

1 Priming of soil organic matter decomposition scales linearly with microbial
2 biomass response to litter input in steppe vegetation

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23

1 **Abstract**

2 Fresh plant litter inputs accelerate soil organic matter (SOM) decomposition through a
3 ubiquitous mechanism called priming. Insufficient priming has been suggested as a stabilization
4 mechanism of SOM at depth, as well as the long-term persistence of some highly degradable
5 organic compounds in soils. Priming therefore plays a crucial, albeit unquantified and commonly
6 neglected, role in the global carbon cycle. Because priming intensity is likely to be altered by
7 global change-induced changes in net primary productivity, it casts substantial uncertainty to
8 future projections of the climate-carbon cycle feedback. Using results from a large field litter
9 manipulation experiment in Mongolian steppe, we here show that priming intensifies with
10 increasing litter inputs, but at a decreasing efficiency: the stimulation per unit litter added
11 declines with increasing litter inputs. This non-linear behavior originates from two antagonistic
12 responses to fresh litter inputs: a stimulation of microbial activity versus a shift in microbial
13 community composition (more fungi) associated to substrate shift from SOM to litter. Despite all
14 complexity, however, the priming effect on SOM decomposition scaled linearly with the
15 response of microbial biomass across the entire range of plant litter addition (60-480 g C m⁻²),
16 suggesting that priming could be modeled effectively as a function of the response of microbial
17 biomass to litter inputs. Incorporating the priming mechanism in Earth System models will
18 improve their estimates of the SOM-climate feedback and appears to be best addressed by
19 explicitly representing microbial biomass in the models.

20

21

1 **Introduction**

2 Mineralization of SOM is one of the largest fluxes in the global carbon cycle (Schlesinger and
3 Andrews 2000) and important uncertainties on its controlling mechanisms still remain (Schmidt
4 et al. 2011). Relatively small changes in the rate of SOM mineralization in response to
5 environmental conditions (such as climate change) could have major impacts on atmospheric
6 CO₂ concentration with positive feedbacks on the Earth's climate (Jenkinson et al. 1991, Jones et
7 al. 2003). Moreover, given the complexity to disentangle CO₂ efflux from root respiration and
8 mineralization in soil (Hanson et al. 2000), the CO₂ efflux originating from SOM mineralization
9 (commonly referred to as ecosystem heterotrophic respiration) is also one of the most poorly
10 constrained. Uncertainty about its temperature sensitivity rendered SOM decomposition one of
11 the largest sources of uncertainty in Earth System models (Friedlingstein et al. 2006) and has
12 therefore received a lot of attention (Davidson and Janssens 2006, Craine et al. 2010). However,
13 current understanding has revealed a substantial role for a mechanism called priming as a driver
14 of SOM decomposition (Kuzyakov 2002, Fontaine et al. 2004, Guenet et al. 2010a, Subke and
15 Bahn 2010, Schmidt et al. 2011), and thereby also as a source of uncertainty in future SOM
16 dynamics as simulated by Earth System models.

17 Priming is a modification of SOM decay by fresh organic matter inputs (litter, root
18 exudates, etc.) relative to the SOM decomposition rate in the absence of the added fresh organic
19 matter. Such inputs may reduce or increase the SOM decomposition rate but generally, they
20 stimulate microbial activity increasing SOM decomposition rate (Blagodatskaya and Kuzyakov
21 2008). Priming is ubiquitous; evidence for the occurrence of priming has been shown for most
22 terrestrial biomes, including forests, grasslands and even croplands (see Blagodatskaya and
23 Kuzyakov 2008 for a review), but priming has also been suggested as an important process

1 impacting organic matter decomposition in freshwater ecosystems and even in oceans (Guenet et
2 al. 2010a, Bianchi 2011). Nevertheless, the mechanisms controlling priming are not fully
3 understood and consequently predicting the priming response in function of soil conditions and
4 of fresh organic matter inputs and composition is not straightforward (Kuzyakov et al. 2000,
5 Kuzyakov 2002, Fontaine et al. 2003, Guenet et al. 2010b). Priming is considered as the
6 consequence of different mechanisms such as N mining, interactions between different microbial
7 groups, co-metabolism, etc. (Kuzyakov et al. 2000, Fontaine et al. 2003, Blagodatskaya and
8 Kuzyakov 2008, Guenet et al. 2010a) but the balance between these mechanisms is not fully
9 understood. In particular, most of the priming studies were performed in laboratory conditions,
10 providing insight in the priming mechanism, but only limited quantitative information for model
11 parameterization and on the occurrence of priming in field conditions.

12 With climate change and increases in human land use, substantial increases in net primary
13 production and thus in soil carbon inputs are projected by coupled carbon cycle-climate models
14 (Qian et al. 2010). These enhanced soil carbon inputs are likely to strengthen the priming effect
15 and to render some currently inert soil carbon vulnerable to microbial decay (Fontaine et al.
16 2007). Therefore, projecting current SOM dynamics to estimate future climate-SOM feedbacks
17 can only be accurate if priming is implicitly or explicitly accounted for. However, only few
18 studies have focused on the dose-response relationship between priming intensity and the amount
19 of fresh organic matter inputs (Blagodatskaya and Kuzyakov 2008, Guenet et al. 2010b). It is
20 therefore crucial to better understand and to estimate relationship between priming intensity and
21 the amount of fresh organic matter inputs.

22 In this study, we applied a gradient in plant litter additions in a field experiment. The
23 objective was not to confirm the existence of priming at yet another terrestrial study site, but to

1 test whether or not the priming effect strengthens linearly with plant litter additions, to elucidate
2 the underlying mechanisms, and especially to study how the priming response could best be
3 approximated by Earth system models.

4

5 **Material and Method**

6 **Experimental facilities**

7 This study was conducted at a field site of the Duolun Restoration Ecology Experimentation and
8 Demonstration Station of the Institute of Botany, the Chinese Academy of Sciences, located in
9 the southeast of Inner Mongolia, northern China (Latitude 42°02'N, longitude 116°16'E,
10 elevation 1350 m a.s.l.). Long-term mean annual temperature at the site is 2.1°C, with monthly
11 mean temperature ranging from -17.5°C in January to 18.9°C in July. Mean annual precipitation
12 is about 380mm, with 90% of the precipitation falling in the growing season between May and
13 October.

14 Total precipitation during the experiment, between October 1st 2008 and September 30th
15 2009, was 219 mm, 75% of which (164 mm) fell between May 1st and September 30th 2009,
16 coinciding with the highest temperatures of the year. Mean annual air temperature in this period
17 was 3.5°C (Fig. S1). Soil temperature and soil water content (SWC) varied significantly with
18 time ($P < 0.001$; Fig. S2), but enhanced plant litter inputs did not affect them (Figure S2; $P >$
19 0.05), nor were there any significant interaction terms (between enhanced soil C inputs and time)
20 on soil temperature and SWC. Soil type was classified as chestnut soil (Chinese classification) or
21 Calcic Luvisols according to the FAO Classification (FAO 1974). Soils are composed of 63%
22 sand, 20% silt, and 17% clay (Niu et al. 2010), and concentration of soil organic C, N and P was
23 1.55%, 0.17% and 0.03%, respectively. The native vegetation was represented by typical steppe

1 communities, where *Stipa krylovii* Roshev., a perennial bunchgrass, dominates the vegetation.
2 Other common species include *Leymus chinensis* (Trin) Tzvel, *Cleistogenes squarrosa* (Trin.)
3 Keng, *Agropyron cristatum* (L.) Gaertner, *Artemisia frigid* Willd., *Potentilla acaulis* L., and
4 *Carex duriuscula* CA Mey. Total vegetation cover was relatively sparse, ranging from 85 to 90%.
5 Annual plant biomass production at the site was c.400g C m⁻² year⁻¹ (Li et al. 2004).

