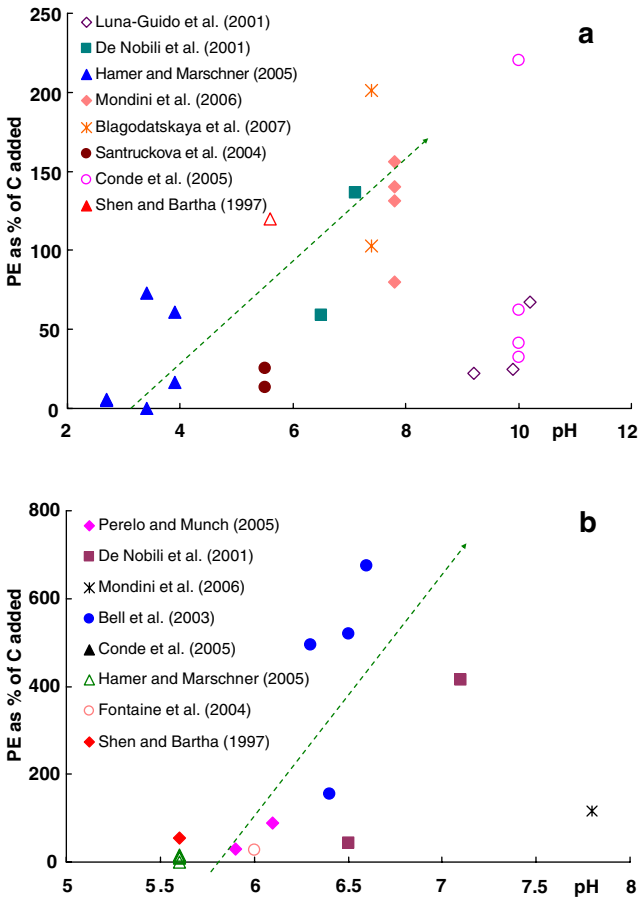

Fig. 5 Relationship between primed CO_2–C efflux expressed as percent of added C and soil pH: easily available substrate (**a**) and plant residues (**b**)

Most studies on priming effects are based on CO_2 measurements, and artifacts can occur if the effect of soil pH on the solubility of CO_2 is neglected. Indeed, the equilibrium among H_2CO_3, CO_3^{2-}, and HCO_3^-, all derived from CO_2, in soil solution is pH dependent, and thus, the soil pH should be considered when interpreting results from CO_2 evolution. Possible mis-estimates of CO_2 evolution do not occur at pH values <5 because H_2CO_3 is the prevailing (about 98%) form that dissociates immediately into CO_2 and H_2O, and thus, all CO_2 produced by soil microorganisms can be freely evolved as CO_2. At pH between 5 and 5.5, the contribution of H_2CO_3 decreases to 70%, and some CO_2 can occur in soil solution as HCO_3^-; this can give small underestimation of the produced CO_2. As the total amount of soluble CO_2 species is less than 0.03 mM l^{-1} at pH 5–5.5, such underestimation does not exceed 0.1 μg CO_2–C g^{-1} (Table 3); however, this value can be neglected considering that the CO_2 production rate in most unamended mineral soils is 1–2 μg CO_2–C g^{-1} h^{-1}.

The prevailing forms of carbonate at pH values higher than 6.8 are HCO_3^- and CO_3^{2-}. Therefore, CO_2 produced by microbial respiration will initially dissolve in soil water until the saturation capacity of the solution is reached. In such case, CO_2 evolution depends on the volume of soil solution. Assuming the soil water content to be 30% of dry weight with a CO_2 evolution rate of about 30–40 μg CO_2–C g^{-1} day^{-1}, the CO_2 evolution from soil can be underestimated by 10% to 100% at soil pH values of 8 and 9, respectively (Table 3). This may explain the one-day delay in the PE of alkaline soils (pH of 9.8–11.7) promoted by the addition of glucose or maize (Conde et al. 2005). Probably, CO_2 produced during first day after treatment was accumulated as HCO_3^- and CO_3^{2-} in soil solution until the CO_2 saturation was reached; then, the excess was evolved as CO_2. Therefore, the short-term PEs in soils at pH>8 should be interpreted with caution. In soils with very high pH (9–11), the PE estimation is even more difficult because of carbonate re-crystallization (Kuzyakov et al. 2006).

