

# Fast mineralization of land-born C in inland waters: first experimental evidences of aquatic priming effect

Bertrand Guenet · Michael Danger · Loïc Harrault · Béatrice Allard ·  
Marta Jauset-Alcala · Gérard Bardoux · Danielle Benest · Luc Abbadie ·  
Gérard Lacroix

Received: 29 March 2013 / Revised: 17 July 2013 / Accepted: 20 July 2013 / Published online: 9 August 2013  
© Springer Science+Business Media Dordrecht 2013

**Abstract** In the context of global change, eroded soil carbon fate and its impact on aquatic ecosystems CO<sub>2</sub> emissions are subject to intense debates. In particular, soil carbon mineralization could be enhanced by its interaction with autochthonous carbon, a process called priming effect, but experimental evidences of this process are scarce. We measured in a microcosm experiment simulating oligo-mesotrophic and eutrophic aquatic conditions how quickly soil organic matter (SOM) sampled in diverse ecosystems was mineralized as compared to mineralization within soil horizons. For both nutrient loads, <sup>13</sup>C-glucose was

added to half of the microcosms to simulate exudation of labile organic matter (LOM) by phytoplankton. Effects of LOM on soil mineralization were estimated using the difference in δ<sup>13</sup>C between the SOM and the glucose. After 45 days of incubation, the mean SOM mineralization was 63% greater in the aquatic context, the most important CO<sub>2</sub> fluxes arising during the first days of incubation. Nutrients had no significant effect on SOM mineralization and glucose addition increased by 12% the mean SOM mineralization, evidencing the occurrence of a priming effect.

**Keywords** Soil erosion · Oligo-mesotrophic systems · Eutrophic systems · Carbon cycle · Aquatic priming effect · Freshwater ecosystems

Handling editor: Luigi Naselli-Flores

**Electronic supplementary material** The online version of this article (doi:10.1007/s10750-013-1635-1) contains supplementary material, which is available to authorized users.

B. Guenet (✉) · L. Harrault · M. Jauset-Alcala ·  
D. Benest · L. Abbadie · G. Lacroix  
UPMC-BIOEMCO, UMR 7618, 46, rue d'Ulm,  
75230 Paris Cedex 05, France  
e-mail: bertrand.guenet@ua.ac.be

B. Guenet  
Onderzoeksgroep Planten- en Vegetatie- ecologie,  
Antwerp University, Campus Drie Eiken – Gebouw C –  
lokaal C2.03, Universiteitsplein 1, 2610 Wilrijk, Belgium

M. Danger  
Laboratoire Interdisciplinaire des Environnements  
Continentaux (LIEC), Université de Lorraine – Metz,  
CNRS UMR, 7360 Metz, France

L. Harrault · B. Allard  
CNRS-BIOEMCO, UMR 7618, Tour 56/66,  
4 place Jussieu, 75005 Paris, France

G. Bardoux  
CNRS-BIOEMCO, UMR 7618, Bâtiment EGER,  
78850 Thiverval Grignon, France

## Introduction

Although global stocks of carbon are relatively well known, their dynamics are still poorly understood (Houghton, 2007). Soils represent the largest continental stock of carbon with 1,700 Tg of C (Berhe et al., 2005). The annual rate of soil erosion varies between 36 and 75 Tg and affects more than 1.1 billion hectares around the Earth with 1.5–5.0% C-content corresponding to a range from 0.54 to 3.75 Tg of organic C (OC) year<sup>-1</sup> which can ultimately end up as dissolved (DOC) or particulate organic carbon (POC) in aquatic ecosystems (Berhe et al., 2005). Soils are eroded by wind, tillage, or water runoff. Between 20 and 25% of the eroded soil organic matter (SOM) is transferred into aquatic systems at a global scale with wide variations across soil types (Lal, 2003). The external supply of organic matter, such as eroded SOM, plays a major role in the C cycle of aquatic ecosystems (del Giorgio et al., 1997; Dodds & Cole, 2007). Nevertheless, whether eroded SOM is a C-sink through burial in aquatic sediments (Smith et al., 2001; Van Oost et al., 2007) or a C-source due to rapid outgassing (Lal, 2003, 2005; Lal & Pimentel, 2008) is still strongly debated. Inland waters are now considered as important sources of CO<sub>2</sub> exchange (Aufdenkampe et al., 2011) and in particular mineralization of allochthonous organic matter is an important fraction of the CO<sub>2</sub> emitted by inland waters (25–33% of the total CO<sub>2</sub> emission, McCallister & Del Giorgio, 2012).

Erosion is a complex phenomenon composed by different steps. For water erosion, it starts when raindrops impact the surface. If the energy of the impact is sufficient, a part of soil may be blown off. Then, the soil is transported by runoff and may be buried through sedimentation or may be transported to the sea. During all this process, a part of SOC could be mineralized (Terrence et al., 2002). Cole et al. (2007) estimated that ca. 43% of the allochthonous organic matter was mineralized in the aquatic ecosystems whereas only 10% was buried in inland waters and 47% was transported to the sea. The great majority of the studies about the C emission due to erosion are focused on the last steps of the erosion processes (Smith et al., 2001; Van Oost et al., 2007) whereas the first step when soil is blown off and is transferred into shallow aquatic systems is far less known. Yet, it represents a huge modification of the environmental

conditions for the microbial community potentially leading to strong changes in soil mineralization rates.

