Soil organic carbon decomposition rates in river systems: effect of experimental conditions

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Abstract. Rivers receive large amounts of terrestrial soil organic carbon (SOC) due to the action of different erosion processes. Mounting evidence indicates that a significant fraction of this SOC, which is often very old, is rapidly decomposed after entering the river system. The mechanisms explaining this rapid decomposition of previously stable SOC still remain unclear. In this study, we investigated the relative importance of two mechanisms that possibly control SOC decomposition rates in aquatic systems: (i)

10   in the river water SOC is exposed to the aquatic microbial community which is able to metabolise SOC much more quickly than the soil microbial community and (ii) SOC decomposition in rivers is facilitated due to the hydrodynamic disturbance of suspended sediment particles. We performed different series of short-term (168 h) incubations quantifying the rates of SOC decomposition in an aquatic system under controlled conditions. Organic carbon decomposition was measured continuously through monitoring dissolved O_2 (DO) concentration using a fiber-optic meter (FirestingO$_2$, PyroScience). Under both shaking and standing conditions,

15   we found a significant difference between SOC with aquatic microbial organisms (SOC+AMO) and without aquatic microbial organisms (SOC-AMO). The presence of an aquatic microbial community enhanced the SOC decomposition process by 70 %– 128 % depending on the soil type and shaking/standing conditions. While some recent studies suggested that aquatic respiration rates may have been substantially underestimated by performing measurement under stationary conditions, our results indicate that this effect is relatively minor, at least under the temperature conditions, the soil type and for the suspended matter concentration

20   range used in our experiments. We propose a simple conceptual model explaining these contrasting results.

1 Introduction

Rivers play an important role in the global carbon cycle by linking terrestrial and aquatic ecosystems. Each year, rivers receive and deliver large amounts of terrestrial organic carbon to the oceans (Raymond & Bauer, 2001;Ward et al., 2017). However, rivers do not just transport OC. In 2007, Cole et. al published the idea of "rivers as an active pipe", which highlights the fact that rivers

25   do not only transport but also process large amounts of OC (Cole et al., 2007). Mounting evidence indicates that a significant fraction of the OC processed in rivers is soil organic carbon (SOC), which is often very old (in the range of 1000–5000 y B.P.) (Raymond & Bauer, 2001; Dodds & Cole, 2007; Mccallister & Paul, 2012). When old SOC is delivered to a river, e.g. by surface erosion processes, part of this SOC can be rapidly mineralized and emitted back to the atmosphere (Lapierre et al., 2013; Wilkinson et al., 2013; Mayorga et al., 2005). Understanding the mechanisms that contribute to this active mineralization process of SOC is

30   essential for understanding the role of rivers in the global carbon cycle and for assessing how the carbon metabolism of rivers may respond to environmental perturbations such as an increase or decrease of terrestrial carbon delivery to the river system and/or changes in hydrology and climate. Indeed, the amount of SOC imported into riverine ecosystems is often large compared to the autochthonous within-river primary production (Cole and Caraco, 2001). It is therefore important to understand the fate of this terrestrial SOC as this does not only affect the global carbon cycle but also strongly regulates the ecological functioning of river

35   ecosystems (Kling, 1995). A key question is then which factors control the decomposition of terrestrial SOC, which has been stable in soil for decades to centuries, when it enters into a river system.

In recent years, the mechanisms controlling this rapid riverine mineralization process have gained increasing attention (Aufdenkampe et al. 2011; Guenet et al. 2014; Ward et al. 2017). While in transit, SOC can be degraded by microbial degradation (Ward et al., 2013) and photochemical oxidation (Spencer et al., 2009). As can be expected, these processes are closely associated

40   with a suite of factors such as temperature (Lapierre et al., 2013; Gudasz et al., 2015), the availability of oxygen (Koehler et al., 2012), and the presence and composition of microbial communities (Ward et al., 2019), along with physical river properties, such as river velocity and hydrodynamic conditions (Ward et al., 2018). For example, Ward et al. (2018) incubated river water and sediment under three rotation regimes to mimic river flow and found respiration rates were 1.4 (under 0.22 m·s^{-1}) and 2.4 times (under 0.66 m·s^{-1}) higher compared with stationary conditions. The physical breakup of large particles induced by river water