6 On October 1st 2008, we established twenty-five 2m ×1m treatment plots. Treatments
7 involved the addition of fresh organic matter to the soil, in the 10 – 20 cm, layer under a *S.*
8 *krylovii* steppe community, at rates equivalent to 0 (control treatment), 60, 120, 240 and 480 g C
9 m⁻², and all arranged as a complete randomized-block design with five replications. This
10 corresponds to increases in litter inputs of 15, 30, 60 and 120%, respectively. It must also be
11 noted that net primary production is assumed to increase between 10 and 60% at the end of the
12 21st century (Arora and Boer, 2014; Todd-Brown et al., 2014), and our selected treatments
13 covered this expected range quite well. The applied fresh organic matter consisted of senescent
14 above-ground tissues from an abundance-weighted mix of plant species occurring at the site. For
15 this purpose, senescent plant biomass was harvested from an adjacent field, air-dried,
16 fragmented, and passed through a sieve with 2-mm mesh size. This plant litter had a C
17 concentration of 40.08 % (standard error (SE) =0.13%, n=5), a N concentration of 0.972% (SE
18 =0.004%, n=5), a P concentration of 0.0768% (SE =0.001%, n=5), and a lignin concentration of
19 19.09% (SE =0.09 %, n=5).

20 Adding the plant litter to the uppermost soil layers was impossible without drastically
21 disturbing the soils. To minimize disturbance, we carefully removed the top 10-cm soil blocks,
22 containing 60% of the root system (Zhou et al. 2007), with a sharp spade, keeping the soil blocks
23 as intact as possible and keeping the vegetation intact. The soil underneath was loosened to a

1 depth of 20 cm, and a predetermined quantity of plant litter was mixed homogeneously with the
2 soil in the 10–20 cm layer. The surface soil blocks were then placed back into their original
3 positions. Remaining fissures between the soil blocks were carefully filled with soil from the 0–
4 10 cm soil layer and gently compacted by hand. To create consistent soil disturbance across
5 treatments, the plots with zero plant litter addition were processed in the same manner as the
6 plots that received plant litter.

7

8 **Soil respiration**

9 One PVC soil collar (8-cm diameter, 5-cm height) was inserted 3 cm into the soil at the center of
10 each measurement plot on October 1st 2008. All living aboveground plants were manually
11 removed inside the soil collars at least one day prior to the measurements to exclude
12 aboveground plant respiration from the measurements. Soil temperature at 20 cm depth was
13 measured concurrently with the LI-8100 portable soil CO₂ flux system, and soil water content
14 (SWC) at 20 cm depth was measured gravimetrically from the difference between fresh weight
15 and oven-dried weight of soil samples. Soil temperature and moisture were measured daily. Soil
16 respiration (R_s) was measured in each plot once in October and November 2008, and in January,
17 March, April and May 2009, and fortnightly from June to September 2009. We used a portable
18 soil CO₂ flux system (LI-8100; Li-Cor, Lincoln, NE, USA). During winter, when there was a
19 snow layer, the gas analyzer was kept in an isolated and heated container to avoid condensation.

20 Based on these measurements and considering that temperature changed between two
21 respiration measurement events, we estimated the annual soil CO₂ efflux respiration for the
22 period October 1st 2008 to September 30th 2009 by fitting the exponential Q₁₀function (Van't
23 Hoff 1899) to the data across all measurement dates, using the average of R_s in each treatment

1 per date:

$$2 \quad R_S = R_{S10^\circ\text{C}} Q_{10}^{((T-10)/10)}$$

3 where T is the soil temperature at 10 cm depth ($^\circ\text{C}$), and $R_{S10^\circ\text{C}}$ (the basal rate of R_S) and
4 Q_{10} (the increase in R_S for every 10°C increase in temperature) are fitted from regression
5 analyses. R_S was then calculated for each temperature measured and averaged on an annual
6 basis.

7 The temporal correlation between SWC and soil temperature ensured very good fits in all
8 treatments between the temperature-only driven soil respiration models and the measured fluxes
9 (Fig. S3), increasing our confidence in the interpolated total annual soil respiration.

10

11 **Root production**

12 Root (≤ 2 mm) production during the growing season was estimated with a modified in-growth
13 core technique (Lund et al. 1970). One hole (5 cm diameter, 20 cm depth) was created in each
14 plot and refilled early Oct 1st2008 with native, root-free soil. The boundaries of the in-growth
15 cores were marked with sticks. The in-growth cores were harvested on September 30th 2009, and
16 all live and dead roots were manually removed from the soil samples and washed. Total root
17 production was estimated as the sum of live and dead roots present in the in-growth core in
18 September 30th 2009.

19

20 **Stocks and decomposition of native and added organic matter**

21 Organic carbon was measured on one sample each of the 0–10 cm and the 10–20 cm layers of
22 each plot, collected on October 1st 2008 (before adding fresh plant litter) and September 30th
23 2009 (after one year) with a soil sampler of 3 cm inner diameter. In the lab, all live and dead

1 roots were manually removed and samples were acidified with HCl (2 M) for 24 hour to remove
2 all inorganic C. Then the (organic) C content of the soil samples was measured using an
3 automatic elemental analyzer Vario EL III (Elementar Analysensysteme Comp., Hanau,
4 Germany). Additionally, soil bulk densities of the 0-10 cm and the 10-20 cm layers of each plot
5 were determined concurrently with soil sampling by a special coring device for the determination
6 of bulk density (volume = 100.0 ml).

7 Quantification of priming effects on decomposition of native soil organic matter require that
8 the latter can be measured, which is unfortunately not possible in situ in the presence of
9 simultaneous litter inputs, exudation and respiration by vegetation. However, it can be
10 approximated by using isotopic techniques adapted from Balesdent et al. (1987) which was
11 already used successfully used for field studies (Van Kessel et al. 2000, Veldkamp et al. 2003,
12 Gielen et al. 2005). Therefore, in our study plots we introduced cores filled with soil originating
13 from C₄ vegetation. This allowed us to determine how much of the added plant litter and of the
14 natural vegetation litter inputs remained in the soil after one year.

15 In practice, at the time of addition of the plant litter to all plots on October 1st 2008, two soil
16 cores sampled under C₄ vegetation per plot were installed in cylindrical nets (diameter of 4 cm,
17 depth of 20 cm, mesh of 2 mm). We used soil that had been under maize cultivation for 18 years,
18 but with white sand mixed in to lower its nutrient content as well as to obtain a more similar
19 texture to that in the surrounding soil. The texture of the soil was 63.4±0.2% sand, 19.5±0.2%
20 silt, and 17.1±0.1% clay. The δ¹³C signature of the C₄ soil cores after acidification to remove
21 carbonates was -20.93‰ (SE = 0.09‰, n = 5); its organic C concentration was 1.54% (SE =
22 0.01%, n = 5, compared to 1.55% in the native C₃ soil); the N concentration in the C₄ cores was
23 0.18% (SE = 0.01%, n = 5, compared to 0.17% in the native C₃ soil); last also the P

1 concentration was very similar: 0.030% (SE = 0.0004%, n = 5, compared to 0.029% in the native
 2 C₃ soil). Each plot had two C₄-soil cores, one with plant litter added in exactly the same amounts
 3 as the plot it was installed in, and one without plant litter added (a control). This technique
 4 allowed us to estimate litter decomposition and use it as a surrogate for the litter decomposition
 5 in the plots with C₃ soil (see below for details). All soil cores were taken to the lab and acidified
 6 to remove carbonates as described above. Total soil organic carbon and its δ¹³C were measured
 7 by using an automatic elemental analyzer Vario EL III (Elementar Analysensysteme Comp.,
 8 Hanau, Germany) and Delta plus XP (Thermo Finnigan, MAT DELTA plus XP, Germany). The
 9 fraction of new SOC in the soil derived from root production was calculated with a simple
 10 mixing model (Balesdent et al. 1987) in the control plot without any litter additions (see below
 11 for details). The δ¹³C content of root litter and added plant litter were highly similar: -28.20‰
 12 (SE = 0.11‰, n = 5) and -28.16‰ (SE = 0.10‰, n=5), respectively. Finally, we estimated the
 13 native C-SOM decomposition using equations (1) to (4):

14 (1) $TOC_{Final} = SOC_{Final} + C_{Litter} + C_{Root}$

15 Where TOC_{Final} is the total organic carbon content at the end of the experiment (without
 16 visible live or dead roots), C_{Litter} the remaining C from the added litter, C_{Root} the remaining C
 17 from root in-growth and SOC_{Final} the remaining soil organic carbon from the native soil at the
 18 end of the experiment.