Table 3 Solubility sum of all CO_2 species (H_2CO_3, CO_3^{2-}, and HCO_3^-) in water and content in a soil (30% water in dry weight) as affected by pH

pH	Solubility of CO_2 in H_2O (M l^{-1})	Amount of soluble CO_2 (μg C g^{-1})	Possible errors
4–5	0.00002	0.07	To be neglected
6.1	0.00004	0.14	To be neglected
7.2	0.0001	0.36	To be considered
8.3	0.001	3.6	High
9.2	0.01	36	Very high
10	0.1	360	Extreme high

Aggregate and particle size fractions

The accumulation and physical protection of SOM and its mineralization depends on soil aggregate size (Denef et al. 2001; Six and Jastrow 2002), and the mean residence time of C in macroaggregates is shorter than that of C in microaggregates (Six and Jastrow 2002). This indicates that the PE due to the response of microorganisms to substrate addition may vary in aggregates with different size. Indeed, Degens and Sparling (1996) observed that only the middle-size fraction (1–2 mm) showed a significant and positive PE as a quick response to glucose addition, and this PE was apparently related to microbial biomass. Both larger (>2 mm) and smaller (<0.25 mm) particles showed PE long (21 days) after glucose application, and probably, both PEs were real because they were not due to microbial turnover (Degens and Sparling 1996). The intermediate aggregate size fractions (0.25–1 mm) showed a negative PE. Probably, the different response of different aggregates was due to the different localization of soil microorganisms; generally, fungi predominate in macroaggregates, whereas bacteria predominate in microaggregates (Guggenberger et al. 1999).

Silt mainly contributes to the formation of microaggregates, while sand particles are mostly associated with macroaggregates. According to Kandeler et al. (1999), invertase activity, mainly due to intracellular activity, was abundant in the silt fraction, whereas extracellular enzyme activities were distributed among particle size fractions; thus, xylanase activity was associated with sand, alkaline phosphatase activity with silt and clay, and protease activity with sand and clay. It is important to underline that, since the present enzyme assays do not allow distinguishing the intracellular and extracellular enzyme activities, these are just hypotheses.

No differences in PE promoted by fructose and alanine were observed between soil fractions (sand, silt, and clay) obtained by mild sonication followed by wet sieving and sedimentation (Ohm et al. 2007). It is important to underline that available fractionation procedures can give artifacts. Sonication can promote not only disaggregation but also desorption of microbial cells from soil particles, and these desorbed microbial cells can re-distribute during wet sieving to smaller size fractions. This can smooth the differences in microbial activity between fractions. The dry sieving method used by Degens and Sparling (1996) seems to yield a more realistic distribution of microbial activity among the soil particles.

Succession of mechanisms in the priming effect

In most studies, only one mechanism has been suggested to explain the observed positive or negative PE. However, as

already mentioned, adding easily available substrates can activate microorganisms, and depending on the added amount, a succession of processes can occur (Fontaine et al. 2003; Kuzyakov and Bol 2006). The PE data should be interpreted considering the size, duration, and timing of PE, the sources of primed or conserved C, and the microbial species or groups inducing the effect. The following sequence of processes can occur during the PE (Fig. 6):

1. The addition of a substrate, which is more easily available than native SOM, will stimulate the growth of the most active part of the microbial community (r strategists). These microorganisms may prolong their activity by using native substrates once the added substrate has been completely utilized. This phase depends on the amount of added substrate and the microbial biomass C content. We can have three different situations (Fig. 6):
   (a) If the amount of added substrate is much less than the microbial biomass C, we obtain a triggering effect with a small, brief increase of CO_2 output from soil microorganisms without any changes in the size and in the composition of microbial community, without additional extracellular enzyme production or SOM decomposition.
   (b) If the amount of added substrate is less but comparable with the microbial biomass C, then the evolved CO_2 may be caused by the pool substitution after the triggering action, and it is mainly released from soil microorganisms without additional SOM decomposition at this stage.
   (c) If the added substrate C is higher than microbial biomass C, then soil microorganisms use the fresh added substrate. This phase, termed as preferential substrate utilization (Billes et al. 1988; Cheng 1999; Sparling et al. 1982), may lead to a temporary decrease in the decomposition of the recalcitrant organic substrates including SOM. The decrease of SOM decomposition by preferential substrate utilization is comparable with the increase of CO_2 evolution from soil microorganisms caused by the pool substitution. Therefore, the preferential substrate utilization (negative PE) can be counterbalanced by pool substitution (positive apparent PE).