45   disturbance may increase the accessibility of microbial enzymes to SOC, thus enhance SOC mineralization (Lal 2003; Richardson et al. 2013). Interactions of microbial community and SOC decomposition have been extensively studied in terrestrial ecosystems (Cleveland et al., 2007; Hu et al. 2014; Tian et al., 2016). Microbial organisms influence SOC cycling not only via decomposition but also because microbial products are themselves important components of soil organic matter (Kögel-Knabner, 2002). Ward et al. (2019) illustrated the potential importance of the composition of the aquatic microbial community by showing that mixing the

50   water of two lowland tributaries (the Tapajós and Xingu rivers) and the Amazon main river resulted in enhanced respiration rates, which they attributed to the fact that such mixing resulted in a more diverse microbial community capable of digesting more OC. While these recent studies clearly indicate that different mechanisms do indeed control OC processing in rivers, important knowledge gaps do remain. One of the reasons for the latter is that, hitherto, very few experiments were carried out with a factorial design allowing to investigate the relative importance and potential interaction of different individual factors.

55   The main objective of this study is to shed light on the fate of SOC in river systems by investigating the relative importance of two key mechanisms that were previously suggested to potentially contribute to the rapid decomposition of previously stable SOC in aquatic systems: (i) in the river water, SOC is exposed to an aquatic microbial community which may be able to metabolise SOC much more quickly than the soil microbial community and (ii) SOC decomposition in rivers is facilitated due to the hydrodynamic disturbance of sediment. We base this work on continuous measurements of DO consumption during a series of lab incubation

60   experiments and the characterisation of the evolution of SOC, Particulate organic carbon (POC) and Dissolved organic carbon (DOC) characteristics throughout the incubation period. By doing so we were able to quantify the importance of these mechanisms both in absolute and relative terms.

2 Material and methods

2.1 Site description

65   River water was collected from the Dijle river about 2 km upstream of the city of Leuven, which is situated in the central Belgian loess belt (Fig. 1). The relief of the 700 km^2 large Dijle river catchment varies from about 25 m above sea level (a.s.l) in the north to ca.165 m a.s.l in the south. The Dijle catchment is characterized by an undulating plateau in which several rivers are incised. Slope gradients are usually less than 5 %, although maximum slopes of ca. 50 % can be found along the valleys. The majority of the soils are Luvisols, developed in the loess deposits (FAO, 1998). The land use in the catchment is mainly cropland, which is

70   particularly vulnerable to erosion during spring and early summer when vegetation cover is low and rainfall intensity is high (Takken et al., 1999). The contemporary land use in the floodplain is dominated by grassland, plantation forests and built-up area (Broothaerts et al., 2014).

2.2 Experimental approach

Experimental setup

75   Our measurement approach involved collecting a large volume of river water, subsequently treated by removing all suspended particles via filtration and dispensing the filtrate into a series of 320 ml glass incubation bottles. The treatments included (1) SOC with rotation, (2) SOC under stationary condition, (3) DOC control with rotation, (4) DOC control under stationary condition, (5) SOC with rotation without AMO, and (6) SOC under stationary condition without AMO (see Table 1 for details). We set six repetitions for each treatment. For each experiment, 4 incubation bottles with the same treatment were incubated, which allowed

80   us to sample for POC and DOC during the course of the experiment (at t = 24 h, 48 h, 96 h, 168 h). Mechanical breakup was simulated by a custom-designed swing system (Fig. 2), which kept the particles in suspension during the incubation. Incubation bottles were fixed in the PVC clip brackets, and rotated at about 7 rounds per minute. Since the bottles were connected with FirestingO$_2$ fibre, the swing system was rotated 180° anticlockwise and 180° clockwise back, which counted as one round. In order to get a higher disturbance intensity, 8 g glass beads (diameter: 2 mm) were added to the bottle before adding soil and water

85   samples. The glass beads were pre-sterilized at 450 °C for 1 hour to avoid contamination.