In terrestrial ecosystems, SOM stability is controlled by three key mechanisms (vön Lutzow et al., 2006) whose relative importance remains poorly understood. Firstly, SOM is stabilized by interactions with mineral surfaces and metal ions. In aquatic ecosystems, stabilized SOM can be buried into the sediments and potentially acts as a sink of carbon (Dean & Gorham, 1998). However, re-suspension is important in shallow aquatic ecosystems (Hamilton & Mitchell, 1996), potentially reducing the sink capacity. Secondly, SOM may be protected from microbial enzymes by occlusion in soil aggregates. Most continental aquatic ecosystems are shallow (Wetzel, 1990) with turbulent mixing. Thus, SOM decomposition should be enhanced in water by physical breakdown of aggregates (Lal, 2003). Thirdly, some complex macromolecules in SOM may be recalcitrant to mineralization because microorganisms are unable to synthesize the enzymes that would degrade them. However, recent studies (Hamer et al., 2004; Fontaine et al., 2007) have discussed the capabilities of soil microorganisms to increase the mineralization rate of recalcitrant organic matter if extra energy is supplied as labile organic matter (LOM). Such a phenomenon is known as the priming effect (PE) (Kuzyakov et al., 2000). PE may be negative (LOM amendment reduces SOM mineralization) but positive PE (LOM amendment increases SOM mineralization) is generally observed (Kuzyakov et al., 2000). PE is controlled by several and sometimes antagonistic mechanisms such as microbial biomass increase due to extra-LOM supply, nutrient mining induced by stoichiometric imbalances between the microorganisms and their substrates, or preferential use of LOM instead of SOM (Guenet et al., 2010a).

In terrestrial systems PE is quantitatively important as it can induce a negative net balance between the input of LOM and the C lost through CO<sub>2</sub> emissions (Fontaine et al., 2004). Although many studies have detected PE in soils, the occurrence of PE is much less obvious in aquatic ecosystems (Farjalla et al., 2009; Nugteren et al., 2009). Yet, some observations suggest the existence of a PE in aquatic systems. For instance, De Haan (1977) showed that the addition of benzoate considered as a labile substrate increased the decomposition of fulvic acids by an *Arthrobacter* strain from Tjeukemeer lake (The Netherlands). Shimp &

Pfaender (1985) observed that addition of amino acids stimulated the mineralization of phenols by a natural microbial lacustrine community. Danger et al. (2013) showed that fungi are more efficient to decompose litter in presence of algae compared to controls without algae. Such effect was observed only when nutrient concentrations were low. Finally, PE might be expected to be of major importance in freshwater and marine ecosystems, where LOM is produced in substantial amounts as for example phytoplankton exudates or labile fractions of plant detritus, but only few observations have been reported (Guenet et al., 2010a). Moreover, to our knowledge no study dealt with the occurrence of PE on soils when these latter are introduced in aquatic systems through erosion for example.

In this study, we first compared the amount of mineralization of SOM, originating from four contrasting soils, in aquatic and terrestrial context. The occurrence of an aquatic PE was also investigated by adding  $^{13}\text{C}$ -labeled glucose as LOM. Finally, the influence of trophic conditions on SOM mineralization in aquatic context and on aquatic PE was studied by adding two different amounts of mineral nutrients (nitrogen and phosphorus) in microcosms to simulate oligo-mesotrophic and eutrophic conditions. We first hypothesize that SOM mineralization rates may be higher in aquatic than in terrestrial systems due to aggregate breakdown, particularly in shallow systems with turbulent mixing. Secondly, we hypothesize that PE should occur in aquatic systems, contributing significantly to the acceleration of SOM mineralization. Since PE intensity is driven by the nutrient availability (Guenet et al., 2010a), nutrient load and SOM C:nutrient ratios are likely to be major drivers of the phenomenon. In terrestrial context, high nutrient loads reduce the PE intensity but it is generally not the case in aquatic context (Guenet et al., 2010a). We therefore assume that high nutrient loads may increase the PE intensity.

## Materials and methods

### Soils

To broaden our conclusions and to avoid pseudo-replication, we chose to integrate a large range of SOM quality through the selection of four contrasting

soils as independent replicates, three representative of SOM from major agro-ecosystems in Europe (cropland, grassland and forest) and one long-term bare fallow soil considered as a surrogate of stabilized SOM (Balabane & Plante, 2004; Barré et al., 2010). The four highly contrasted SOM were used as independent replicates. The first two were cambisols (WRB), and were sampled at the Centre de Recherche en Ecologie Expérimentale et Prédictive (CEREPE- Ecotron Ile de France, ENS, St Pierre Les Nemours, France). One was sampled under deciduous forest dominated by *Quercus robur*, *Quercus petraea* and *Carpinus betulus L* and is called “Forest soil.” The second was sampled under grassland dominated by gramineae and is called “Grassland soil.” The two last soils used were Eutric cambisols (WRB) and were sampled at two long-term experimental sites at the Institut National de la Recherche Agronomique (Versailles, France). The first one was sampled at the Closeaux site. The Closeaux experiment is a chronosequence of C4 crops planted on a soil with an organic matter of C3 origin. The site is managed as classical cropland sites in this area. We used the control plot, which has always been under C3 plant rotation and under wheat monoculture since 1993. This soil is called “Cropland soil.” The second soil was a bare fallow soil, sampled from the control plots of the “42 plots experiment.” The control plot of this long-term experiment is a fallow soil that has been maintained bare and without addition of organic matter or other fertilizer since 1929. This soil is called “Bare fallow soil.” The plots were initially weeded manually and, since 1967, herbicides have been applied to prevent weed growth. In this study, we consider that this particular SOM as a surrogate for stable SOM as has been proposed in previous studies (Balabane & Plante, 2004; Barré et al., 2010; Guenet et al., 2010b) even if effect of herbicides on soil decomposers is not well understood (Johnsen et al., 2001). The four soil samples were collected using a shovel in the surface layer (0–20 cm), air-dried during 48 h and sieved (2 mm). The size of the layer is relevant in the context of erosion since it corresponds to the tilled layer, which is the layer impacted by erosion. Furthermore it must be noted that we used only soils without carbonates to avoid noise in our respiration signal due to carbonates dissolution (Chenu et al., unpublished data; Raynaud et al., unpublished data).

