Biology and Fertility of Soils (2019) 55:339–350
https://doi.org/10.1007/s00374-019-01351-0

ORIGINAL PAPER

Priming alters soil carbon dynamics during forest succession

Na Qiao[1,2] · Juan Wang[3] · Xingliang Xu[1] · Youxin Shen[2] · Xi'en Long[4] · Yuehua Hu[2] · Douglas Schaefer[2] · Shenggong Li[1] · Huimin Wang[1] · Yakov Kuzyakov[1,5,6,7]

Received: 22 February 2018 / Revised: 23 February 2019 / Accepted: 27 February 2019 / Published online: 13 March 2019

Abstract

The mechanisms underlying soil organic carbon (C) dynamics during forest succession remain challenged because amount, quality, and composition of C inputs change with tree growth and species composition. Soils were collected from two stages (grassland and young secondary forest) of secondary succession after the clear-cut of primary old-growth forest due to land-use change, with a native old-growth forest (undisturbed for more than 200 years) as the reference. Soil samples were incubated for 170 days and the priming effects were quantified by one pulse addition of ^{13}C-labeled glucose. ^{13}C-PLFAs (phospholipid fatty acids) were analyzed to identify microbial functional groups utilizing glucose and to explore their accordance with SOM priming during succession. Soil C was primed much more strongly in young secondary forest than in grassland or old-growth forest. Priming resulted in large C losses (negative net C balance) in young-forest soil, whereas C stocks increased in grassland and old-growth forest (positive net C balance). Microbial composition assessed by PLFA and utilization of easily available organics (^{13}C-PLFA) indicated that fungi were mainly responsible for priming in young-forest soil. Consequently, labile C released by litter decomposition and root exudation together with the availability of soil nutrients determines microbial functional groups that decompose soil organic matter during initial succession. These findings provide novel ecological connections between soil organic matter dynamics and C (de)stabilization with microbial functioning during forest succession and show that priming direction and intensity is important to distinguish soil C dynamics in young- and old-growth forests.

Keywords ^{13}C-PLFA · Forest succession · Old-growth forest · Priming effect · Soil carbon dynamic · Young secondary forest

Introduction

Soil organic matter (SOM) consists of plant and animal residues at various stages of decay, microbial necromass, and new substances synthesized and released by microbes into the soil (Miltner et al. 2012). Microbial processing and physicochemical interactions lead to gradual physical and biochemical protection of SOM from microbial decomposition (Ladd et al. 1996; Schmidt et al. 2011; Kleber et al. 2015; Pronk et al. 2017). Globally, the SOM pool contains four times more C

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-019-01351-0) contains supplementary material, which is available to authorized users.

✉ Xingliang Xu
xuxingl@hotmail.com

[1] Key Laboratory of Ecosystem Network Observation and Modeling, Chinese Academy of Sciences (CAS), Institute of Geographic Sciences and Natural Resources Research, 11A Datun Road, Chaoyang District, Beijing 100101, China

[2] Key Laboratory of Tropical Forest Ecology, Chinese Academy of Sciences, Xishuangbanna Tropical Botanical Garden, Menglun, Mengla 666303, Yunnan, China

[3] Institute of Urban Environment, Chinese Academy of Sciences, 1799 Jimei Road, Xiamen 361021, China

[4] School of Geographic Sciences, Nantong University, Nantong 226007, China

[5] Department of Soil Science of Temperate Ecosystems, Department of Agricultural Soil Science, University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[6] Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia 420049

[7] Agro-Technological Institute, RUDN University, Moscow, Russia 117198

than the atmosphere (Tarnocai et al. 2009) with small changes having large effects on atmospheric CO_2 and potentially causing climate feedbacks (Heimann and Reichstein 2008). Even though SOM structures, properties, and functions have been extensively studied for more than a century, many processes driving SOM destabilization and dynamics remain unclear (Schmidt et al. 2011).

Forests occupy about one-third of the Earth's land surface (Bloom et al. 2016). Global forest C stock totals approximately 861 Pg C above and below ground. Approximately 44% of which is stored in forest soils to 1-m depth (Pan et al. 2011). To better understand the processes controlling SOM destabilization and dynamics, a chronosequence approach, together with succession theories, has been developed (Pregitzer and Eusckirchen 2004; Walker et al. 2010). In this respect, two main succession types, primary and secondary, are often investigated to understand SOM development and dynamics. Primary succession is the series of community changes, which take place on a completely new habitat, which has never been colonized before. It begins on soil parent materials and is ongoing simultaneously with soil development. The C stocks increase through time, culminating in old-growth forests (Field and Kaduk 2004; Gleixner et al. 2009) (Fig. 1), in which annual C accumulation may approach zero because the C gain through photosynthesis is balanced by the C loss due to respiration (and erosion). Such temporal patterns were well documented in the classical studies on ecosystem succession (Olson 1958), predicting that old-growth forests should be neutral C sinks (Odum 1969). However, recent studies showed that old-growth forests can persist as C sinks in terms of both vegetation and soils (Luyssaert et al. 2008; Gleixner et al. 2009; Fontaine et al. 2017; Stahl et al. 2017). Soil C stocks, representing 26 to 33% of the total C in old-growth forests, still increase with time (Fredeen et al. 2005; Zhou et al. 2006). In contrast to primary succession, secondary succession is the series of community changes, which occur on a previously colonized but disturbed habitat. It often starts from a natural disturbance (e.g., a storm, a fire, or a flood) or an anthropogenic land-use change. The pattern of secondary succession is much more complicated because (1) various disturbances leave some organic C, plant seeds, and living organisms in the remaining soil (Bormann and Likens 1979) and (2) surrounding vegetation, climate, and topography can strongly affect succession. Such successions generally include the following stages, grasses and herbs, small bushes, young trees, and old-growth forests.

Empirical studies have demonstrated that the loss of the remaining soil organic C via decomposition often exceeds the C gained by the growth of young trees. This leads to rapid loss of soil C derived from old forests, often in the early stages of secondary forest succession (Harmon et al. 1990; Zak et al. 1990; Pregitzer and Euskirchen 2004; Yang et al. 2011) (Fig. 1). Several explanations have been put forward for this rapid loss of soil C from young forests and accumulation in old-growth forests, e.g., the imbalance between decomposition and inputs, as well as, stoichiometric imbalances (Vitousek et al. 1988). Specifically, low litter input with strong SOM decomposition resulted in rapid loss of soil C under young secondary forest (Georgiadis 2011). Resource stoichiometry exerts a great impact on microorganisms and decomposition and thus soil C transformation and accumulation (Zechmeister-Boltenstern et al. 2015; Qiao et al. 2016). C

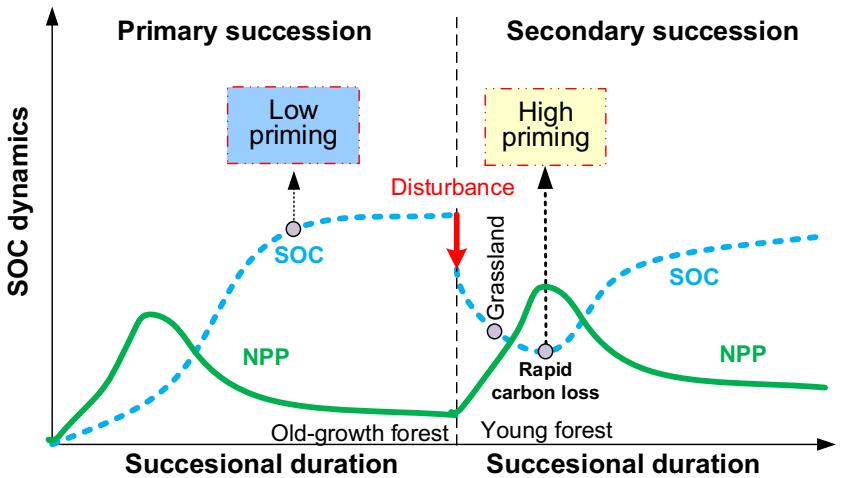

Fig. 1 Dynamics of soil organic matter (SOM) content, net primary production (NPP), and priming effects during primary and secondary vegetation succession (Odum 1969; Harmon et al. 1990; Harmon 2001; Trumbore et al. 2015). Litter production and root exudates have a similar pattern as NPP (green continuous line) (Chapin et al. 2012). Old-growth forest accumulates high soil organic C (SOC, blue dashed line) via long-term succession. Removal of vegetation due to land-use change leads to secondary succession, e.g., grassland and young secondary forest. There is rapid SOC loss at the stage of young secondary forests. Gray dots refer to the stages of ecosystem succession we used in this study. Low priming effects are hypothesized at low NPP (corresponding to low litter and rhizodeposition input) due to a stable level of SOC during the steady state of primary vegetation (old-growth forest). Intensive priming effects are expected at high NPP during by new vegetation (after disturbance) in grassland or young secondary forest