19 From equation (1) we obtained equation (2):

20 (2) $SOC_{Final} = TOC_{Final} - C_{Litter} - C_{Root}$

21 The term TOC_{Final} is measured at the end of the experiment. In the control treatment (no
 22 litter added), the term C_{Litter} is null and we can therefore estimate the term C_{Root} using the
 23 difference in the δ¹³C between the C₃ grasses and the C₄ soil using equation (3).

1 (3)
$$\delta^{13}C_{Final} \times C_{Root} = \delta^{13}C_{NativeSOC} \times (TOC_{Final} - C_{Root}) + \delta^{13}C_{Root} \times C_{Root}$$

2 where $\delta^{13}C_{Final}$ is the $\delta^{13}C$ of the total organic content at the end of the experiment, $\delta^{13}C_{Root}$
 3 is the $\delta^{13}C$ of the roots produced during the experiment and $\delta^{13}C_{NativeSOC}$ the $\delta^{13}C$ of the C₄ soil
 4 organic carbon before the experiment. The term SOC_{Final} is substituted by $(TOC - C_{Root})$ following
 5 equation (2) without litter amount. Thus, we obtained equation 3'

6 (3')
$$C_{Root} = \frac{\delta^{13}C_{Final} \times TOC_{Final} - \delta^{13}C_{NativeSOC} \times TOC_{Final}}{\delta^{13}C_{Root} - \delta^{13}C_{NativeSOC}}$$

7 Extrapolating root production in the C₄ soil cores to the C₃ soil hinges on the assumption
 8 that no differences in root growth occurred. Because we observed only small differences in root
 9 production across all sampled plots (different litter addition), we assume that root production was
 10 similar in all plots, including the C₄ plots (Table S1).

11 Then the C_{Litter} term of equation (2) was estimated using equation (4), based on the
 12 difference in $\delta^{13}C$ between C₃-Litter and the soil originally cover by C₄ vegetation.

13 (4)
$$\delta^{13}C_{Litter} \times C_{Litter} = \delta^{13}C_{Final} \times TOC_{Final} - \delta^{13}C_{NativeSOC} \times (SOC_{Final} - C_{Root}) + \delta^{13}C_{Root} \times C_{Root}$$

14 where $\delta^{13}C_{Litter}$ is the $\delta^{13}C$ of the litter added. The term SOC_{Final} is substituted by $(TOC -$
 15 $C_{Litter} - C_{Root})$ following equation (2). Thus, we obtained equation 4'

16 (4')
$$C_{Litter} = \frac{(\delta^{13}C_{Final} - \delta^{13}C_{NativeSOC}) \times TOC_{Final} + (\delta^{13}C_{NativeSOC} - \delta^{13}C_{Root}) \times C_{Root}}{\delta^{13}C_{Litter} - \delta^{13}C_{NativeSOC}}$$

17 This approach implicitly assumes that litter mineralization rates were the same in the C₃ and
 18 in the C₄ soil. The main driving factors of controlling the microbial community structure,
 19 biomass and activity and in fine the mineralization are temperature, moisture, nutrients
 20 availability and interaction with soil texture. Soil temperature was equivalent in both soil as well
 21 as soil moisture (Fig. S2). Moreover, we adjusted the SOM, N and P content of the C₄ soil (by

1 diluting with sand) to the values measured in the C₃ soil. Hence, nutrient availability was
2 equivalent in both soils. The textures of the two soils were also quite similar, inducing similar
3 interaction with clay. Microbial biomass, microbial community structure and microbial activity
4 were measured (see details below) in both soils and no significant differences were observed
5 between C3 and C4 soils at any treatment (Table S1). Finally, isotopic method used here to
6 evaluate the SOC formed from litter decomposition was evaluated using litterbags methods (see
7 details below) and no effect of the method was detected by the Duncan test at any treatment (Fig.
8 1). Given the high similarities between the soils for the main driving factors controlling the
9 mineralization we are convinced that the relative patterns reported below are robust.

10 Finally, using equation (3') and (4') in equation (2) we were able to estimate the final SOC
11 content of each treatment and calculate priming effect using equation (5)

12 (5) Priming effect = $(SOC_{Final} - SOC_{Initial})_{Treatment} - (SOC_{Final} - SOC_{Initial})_{Control}$

13

14 **Decomposition of added organic matter**

15 To check the estimation of added litter decomposition using isotopic techniques, we also
16 determined the decomposition of added litter using the litterbag method (Arunachalam et al.,
17 1996, Xiao et al., 2010). Each litterbag had a dimension of 10 cm×10 cm and a mesh of 48µm.
18 1.5, 3, 6 and 12 g dry mass of litter were placed in litterbag corresponding to plant litter amount
19 of 60, 120, 240 and 480 g C m⁻². Then, litterbags were heat-sealed and placed in the 10-20 cm
20 soil layer on October 1st 2008, respectively. All litterbags were collected on September 30th 2009.
21 The non decomposed litter was oven-dried at 65°C and C content of the added organic matter
22 samples was measured using an automatic elemental analyzer Vario EL III (Elementar
23 Analysensysteme Comp., Hanau, Germany).

1

2 **Soil microbial biomass, community composition and microbial respiration**

3 To better understand how soil microorganisms were affected by the litter additions we measured
4 total microbial biomass, relative contents of phospholipid fatty acids (PLFAs) and microbial
5 respiration in optimal conditions. Soil samples were collected from three random locations in
6 each plot for determination of microbial biomass carbon and microbial activity. These soil
7 samples were taken on September 30th 2009 with a cylindrical soil sampler (3 cm inner diameter)
8 from the 0-20 cm layers, and stored in a cooler for transportation to the laboratory for further
9 analysis. There, fresh samples were processed with a 2-mm sieve and manually cleaned of any
10 visible plant tissues. The three samples from each plot were pooled and treated as a single sample
11 for analysis.

12 Soil microbial biomass carbon was measured using the fumigation–extraction method
13 (Vance et al., 1987). Fresh soil samples were carefully adjusted to 60% of water-holding capacity
14 in the dark and incubated for one week at 25°C. After fumigation, 20.0 grams (dry weight
15 equivalent) of fumigated and non-fumigated soil samples were extracted with 0.5 M K₂SO₄. Soil
16 microbial biomass carbon was calculated as the difference in extractable carbon of fumigated and
17 non-fumigated soil samples. To correct for incomplete extractability, a conversion factor of 0.38
18 was used to estimate microbial biomass carbon (Lovell et al. 1995).

19 Soil microbial community structure was assessed by measuring relative contents of PLFAs
20 (Bardgett et al. 1999; Fierer et al. 2003). PLFAs were extracted and quantified from 8.0 g (dry
21 weight equivalent) of soil using a procedure described by Bossio and Scow (1998). The
22 separation and identification of extracted PLFAs were carried out according to the standard
23 protocol of the Sherlock Microbial Identification System V_{4.5} (MIDI), using a Gas

1 Chromatograph (Hewlett Packard 6850, USA). Fatty acid nomenclature used in this study was as
2 defined by Bossio and Scow (1998). The fatty acids 14:00, 15:00, i15:0, a15:0, 16:00, i16:1 G,
3 i16:0, 16:1 2OH, 16:1 ω 9c, 16:1 ω 7c, 16:1 ω 5c, i17:0, a17:0, cy17:0, 18:1 ω 5c were chosen to
4 represent the PLFAs of the bacterial group (Frostegård et al. 1993, Zhang et al. 2009). PLFAs
5 18:3 ω 6c and 18: 2 ω 6. 9c were chosen to represent the fungal group (Zhang et al. 2005, 2009).
6 Data from the PLFAs was presented as the percentage of the total PLFAs detected within a
7 sample.