## Soil characterization

C and N contents were measured using a CHN analyzer (Carlo Erba, NA 1500, AS 128) with acetanilide as standard. For each soil, hydrophilic and hydrophobic fractions were extracted from a homogenized subsample (60–110 mg) using an accelerated solvent extractor (ASE 100, Dionex) at 100°C and 10<sup>7</sup> Pa. Hydrophilic compounds were first extracted with H<sub>2</sub>O (3 × 5 min), concentrated under reduced pressure, freeze-dried, and weighed. The hydrophobic fraction was subsequently extracted using methanol (MeOH, 1 × 5 min) followed by extraction with a mixture of dichloromethane (DCM):MeOH (93:7, v/v; 3 × 5 min). The MeOH and DCM/MeOH extracts were pooled and dried over Na<sub>2</sub>SO<sub>4</sub>. Solvents were removed under reduced pressure and hydrophobic compounds weighed.

The freeze-dried hydrophilic extracts were dissolved in a known volume of H<sub>2</sub>O and assayed for sugars and proteins. Sugar contents were determined by the phenol–sulfuric acid colorimetric method (Dubois et al., 1956) with glucose as standard. The protein contents were determined by the colorimetric method of Lowry (Lowry et al., 1951) with bovine serum albumin (BSA) as standard. Sugars and proteins were respectively expressed as mg of glucose and BSA equivalent per gram of C-SOM.

## δ<sup>13</sup>C-glucose

Uniformly <sup>13</sup>C-labeled glucose was obtained from Sigma-Aldrich (St Louis, USA). The initial δ<sup>13</sup>C value was 8,809,023‰. It was diluted (1:20,000) with non-labeled glucose (Sigma-Aldrich, St Louis, USA), in order to respect technical constraints of the isotope ratio mass spectrometer. The final δ<sup>13</sup>C value of glucose was –7‰. Controls were performed to check the stability of the δ<sup>13</sup>C-CO<sub>2</sub> deriving from glucose mineralization by incubating a microbial community in a mineral medium with glucose as the sole carbon source.

## Experimental unit

Incubations were performed in 120-ml glass flasks closed with an airtight septum. The terrestrial system was simulated by incubating 20 g of dried soil re-moistened at 80% of the field capacity. Soil incubations are classically performed at such moisture levels

(Gastine et al., 2003; Loranger-Merciris et al., 2006). The aquatic system was simulated by incubating 50 mg C-soils completed with different amounts of pure Fontainebleau sand to finally obtain the same soil weight in each flask and the same ratio between the C added from the soils and the nutrients available in the COMBO medium, 1 ml of lake water (Créteil, France) filtered through a 0.7 μm GF/F filter to exclude potential microorganism predators, and 2 ml of mineral medium. Water (Milli-Q) added to a final volume of 21 ml. The amount of C in the terrestrial system is higher than the in the aquatic systems but the volume occupied by the soils in terrestrial system is equivalent to volume occupied by the mix in the aquatic systems and therefore the oxygen availability is similar. Since the volume of air in the headspace is equivalent and since we expressed the results by g C added, we assume that such differences did not impact our results. Therefore the flask headspace corresponded to a volume of 99 ml. Two types of modified COMBO (Kilham et al., 1998) (NH<sub>4</sub>NO<sub>3</sub> replacing NaNO<sub>3</sub> as N source) were used as mineral medium to simulate the low (3.8 μM of N and 0.11 μM of P) and the high (38 μM of N and 1.1 μM of P) amount of N and P. It corresponded to a moderate load of nutrients similar to that observed in oligo-mesotrophic ecosystems or to a high nutrient load, similar to that observed in eutrophic ecosystems (Carlson & Simpson, 1996). The pH of the COMBO was 6.5 and since the buffering capacity of the COMBO is quite high, we assumed that the pH was constant and around 6.5 during the experiment. In addition, to investigate a possible ‘PE’, i.e., acceleration of SOM mineralization in the aquatic system, we performed extra-treatments by adding a uniformly <sup>13</sup>C-labeled glucose amendment to each nutrient load. These treatments mimic the availability of LOM in aquatic systems such as algal exudates that could accelerate mineralization of SOM (Myklesstad, 1995). In the aquatic system, 1 ml of glucose solution (2.5 g C-glucose l<sup>-1</sup>, δ<sup>13</sup>C = –7‰) corresponding to a final concentration of 50 mg C-glucose g<sup>-1</sup> C-soil (120 mg C-glucose l<sup>-1</sup>) was added to the flasks. This amount of glucose is comparatively high to what is observed in aquatic ecosystems (Goodman et al., 2011) but corresponds to a classical input in priming effect experiments in terrestrial context (Fontaine et al., 2004; Guenet et al., 2010b). Controls with low and high nutrient loads and without glucose were performed to calculate the effect of glucose addition on the SOM mineralization. The flasks were incubated in

the dark during 45 days at 20°C. For aquatic systems, the flasks were gently mixed using a mixing machine at 60 round per minute during the incubations to simulate turbulent mixing. Measurements of CO<sub>2</sub> and δ<sup>13</sup>C were carried out after 2, 8, 15, 22, 29, 36, and 45 days. Finally, we have five modalities: terrestrial context, aquatic context with low nutrient load, aquatic context with low nutrient load and with glucose, aquatic context with high nutrient load and aquatic context with high nutrient load and with glucose. As previously explained, we used the different soils as independent replicates to avoid pseudo-replications and to broaden our conclusions.

### CO<sub>2</sub> and δ<sup>13</sup>C measurements

For each simulated system, the CO<sub>2</sub> concentration was determined using MICROGC (Agilent 3000A, Santa Clara, CA, USA) and carbon isotope ratios were measured using an automatic unit that coupled a Gas Chromatograph (Hewlett-Packard, 58900) with an isotope ratio mass spectrometer (Isochrom III, Micro-mass-GVI Optima). At the start of the experiment and after each CO<sub>2</sub> measurement, all flasks were flushed with reconstituted, moistened and CO<sub>2</sub>-free air.