Collection and incubation

River water was filtered on 0.7 μm glass fiber filters to remove all suspended particles. For treatments without aquatic microbial communities, river water was then filtered through 0.2 μm syringe filters. Since filtration at 0.7 μm already removed the majority of microbial organisms, 4 ml unfiltered river water was added to serve as an inoculum for treatments with aquatic microbial

90   organisms. Soil samples were collected from a Belgian Loess Belt near Leuven: one from arable land (50°48'31.6″N, 4°35'16.9″E); the other from Bertem forest (50°52'59.8″N, 4°38'24.2″E). These soils were oven dried at ~55 °C and then sieved through 2 mm to remove all roots and stones. In order to have a detectable oxygen consumption from POC, POC concentration was controlled at 10–12 mg L^{-1} by adding 160 mg arable land soil and 60 mg forest soil (details in Table 2). The bottles were subsequently closed without headspace. For the suspended condition treatments, bottles were fixed on the swing systems (Fig. 2). The remaining bottles

95   were placed on a shelf to keep particles settled in each bottle. To avoid the influence of temperature and light (both potentially influencing OC mineralization), all bottles were wrapped with aluminium foil and incubated in a temperature-controlled room at ~20 °C. Six experimental runs for each soil type were conducted to investigate the effect of water disturbance and the presence of an aquatic microbial community on SOC decomposition.

Given that the soil samples were oven-dried at ~55 °C before the incubation, a certain amount of soil microbial organisms might

100   have been eliminated. In order to test this, we carried out a series of supplementary experiments whereby air dried and oven dried soil material was used under stationary and rotation conditions. Soil material was collected from the same arable land (OC%: 1.5 %). The soil was divided into two parts and dried with two methods: one part was oven dried at ~55 °C, the other was air dried. The incubations followed the same procedures mentioned above.

DO, POC and DOC measurements

105   DO was measured continuously for 168 hours using an optical oxygen meter (FireStingO$_2$), and we used the DO data to derive the total amount of C mineralized in each incubation series, using a procedure similar to the one used in previous studies (Berggren et al., 2012; Richardson et al., 2013). It is known that, when OC is decomposed, the O$_2$: CO$_2$ ratio is not constant but depends on the composition of the substrate and the characteristics of the bacterial community (Berggren et al., 2012). Richardson et al. (2013) took the elemental composition of organic matter into account, and reported an O$_2$: CO$_2$ ratio of 1.04–1.2 for detrital organic matter

110   and 1.0 for DOC. In this study, we used an O$_2$: CO$_2$ ratio equal to 1.0 for both SOC and DOC, thus assuming that the mineralization

of 1 mole C consumed 1 mol O_2. Using a respiration ratio of 1.0 might result in an overestimate of the total amount of C mineralized, but since all treatments were calculated with the same respiration ratio and the same soil samples were used throughout the experiments, the relative variations will not be affected by this choice.

After the incubation period, the entire volume of ~320 ml water was filtered for later determination of total POC, particulate
115   nitrogen (PN) and the stable C isotope ratios (δ^{13}C) of POC on pre-combusted 25 mm Whatman GF/F filters (pore size: 0.7 μm). Due to the high concentrations of soil materials in each bottle, each water sample was filtered on 2–5 filter papers. For arable land soil incubations, water samples were filtered on 5 filter papers, we measured all filter papers for 2 repetitions, and 2 of 5 filters papers for the other 4 repetitions. Then we used the suspended sediment weight and POC concentrations measured on this subsample to calculate the POC and PN content as well as the δ^{13}C signature of the entire sample; for forest soil incubations, water
120   samples were filtered on 2–5 filter paper, all of the filter papers were oven dried at 50 °C and preserved for POC, PN and δ^{13}C measurements. Inorganic C was removed from the filters by exposing them to HCl fumes overnight in a desiccator. Subsequently, the dried filters were packed in Ag cups for analysis on an elemental analyser-isotope ratio mass spectrometer (EA-IRMS, ThermoFinnigan Flash HT and Delta V Advantage). Certified (IAEA-600, caffeine) and in-house laboratory standards (leucine and tuna tissue) were analysed throughout each run.

125   To determine the DOC concentration and its stable isotope composition, 40 ml filtered water samples (0.2 μm) were collected and stored in glass vials with Teflon-coated screw caps and 100 μL of H_3PO_4 was added for preservation. Analysis of DOC and $\delta^{13}C_{DOC}$ was performed on a wet oxidation TOC analyzer (IO Analytical Aurora 1030W) coupled with an isotope ratio mass spectrometer (ThermoFinnigan Delta V Advantage). Quantification and calibration were performed with IAEA-C6 (δ^{13}C = –10.4 ‰) and an internal sucrose standard (δ^{13}C = –26.99 ± 0.04 ‰).

130   **2.3 Statistical analysis**

Statistical tests were performed in R. The normality of data was tested with the Shapiro–Wilk test. Paired sample t test was applied to test for differences in DO consumption rates, total amount of C mineralized and POC loss between treatments under rotation and stationary conditions, and between treatments with and without the presence of AMO. Average values are given ± the standard deviation.