8 Soil microbial activity was estimated by determining CO₂ evolution over a two-week
9 incubation period. First, 20.0 g (dry weight equivalent) of soil was brought to 60% of the water
10 holding capacity and incubated at 25°C for two weeks. Respired CO₂ was captured in 5.0 ml of
11 0.5 M NaOH suspended inside a Mason jar (Hu and Van Bruggen, 1997), and the NaOH solution
12 was subsequently titrated to determine the amount of CO₂ evolved.

13

14 **Statistical analysis**

15 Data management and statistical analyses were performed using the SPSS software package
16 (SPSS, Chicago, IL, USA). Two-way ANOVA was used to detect the effects of plant litter
17 addition and sample time on soil temperature and SWC. One-way analysis of variance was used
18 to detect the effects of plant litter addition on root production, soil microbial biomass C, soil
19 microbial activity, ratio of fungal to bacterial biomass, annual soil respiration, decomposition of
20 native C and of added plant litter, R_{S10°C} and Q₁₀. Multiple comparisons were also performed to
21 permit separation of effect means using the Duncan test at a significance level of P < 0.05. We
22 used a nonlinear least square fitting to determine the temperature response of R_S.

23 The corrected Akaike Information Criterion (cAIC) was used to determine which function

1 was best suited to fit the priming-microbial biomass relationship:

$$2 \quad (6) \quad cAIC = n \times \ln\left(\frac{RSS}{n}\right) + 2 \times k + \frac{2 \times k \times (k+1)}{n-k-1}$$

3 where n = number of samples, k = number of parameters in the functions, and RSS =
4 residual sum of squares, calculated as:

$$5 \quad (7) \quad RSS = \sum_i (y_i - f(x_i))^2$$

6 where y_i is the observed respiration and $f(x_i)$ is the respiration predicted by the models.

7 The corrected Akaike Information Criterion is to be preferred when n is small, as in our
8 study with only 5 replicates and 5 different treatments. We found that linear functions were best
9 suited. Therefore, linear regressions were used to relate plant litter inputs to annual soil
10 respiration, and priming of soil organic matter decomposition to microbial biomass C.

11

12 **Results**

13 Our methods to estimate priming in our experiment were based on two main assumptions: that
14 root production and litter decomposition were equal in the C3 and C4 soils. Root production was
15 easily measured in both soils using the modified in-growth core technique (Lund et al. 1970).
16 Table S1 clearly shows that the assumption of equal root growth was respected, since no effect of
17 soil on root production was observed. Furthermore, we modified the C4 soil to obtain
18 characteristics as close as possible to the original C3 soil. As a consequence, soil microbial
19 biomass, activity, as well as the microbial community structure were not significantly different
20 between the C3 and C4 soil (Table S1). Although we did not measure litter decomposition
21 directly, the fact that these three variables did not differ between the different soil types made us
22 confident that litter decomposition also was equivalent in C3 and C4 soils. We therefore consider
23 that the two important assumptions underlying our method used to estimate priming were valid.

1 In addition to the support for the basic assumptions, we also compared the calculated litter
2 decomposition rates using the isotopic method with those calculated with the more traditional
3 litterbag method. Both methods give similar results (Fig. 1), with no effect of the method used on
4 the decomposition rates estimated for any treatment.

5 Higher plant litter inputs clearly resulted in larger microbial biomass and stimulated
6 microbial activity (Fig. 2a, 2b). At higher litter inputs, fungal phospholipid fatty acids (PLFAs)
7 increased relative to bacterial PLFAs (Fig. 2c), which was primarily attributable to increased
8 fungal abundance; bacterial PLFAs were only marginally altered (Table S1).

9 Total annual soil respiration increased substantially (at $P < 0.05$) and linearly with
10 increasing plant litter additions (Fig. 3a). Across all treatments, roughly half of the added litter
11 was lost during the first year following addition. Remarkably, however, the fraction of the added
12 plant litter that was decomposed within one year increased with higher amounts of added plant
13 litter (from 0.42 to 0.57; see the arrows in Fig. 3a). With increasing litter inputs, plant litter thus
14 progressively became the preferred substrate for microbial decay (Fig. 4).

15 As plant litter inputs increased, also mineralization of SOM was enhanced; i.e. plant litter
16 additions primed the decomposition of SOM (Fig. 3b). Adding 480 g C m^{-2} of plant litter primed
17 SOM decomposition by 18% (extra mineralization of $67 \text{ g SOM-C m}^{-2} \text{ year}^{-1}$), compared to the
18 control without additional litter inputs. However, the priming efficiency of plant litter additions
19 strongly declined with increasing amounts of litter inputs (see arrows in Fig. 3b). Whereas an
20 extra litter input of 60 g C m^{-2} in the lowest treatment primed the decomposition of native SOM
21 by $24 \text{ g C m}^{-2} \text{ year}^{-1}$ (a priming response equivalent to 40% of the added C), an input of 480 g C
22 m^{-2} litter inputs induced an increase of $67 \text{ g C m}^{-2} \text{ year}^{-1}$ in SOM decomposition (or a priming
23 response equivalent to 14% of the additional litter C inputs). These results suggest the absence of

1 simple linear relationship between priming effect and litter inputs. Nonetheless, our study did
2 show that across a large gradient in litter addition, priming was linearly correlated with total
3 microbial biomass (Fig. 5, Table S2).

4

5 **Discussion**

6 To our knowledge, priming responses to a gradient in plant inputs were previously studied
7 only in a laboratory soil incubation experiment in which the effects of different amounts and
8 different chemical compositions of plant litter additions on SOM decomposition were studied
9 (Guenet et al. 2010b). Despite being a laboratory soil incubation experiment and not a field
10 study, these authors reported strikingly similar response patterns to those observed here, i.e. a
11 declining efficiency of the priming effect at higher litter inputs.

12 Several processes have been proposed to underlay the priming mechanism (Kuzyakov 2002,
13 Guenet et al. 2010b), but the most commonly accepted hypothesis states that the input of easily
14 degradable substrates (high energy yield per unit energy/nutrients invested) provides microbes
15 with the energy required to sustain more active biomass, whose enhanced production of extra-
16 cellular enzymes not only degrades more of the fresh plant inputs but also part of the already
17 present, less-energy (but more nitrogen) yielding SOM (Broadbent 1948, Bingeman et al. 1953,
18 Wu et al. 1993, Kuzyakov et al. 2000, Fontaine et al. 2003, Blagodatskaya and Kuzyakov 2008).
19 In agreement with this hypothesis, the priming responses observed here were associated with
20 enhanced microbial biomass and activity at higher plant litter inputs. However, this hypothesis
21 alone fails to explain the absence of a linear relationship between litter inputs and priming effect
22 intensity (Fig. 3b), for which other mechanisms must come into play.

23 In addition to the stimulation of microbial biomass, microbial activity and priming of SOM

1 decomposition, we also found that plant litter progressively became the preferred microbial
2 substrate. This suggests that, as litter inputs increased, a growing fraction of the microbial
3 biomass used litter as growth substrate instead of native SOM, thereby partly offsetting the effect
4 of the higher microbial activity on priming. Combined, our observations thus suggest an
5 important role for two antagonistic mechanisms in the priming effect, occurring together and
6 explaining the absence of a linear response of priming to the litter input amounts. The first being
7 an increase in microbial growth and in synthesis of extra-cellular enzymes, increasing the
8 mineralization rate of both fresh plant litter and native SOM. The second mechanism being a
9 change in substrate preference by the decomposer organisms (or at least part thereof), from SOM
10 towards amended litter. This second mechanisms increased the decay of fresh plant litter, but not
11 of SOM, thereby reducing the priming effect on SOM decomposition. Our results indicate that
12 the second mechanism becomes more important at higher litter inputs, explaining the non-
13 linearity of the priming response to litter additions.