The PE was calculated as the difference between CO<sub>2</sub> derived from SOM mineralization when glucose was amended and the CO<sub>2</sub> derived from SOM mineralization without glucose addition.

$$PE = \alpha \times Q_{\text{sample}} - Q_{\text{control}}$$

with  $Q_{\text{sample}}$  is the amount of CO<sub>2</sub> in the flask headspace with glucose and  $Q_{\text{control}}$  is the amount of CO<sub>2</sub> in the control flask headspace

$$\text{and } \alpha = (A_{\text{glucose}} - A_{\text{sample}}) / (A_{\text{glucose}} - A_{\text{control}})$$

$A_{\text{glucose}}$ ,  $A_{\text{sample}}$ , and  $A_{\text{control}}$  represent respectively the <sup>13</sup>C isotopic abundance of glucose, of CO<sub>2</sub> in the sample headspace and of CO<sub>2</sub> in the control headspace. <sup>13</sup>C isotopic abundance is derived from the δ<sup>13</sup>C as follows:

$$\delta^{13}\text{C} = \left[ \left( \frac{R_{\text{sample}}}{R_{\text{pdb}}} \right) - 1 \right] \times 1000 \quad \text{with}$$

$$R_{\text{pdb}} = 0.0112372$$

$$A_{\text{sample}} = R_{\text{sample}} / (1 + R_{\text{sample}})$$

### Statistical analysis

All statistical analyses were performed using R 2.7.1 (R Development Core Team, 2008). We ran linear

mixed effect model with the soil as random effect. We first analyzed the mineralization of the data obtained with the incubation without glucose to test the time and the context effect (aquatic vs. terrestrial) as well as their interaction and then we analyzed the data of the incubation in only aquatic context to test the time, the glucose and the nutrient load effect as well as their interaction. Since we split our dataset we applied Bonferroni corrections to conclude on the significance. Because of non-normality and heteroscedasticity, data were rank-transformed before analysis. We then performed Tukey post hoc tests using the multcomp package of R 2.7.1.

## Results

### Soils

Chemical analyses of soils revealed strong differences in the characteristics of the studied soils (Table 1). Particularly, bare fallow soil contained a very low C-content (4.7 mg C g<sup>-1</sup> soil), whereas the carbon content of forest soil was fourfold higher than that of bare fallow soil. C/N ratio of the forest soil was threefold higher than that of the bare fallow soil. The forest soil was also characterized by a fourfold higher hydrophobic/hydrophilic compounds ratio than the other soils.

### Aquatic conditions versus terrestrial conditions

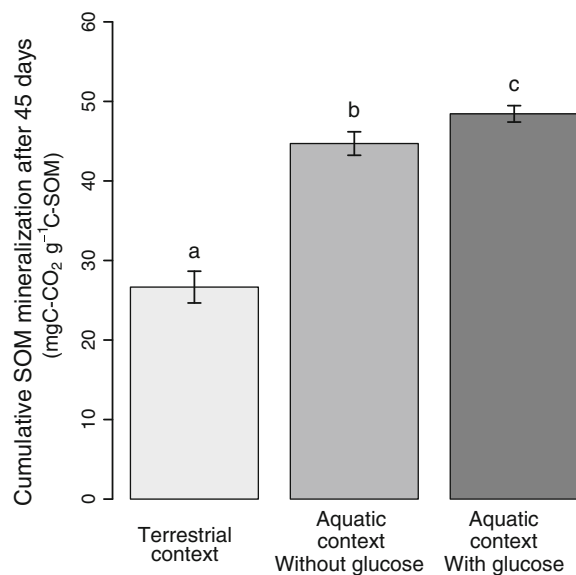
Figure 1 shows the cumulative mineralization at the end of the incubation for terrestrial systems and for aquatic systems with or without glucose. At the end of incubation, the cumulative SOM mineralization was 63% higher in the aquatic system compared to terrestrial (soil) system ( $P < 0.001$ ). Without glucose addition, neither the amount of SOM mineralized ( $P = 0.12$ , data not shown) nor the mineralization dynamics were affected by nutrient availability ( $P = 0.59$ , Fig. 2). The daily mineralization rate was maximal around the second day and then progressively decreased. The daily mineralization rate was always higher in the aquatic context than in the terrestrial context ( $P < 0.01$ ). However, Fig. 2 suggests some differences between soil types. With the exception of the grassland soil, incubation in water resulted in an increase in the mineralization rate from the first days of experiment. For the grassland soil, the

**Table 1** Characteristics of the four soils

Soil	C content (mg C g <sup>-1</sup> )	N content (mg N g <sup>-1</sup> )	C/N	pH (1/3)	Hydrophilic compounds (mg g <sup>-1</sup> C-SOM)	Hydrophobic compounds (mg g <sup>-1</sup> C-SOM)	Hydrophobic/ hydrophilic	$\delta^{13}\text{C}$ (‰)
Bare fallow	4.7 ± 0.1	0.54 ± 0.04	8.65 ± 0.83	4.0	177.8 (5.1, 18.7) <sup>a</sup>	44.4	0.2	-24.6
Cropland	11.7 ± 0.1	1.04 ± 0.04	11.23 ± 0.41	6.1	129.5 (15.4, 23.1) <sup>a</sup>	32.6	0.3	-24.9
Grassland	10.2 ± 0.1	0.84 ± 0.01	12.09 ± 0.08	5.6	193.2 (35.3, 37.3) <sup>a</sup>	58.8	0.3	-26.7
Forest	20.0 ± 0.8	0.78 ± 0.06	25.34 ± 1.35	3.7	145.1 (42.1, 37.7) <sup>a</sup>	173.8	1.2	-24.0