demand drives microbial mineralization of organic phosphorus (P) during the early stage of soil development (Wang et al. 2016) and faster litter decomposition in the earlier successional phase than later stages of succession ascribed to less P limitation (Vitousek and Farrington 1997; Xuluc-Tolosa et al. 2003; Menge et al. 2012). The reason is that ecosystems shift during succession from N limitation on young soils to P limitation on old soils (Menge et al. 2012). Such stoichiometric imbalance will affect SOC accumulation mainly through altering the quality of litter and element use efficiencies during succession (Schulz et al. 2013; Mooshammer et al. 2014). Because biological communities drive ecosystem properties and development (Schaaf et al. 2011), the stoichiometric imbalance is a result of biotic–abiotic interactions over the courses of ecosystem succession (Schulz et al. 2013). This indicates that temporal variation in driving variables play an important role for SOM patterns during succession (Harmon 2001), especially for the rapid loss of soil C from young forests versus C accumulation in older forests due to the vegetation development and nutrients' availability. Our knowledge of these mechanisms responsible for soil C stabilization and accumulation over time is still very limited (Wirth et al. 2009).

Microbial decomposition of SOM is primarily modified by available C input (Blagodatskaya and Kuzyakov 2013), defined as priming effects (e.g., Kuzyakov et al. 2000; Cheng et al. 2003). Priming effects can accelerate SOM decomposition and have great potential to change soil C dynamics (Blagodatskaya and Kuzyakov 2008; Kuzyakov 2010; Cheng et al. 2014; Huo et al. 2017). Priming effects are often observed in the rhizosphere and detritusphere, hotspots with high input of labile organics via root exudation, and litter decomposition (Kuzyakov and Blagodatskaya 2015). Root exudation and litter are correlated with net primary productivity (NPP, Norby and Zak 2007) and have a very tight temporal coupling (Kuzyakov and Gavrichkova 2010). Many young secondary forests can have higher rates of NPP than do old-growth forests (Harmon 2001; He et al. 2012; Poorter et al. 2016). Compared to older plants, which allocate the newly assimilated C to the shoots, young plants allocate more C to roots for acquiring more nutrients and water to support biomass increase (Gregory and Atwell 1991; Palta and Gregory 1997; Kuzyakov 2001). Given higher NPP in young secondary forests, exudation decreases per unit root biomass of older trees in old-growth forests (Nguyen 2003). Also, the contribution of roots to SOM is higher than that of the above ground litter (Berhongaray et al. 2018). This indicates that roots play different roles in SOM formation in young and old forests. However, the role of priming effects caused by root exudation in SOM decomposition and C accumulation remains unclear.

Priming effects of SOM decomposition are a result of interactions between living and dead organic matter (Kuzyakov 2010). The change in plant species composition and tree aging with time affects the quality and quantity of labile organics incorporated to the soil through root exudation and litter decomposition, along with distinct nutrient requirements. The old and physically protected SOM is exposed to living roots and litterfall in young secondary forests because the topsoil is often lost via erosion by high rainfall under low vegetation coverage conditions (Guillaume et al. 2015). As a result, labile C derived from root exudation and litter decomposition have high chance to access such SOM fraction in the young secondary forests. Based on the previous observations that rapid soil C losses occurred in many young secondary forests, we hypothesize that strongly positive priming in young forests leads to substantial SOM depletion and thus soil C losses, whereas the remaining of added labile C compensates soil C loss caused by priming in old-growth forests, resulting in C sequestration (Fig. 1). Considering relatively stable vegetation biomass as well as shallow root system in grasslands, we additionally hypothesize that priming in grasslands could be the highest due to high root exudation. To test the hypotheses above, we selected two early stages (grassland, approximately 20 years and young secondary forest, about 40 years) of a secondary succession, which started after the clear-cut of trees due to land-use change, and used a native old-growth forest (undisturbed for more than 200 years) as the reference (Fig. 1). Soils were incubated under controlled conditions for 170 days and the priming effects were quantified by addition of uniformly ^{13}C-labeled glucose. Following incubation, ^{13}C-PLFAs (phospholipid fatty acids) were analyzed to identify microbial functional groups utilizing glucose (Yao et al. 2014) and to explore their accordance with SOM priming through succession.

Material and methods

Site description and soil collection

The research site is located in the Shilin Stone Forest Geological Park (24° 38′–24° 58′ N, 103° 11′–103° 29′ E) in the Yunnan Province of southwestern China. Annual precipitation averaged 970 mm, ranging from 800 to 850 mm, with approximately 80% between May and October. The annual temperature averaged 16.2 °C, with the minimum temperature of 8.2 °C in the coldest month (January) and the mean maximum temperature of 20.7 °C in the warmest month (July). Three vegetation successional stages, i.e., grassland, secondary young-growth forest, and primary old-growth forest at the same altitude, were selected to represent a succession series. In the past several decades, the primary forests have been destroyed due to human activities, e.g., the harvest of firewood and land clearing for crops and animal grazing (Shen et al. 2007). Consequently, some of the grassland sites are about 20 years old with low content of soil organic C, while some of the young forest sites are approximately 40 years old

with most of the trees 20 years old. The grassland is dominated by *Heteropogon contortus* (L.) P. Beauv. ex Roemer, *Oplismenus compositus* (L.) P. Beauv., *Schizachyrium delavayi* (Hack) Bor, and *Eupatorium adenophorum* Spreng (Shen et al. 2007). Some bushes and small trees were sparsely scattered in these grasslands. The young secondary forest is dominated by *N. homilantha* with a low tree density. The old-growth forest (more than 200 years old) site was an evergreen broadleaf forest with few deciduous species: *Cyclobalanopsis glaucoides* Schottky, *Olea yunnanensis* Hand-Mazz, *Pistacia weinmannifolia* J. Poisson ex Fr., *Pistacia chinensis* Bunge, *Neolitsea homilantha* Allen, and *Albizia mollis* (Wall.) Boiv (Shen et al. 2005). The soil is silty clay loam (WRB 2014) derived from limestone and weakly metamorphosed marine sediments. The soil contains about 50% silt, 30% clay, and 20% sand.

In each vegetation type, four plots (80 m × 50 m) were established. In each plot, mineral soil was randomly collected from the top 10-cm depth after litter removal, at five sampling points (approximately 20-m distance between them, Fig. S1). These soil samples from the same plot were combined as a single replicate. The soils were stored at 4 °C before analysis. Soil total phosphorus (P) content was measured using an optical emission spectrometry (Optima 5300DV; PerkinElmer, Shelton, USA) after nitric-perchloric acid digestion (Olsen et al. 1954). Available soil P was determined by the Olsen method (Olsen et al. 1954). Soil organic C was estimated using the dichromate oxidation and titration method (Kalembasa and Jenkinson 1973). Determination of total N in soil was done by the Kjeldahl method (Bremner 1960). Potentially available N in soils was estimated using alkaline hydrolysis approach (Bao 2000). Briefly, the available N was reduced to NH_3 at 40 °C for 24 h after adding $FeSO_4$ powder and a NaOH solution. After then, NH_3 was absorbed in H_3BO_3 and titrated with H_2SO_4 to determine the available N content. Dissolved organic C (DOC) concentrations in the K_2SO_4 extracts were measured with a Dimatec-100 TOC/TIC analyzer (Dimatec Analysentechnik GmbH, Essen, Germany). Soil pH values were determined with a glass electrode using a 1:2 soil-to-water ratio (Jackson 1967). These results are presented in Table 1.