14 The relative shift in substrate use from SOM to plant litter coincided with a relative shift in
15 microbial community composition. At higher litter inputs, fungal phospholipid fatty acids
16 (PLFAs) increased, while bacterial PLFA's were not altered. Our study thus suggests that fungi
17 were the organisms responsible for the observed non-linearity in the priming response and may
18 therefore be considered as main actors in the priming response, as previously suggested (Carney
19 et al. 2007). However, it must be noted that our results were obtained by adding plant litter and
20 that we ignored the priming effect due to root exudation. Moreover, to avoid differential
21 treatment impacts on vegetation by the different amounts of litter added, as well as to avoid
22 issues with horizontal litter losses (e.g. through wind), we added the litter at 10cm depth. We
23 realize that this makes our results not directly extrapolatable to natural systems, where an

1 important fraction of annual litter inputs occurs aboveground. Adding the litter below-ground
2 may have increased the priming effect because the litter surface accessible to the microorganisms
3 is higher than that of litter deposited on the surface. However, we still believe that the main
4 mechanism we report is still valid: that the priming response scales linearly with the microbial
5 response. More studies are therefore still needed to determine whether the relationship between
6 the priming effect and the amount of root exudates is the same as the relationship obtained here
7 with plant litter inputs.

8

9 **Conclusions**

10 In a future warmer and CO₂-enriched world, global plant productivity is expected to
11 increase (Qian et al. 2010) and the associated priming could destabilize organic matter that is
12 currently protected from decomposition (Fontaine et al. 2007), thereby accelerating the increase
13 in atmospheric CO₂ concentrations. Earth system models thus need to incorporate the priming
14 effect on SOM decomposition if they are to accurately simulate trajectories of soil respiration
15 and SOM stocks in response to future changes in climate and plant productivity, as well as their
16 feedbacks to the climate system. However, the shifts in microbial community structure and in
17 substrate preference are unlikely to become embedded in such large scale models in the near
18 future.

19 Nonetheless, our study also showed that across a large gradient in litter addition, priming
20 was linearly correlated with total microbial biomass. The priming response of SOM
21 decomposition to future changes in litter inputs could thus be best incorporated in Earth System
22 model structures by explicit representation of microbial biomass, improving their simulations of
23 SOM decomposition under conditions not currently encountered. Because microbial biomass

1 carbon is easily measured and ample data are available from field studies and climate
2 manipulation experiments, a wealth of model validation data is already available and waiting to
3 be exploited. Predicting microbial biomass mechanistically would involve representation of
4 microbial community structures and substrate preferences, and these detailed microbial processes
5 are currently far beyond the remit of these models.

6 A more straightforward representation of the priming mechanism in Earth System models
7 could involve the introduction of a direct control of litter inputs on native SOM mineralization,
8 without explicit representation of microbial biomass. This could be achieved as a simple addition
9 to the classical scheme already used by Earth system models, in which SOM mineralization is
10 controlled only by the amount of SOM present, temperature and soil moisture (Friedlingstein et
11 al. 2006). Nevertheless, this simple yet purely empirical approach is currently hampered by lack
12 of data linking priming intensities to litter inputs. More litter-gradient studies in different biomes
13 are needed to assess the potential of the latter approach to embed the priming mechanism in
14 Earth System models.

15

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5

1 **References**

- 2 Arora, V. K. and Boer, G. J. 2014. Terrestrial ecosystems response to future changes in climate
3 and atmospheric CO₂ concentration. – *Biogeosciences Discussions* 11: 3581–3614.
- 4 Arunachalam, A. et al. 1996. Fine root decomposition and nutrient mineralization patterns in a
5 subtropical humid forest following tree cutting. – *Forest Ecol. Manag.* 86: 141–150.
- 6 Balesdent, J. et al. 1987. Natural C⁻¹³ abundance as a tracer for studies of soil organic matter
7 dynamics. – *Soil Biol. Biochem.* 19: 25–30.
- 8 Bardgett, R. D. et al. 1999. Below-ground microbial community development in a high
9 temperature world. – *Oikos* 85: 193–203.
- 10 Bianchi, T. S. 2011. The role of terrestrially derived organic carbon in the coastal ocean: A
11 changing paradigm and the priming effect. – *P. Natl. Acad. Sci USA* 108: 19473–19481.
- 12 Bingeman, C. W. et al. 1953. The effect of the addition of organic materials on the
13 decomposition of an organic soil. – *Soil Sci. Soc. Am. J.* 17: 34–38.
- 14 Blagodatskaya, E. and Kuzyakov, Y. 2008. Mechanisms of real and apparent priming effects and
15 their dependence on soil microbial biomass and community structure: critical review. – *Biol.*
16 *Fert. Soils* 45: 115–131.
- 17 Bossio, D. and Scow, K. 1998. Impacts of carbon and flooding on soil microbial communities:
18 phospholipid fatty acid profiles and substrate utilization patterns. – *Microb. Ecol.* 35: 265–
19 278.
- 20 Broadbent, F. 1948. Nitrogen release and carbon loss from soil organic matter during
21 decomposition of added plant residues. – *Soil Sci. Soc. Am. J.* 12: 246–249.
- 22 Carney K.M. et al. 2007. Altered soil microbial community at elevated CO₂ leads to loss of soil
23 carbon. – *P. Natl. Acad. Sci. USA* 104: 4990–4995.

- 1 Craine, J.M. et al. 2010. Widespread coupling between the rate and temperature sensitivity of
2 organic matter decay. – *Nat. Geosci.* 3: 854–857.
- 3 Davidson, E.A. and Janssens, I.A. 2006. Temperature sensitivity of soil carbon decomposition
4 and feedbacks to climate change. – *Nature* 440: 165–173.
- 5 FAO. 1998. World reference base for soil resources (World Soil Resources report no. 84). – FAO,
6 Rome.
- 7 Fierer, N. et al. 2003. Variations in microbial community composition through two soil depth
8 profiles. – *Soil Biol. Biochem.* 35: 167–176.
- 9 Fontaine, S. et al. 2003. The priming effect of organic matter: a question of microbial
10 competition? – *Soil Biol. Biochem.* 35: 837–843.
- 11 Fontaine, S. et al. 2004. Carbon input to soil may decrease soil carbon content. – *Ecol. Lett.* 7:
12 314–320.
- 13 Fontaine, S. et al. 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon
14 supply. – *Nature* 450: 277–280.
- 15 Friedlingstein, P. et al. 2006. Climate-carbon cycle feedback analysis: Results from the C4MIP
16 model intercomparison. – *J. Climate* 19: 3337–3353.
- 17 Frostegård, Å. and Bååth, E. 1996. The use of phospholipid fatty acid analysis to estimate
18 bacterial and fungal biomass in soil. – *Biol. Fert. Soils* 22: 59–65.
- 19 Frostegård, Å. et al. 1993. Phospholipid fatty acid composition, biomass, and activity of
20 microbial communities from two soil types experimentally exposed to different heavy
21 metals. – *Appl. Environ. Microb.* 59: 3605–3617.
- 22 Gielen, B. et al. 2005. Net carbon storage in a poplar plantation (POPFACE) after three years of
23 free-air CO₂ enrichment. – *Tree Physiol.* 25: 1399–1408.