C, N content and C/N values are mean ± SE ( $n = 3$ )

<sup>a</sup> Values in parentheses indicate the estimated content of proteins and sugars (mg g<sup>-1</sup> C-SOM), respectively



**Fig. 1** Soil organic matter cumulative mineralization after 45 days of incubation for different systems expressed as mg C-CO<sub>2</sub> g<sup>-1</sup> of C-SOM (mean ± SE). For the aquatic systems, the bars represent the mean over the oligo-mesotrophic and the eutrophic systems. Standard errors were corrected by the random effect due to the soils. Different letters indicate significant differences ( $P < 0.05$ )

mineralization seemed similar for aquatic and terrestrial systems during the first ten days and after, it tended to be higher in the aquatic system until the end of the incubation. Mineralization rates in forest samples tended to be higher in the aquatic system than in the terrestrial one during the entire incubation period (Fig. 2). By contrast, after 21 days, the mineralization rates in cropland and bare fallow samples tended to reach a similar level in aquatic and terrestrial systems (Fig. 2).

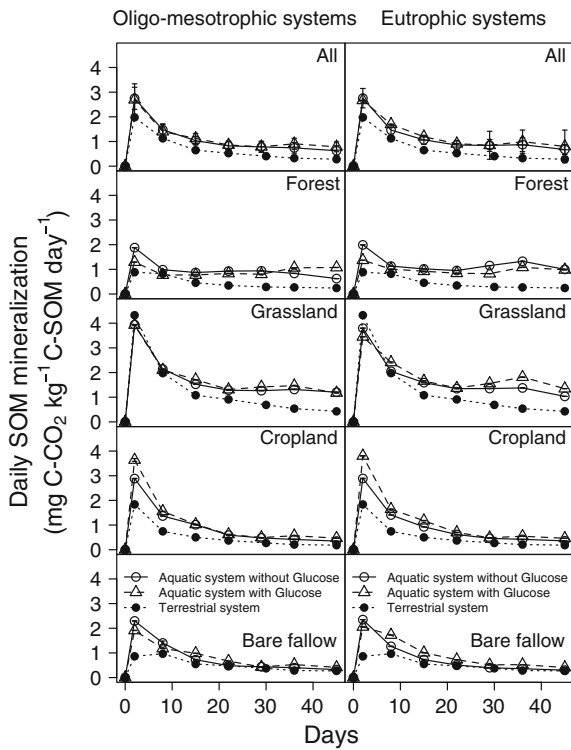
### Priming effect in aquatic conditions

Glucose addition in the oligo-mesotrophic systems (Fig. 3) increased the SOM mineralization at the end of the incubation by 12% ( $P < 0.05$ ), compared to the same soils without glucose. It corresponds to a SOM mineralization increase of 75% compared to the same soils in terrestrial conditions ( $P < 0.001$ ). For the eutrophic systems, we failed to detect any statistical effect of glucose addition on the SOM mineralization. However, this might be due to a high sensitivity of the forest soil to the combined addition of glucose and nutrients (Fig. 3). Depending on the nutrient load, accumulated PE for forest SOM at the end of the incubation period tended to be negative when nutrient load was high and positive when nutrient load was low (Fig. 3). A greater positive PE was observed for grassland, cropland, and bare fallow soils in high nutrient conditions (Fig. 3). For the bare fallow soil in low nutrient conditions, a negative PE occurred during about 2 weeks followed by a positive PE (Fig. 3). Furthermore, the PE observed for the bare fallow soil under nutrient enriched conditions tended to be positive.

### Discussion

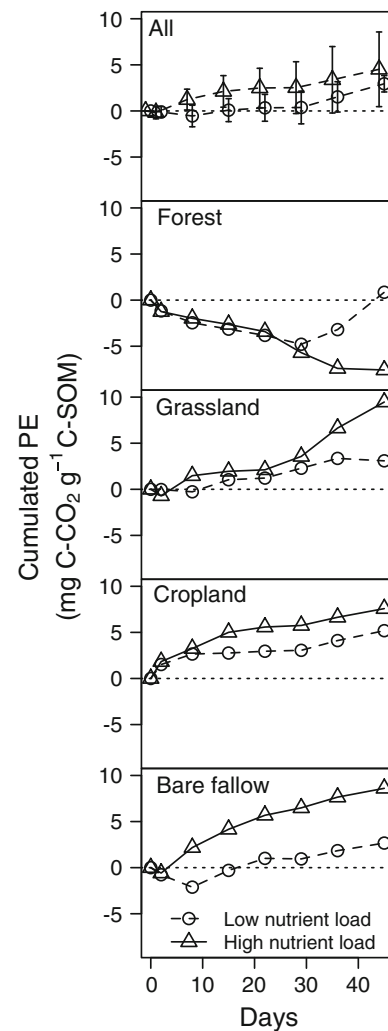
#### Aquatic conditions versus terrestrial conditions

In terrestrial ecosystems, carbon is taken up by autotrophic organisms and sequestered in soils and vegetation. A part of this carbon is eroded and either redeposited on land or is transported by streams to