The first two situations may give an apparent PE and are completed within 1–5 days without any SOM decomposition, whereas the third situation can cause the following processes (Fig. 6):

2. The most active part of the microbial community benefits from the added substrate by increasing its activity, and with a sufficient substrate amount, the active microorganisms can grow. This phase has been called 'microbial activation' (Cheng and Coleman 1990; De Nobili et al. 2001; Helal and Sauerbeck 1984; Sallih and Bottner 1988), and it is followed by changes in the composition of the microbial communities, such as development of the fungal population accompanied by activation of autochthonous bacterial species.
3. When the most easily available substrates are consumed, the activated microorganisms will look for and utilize the substrates of lower availability (Kuzyakov and Bol 2006) producing extracellular enzymes, which may promote additional SOM decomposition by co-metabolism

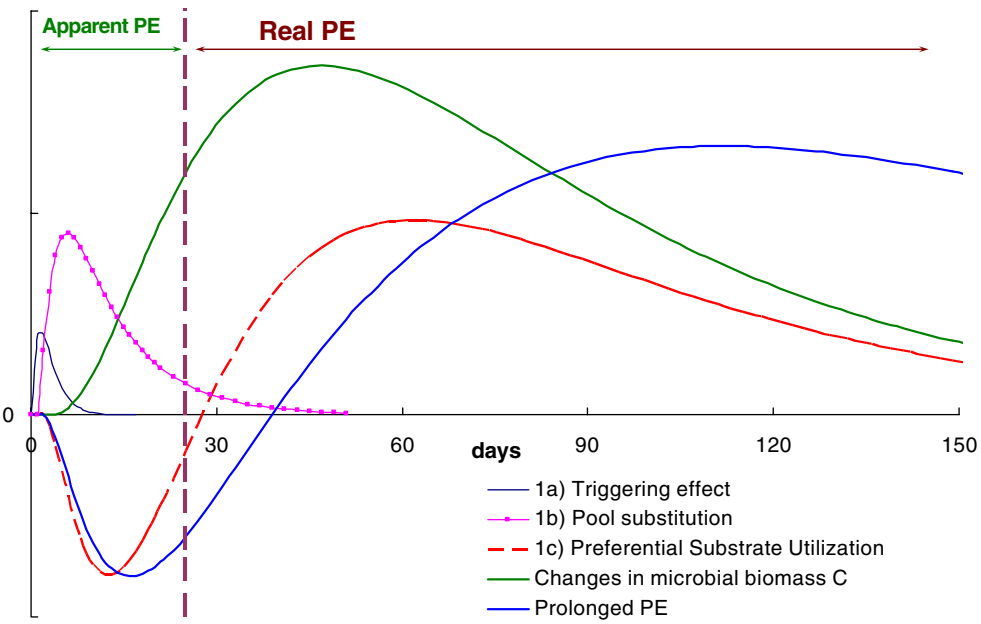

Fig. 6 Sequence of mechanisms during priming effect

especially if some nutrients are present in limiting amounts. As SOM is the most important source of nutrients such as N and P, extracellular enzymes for SOM decomposition will be released. In this case, the real PE occurs.

4. Finally, microbial activity and microbial biomass will decline and return to the initial state, so that the initial equilibrium between the available SOM pool and the microbial community responsible for its utilization will be restored (Stenstrom et al. 2001) within few days or few weeks (Kuzyakov and Bol 2006).

Conclusions and outlook

The amount of added available substrate in relation to the microbial C is a key factor affecting the direction and the type of the priming effect. If the amount of added C is lower than about 15% of microbial biomass C, there is positive correlation between the amounts of added and primed CO_2–C. However, most of the short-term (hours to days) PEs are apparent due to the higher microbial turnover. Real priming requires longer-term (weeks to months) incubations than apparent PE. Above 50% of microbial C, the added substrate C stimulates microbial growth and may alter the composition of microbial community, with stimulation of the syntheses of extracellular enzymes with real SOM decomposition. In contrast, a surplus of available C, combined with a surplus of other available nutrients, may lead to preferential substrate utilization and to a decreased SOM decomposition.

An increase in the syntheses of intracellular enzymes without simultaneous/subsequent increase in the syntheses and release of extracellular enzymes is mainly connected with apparent PEs, that is higher internal microbial metabolism without stimulation of SOM decomposition.

The following future research is required to verify our proposed hypotheses:

- The different soils should be tested by applying C substrates in amounts calculated considering the value of soil microbial biomass C.
- Extracellular and intracellular enzyme activities, microbial biomass, and microbial community structure should be monitored so as to evaluate real versus apparent PE. Molecular methods for determining composition of microbial communities should be chosen by considering the ecological functions of soil microorganisms. As the structure of such communities is functionally highly redundant, the sole estimation of the structure of microbial communities may be not significant to better understand the underlying mechanisms of priming effects.
- Budgeting the C remaining from the added C substrate versus extra CO_2 is crucial for drawing conclusions about real versus apparent PE and for understanding the relative mechanisms. Without applying ^{14}C or ^{13}C, the PE can be accepted as real only if the amount of extra CO_2 exceeds both the amount of C added and the amount of microbial biomass C. If the extra CO_2 does not exceed microbial biomass C, no direct conclusions about the source of extra CO_2 can be reached in studies without applying labeled C substrates.

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