135   **3 Results**

3.1 Effect of water disturbance and AMO on DO consumption rates

In all treatments, DO concentrations followed a decreasing trend, and the DO consumption rates were relatively constant over time (Fig. 3). For incubations where soil was present, cumulative DO consumption ranged between 0.7 and 2.6 mg L^{-1} during the 168-h incubation period. The highest DO consumption occurred in the SOC+AMO treatment with an average decrease rate at 0.015
140   mg O_2 L^{-1} h^{-1} (arable soil) and 0.010 mg O_2 L^{-1} h^{-1} (forest soil). DO consumption was lowest for treatments where soil was present without AMO. We found that keeping soil particles in suspension resulted in a relatively small acceleration of the OC decomposition process. With the presence of aquatic microbial organisms (SOC+AMO), DO consumption rate was increased by 13 % ($p < 0.05$) for the arable soil while no significant effect was found for forest soil ($p > 0.05$).

The addition of AMO, on the other hand, had a much stronger stimulation effect on OC decomposition. Compared with SOC-
145   AMO treatments, DO consumption rates were approximately doubled by the presence of AMO for both soil types. This increase was present under both rotation (arable land soil: 0.015 *vs.* 0.007 mg O_2 L^{-1} h^{-1}, $p < 0.01$; forest soil: 0.010 *vs.* 0.005 mg O_2 L^{-1} h^{-}

^{1}, $p < 0.01$) and stationary conditions (arable land soil: 0.014 *vs.* 0.007 mg O_2 L^{-1} h^{-1}, $p < 0.01$; forest soil: 0.009 *vs.* 0.004 mg O_2 L^{-1} h^{-1}, $p < 0.01$).

3.2 Total amount of C mineralized

150   We calculated the total amount of C mineralized in each treatment, using an O_2: CO_2 ratio of 1.0 and expressed mineralisation rates on a per carbon basis. Obviously, trends in total C mineralisation are similar to those in oxygen consumption. For SOC+AMO incubations with soil from arable land, keeping particles in suspension increased total amount of C mineralized by 13 % ($p < 0.05$, Fig. 4). Also for forest soil a small increase was noted (11 %) but this was not statistically significant ($p > 0.05$). Compared with SOC-AMO, the presence of aquatic microbial organisms (SOC+AMO) significantly stimulated OC decomposition. Specifically,

155   for soil from arable land, AMO addition led to 91 % (under rotation conditions)–128 % (under stationary conditions) more C mineralized by the end of the incubation, and 116 % (under rotation conditions)–118 % (under stationary conditions) with soil from forest depending on rotation and stationary conditions.

    In addition, when comparing the mineralisation of oven-dried and air-dried soil without AMO addition, oven-dried soil incubation showed a 40 % lower C loss in comparison to air dried soil incubation at the end of the incubation (Fig. 5). However, the addition

160   of AMO resulted in higher C loss with both treatments (air drying: 70 %; oven drying:165 %).

3.3 Particulate and dissolved organic carbon concentrations and $\delta^{13}C_{POC}$, $\delta^{13}C_{DOC}$ values

    Measurements of final POC concentrations showed a reduction of POC at the end of the incubations, where 5 %–13 % of POC was mineralized for incubations with soil from arable land, and 1 %–11 % for incubations with forest soil (Fig. 6, Table 3). Keeping particles in suspension resulted in ca. 4 % more POC loss in SOC+AMO incubation series for both soil types (arable land: 13 %

165   *vs.* 9 %, $p < 0.05$; forest: 11 % *vs.* 7 %, $p > 0.05$). Conversely, keeping particles in suspension in SOC-AMO incubation series showed negative effects with 1 % (arable land soil)–4 % (forest soil) less POC loss, but this was not statistically significant ($p > 0.05$). The presence of AMO led to ca. 8–10 % (with rotation) and 2–3 % (under stationary conditions) more POC loss for both soil types. These trends are generally consistent with the patterns derived from the oxygen consumption measurements, i.e. a larger reduction in POC when AMO is present and a limited effect of hydrodynamic disturbance. However, it is also clear that variations

170   in residual POC are less consistent than those observed from oxygen consumption rates. In 7 of 12 incubation series, the combined POC and DOC losses exceeded the total amount of C mineralized calculated from DO consumption (Table 3).