Soil incubation and CO_2 analysis

Thirty grams of air-dried, 2-mm-sieved, root-picked soil was added to 250-mL Schott bottles and adjusted to 60% of water-holding capacity (WHC). All soils were pre-incubated at 20 °C for 7 days. After pre-incubation, one pulse of ^{13}C-labeled glucose was added into the soil. Glucose was used in this study because it is a major component of root exudates and product of litter decomposition (Derrien et al. 2014; Gunina and Kuzyakov 2015). Water or ^{13}C uniformly labeled glucose (5.97% atom ^{13}C) solutions were added to the soil surface, dropwise using a pipette, to obtain uniform distribution and moisture 70% of WHC, which was maintained throughout the experiment. Total glucose C additions were equal to 2% of the SOC in each soil (i.e., 20 mg C g^{-1} SOC, equal to 0.68 mg C g^{-1} soil for grassland, 1.68 mg C g^{-1} soil for young secondary forest, and 2.27 mg C g^{-1} soil for old-growth forest), which is comparable to microbial biomass-C (MBC) and adequate to stimulate microbial activity (Blagodatskaya and Kuzyakov 2008). All incubations were conducted at 20 °C. Five milliliters of 1 M NaOH was placed in small cups within each incubation bottle to trap CO_2 and was replaced weekly. CO_2 was trapped from each incubation bottle and analyzed for C content and $\delta^{13}C$. During the incubation, four empty bottles were used as blanks to consider the atmospheric CO_2. Soil samples were destructively harvested for analysis after incubation for 170 days.

To measure CO_2 absorbed in NaOH solutions, 4 mm of 0.5 M $SrCl_2$ was added to precipitate carbonate. Unreacted NaOH was titrated with 0.2 M HCl against the phenolphthalein endpoint (Zibilske 1994). Precipitated $SrCO_3$ was centrifuged three times at 1200 *g* for 10 min, followed each time by rinsing with degassed, deionized water. The $SrCO_3$ was then dried at 105 °C and weighed into tin capsules for analysis of organic C and $^{13}C/^{12}C$ ratios by continuous-flow gas isotope ratio mass spectrometry (MAT253, Finnigan MAT, Germany), coupled by ConFlo III device (Finnigan MAT, Germany) to an elemental analyzer (EA 1112, CE Instruments, Italy).

Table 1 Topsoil (upper 10 cm) properties in grassland, young secondary forest, and old-growth forest (succession stages). Values represent means ± 1 SE of four replicates. Small letters in the same column indicate significant differences ($p < 0.05$) among grassland, young secondary forest soil and old-growth forest soil. *SOC*, soil organic C; *DOC*, dissolved organic C

Succession stages	pH (H_2O)	Bulk density (g cm^{-3})	SOC (%)	DOC (μg g^{-1} soil)	Total N (%)	Available N (μg g^{-1} soil)	Total P (%)	Available P (μg g^{-1} soil)
Grassland	6.7 ± 0.4	1.30 ± 0.15	3.42 ± 0.34c	229 ± 12.3c	0.22 ± 0.02c	162.8 ± 5.1c	0.029 ± 0.003c	2.10 ± 0.25
Young forest	6.0 ± 0.4	1.18 ± 0.21	8.41 ± 0.54b	542 ± 10.5b	0.50 ± 0.03b	237.6 ± 13.9b	0.076 ± 0.005b	2.79 ± 0.39
Old-growth forest	5.9 ± 0.3	1.08 ± 0.10	11.33 ± 0.80a	838 ± 22.7a	0.83 ± 0.08a	323.7 ± 13.9a	0.104 ± 0.008a	3.15 ± 0.43

Lipid extraction and ^{13}C PLFA analysis

Lipid extraction and PLFA analyses were performed according to (Frostegård et al. 1993; Wang et al. 2014). Briefly, 2.0-g freeze-dried soil was extracted twice using 22.8 mL one-phase mixture (1:2:0.8 *v*/*v*/*v*) of chloroform, methanol, and citrate buffer (0.15 M, pH 4.0). Four replications were used for PLFA analysis. For gas chromatography (GC) analysis, PLFAs were derivatized to their fatty acid methyl esters. The $^{13}C/^{12}C$ ratios of individual PLFAs were analyzed by GC–C–IRMS using a Trace GC Ultra gas chromatograph with combustion column, attached via a GC combustion III to a Delta V Advantage isotope ratio mass spectrometer (Thermo Finnigan, Germany) (Thornton et al. 2011). Monounsaturated (16:1u7c, 18:1u7c) and cyclopropyl-saturated PLFAs (cy17:0, cy19:0) were considered as markers for Gram-negative bacteria (G−); terminal-branched saturated PLFAs (a15:0, i15:0, i16:0, i17:0, a17:0) were used to indicate Gram-positive bacteria (G+); PLFAs (18:2u6,9, 18:1u9c) were used to represent fungi (Frostegård and Bååth 1996). Midchain branched saturated PLFAs (10Me16:0, 10Me17:0, 10Me18:0) were considered as markers for actinomycetes; short- or odd-chain saturated PLFAs (14:0, 15:0, 16:0, 17:0) were used to indicate non-specific makers (refer as to the whole microbial community) present in all microbes (Zelles 1999).

Calculations and statistics

The soil-derived CO_2-C (C_{SOC}) was separated from glucose-derived CO_2-C (C_G) using mass balance equations:

$$C_{SOC} + C_G = C_t \quad (1)$$

$$C_{SOC} \times \delta^{13}C_{CK} + C_G \times \delta^{13}C_G = C_t \times \delta^{13}C_t \quad (2)$$

where $\delta^{13}C_{CK}$ is the isotopic signature (‰) of soil-derived CO_2-C measured in control soils, $\delta^{13}C_G$ is the isotopic signature of added glucose, C_t is the total CO_2 released by the amended soil on the specific day of measurement, and $\delta^{13}C_t$ is its isotopic signature.

Primed C was calculated as the following equation (Qiao et al. 2014):

$$\text{Primed C} = C_t – C_{soc} – C_{CK} \quad (3)$$

where C_{SOC} is SOC-derived C-CO_2 under glucose addition treatments and C_{CK} is total C-CO_2 from the soil receiving only water.

Glucose release (%) was C_G divided by total glucose added, and net C balance was calculated as the difference between primed C and retention of added glucose C (Qiao et al. 2014).

The $\delta^{13}C$ of each PLFA molecule was corrected for the C added during derivatization using a mass balance as follows (Dungait et al. 2011; Wang et al. 2014):

$$n_{cd}\delta^{13}Ccd = n_c\delta^{13}C_c + n_d\delta^{13}C_d \quad (4)$$

where n is the number of C atoms, c is to the compound of interest, d is the derivatizing agent (methanol: $n_d = 1$ and $\delta^{13}C = -29.33‰$), and cd is the corresponding derivatized compound of interest.

The amount of glucose-derived labeled C in each PLFA (Pi) was estimated by the below equation (Dungait et al. 2011; Wang et al. 2014):

$$Pi = Mi \times \left(\delta^{13}C_c – \delta^{13}C_{control}\right) / \left(\delta^{13}C_{glucose} – \delta^{13}C_{control}\right) \quad (5)$$

where Mi refers to the molecular C content of the individual PLFAs; $\delta^{13}C_c$ and $\delta_{13}C_{control}$ indicate the $\delta^{13}C$ of individual PLFAs in the samples added without or with glucose, respectively. $\delta^{13}C_{glucose}$ is the $\delta^{13}C$ of added glucose. The total ^{13}C enrichment of PLFAs in each soil was calculated dividing the sum of the amount of glucose-derived labeled C in each PLFA (Pi) by the sum of the molecular C content of the individual PLFAs (Mi) (Wang et al. 2014).

Shapiro–Wilk tests showed that all data were normally distributed. The significant difference in primed C and net C balance between three successional stages (grassland, young-growth forest, and old-growth forest) was examined by one-way analysis of variance (ANOVA) followed with post hoc Tukey honest (HSD) tests using SPSS 21.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was performed to test the significant difference in ^{13}C incorporation into each active microbial community (as a percentage) between successional stages. The lowest Akaike information criterion model was used for the multiple linear regression to represent the correlation between active microbial communities and priming using R 3.2.1 with a MuMIn package (Barton 2012). Redundancy analysis on soil microbial communities in three successional stages constrained by environmental variables was performed by R 3.2.1 (Borcard et al. 2011). All differences were tested for significance at $p < 0.05$.