**Fig. 2** Daily soil organic matter mineralization rate in the terrestrial system (*filled circle*), in the aquatic system with glucose (*triangle*) and in the aquatic system without glucose (*open circle*), expressed as  $\text{mg C-CO}_2 \text{ g}^{-1}$  of C-SOM per day. Oligo-mesotrophic aquatic systems and eutrophic aquatic systems are represented on the *left* and on the *right side*, respectively. “All” corresponds to the mean of the four soils following by each single soil. Standard errors were corrected by the random effect due to the soils when mean of all soils are represented. For single soil, error bars represent measurement variability. Since measurements variability was small and sometimes standard errors also, error bars are not visible

rivers, lakes and seas (Terrence et al., 2002). Very high sedimentation rates have been reported leading to the preliminary conclusion that inland waters are a very important C-sink, sequestering mainly C from terrestrial origins (Smith et al., 2001; Van Oost et al., 2007). However, we observed that when soil is transferred in aquatic systems during the eroding process, the mineralization rate is largely higher compared to terrestrial systems. This mineralization occurred mainly during the first days indicating that the re-deposition of eroded C in water could largely contribute to carbon emission from inland waters. This suggests that an important amount of  $\text{CO}_2$  may be emitted during the very first step of the erosion process. Consequently, high sedimentation rates do not ensure



**Fig. 3** Cumulative PE on the four-studied soil with high (*triangle*) and low (*circle*) nutrient load, expressed as  $\text{mg C-CO}_2 \text{ g}^{-1}$  of C-SOM. “All” corresponds to the mean of the four soils following by each single soil. Standard errors were corrected by the random effect due to the soils when mean of all soils are represented

sequestration as land-born C can be rapidly mineralized. This result is in accordance with the recent estimation of terrestrial C contributions to  $\text{CO}_2$  efflux from inland waters suggesting that almost half of the terrestrial C deposited in inland waters is mineralized before entering the ocean (Cole et al., 2007).

Furthermore, we observed that the effect of aquatic conditions may be different depending on the soils properties. The less sensitive soils to the aquatic conditions during the first days seemed to be the forest and the grassland soils, which are also those which

were the least managed by humans. The physical structure of forest and grassland soils has remained undisturbed by anthropogenic actions for several decades. In contrast, the cropland and bare fallow experimental sites were tilled every year, thus disrupting soil structure and aggregate stability (Lal, 2005). Moreover, the weak stability of soil aggregates from the bare fallow soil has been demonstrated previously (Balabane & Plante, 2004). Thus, as expected, shallow and mixed aquatic conditions increase SOM mineralization compared to terrestrial conditions and the lags observed in C release could probably increase with aggregate stability.

#### Priming effect in aquatic conditions

In this study, we tested for the first time the occurrence of PE in aquatic systems and as hypothesized, PE occurred in aquatic systems and this PE was, at least partly, driven by nutrient load. The mean PE being generally higher in the eutrophic systems compared to the oligo-mesotrophic systems even if any significant effect was detected due to strong negative PE observed with the forest soil. This could be explained by an increase in microbial biomass and/or exoenzyme production due to glucose and nutrient supply (Kuz'yakov et al., 2000; Nugteren et al., 2009), leading to a SOM mineralization increase. However, in terrestrial context, an opposite effect of the nutrients availability is generally observed (Martin-Olmedo et al., 2002; Fontaine et al., 2004; Blagodatskaya et al., 2007; Guenet et al., 2010c). On the contrary, when PE occurrence has been suggested in aquatic context, an enhancement of bacterial respiration has also been observed when nutrients and LOM were both amended (Carlson et al., 2002; Farjalla et al., 2009). Such discrepancies between terrestrial and aquatic conditions may be due to the high heterogeneity of soils that limits nutrient mobility. Indeed, in soils, interactions of SOM and/or enzymes with mineral matrix, low water content, or physical structuration due to aggregation, result in the formation of several microniches for microbial activity almost independent where nutrient and C availability may be quite different (Nunan et al., 2003; Lehmann et al., 2008). Therefore, nutrient addition may lead to high concentration of nutrients in some of the most active niches. In such niches, the nutrient availability might be sufficient to avoid enzyme synthesis to 'mine' the nutrients stored in

SOM and thus reduce the SOM mineralization (Craine et al., 2007). The high reduction of SOM mineralization in such niches may not be compensated by the enhancement effect of nutrients on SOM mineralization in the niches where nutrients are less available. In contrast, aquatic systems and in particular the shallow ones as simulated in our microcosms may probably be more homogenous because of the turbulent mixing and nutrient availability may be not sufficient to reduce the nutrient mining. Except for forest soil in eutrophic microcosm, we observed positive PE (i.e., SOM mineralization increase due to glucose addition). Our results will certainly require further confirmation using other soils in microcosm and field experiments, but if confirmed, positive PE might substantially reduce the C storage due to SOM burying. Furthermore, a positive PE is observed for the bare fallow soil, which is considered as a surrogate for the stable SOM pool. This suggests that PE might even accelerate mineralization of very stable SOM, PE being greater with increased nutrient loads. It must be noted that the amount of glucose added in this study is equivalent to what is amended in the PE studies performed on soils in a terrestrial context (Fontaine et al., 2004; Guenet et al., 2010b) but is high compared to what is generally observed in natural aquatic ecosystems (Goodman et al., 2011). Moreover, the link between PE intensity and the LOM amount is not straightforward (Guenet et al., 2010c). Thus, the quantitative importance of PE in aquatic ecosystems should be estimated with more realistic LOM amounts.

Negative PE values observed for forest SOM might not be due to a higher recalcitrance of forest SOM compared to the others, as bare fallow soil was only composed by stabilized C (Barré et al., 2010). Moreover, this soil had the highest C:N ratio (Table 1). According to the preferential substrate utilization hypothesis (Cheng, 1999), this high C:N ratio might have favored preferential utilization of LOM because N-limited microorganisms should have mineralized more C from SOM to acquire enough N for their growth. This phenomenon is known as N mining (Moorhead & Sinsabaugh, 2006) and assume that N starvation might induce a higher enzymatic production by microorganisms to mineralize the SOM and release N in the system. As a consequence, labile degradation products are also released and might be used as substrates inducing extra-CO<sub>2</sub> emissions. Another factor explaining this negative PE could be



the high concentration of tannins in forest soils. These compounds are known to complex proteins and deactivate microbial exoenzymes (Kraus et al., 2003). The inactivation of exoenzymes responsible for SOM mineralization could favor a preferential LOM degradation, and result in a negative PE.