    $\delta^{13}C_{POC}$ values showed similar variation patterns for both soil types (Fig. 6): firstly, an increase occurred during the first 24 h (for forest soil) or 48 h (arable land soil). The increase in $\delta^{13}C_{POC}$ was more important for forest soil (0.4 ‰–0.6 ‰) in comparison to arable land soil (0.2 ‰–0.4 ‰). After this initial period, $\delta^{13}C_{POC}$ values stabilised.

175   DOC concentrations were relatively stable during the 168 h incubation (Fig. 7; Table 3). Initial $\delta^{13}C_{DOC}$ was lower in arable land soil experiments where no AMO was present. When AMO was present the initially higher $\delta^{13}C_{DOC}$ values declined to similar values as those observed in experiments without AMO in the first 48 h. In the experiments with forest soils initial $\delta^{13}C_{DOC}$ were ca. 0.6 ‰ higher than the initial $\delta^{13}C_{DOC}$ values for arable land soils without AMO. $\delta^{13}C_{DOC}$ were stable throughout the incubation period for all forest soil experiments.

180   For both soil types the equilibrium $\delta^{13}C_{DOC}$ values are somewhat lower than the equilibrium $\delta^{13}C_{POC}$ values: this difference is more pronounced for the forest soil (ca. 1 ‰ in comparison to ca. 0.5 ‰ on average) ($p > 0.05$).

4 Discussion

Our results revealed that terrestrial SOC can indeed be mineralized relatively quickly when introduced in an aquatic environment. With the presence of aquatic microbial organisms (SOC+AMO), up to 0.58 (forest soil) or 0.97 (arable land soil) mg C L^{-1} was

185 mineralized within the 168 h incubation period, equivalent to 83–139 μg C^{-1} d^{-1}. Comparable respiration rates of 20–80 μg C L^{-1} d^{-1} were reported under similar temperature conditions by Berggren et al. (2010). Similar incubation experiments with water samples collected from northern temperate lakes and streams reported respiration rates from 16 to 54 μg C L^{-1} d^{-1} (Mccallister and Paul, 2012). We found that C mineralization rates were quite significant: in the presence of an AMO (which is always the case in natural conditions), 4 to 6 % of the OC present at the initiation of the experiment was mineralized during the experiment (Fig. 4).

190 Typical soil incubation experiments show a loss of max. 2 % of the SOC in the first two weeks: Gillabel et al., (2010) incubated soil samples under 25 °C and found ca. 2 % SOC was respired in 20 days with a mineralization rate at around 1 μg C per mg C per day; Angst et al., (2019) conducted similar soil incubation and found a cumulative respiration of 20 mg CO$_2$ (g C soil)$^{-1}$ in 14 days, equivalent to around 0.4 μg C per mg C per day. Li et al., (2018) incubated soil with fertilization and straw application, and found a highest cumulative respiration at around 800 mg CO$_2$-C kg^{-1} soil in 20 days, equivalent to around 2 μg C per mg C per day.

195 These mineralization rates are generally much lower than those observed in our study, where 6–9 μg C is mineralized per mg C per day. This suggests that SOC indeed decomposes more rapidly in aquatic systems than in the terrestrial environment because SOC is not as recalcitrant as preciously thought in aquatic systems (Mayorga et al., 2005; Mccallister & Paul, 2012). Several studies already suggested that the transition from terrestrial to aquatic conditions likely facilitated SOC decomposition rates because of potential shifts in environmental conditions (Gurwick et al., 2008; Butman & Raymond, 2011; Mccallister & Paul,

200 2012). In soils, sorption of OC to mineral surfaces and encapsulation of C within soil aggregates may protect SOC from complete mineralization (Bianchi et al., 2011; Schmidt, et al., 2011). This results in the accumulation of older SOC in pools that are less accessible to decomposers and their extracellular enzymes (Marín-Spiotta et al., 2014). When SOC enters aquatic systems, a disruption of the mechanisms protecting C from mineralization, such as a physical disturbance due to the physical action of transport in water but also due to aggregate slaking (Le Bissonnais, 1996), may lead to the exposure of these protected pools to

205 decomposers and therefore to an increase of the SOC decomposition rate. Alternatively, SOC decomposition may be accelerated due to the fact that, in an aquatic environment, a population of possible consumers is present that is different from that in the soil and that may be capable of rapidly mineralizing SOC that is otherwise preserved over long-time scales in a soil environment. However, based on DO measurements, our results indicate that physical disturbance, which was simulated by rotation in our experiments, had a relatively minor effect on SOC decomposition (arable land soil: $p < 0.05$; forest soil: $p > 0.05$) (Fig. 3, Fig. 4).