- 1 Guenet, B. et al. 2010a. Priming effect: bridging the gap between terrestrial and aquatic ecology.
2 – Ecology 91: 2850–2861.
- 3 Guenet, B. et al. 2010b. Is there a linear relationship between priming effect intensity and the
4 amount of organic matter input? – Appl. Soil Ecol. 46: 436–442.
- 5 Hanson, P. et al. 2000. Separating root and soil microbial contributions to soil respiration: a
6 review of methods and observations. – Biogeochemistry 48: 115–146.
- 7 Hu, S. and Van Bruggen, A. 1997. Microbial dynamics associated with multiphasic
8 decomposition of ¹⁴C-labeled cellulose in soil. – Microb. Ecol. 33: 134–143.
- 9 Jenkinson, D. et al. 1991. Model estimates of CO₂ emissions from soil in response to global
10 warming. – Nature 351: 304–306.
- 11 Jones, C. D. et al. 2003. Uncertainty in climate–carbon-cycle projections associated with the
12 sensitivity of soil respiration to temperature. – Tellus B 55: 642–648.
- 13 Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. – J. Plant Nutr. Soil
14 Sc. 165: 382–396.
- 15 Kuzyakov, Y. et al. 2000. Review of mechanisms and quantification of priming effects. – Soil
16 Biol. Biochem. 32: 1485–1498.
- 17 Li, L. H. et al. 2004. Soil carbon budget of a grazed *Leymus chinensis* steppe community in the
18 Xilin River Basin of Inner Mongolia. – Acta Phytoecol. Sin. 28: 312–317. (In Chinese)
- 19 Lovell, R. et al. 1995. Soil microbial biomass and activity in long-term grassland: effects of
20 management changes. – Soil Biol. Biochem. 27: 969–975.
- 21 Lund, Z. F et al. 1970. An implanted soil mass technique to study herbicide effects on root
22 growth. – Weed Sci. 18: 279–281.
- 23 Niu, S. L. et al. 2010. Nitrogen effects on net ecosystem carbon exchange in a temperate steppe.

- 1 – Global Change Biol. 16: 144–155.
- 2 Qian, H. et al. 2010. Enhanced terrestrial carbon uptake in the northern high latitudes in the 21st
3 century from the Coupled Carbon Cycle Climate Model Intercomparison Project model
4 projections. – Global Change Biol. 16: 641–656.
- 5 Schlesinger, W.H. and Andrews, J.A. 2000. Soil respiration and the global carbon cycle. –
6 Biogeochemistry 48: 7–20.
- 7 Schmidt, M. W. I. et al. 2011. Persistence of soil organic matter as an ecosystem property. –
8 Nature, 478: 49–56.
- 9 Subke, J. A. and Bahn, M. 2010. On the ‘temperature sensitivity’ of soil respiration: Can we use
10 the immeasurable to predict the unknown? – Soil Biol. Biochem. 42: 1653–1656.
- 11 Todd-Brown, K. E. O. et al. 2014. Changes in soil organic carbon storage predicted by Earth
12 system models during the 21st century. – Biogeosciences, 11: 2341–2356
- 13 Van Kessel, C. et al. 2000. Net soil carbon input under ambient and elevated CO₂ concentrations:
14 isotopic evidence after 4 years. – Global Change Biol. 6: 435–444.
- 15 Vance, E. et al. 1987. An extraction method for measuring soil microbial biomass C. – Soil Biol.
16 Biochem. 19: 703–707.
- 17 Van't Hoff, J. 1899. Chemical dynamics. In: Lectures on theoretical and physical chemistry. –
18 Edward. Arnold, London, UK.
- 19 Veldkamp, E. et al. 2003. Substantial labile carbon stocks and microbial activity in deeply
20 weathered soils below a tropical wet forest. – Global Change Biol. 9: 1171–1184.
- 21 Wu, J. et al. 1993. Formation and destruction of microbial biomass during the decomposition of
22 glucose and ryegrass in soil. – Soil Biol. Biochem. 25: 1435–1441.
- 23 Xiao, C. W. et al. 2010. Belowground carbon pools and dynamics in China’s warm temperate

1 and sub-tropical deciduous forests. – *Biogeosciences* 7: 275–287.

2 Zhang, Q. et al. 2009. The diversity of phospholipid fatty acid (PLFA) biomarker for the

3 microbial community in soil. – *Acta Ecol. Sin.* 29: 4127–4137.

4 Zhang, W. 2005. Soil microbial responses to experimental warming and clipping in a tallgrass

5 prairie. – *Global Change Biol.* 11: 266–277.

6 Zhou, Z. et al. 2007. Soil carbon and nitrogen stores and storage potential as affected by land-use

7 in an agro-pastoral ecotone of northern China. – *Biogeochemistry* 82: 127–138.

8

1 **Captions to figures**

2 **Figure 1.** Decomposition of added plant litter measured by litterbag and isotopic methods under
3 different amounts of added plant litter in a steppe community of northern China. Vertical bars
4 indicate one standard error about the mean (n=5). Ns represents no significant difference in
5 decomposition of added plant litter measured by between litterbag and isotopic methods.

6 **Figure 2.** Soil microbial biomass C (a), soil microbial activity (b) and ratio of fungal to bacterial
7 biomass (c) for different amounts of added plant litter in a steppe community of northern China.
8 Error bars indicate one standard error about the mean (n=5). Different letters indicate statistically
9 significant differences ($P < 0.05$) according to the Duncan test. The ratio of fungal to bacterial
10 biomass was computed from PLFA measurements as explained in the methods section.

11

12 **Figure 3.** Impact of different plant litter inputs on soil respiration and decomposition fluxes in a
13 steppe community of northern China. Error bars indicate one standard error about the mean
14 (n=5). Panel a: annual soil respiration (solid line) and decomposition of the added plant litter
15 (columns). R^2_{adj} is the adjusted coefficient of determination. The arrows in between the columns
16 indicate the decomposed fraction of the litter that was added on top of that in the previous
17 treatment (e.g. the value next to the arrow between the 240 and 480 g C treatments (0.57 g g^{-1})
18 reflects the difference in added litter decomposition between both treatments (i.e. $136 \text{ g C m}^{-2} \text{ y}^{-1}$)
19 divided by the difference in litter addition (i.e. $240 \text{ g C m}^{-2} \text{ y}^{-1}$). Panel b: Decomposition of
20 native soil organic matter at different litter inputs. Numbers inside the bars represent the increase
21 in decomposition rate of the native soil organic matter relative to the control (i.e. priming).
22 Numbers alongside the arrows represent the efficiency of the priming effect, i.e. the increase in
23 decomposition of native soil organic matter per unit plant litter added. Although the priming

1 effect continues to increase with litter additions in absolute numbers, the efficiency declines
2 sharply.