To our knowledge, this study constitutes the first unequivocal demonstration of PE occurrence on soils in aquatic systems. Yet, despite the huge literature reporting this phenomenon in terrestrial ecosystems, it has often been ignored in aquatic ones (Guenet et al., 2010a). Mineralization of allochthonous organic matter such as eroded SOM might be strongly enhanced in aquatic ecosystems. This preliminary study opens exciting paths of investigation and new perspectives.

- (i) First, we must confirm these results with different soils coming from diverse ecosystems and try to relate the intensity of PE with the SOM chemical composition, the microbial community structure, the soil physical structure, etc.
- (ii) Then, we must understand how interactions between soil and aquatic microbial community may impact the PE.
- (iii) The role of the nutrient loads should also be better understood using for example a laboratory experiment where a gradient of different quantity nutrient are added to a single soil in aquatic and terrestrial context combined with an addition of FOM.
- (iv) We must quantify the quantitative importance of such phenomenon in the C balance of soil erosion using field studies with detailed measurements of the soil C balance.
- (v) If PE plays an important role in the soil erosion C balance and induces substantial fluxes from surfaces to the atmosphere, it must be incorporated in the coupled climate/carbon model used for the future climate predictions.
- (vi) Finally, if PE exist on soils in aquatic systems it probably also occurs with autochthonous OM produced in the aquatic systems.

**Acknowledgments** The authors thank ANR Biofun and R2DS for financial support, J.P. Petraud and the INRA Versailles for maintaining the Closeaux and 42 plots long-term experiments. The authors acknowledge Emma Rochelle-Newall, Philippe Ciais, and Sebastian Luyssaert for providing comments and suggestions. Bertrand Guenet was a visiting international fellow funded by the Fund for Scientific Research-Flanders (FWO).

## References

- Aufdenkampe, A. K., E. Mayorga, P. A. Raymond, J. M. Melack, S. C. Doney, S. R. Alin, R. E. Aalto & K. Yoo, 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. *Frontiers in Ecology and the Environment* 9: 53–60.
- Balabane, M. & A. Plante, 2004. Aggregation and carbon storage in silty soil using physical fractionation techniques. *European Journal of Soil Science* 55: 415–427.
- Barré, P., T. Eglin, B. T. Christensen, P. Ciais, S. Houot, T. Kätterer, F. van Oort, P. Peylin, P. R. Poulton, V. Romanov & C. Chenu, 2010. Quantifying and isolating stable soil organic carbon using long-term bare fallow experiments. *Biogeosciences* 7: 3839–3850.
- Berhe, A. A., E. Carpenter, L. Codispoti, A.-M. Izac, J. Lemoalle, F. Luizao, M. Scholes, P. Tréguer & B. Ward, 2005. Nutrient cycling, Chap. 12. In Hassan, R. et al. (eds) *Ecosystems and Human Well-Being: Current State and Trends. Millennium Ecosystem Assessment*, Island Press, Washington, DC: 722.
- Blagodatskaya, E. V., S. A. Blagodatsky, T. H. Anderson & Y. Kuzyakov, 2007. Priming effects in Chernozem induced by glucose and N in relation to microbial growth strategies. *Applied Soil Ecology* 37: 95–105.
- Carlson R. E. & J. Simpson, 1996. A Coordinator's Guide to Volunteer Lake Monitoring Methods. North American Lake Management Society, Madison: 96 pp.
- Carlson, C. A., S. J. Giovannoni, D. A. Hansell, S. J. Goldberg, R. Parsons, M. P. Otero, K. Vergin & B. R. Wheeler, 2002. Effect of nutrient amendments on bacterioplankton production, community structure, and DOC utilization in the northwestern Sargasso Sea. *Aquatic Microbial Ecology* 30: 19–36.
- Cheng, W., 1999. Rhizosphere feedbacks in elevated CO<sub>2</sub>. *Tree Physiology* 19: 313–320.
- Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg & J. Melack, 2007. Plumbing the global carbon cycle: integrating inland waters into the Terrestrial Carbon Budget. *Ecosystems* 10: 172–185.
- Craine, J. M., C. Morrow & N. Fierer, 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88: 2105–2113.
- Danger, M., J. Cornut, E. Chauvet, P. Chavez, A. Elger & A. Lecerf, 2013. Benthic algae stimulate leaf litter decomposition in detritus-based headwater streams: a case of aquatic priming effect? *Ecology*. doi:10.1890/12-0606.1.
- De Haan, H., 1977. Effect of benzoate on microbial decomposition of fulvic acids in Tjeukemeer (Netherlands). *Limnology and Oceanography* 22: 38–44.
- Dean, W. E. & E. Gorham, 1998. Magnitude and significance of carbon burial in lakes, reservoirs, and peatlands. *Geology* 26: 535–538.
- del Giorgio, P. A., J. J. Cole & A. Cimleris, 1997. Respiration rates in bacteria exceed phytoplankton production in unproductive aquatic systems. *Nature* 385: 148–151.
- Dodds, W. K. & J. J. Cole, 2007. Expanding the concept of trophic state in aquatic ecosystems: it's not just the autotrophs. *Aquatic Sciences* 69: 427–439.
- Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers & F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350–356.