210 This result is different from that reported in two previous studies with similar incubation approaches. Richardson et al (2013) and Ward et al (2018) found that keeping sediment particles in suspension increased river-borne organic matter decomposition by 40 to 140 %, depending on the rotation speed. At present, we cannot fully explain why our results are different from those obtained by the latter two studies. However, our data do indicate that the relative role of physical disturbance on our test soil (diameter < 2 mm) vs. a priming effect due to a different microbial community may vary between different ecosystems. There are two possible

215 reasons as to why we did not find a strong effect of physical disturbance on SOC decomposition. It is hypothetically possible that the level of physical protection of (a large part of) the SOC that is present is such that it is not disrupted by the physical disturbance that we imposed. Alternatively, the level of physical protection was so weak that the mere immersion of soil particles in water was sufficient to destroy most of it. Given the fact that loess soils are known for their very low structural stability (Le Bissonnais, 1996) and the high POC mineralisation rates we observed, we propose that the latter is more likely in our experiments. The results of our

220 last series of experiments (Fig. 5) also show, however, that, when SOC is introduced into an aquatic environment where no aquatic microbial community is present, there is also a significant degree of SOC decomposition. Again, this suggests that the simple

immersion into water results in the breakdown of the physical protection of SOC, so that a similar microbial community becomes much more effective in decomposing SOC in an aqueous rather than a soil environment.

The presence of an aquatic microbial community caused a much more rapid mineralization of SOC (Fig. 3, Fig. 4). In all experimental runs that we performed (n = 12), there was a significant difference observed between treatments with and without aquatic microbial organisms ($p < 0.05$). Given that the soil samples were oven-dried at ~55 °C before the incubation, the effect of inoculation with AMO may at least partly be explained by the fact that the soil microbial community was killed by the drying process. Comparing the results of oven-dried and air-dried soil without AMO addition, it is clear that oven drying indeed led to a 40 % decrease in the total amount of C mineralized in comparison to air dried soil, indicating that oven drying indeed eliminated an important fraction of OC-consuming soil microbial organisms (Fig. 5). However, the addition of AMO resulted in clearly higher C losses with both soil treatments (air drying: ca.70 %; oven drying: ca.165 %), indicating that aquatic microorganisms indeed have the capacity to rapidly consume SOC that is not readily mineralized by soil microorganisms. This is not surprising. It is well known that a significant fraction of the SOC is very old (Trumbore, 2000). The presence of such old fractions in the soil is only possible if the soil ecosystem does contain no or only a very small number of consumers capable of mineralizing this POC fraction. Clearly, some consumers in aquatic ecosystems do have this capability so that significantly more POC can be consumed. The higher POC consumption rates observed when an AMO is present may, of course, partly be due to the fact that more microbes were present in those experiments where AMO were introduced. We compared the initial population of bacteria with and without the addition of AMO. The addition of AMO led to 20–30 % more bacteria present in the water at the beginning of the experiment (arable land: 4.30×10^5 *vs.* 3.52×10^5 cells ml^{-1}; forest soil: 3.67×10^5 *vs.* 2.86×10^5 cells ml^{-1}). This larger initial population could partly explain the higher SOC decomposition rates with the addition of AMO, but the fact that the addition of AMO increases SOC decomposition rates by 70–165 % rather than 20–30 % does suggest that the aquatic microbial community is indeed capable of attacking old, stable SOC more effectively than the soil microbial community. Although the microbial community is considered to play a central role in shaping OC reactivity in both terrestrial and aquatic systems (Schmidt et al., 2011), such strong stimulation effect of the addition of AMO on SOC has rarely been reported.