Results

Soils without glucose (i.e., control) from young (157 ± 10.2 μg C g^{-1} SOC d^{-1}) and old-growth forest (140 ± 5.4 μg C g^{-1} SOC d^{-1}) showed similar CO_2 efflux rates on an SOC-specific basis during the 170-day incubation (Fig. 2). These CO_2 fluxes were lower than those from grassland soil (180 ± 8.5 μg C g^{-1} SOC d^{-1}). Labile C input strongly increased total CO_2 flux rates in all three soils, especially during the first week (Fig. 2).

All three soils showed positive priming effects, but with distinct patterns (Fig. 3a). The grassland and young-forest soils demonstrated similar priming in the first month, but higher than in old-growth forest soil. Subsequently, priming

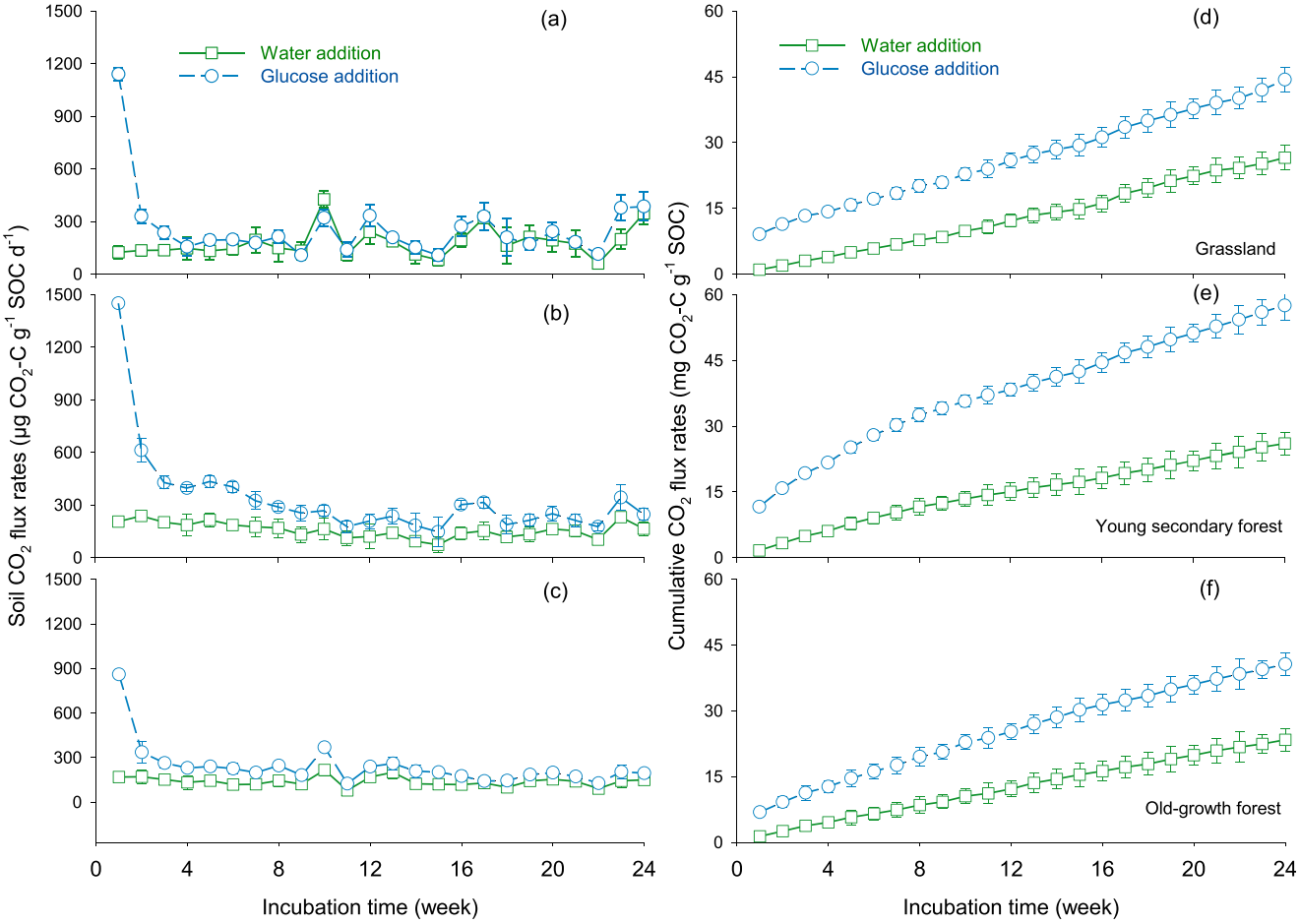

Fig. 2 Total CO_2 efflux rates (left) and cumulative total CO_2 efflux (right) from soil during the 170-day incubation period. Soils were collected from three successional stages, i.e., grassland (**a**, **d**), young secondary forest (**b**, **e**), and old-growth forest (**c**, **f**). Values represent means ± 1 SE of four replicates

increased substantially in the young-forest soil, but it gradually converged in both grassland and old-growth forest soils (Fig. 3a). Cumulative priming after 170 days in the young-forest soil was 2 times higher than in grassland or old-growth forest soil (Table 2; Fig. 3a). At the end of 170-day incubation, more than 12.0 mg C g^{-1} SOC derived from added glucose remained in grassland and old-growth forest soils, but only 6.6 mg C g^{-1} SOC remained in the young secondary forest soil (Table 2). Taking the remaining of C from added glucose, the grassland and old-growth forest soils had positive net C balances (normalized to SOC amount), whereas net C balance in the young-forest soil was strongly negative although priming accelerated C loss in all three soils (Fig. 3b).

At the end of 170-day incubation, old-growth forest soil showed the highest total PLFAs as an indicator of microbial biomass, followed by young secondary forest and grassland soil (Fig. 4). The dominance of microbial groups and the groups incorporating the most glucose C differed among three soils (Fig. 4). The utilization of glucose by G+ bacteria decreased while that by the whole microbial community (represented by non-specific PLFA makers) increased along the secondary succession from grassland to old-growth forest. G– bacteria showed low ^{13}C incorporation in young-forest soil, but high ^{13}C incorporation in old-growth forest soil (Fig. 4). Actinomycetes demonstrated the lowest ^{13}C incorporation in grassland soil. By comparison, fungi were more active for glucose C retention in the young-forest soil than in the other two soils (Fig. 4). Redundancy analysis (RDA) in three soil types demonstrated specific patterns: domination of fungal activities within the young-forest soil; but the whole microbial community (represented by non-specific PLFA makers) within the old-growth forest (Fig. 5). Multiple linear regression between ^{13}C-PLFA in microbial groups and intensity of priming effect showed that ^{13}C incorporation from glucose into fungi was positively correlated with the acceleration of SOM decomposition ($p < 0.005$) (Table S1).

Discussion

A classical theory of ecosystem succession suggests that old-growth forests are a neutral C sink (Odum 1969).

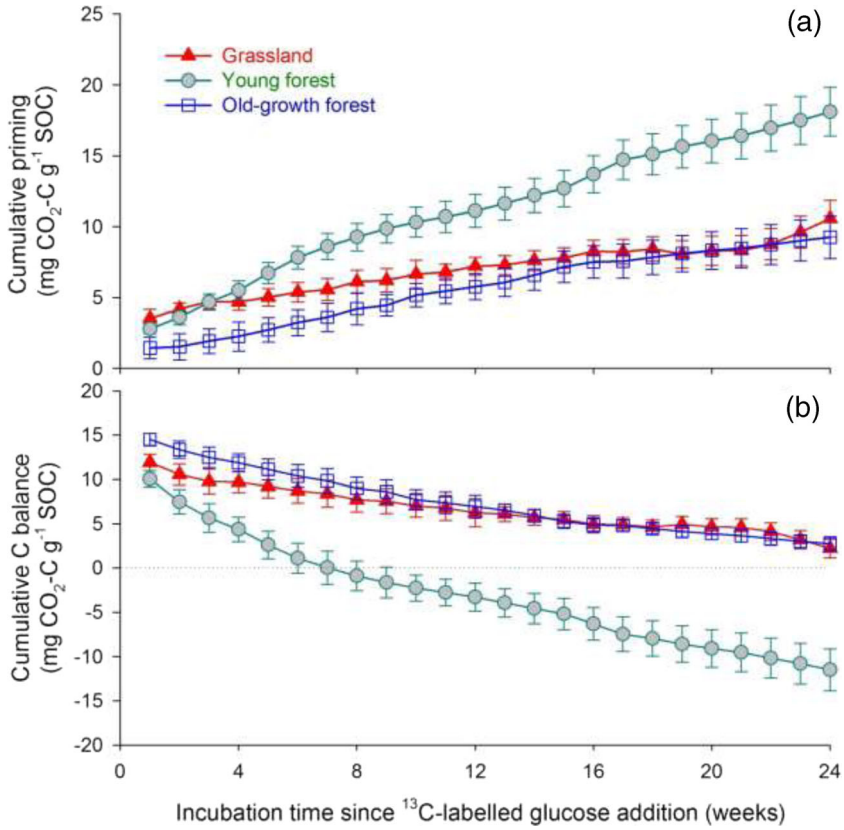

Fig. 3 Cumulative priming (**a**) and net carbon (C) balance between primed SOC and remained glucose C (**b**) in soils collected from three successional stages (i.e., grasslands, young-growth forests, and old-growth forests) during the 170-day incubation period. Values represent mean ± 1 SE of four replicates