- Farjalla, V., C. C. Marinho, B. M. Faria, A. M. Amado, F. A. Esteves, R. L. Bozelli & D. Giroldo, 2009. Synergy of fresh and accumulated organic matter to bacterial growth. *Microbial Ecology* 57: 657–666.
- Fontaine, S., G. Bardoux, L. Abbadie & A. Mariotti, 2004. Carbon input to soil may decrease soil carbon content. *Ecology Letters* 7: 314–320.
- Fontaine, S., S. Barot, P. Barré, N. Bdioui, B. Mary & C. Rumpel, 2007. Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450: 277–280.
- Gastine, A., M. Scherer-Lorenzen & P. W. Leadley, 2003. No consistent effects of plant diversity on root biomass, soil biota and soil abiotic conditions in temperate grassland communities. *Applied Soil Ecology* 24: 101–111.
- Goodman, K. J., M. A. Baker & W. A., Wurtsbaugh, 2011. Lakes as buffers of stream dissolved organic matter (DOM) variability: temporal patterns of DOM characteristics in mountain stream-lake systems. *Journal of Geophysical Research* 116:G00N02.
- Guenet, B., M. Danger, L. Abbadie & G. Lacroix, 2010a. Priming effect: bridging the gap between terrestrial and aquatic ecology. *Ecology* 91: 2850–2861.
- Guenet, B., J. Leloup, X. Raynaud, G. Bardoux & L. Abbadie, 2010b. Negative priming effect on mineralization in a soil free of vegetation for 80 years. *European Journal of Soil Science* 61: 384–391.
- Guenet, B., C. Neill, G. Bardoux & L. Abbadie, 2010c. Is there a linear relationship between priming effect intensity and the amount of organic matter input? *Applied Soil Ecology* 46: 436–442.
- Hamer, U., B. Marschner, S. Brodowski & W. Amelung, 2004. Interactive priming of black carbon and glucose mineralization. *Organic Geochemistry* 35: 823–830.
- Hamilton, D. & S. Mitchell, 1996. An empirical model for sediment resuspension in shallow lakes. *Hydrobiologia* 317: 209–220.
- Houghton, R. A., 2007. Balancing the global carbon budget. *Annual Review Earth and Planetary Sciences* 35: 313–347.
- Johnsen, K., C. S. Jacobsen, V. Torsvik & J. Sørensen, 2001. Pesticide effects on bacterial diversity in agricultural soils – a review. *Biology and Fertility of Soils* 33: 443–453.
- Kilham, S. S., D. A. Kreeger, S. G. Lynn, C. E. Goulden & L. Herrera, 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. *Hydrobiologia* 377: 147–159.
- Kraus, T. E. C., R. A. Dahlgren & R. J. Zasoski, 2003. Tannins in nutrient dynamics of forest ecosystems – a review. *Plant and Soil* 256: 41–66.
- Kuzyakov, Y., J. K. Friedel & K. Stahr, 2000. Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry* 32: 1485–1498.
- Lal, R., 2003. Soil erosion and the global carbon budget. *Environment International* 29: 437–450.
- Lal, R., 2005. Soil erosion and carbon dynamics. *Soil and Tillage Research* 81: 137–142.
- Lal, R. & D. Pimentel, 2008. Soil erosion: a carbon sink or source? *Science* 319: 1040–1041.
- Lehmann, J., D. Solomon, J. Kinyangi, L. Dathe, S. Wirick & C. Jacobsen, 2008. Spatial complexity of soil organic matter forms at nanometre scales. *Nature Geoscience* 1: 238–242.
- Loranger-Merciris, G., L. Barthes, A. Gastine & P. W. Leadley, 2006. Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. *Soil Biology and Biochemistry* 38: 2336–2343.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193: 265–275.
- Martin-Olmedo, P., R. M. Rees & J. Grace, 2002. The influence of plants grown under elevated CO<sub>2</sub> and N fertilization on soil nitrogen dynamics. *Global Change Biology* 8: 643–657.
- McCallister, S. L. & P. A. Del Giorgio, 2012. Evidence for the respiration of ancient terrestrial organic C in northern temperate lakes and streams. *Proceedings of the National Academy of Sciences of the United States of America* 109: 16963–16968.
- Moorhead, D. & R. Sinsabaugh, 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs* 76: 151–174.
- Myklesstad, S., 1995. Release of extracellular products by phytoplankton with special emphasis on polysaccharides. *Science of the Total Environment* 165: 155–164.
- Nugteren, P., L. Moodley, G.-J. Brummer, C. H. Heip, P. M. J. Herman & J. J. Middelburg, 2009. Seafloor ecosystem functioning: the importance of organic matter priming. *Marine Biology* 156: 2277–2287.
- Nunan, N., K. Wu, I. M. Young, J. W. Crawford & K. Ritz, 2003. Spatial distribution of bacterial communities and their relationships with the micro-architecture of soil. *FEMS Microbiology Ecology* 44: 203–215.
- R Development Core Team, 2008. R: A Language and Environment for Statistical Computing R Foundation for Statistical Computing. Vienna, Austria.
- Shimp, R. & F. K. Pfaender, 1985. Influence of naturally occurring humic acids on biodegradation of monosubstituted phenols by aquatic bacteria. *Applied and Environmental Microbiology* 49: 402–407.
- Smith, S., W. Renwick, R. Buddemeier & C. Crossland, 2001. Budgets of soil erosion and deposition for sediments and sedimentary organic carbon across the conterminous United States. *Global Biogeochemical Cycles* 15: 697–707.
- Terrence, J. T., G. R. Foster & K. G. Renard, 2002. *Soil Erosion: Processes, Prediction, Measurement, and Control*. Wiley, New York.
- Van Oost, K., T. A. Quine, G. Govers, S. De Gryze, J. Six, J. W. Harden, J. C. Ritchie, G. W. McCarty, G. Heckrath, C. Kosmas, J. V. Giraldez, J. R. M. da Silva & R. Merckx, 2007. The impact of agricultural soil erosion on the global carbon cycle. *Science* 318: 626–629.
- von Lutzow, M., I. Kogel-Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner & H. Flessa, 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions – a review. *European Journal of Soil Science* 57: 426–445.
- Wetzel, R. G., 1990. Landwater interfaces: metabolic and Limnological regulators. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie* 24: 6–24.