3

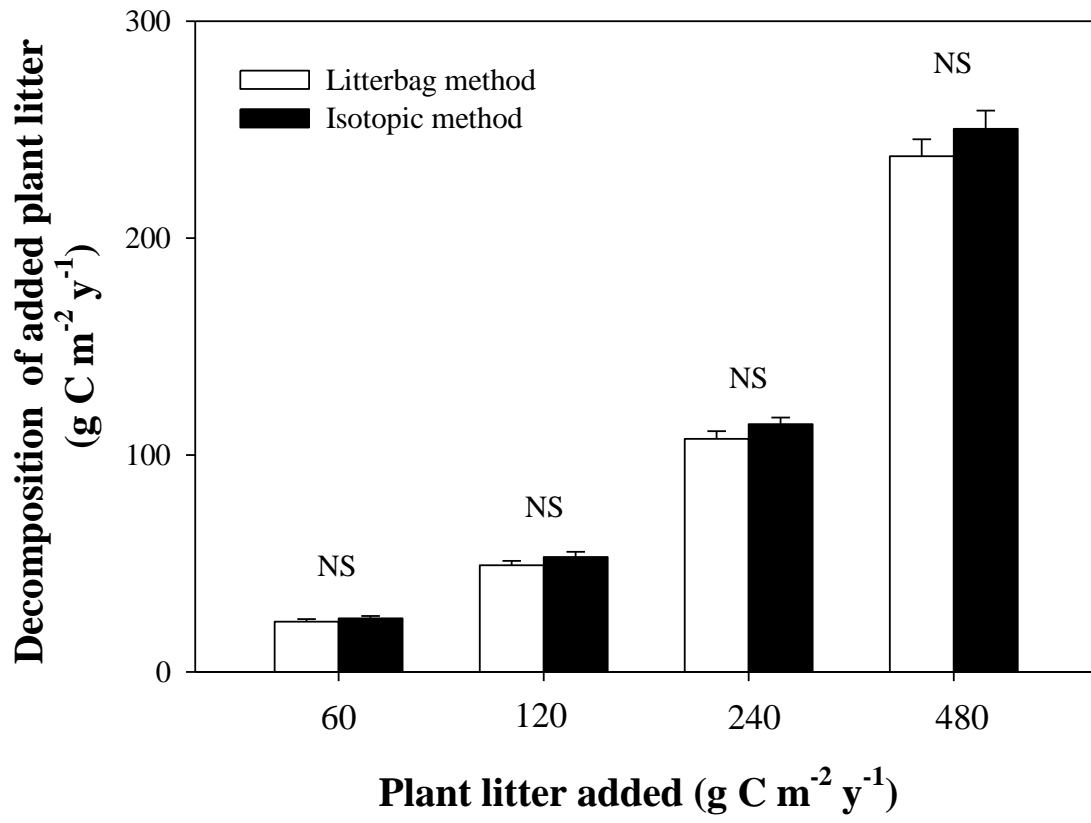
4 **Figure 4.** Relative contribution of substrates (soil organic matter (priming) and added plant
5 litter) to the increases in heterotrophic respiration between different amounts of added plant litter.
6 The increase in heterotrophic respiration with litter inputs in the low treatments was supported
7 for 50% by both priming and decomposition of the added litter. In contrast, the increase in
8 heterotrophic respiration with additional litter inputs in the higher treatments was supported for
9 almost 90% by decomposition of the added litter, indicating a relative shift in substrate use.

10

11 **Figure 5.** Priming of soil organic matter decomposition as a function of microbial biomass in a
12 Mongolian steppe ecosystem.

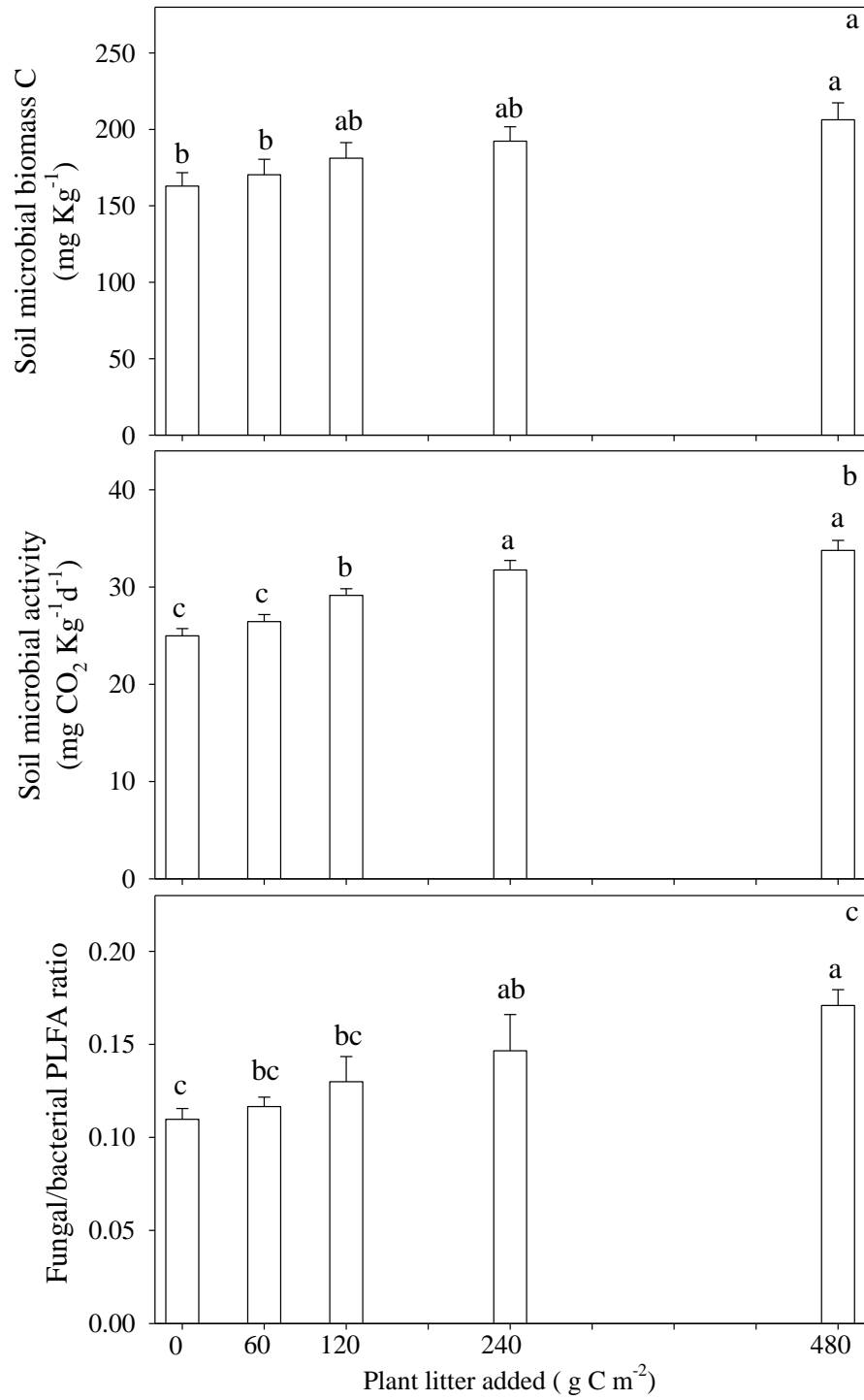
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1 **Figure 1**



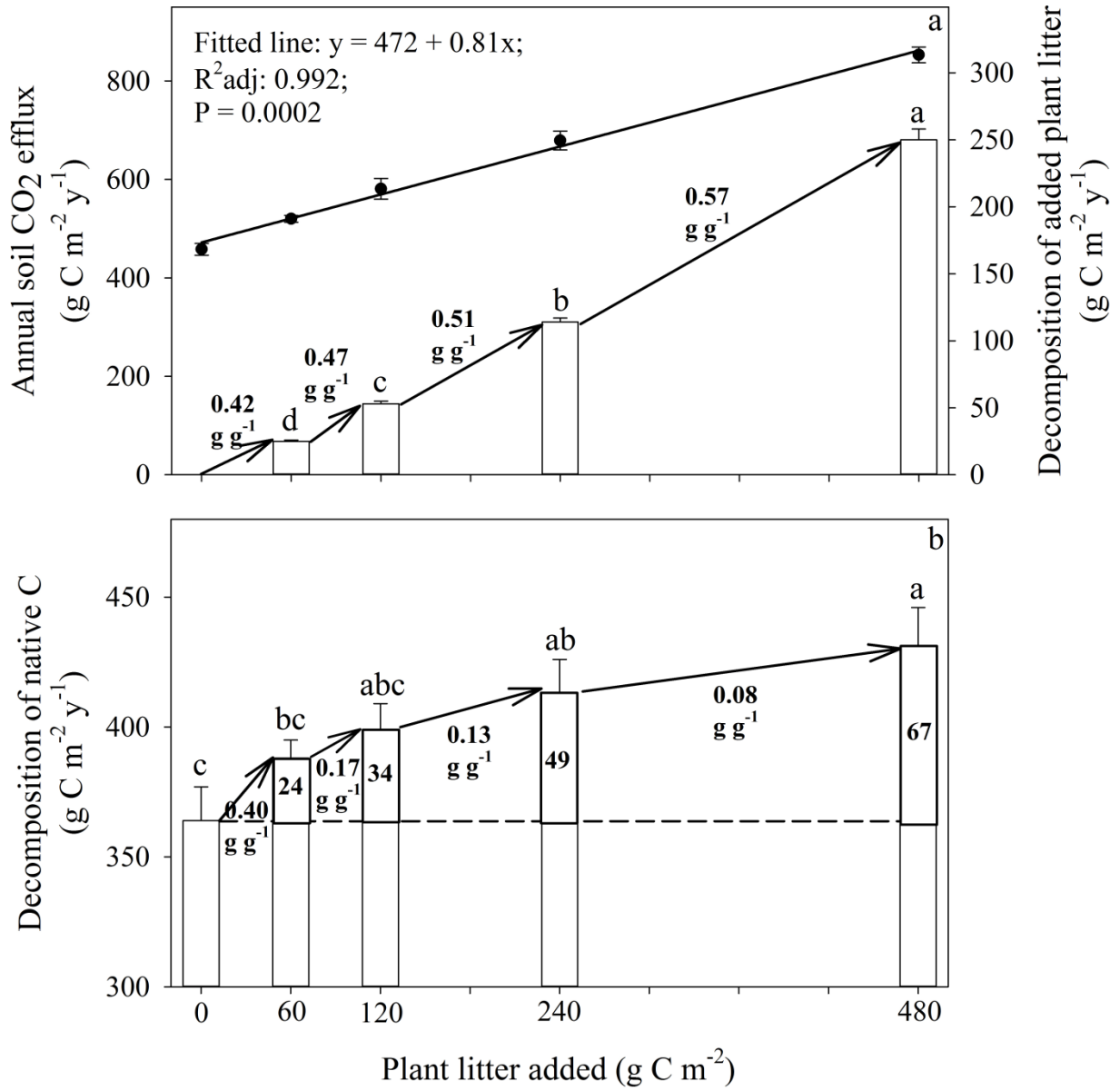
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1 **Figure 2**