However, recent studies have demonstrated that old-growth forests in many climates still sequester C in both vegetation and soils (Phillips et al. 1998; Zhou et al. 2006; Luyssaert et al. 2008; Lewis et al. 2009; Pan et al. 2011). Soil C often suffers from rapid losses during the early stages of secondary forest succession (Guillaume et al. 2015), which starts from the disturbed old-growth forests (Harmon et al. 1990; Zak et al. 1990; Pregitzer and Euskirchen 2004; Yang et al. 2011). Here, we present a novel finding that the differing soil C dynamics during vegetation succession might be explained by priming. The ^{13}C-PLFA results suggest that the incorporation of labile substrates by fungi is related to positive SOM-priming in young forests (Garcia-Pausas and Paterson 2011; Fontaine et al. 2011).

After the clear-cut of old-growth forest, the secondary succession started and the plants are very sparse at the early stages. As a result, surface soils derived under old-growth forest are frequently lost via erosion due to high precipitation and a lack of vegetation protection (Guillaume et al. 2015). Litter C input in young forests is less than in old-growth forests, but the decomposition rates are nearly the same, or even higher (Ostertag et al. 2008; Zhang et al. 2013). More exudation per unit soil volume is often expected in the young secondary forest due to higher NPP. Labile C, derived from root exudation and litter decomposition, will boost microbial activity (Blagodatskaya and Kuzyakov 2013) (e.g., for fungi) to produce more extracellular enzymes for SOM decomposition (Fontaine et al. 2011; Chen et al. 2014) (Fig. 6). As a result, more nutrients due to increased mineralization are released to

Table 2 Carbon derived from added glucose remaining in the soil, primed C, and net C balance per gram of SOC at the end of an incubation period of 170 days. Values represent means ± 1 SE of four replicates. Small letters in the same column indicate significant difference ($p < 0.05$) between successional stages

Successional stages	mg C g^{-1} SOC		
	Remaining of added glucose	Primed C	Net C balance
Grassland	12.8 ± 0.4a	10.6 ± 1.3b	2.2 ± 1.1a
Young forest	6.6 ± 0.5b	18.1 ± 1.7a	− 11.5 ± 2.4b
Old-growth forest	12.0 ± 0.4a	9.3 ± 1.5b	2.8 ± 0.8a

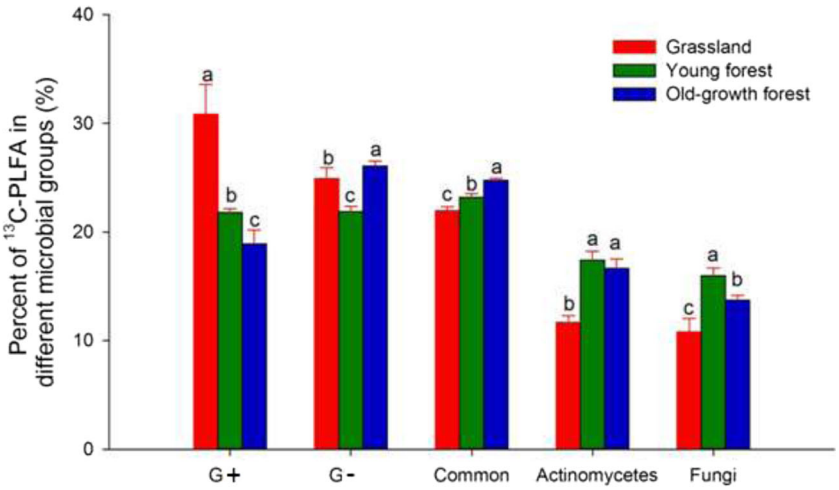

Fig. 4 Distribution of ^{13}C-PLFA in microbial groups (i.e., G+ bacteria, G– bacteria, the whole microbial community (common) represented by non-specific PLFA makers), actinomycetes and fungi in grassland, young-growth forest soil, and old-growth forest soil at the end of 170-day incubation. Values represent mean ± 1 SE of four replicates. Small letters above bars within each microbial group indicate significant difference among grassland, young-, and old-growth forest soils at $p < 0.05$ level

meet the higher requirements of young growing trees for rapid growth (Guariguata and Ostertag 2001; Fontaine et al. 2011).

It has been debated whether old-growth forests are C neutral or C sinks. The main issue is whether they achieve steady-state soil organic C that may require vegetation succession of centuries or longer (Odum 1969; Luyssaert et al. 2008). Because age thresholds for old-growth forests are conventionally regarded as 200 years (Pregitzer and Euskirchen 2004), we used a forest site with more than 200 years old to represent the old-growth forest. Compared to young secondary forest, old-growth forest presented higher C and N availability but similar P availability (Table 1). This indicates that microorganisms in the young secondary forest are mainly limited by labile C and N, while they are more limited by available P in old-growth forest (Vitousek and Farrington 1997; Xuluc-Tolosa et al. 2003; Menge et al. 2012). Consequently, the input of labile C stimulate microbial growth in young secondary forest soil and produced more extracellular enzymes for mining of N from SOM, which induced strong positive priming. By comparison, the input of labile C stimulated microbial growth (e.g., microbial growth of G– bacteria and the whole microbial community as shown by non-specific PLFA, Fig. 4) in old-growth forest soil, but microorganisms produced less extracellular enzymes than fungi for mining of N from SOM. Due to P limitation in old-growth forest, microorganisms invested more C and energy for mobilization of inorganic and organic P, but this process did not induce priming of SOM decomposition (Dijkstra et al. 2013), partly because P will be mobilized from mineral sources. As a result, smaller priming effects occurred in old-growth forest soil (Fig. 6), leading to C accumulation due to high retention of labile C (Qiao et al. 2014). Compared to young and old forests, microorganisms in grassland are co-limited by C, N, and P availability (Table 1). The input of labile C-stimulated microbial growth of G+ bacteria in grassland soil, but induced comparable priming as shown in old-growth forest soil. This could be ascribed to distinct nutrients availability as well as the quality and quantity of labile C input exerting an impact on microbial turnover (Sullivan and Hart 2013; Chen et al. 2019).

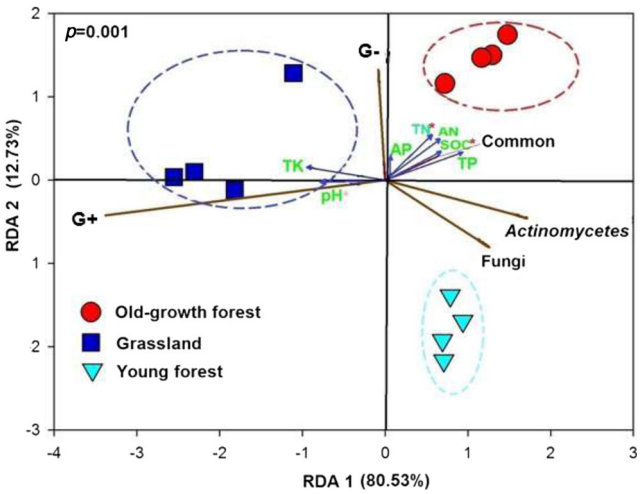

Fig. 5 RDA triplot of soil microbial communities (as ^{13}C-PLFA data) of three successional stages (i.e., grasslands, young-growth forests, and old-growth forests) constrained by environmental and soil variables (blue arrows); microbial communities are represented by brown lines. G+ refers to Gram-positive bacteria, G− refers to Gram-negative bacteria, common refers to all microbes with non-specific PLFA makers, AN refers to available N, AP refers to available P, TN refers to total N, SOC refers to soil organic C, and pH refers to soil pH. The variables with a red asterisk indicate significant effects at $p < 0.05$ level

Microbes play a central role in SOM decomposition (Schmidt et al. 2011; Schimel and Schaeffer 2012) and microbial community composition is a determinant of SOM mineralization, e.g., fungi play an important role in priming effect (Garcia-Pausas and Paterson 2011; Fontaine et al. 2011; Shahzad et al. 2012). This study finds that the forest succession is accompanied by the microbial succession with distinct functional roles in decomposition: Gram-positive bacteria in grassland soil, fungi in young-forest soil, and the whole microbial community in the old-growth forest soil. However, a

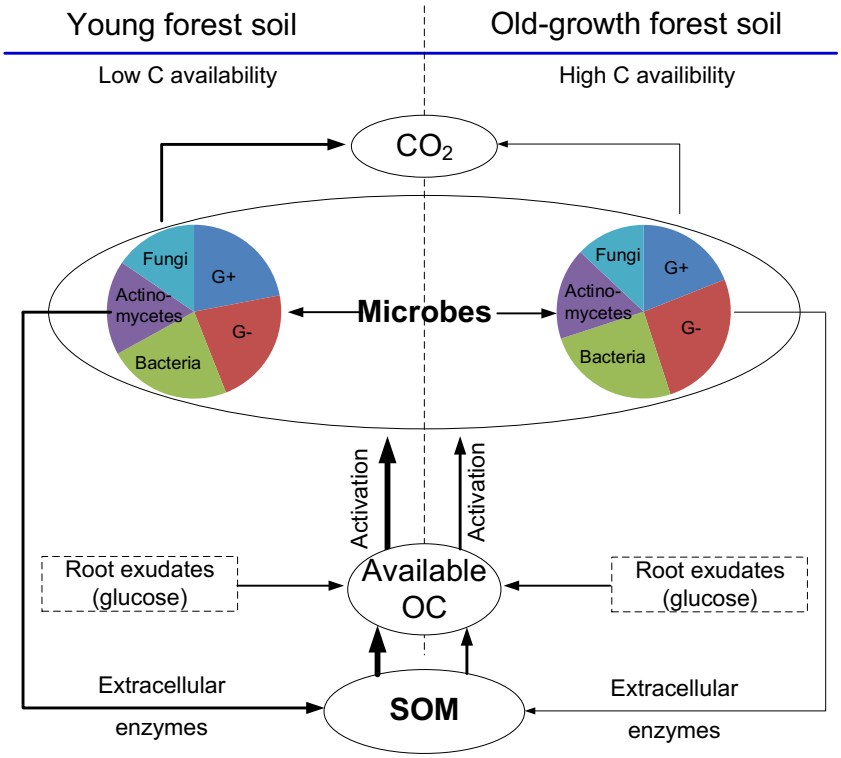

Fig. 6 Mechanisms responsible for priming effects in young-forest soils (left) and old-growth forest soils (right). Line thickness represents the strength of the process. See the main text for further explanations

previous study reported that utilization of labile substrate by G– bacteria did not promote positive SOM-priming (Garcia-Pausas and Paterson 2011). This indicates that the mechanisms of priming effects could be much more complicated than previously expected. Priming of SOM decomposition is a result of interactions between living and dead organic matter (Kuzyakov 2010), but microbial functional groups involved in priming effects could be strongly regulated by the amount and stoichiometric ratios in the labile resources as well as plant uptake (Shahzad et al. 2012; Chen et al. 2014; Perveen et al., 2014; Qiao et al. 2016; Wang et al. 2016; Chen et al. 2019).

Carbon sequestration is an ecosystem process (Schmidt et al. 2011) primarily mediated by plants and soil microbes (Miltner et al. 2012). The growth of plants and various microbial processes have seasonal and annual dynamics. Using three different succession stages of ecosystems, we present experimental evidence that priming of SOM decomposition is much stronger in secondary young-forest soil than in old-growth forest and grassland soils, which strongly affects C sequestration and SOM dynamics. Previous studies showed that apparent priming effects can contribute to CO_2 efflux through microbial turnover (i.e., microbial biomass replaced by added C) but not contribute to SOM decomposition (Fontaine et al. 2003, 2007; Blagodatskaya and Kuzyakov 2008). However, apparent priming effects mainly occur at the initial stages (within several weeks) after substrate addition (Blagodatskaya and Kuzyakov 2008). Over 24-week incubation, real priming dominated in priming of SOM (Qiao et al. 2014). This indicates that the soil C losses caused by priming in this study are mainly derived from native SOM decomposition.

We conclude that priming is one of the potential mechanisms for distinct soil C dynamics in young- and old-growth forests. Our findings provide novel ecological connections between SOM dynamics and C stabilization with microbial functioning during forest succession. The intensity of priming and the direction of soil C balance depend on the vegetation development (plant biomass at a steady state such as in grassland or old-growth forest versus a continuous increase of plant biomass such as in a secondary young forest). The soil functions as a bank of nutrients (Fontaine et al. 2011; Shahzad et al. 2012; Perveen et al. 2014). When plant biomass increases, the continuous nutrients uptake intensifies the rhizosphere priming effect relative to the microbial building of SOM. The net decomposition of SOM releases the N (and C) required for plant growth. When plant biomass accumulation stops or decreases (old-growth forest and grassland), soluble N increases in ecosystems (atmospheric deposition, symbiotic fixation, etc.). This decreases rhizosphere priming relative to SOM building resulting in SOM accumulation. However, we have to mention that our results may have some uncertainties because they were obtained under controlled conditions and the ^{13}C-PLFA analysis is based on only one point at the end of the incubation. Some of PLFAs could be secondary utilization of microbial necromass and glucose decomposition products. Additionally, the normalization by soil organic C content did not consider microbial status or functional groups, which could lead to some uncertainty.

Therefore, future research should involve determinations of ^{13}C incorporation into PLFA at shorter periods and of enzyme activities involved in mining N and P from SOM using additional successional sequences in various climates.

Funding information This study was supported by the National Natural Science Foundation of China (41830646, 41601318, 31470560, and 41671031), the general financial grant from the China Postdoctoral Science Foundation (2016M600123) and the national key research and development program of China (2016YFC0502503), and Youth Innovation Research Team Project (LENOM2016Q0004). The publication was supported by the Government Program of Competitive Growth of Kazan Federal University and with the support of the "RUDN University program 5-100."

References

Bao S (2000) Agro-chemical analyses of soils. China Agriculture Press, Beijing, China

Barton K (2012) Package 'MuMIn': multi-model inference. R Package, Version 1 (7), 11

Berhongaray G, Cotrufo FM, Janssens IA, Ceulemans R (2018) Below-ground carbon inputs contribute more than above-ground inputs to soil carbon accrual in a bioenergy poplar plantation. Plant Soil 434: 363–378. https://doi.org/10.1007/s11104-018-3850-z

Blagodatskaya E, Kuzyakov Y (2013) Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem 67:192–211

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115–131

Bloom AA, Exbrayat J-F, van der Velde IR, Feng L, Williams M (2016) The decadal state of the terrestrial carbon cycle: global retrievals of terrestrial carbon allocation, pools, and residence times. PNAS 113: 1285–1290

Borcard D, Gillet F, Legendre P (2011) Numerical ecology with R. Springer New York

Bormann FH, Likens GE (1979) Catastrophic disturbance and the steady state in northern hardwood forests: a new look at the role of disturbance in the development of forest ecosystems suggests important implications for land-use policies. Am Sci 67:660–669

Bremner JM (1960) Determination of nitrogen in soil by the Kjeldahl method. J Agri Sci 55:11–33

Chapin FS, Matson PA, Vitousek PM (2012) Principles of terrestrial ecosystem ecology, 2nd edn. Springer, New York

Chen RR, Mehmet S, Blagodatskaya S, Olga M, Klaus D, Lin XG, Blagodatskaya E (2014) Soil C and N availability determine the priming effect: microbial N mining and stoichiometric decomposition theories. Glob Change Biol 20:2356–2367