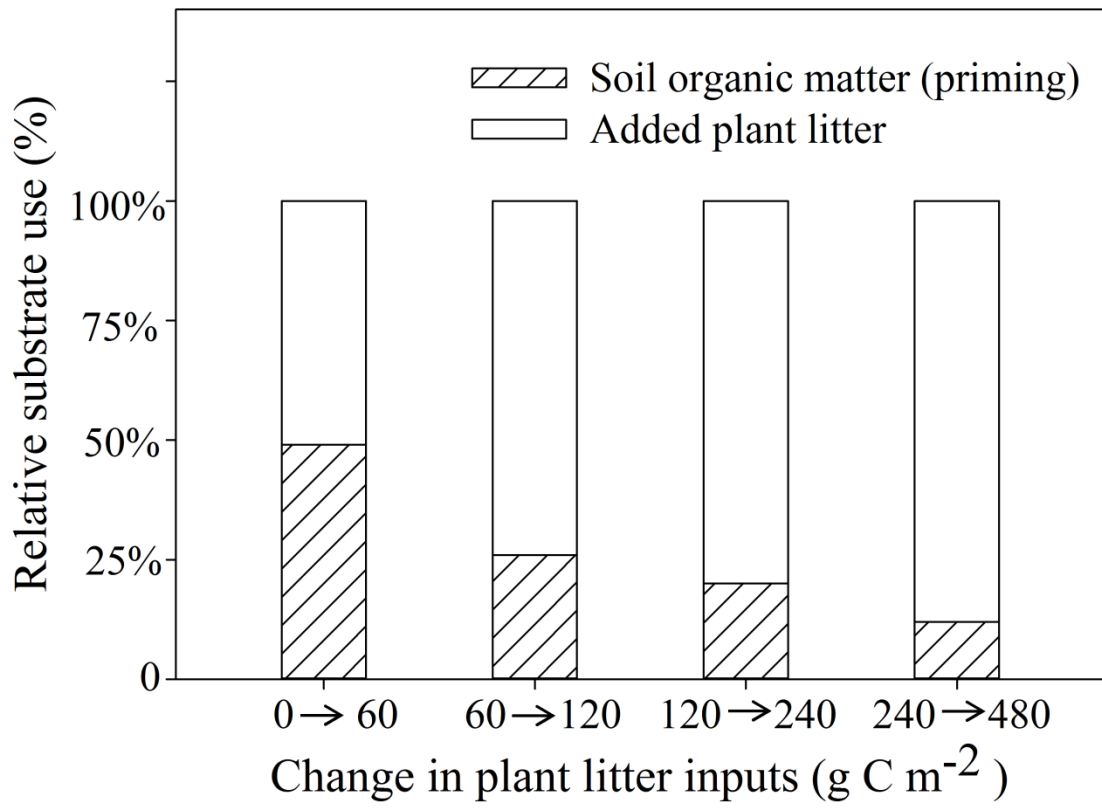
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1 **Figure 3**
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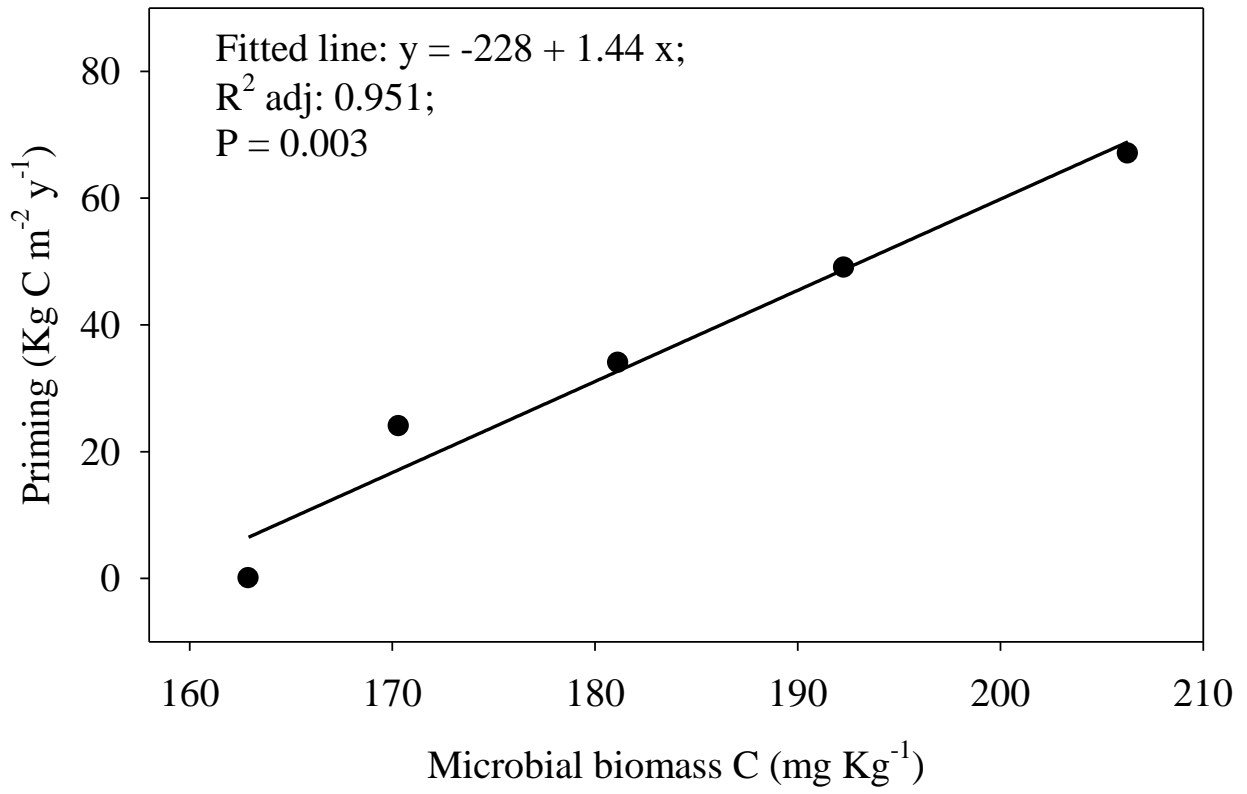
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1 **Figure 4**



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1 **Figure 5**



2
3