Chen J, Seven J, Zilla T, Dippold MA, Blagodatskaya E, Kuzyakov Y (2019) Microbial C: N: P stoichiometry and turnover depend on nutrients availability in soil: a ^{14}C, ^{15}N and ^{33}P triple labelling study. Soil Biol Biochem 131:206–216

Cheng W, Johnson DW, Fu S (2003) Rhizosphere effects on decomposition. Soil Sci Soc Am J 67:1418–1427

Cheng WX, Parton WJ, Gonzalez-Meler MA, Philips R, Asao S, McNickle GG, Brzostek E, Jastrow JD (2014) Synthesis and modeling perspectives of rhizosphere priming. New Phytol 201:31–44

Derrien D, Plain C, Courty PE, Gelhaye L, Moerdijk-Poortvliet TCW, Thomas E, Versini A, Zeller B, Koutika LS, Boschker HTS, Epron D (2014) Does the addition of labile substrate destabilise old soil organic matter? Soil Biol Biochem 76:149–160

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2013) Rhizosphere priming: a nutrient perspective. Front Microbiol 4:216

Dungait JAJ, Kemmitt SJ, Michallon L, Guo S, Wen Q, Brookes PC, Evershed RP (2011) Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using ^{13}C-PLFA analysis. Eur J Soil Sci 62:117–126

Field CB, Kaduk J (2004) The carbon balance of an old-growth forest: building across approaches. Ecosystems 7:525–533

Fontaine S, Barot S, Barré P, Bdioui N, Mary B, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. Nature 450:277–280

Fontaine S, Henault C, Aamor A, Bdioui N, Bloor JMG, Maire V, Mary B, Revaillot S, Maron PA (2011) Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. Soil Biol Biochem 43:86–96

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? Soil Biol Biochem 35: 837–843

Fontaine S, Stahl C, Klumpp K, Picon-Cochard C, Grise MM, Dezécache C, Ponchant L, Freycon V, Blanc L, Bonal D, Burban B, Soussana I-F, Blanfort V, Alvarez G. (2017) Response to editor to the comment by Schipper & Smith to our paper entitled "continuous soil carbon storage of old permanent pastures in Amazonia. Glob Change Biol 24:e732–e733

Fredeen AL, Bois CH, Janzen DT, Sanborn PT (2005) Comparison of coniferous forest carbon stocks between old-growth and young second-growth forests on two soil types in Central British Columbia. Can J For Res 35:1411–1421

Frostegård Å, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22: 59–65

Frostegård Å, Bååth E, Tunlio A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biol Biochem 25:723–730

Garcia-Pausas J, Paterson E (2011) Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biol Biochem 43:1705–1713

Georgiadis P (2011) Accumulation of carbon and nitrogen in Swedish forest soils over stand age. (M.Sc. Thesis), Swedish University of Agricultural Sciences, Uppsala, Sweden

Gleixner G, Tefs C, Jordan A, Hammer M, Wirth C, Nueske A, Telz A, Schmidt UE, Glatzel S (2009) Soil carbon accumulation in old-growth forests. In: Wirth C, Gleixner G, Heimann M (Eds) Old-growth forests: function, fate and value. Ecol Stu 207:231–266

Gregory PJ, Atwell BJ (1991) The fate of carbon in pulse-labelled crops of barley and wheat. Plant Soil 136:205–213

Guariguata MR, Ostertag R (2001) Neotropical secondary forest succession: changes in structural and functional characteristics. For Ecol Manag 148:185–206

Guillaume T, Damris M, Kuzyakov Y (2015) Losses of soil carbon by converting tropical forest to plantations: erosion and decomposition estimated by $\delta^{13}C$. Glob Change Biol 21:3548–3560

Gunina A, Kuzyakov Y (2015) Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol Biochem 90:87–100

Harmon ME (2001) Carbon sequestration in forests: addressing the scale question. J For-Washington 99:24–29

Harmon ME, Ferrell WK, Franklin JF (1990) Effects on carbon storage of conversion of old-growth forests to young forests. Science 247:699–702

He L, Chen JM, Pan Y, Birdsey R, Kattge J (2012) Relationships between net primary productivity and forest stand age in US forests. Global Biogeochem Cy 26:GB3009

Heimann M, Reichstein M (2008) Terrestrial ecosystem carbon dynamics and climate feedbacks. Nature 451:289–292

Huo C, Luo Y, Cheng W (2017) Rhizosphere priming effect: a meta-analysis. Soil Biol Biochem 111:78–84

Jackson ML (1967) Soil chemical analysis. Prentice Hall of India, Pvt. Ltd., New Delhi : 498

Kalembasa SJ, Jenkinson DS (1973) A comparative study of titrimetric and gravimetric methods for determination of organic carbon in soil. J Sci Food Agri 24:1085–1090

Kleber M, Eusterhues K, Keiluweit M, Mikutta C, Mikutta R, Nico PS (2015) Mineral–organic associations: formation, properties, and relevance in soil environments. Adv Agron 130:1–140

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Kuzyakov YV (2001) Tracer studies of carbon translocation by plants from the atmosphere into the soil (a review). Eurasian Soil Science 34:28–42

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: concept & review. Soil Biol Biochem 83:184–199

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32: 1485–1498

Kuzyakov Y, Gavrichkova O (2010) Review: time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. Glob Change Biol 16:3386–3406

Ladd JN, Foster R, Nannipieri P, Oades JM (1996) Soil structure and biological activity. In: Stotzky G, Bollag J-M (eds) Soil biochemistry, vol 9. Marcel Dekker, New York, pp 23–78

Lewis SL, Lopez-Gonzalez G, Sonké B, Affum-Baffoe K, Baker TR, Ojo LO, Phillips OL, Reitsma JM, White L, Comiskey JA, Marie-Noël D, Ewango CEN, Feldpausch TR, Hamilton AC, Gloor M, Hart T, Hladik A, Lloyd J, Lovett JC, Makana J-R, Malhi Y, Mbago FM, Ndangalasi HJ, Peacocl J, Peh KS-H, Sheil D, Sunderland T, Swaine MD, Taplin J, Taylor D, Thomas SC, Votere R, Wöll H (2009) Increasing carbon storage in intact African tropical forests. Nature 457:1003–1006

Luyssaert S, Schulze ED, Börner A, Knohl A, Hessenmöller D, Law BE, Ciais P, Grace J (2008) Old-growth forests as global carbon sinks. Nature 455:213–215

Menge DN, Hedin LO, Pacala SW (2012) Nitrogen and phosphorus limitation over long-term ecosystem development in terrestrial ecosystems. PLoS One 7(8):e42045

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M (2012) SOM genesis: microbial biomass a significant source. Biogeochemistry 111:41–55

Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter AA (2014) Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Front Microbiol 5:22

Nguyen C (2003) Rhizodeposition of organic C by plant: mechanisms and controls. Agronomie 23:375–396

Norby RJ, Zak DR (2007) Ecological lessons from free-air CO_2 enrichment (FACE) experiments. Ann Rev Ecol Evol Syst 42:181–203

Odum EP (1969) The strategy of ecosystem development. Science 164: 262–270

Olsen SR, Cole CV, Watanabe FS (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular No. 939, US Government Printing Office, Washington DC

Olson JS (1958) Rates of succession and soil changes on southern Lake Michigan sand dunes. Bot Gazette 119:125–170

Ostertag R, Marín-Spiotta E, Silver WL, Schulten J (2008) Litterfall and decomposition in relation to soil carbon pools along a secondary forest chronosequence in Puerto Rico. Ecosystems 11:701–714

Palta JA, Gregory PJ (1997) Drought affects the fluxes of carbon to roots and soil in 13C pulse-labelled plants of wheat. Soil Biol Biochem 29:1395–1403

Pan YD, Birdsey RA, Fang JY, Houghton R, Kauppi PE, Kurz WA, Phillips OL, Shvidenko A, Lewis SL, Canadell JG, Ciais P, Jackson RB, Pacala SW, McGuire AD, Piao SL, Rautiainen A, Sitch S, Hayes D (2011) A large and persistent carbon sink in the world's forests. Science 333:988–993

Perveen N, Barot S, Alvarez G, Klumpp K, Martin R, Rapaport A, Herfurth D, Louault F, Fontaine S (2014) Priming effect and microbial diversity in ecosystem functioning and response to global change: a modeling approach using the SYMPHONY model. Glob Change Biol 20:1174–1190

Phillips OL, Malhi Y, Higuchi N, Laurance WF, Nunez PV, Vasquez RM, Laurance SG, Ferreira LV, Stern M, Brown S, Grace J (1998) Changes in the carbon balance of tropical forests: evidence from long-term plots. Science 282:439–442

Poorter L, Bongers F, Aide TM, Zambrano AMA, Balvanera P, Becknell JM, Boukili V, Brancalion PHS, Broadbent EN, Chazdon RL, Craven D, de Almerida-Cortez JS, Cabral GAL, de Jong BHJ, Denslow JS, Dent DH, Dewalt SJ, Dupuy JM, Durán SM, Espírito-Santo MM, Fandino MC, César RG, Hall JS, Hernandez-Stefanoni JL, Jakovac CC, Junqueira AB, Kennard D, Letcher SG, Juan-Carlos L, Lohbeck M, Marín-Spiotta E, Martínez-Ramos M, Massoca P, Meave JA, Mesquita R, Mora F, Muñoz R, Nunes YRF, Ochoa-Gaona S, de Oliveira AA, Orihuela-Belmonte E, Peña-Claros M, Pérez-García EA, Piotto D, Powers JS, Rodríguez-Velázquez J, Romero-Pérez IE, Ruiz J, Saldarriaga JG, Sanchez-Azofeifa A, Schwartz NB, Steininger MK, Swenson NG, Toledo M, Uriarte M, van Breugel M, van der Wal H, Veloso MDM, Vester HFM, Vicentini A, Vieira ICG, Bentos TV, Williamson GB, Rozendaal DM (2016) Biomass resilience of neotropical secondary forests. Nature 530:211–214

Pronk GJ, Heister K, Vogel C, Babin D, Bachmann J, Ding G, Ditterich F, Gerzabek MH, Giebler J, Hemkemeyer M, Kandeler E, Mouvenchery YK, Miltner A, Poll C, Schaumann GE, Smalla K, Steinbach A, Tanuwidjaja I, Tebbe CC, Wick LY, Woche SK, Totsche KU, Schloter M, Kögel-Knabner I (2017) Interaction of minerals, organic matter, and microorganisms during biogeochemical interface formation as shown by a series of artificial soil experiments. Biol Fertil Soils 53:9–22

Pregitzer KS, Euskirchen ES (2004) Carbon cycling and storage in world forests: biome patterns related to forest age. Glob Change Biol 10: 2052–2077

Qiao N, Schaefer D, Blagodatskaya E, Zou XM, Xu XL, Kuzyakov Y (2014) Labile carbon retention compensates for CO_2 released by priming in forest soils. Glob Change Biol 20:1943–1954

Qiao N, Xu XL, Hu Y, Blagodatskaya E, Liu Y, Schaefer D, Kuzyakov Y (2016) Carbon and nitrogen additions induce distinct priming effects along an organic-matter decay continuum. Sci Rep 6:19865

Schaaf W, Bens O, Fischer A, Gerke HH, Gerwin W, Grűnewald U, Holländer HM, Kőgel-Knabner I, Mutz M, Schloter M, Schulin R, Veste M, Winter S, Hűttl RF (2011) Patterns and processes of initial terrestrial-ecosystem development. J Plant Nutr Soil Sci 174:229–239

Shahzad T, Chenu C, Repinçay C, Mougin C, Ollier J-L, Fontaine S (2012) Plant clipping decelerates the mineralization of recalcitrant soil organic matter under multiple grassland species. Soil Biol Biochem 51:73–80

Schimel J, Schaeffer SM (2012) Microbial control over carbon cycling in soil. Fron Microbiol 3:348

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knaber I, Lehmann J, Manning DA, Nannipieri P, Rasse DP, Weiner S, Trumbore SE (2011) Persistence of soil organic matter as an ecosystem property. Nature 478:49–56

Schulz S, Brankatschk R, Dümig A, Kögel-Knabner I, Schloter M, Zeyer J (2013) The role of microorganisms at different stages of ecosystem development for soil formation. Biogeosciences 10:3983–3996

Shen YX, Liu WY, Cao M, Li YH (2007) Seasonal variation in density and species richness of soil seed-banks in karst forests and degraded vegetation in Central Yunnan, SW China. Seed Sci Res 17:99–108

Shen YX, Liu WY, Li YH, Cui JW (2005) Community ecology study on karst semi-humid evergreen broad-leaved forest at the central part of Yunnan. Guihaia 25:321–326

Stahl C, Fontaine S, Klumpp K, Picon-Cochard C, Grise MM, Dezécache C, Ponchant L, Freycon V, Blanc L, Bonal D, Burban B, Soussana J-F, Burban B (2017) Continuous soil carbon storage of old permanent pastures in Amazonia. Glob Change Biol 23:3382–3392

Sullivan BW, Hart SC (2013) Evaluation of mechanisms controlling the priming of soil carbon along a substrate age gradient. Soil Biol Biochem 58:293–301

Tarnocai C, Canadell JG, Schuur EAG, Kuhry P, Mazhitova G, Zimov S (2009) Soil organic carbon pools in the northern circumpolar permafrost region. Glob Biogeochem Cycl 23:GB2023

Thornton B, Zhang Z, Mayes RW, Hogberg MN, Midwood AJ (2011) Can gas chromatography combustion isotope ratio mass spectrometry be used to quantify organic compound abundance? Rapid Commun Mass Spectrom 25:2433–2438

Trumbore S, Brando P, Hartmann H (2015) Forest health and global change. Science 349:814–818

Vitousek PM, Fahey T, Johnson DW, Swift MJ (1988) Element interactions in forest ecosystems: succession, allometry and input-output budgets. Biogeochemistry 5:7–34

Vitousek PM, Farrington H (1997) Nutrient limitation and soil development: experimental test of a biogeochemical theory. Biogeochemistry 37:63–75

Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies of ecological succession and soil development. J Ecol 98:725–736

Wang J, Thornton B, Yao HY (2014) Incorporation of urea-derived ^{13}C into microbial communities in four different agriculture soils. Biol Fertil Soils 50:603–612

Wang J, Wu Y, Zhou J, Bing H, Sun H (2016) Carbon demand drives microbial mineralization of organic phosphorus during the early stage of soil development. Biol Fert Soils 52:825–839

Wirth C, Messier C, Bergeron Y, Frank D, Fankhänel A (2009) Old-growth forest definitions: a pragmatic view. In: Wirth C, Gleixner G, Heimann M (eds) Old-growth forests. Springer, Berlin, Heidelberg, pp 11–33

WRB (World Reference Base for Soil Resources) (2014) FAO International Soil Classification System for Naming Soils and Creating Legends for Soil Maps 106

Xuluc-Tolosa FJ, Vester HFM, Ramırez-Marcial N, Castellanos-Albores J, Lawrence D (2003) Leaf litter decomposition of tree species in three successional phases of tropical dry secondary forest in Campeche, Mexico. For Ecol Manag 174:401–412

Yang YH, Luo YQ, Finzi AC (2011) Carbon and nitrogen dynamics during forest stand development: a global synthesis. New Phytol 190:977–989

Yao HY, Chapman SJ, Thornton B, Paterson E (2014) ^{13}C PLFAs: a key to open the soil microbial black box? Plant Soil 392:3–15

Zak DR, Grigal DF, Gleeson S, Tilman D (1990) Carbon and nitrogen cycling during old-field succession: constraints on plant and microbial biomass. Biogeochemistry 11:111–129

Zechmeister-Boltenstern S, Keiblinger KM, Mooshammer M, Peñuelas J, Richter A, Sardans J, Wanek W (2015) The application of ecological stoichiometry to plant–microbial–soil organic matter transformations. Ecol Monogr 85:133–155

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. Biol Fertil Soils 29:111–129

Zhang KR, Cheng XL, Dang HH, Ye C, Zhang YL, Zhang QF (2013) Linking litter production, quality and decomposition to vegetation succession following agricultural abandonment. Soil Biol Biochem 57:803–813

Zhou G, Liu S, Li Z, Zhang D, Tang X (2006) Old-growth forests can accumulate carbon in soils. Science 314:1417

Zibilske LM (1994) Carbon mineralization. In Weaver RW, Angle S, Bottomley P, (complete the list of editors) (Eds) Methods of soil analysis, part 2, Microbiological and Biochemical Properties, Soil Science Society of America, Madison, WI, pp. 835–863.