The evolution of POC and DOC concentrations during the experiments is generally in agreement with the patterns derived from the oxygen consumption measurements. However, the variations in residual POC are less consistent than those observed from oxygen consumption. Several reasons might explain the discrepancy between the total C mineralisation as calculated from oxygen consumption in comparison to direct measurements of POC. Firstly, POC and DOC samples were collected from 4 incubation bottles for one treatment. Even though we controlled the initial conditions of each bottle as closely as possible, there might be heterogeneity between different bottles with respect to the OC content of the soil sample that was placed into the bottle. Secondly, we compared POC measurements from 2–5 filter papers for soil from arable land and 5 filter papers for forest soil. For each individual measurement filter weight has to be subtracted from the gross weight of the filter plus the sediment. Given the small quantities of sediment present on the filters, small weighing errors will result in relatively large errors in the calculation of the amount of POC that is remaining. Oxygen measurements are non-intrusive and are not subject to measurement errors related to the weighing of small quantities. We therefore believe that the oxygen consumption measurements provide us with more robust measurements of OC decomposition in comparison to direct measurements of the OC content of the remaining sample. The direct measurements suggest that, overall, POC mineralisation was more important than DOC mineralisation when POC was present. Indeed, DOC concentrations showed little variation during the experiments, despite significant oxygen consumption rates.

The increase of $\delta^{13}C_{POC}$ values during the first 24–48 hours suggests that during this period an isotopically lighter POC fraction was preferentially mineralised. This resulted in the POC in the aquatic environment becoming enriched in ^{13}C by 0.2–0.6 ‰ compared to the POC in the original soil sample. After the initial adjustment period, $\delta^{13}C_{POC}$ remained stable, suggesting the initial

adjustment is indeed due to the preferential consumption of a somewhat lighter, less recalcitrant POC-fraction rather than continued preferential consumption of lighter POC. The fact that, over the whole course of the experiments, the $\delta^{13}C_{DOC}$ values are lower than the corresponding $\delta^{13}C_{POC}$ values, is on the other hand, best explained by a continuous leaching/release of DOC with a

265   somewhat lower $\delta^{13}C$ signature from the soil POC, replacing the mineralised DOC as it is unlikely that the DOC fraction would be entirely stable while POC is continuously mineralised. Mineralisation of the original DOC and its replacement with soil-derived DOC could also explain the drop in $\delta^{13}C_{DOC}$ during the initial phase of the experiments with arable soil and AMO, because the lighter DOC that was originally present in the river water is replaced by soil-derived DOC. However, this drop was not observed in the experiments with forest soil. This may be partly explained by the higher $\delta^{13}C$ value of the forest soil (–28.6 ‰) in comparison

270   to the $\delta^{13}C$ signature of the arable soil (–29.4 ‰) causing the DOC released from the forest soil to have an isotopic signature close to that of the river water.

While the patterns described above would be consistent with the preferential decomposition of isotopically lighter POC, we did not observe an increase in $\delta^{13}C_{POC}$ during our experiments as might be expected by the selective mobilisation of an isotopically lighter soil fraction: this can be explained by the relatively small differences in $\delta^{13}C$ values between POC and DOC in combination

275   with the fact that only a small fraction of the POC is ultimately mineralised, whereby most of this mineralised fraction may have been directly transformed to CO_2. If this mineralization does not selectively affect specific fractions of the POC pool, the $\delta^{13}C_{POC}$ values can be expected to remain more or less constant throughout the incubation period.

Based on our findings, we propose that the relative importance of physical disturbance vs. exposure to a novel microbial community is likely to depend on (i) the level to which the SOC is indeed physically protected and (ii) the extent to which this protection is

280   destroyed when soil particles are introduced in river water. When the protection level is relatively important but, at the same time, sensitive to water immersion, further physical disturbance is unlikely to strongly increase SOC breakdown in aquatic conditions. If, on the other hand, physical protection is strong and resistant to immersion, physical disturbance may be necessary to break down soil aggregates to the extent that is needed to expose a significant fraction of SOC to the microbial community present in water (Fig. 8). The effect of the presence of an aquatic microbial community, on the other hand, will depend on its composition

285   and its vigour. The addition of SOC may shift aquatic microbial metabolisms and make it more prone to SOC decomposition (Lennon and Pfaff, 2005). Priming effects, whereby the exposure of old SOC to a different pool of microbes leads to increased SOC decomposition have also been documented in soil environments (Fontaine et al., 2007). External drivers such as water temperatures may play a role here. However, it is fair to state that our current understanding of the interaction between old SOC and different microbial communities is too limited to develop general principles describing which factors may stimulate or slow

290   the decomposition of SOC exposed to a new microbial community.

5 Conclusions

We investigated the relative importance of physical disturbance vs. exposure to a novel microbial community on SOC decomposition rates in aquatic environments. While some recent studies found that the impact of mechanical disturbance on SOC decomposition rates was very important, we found only a very modest increase in SOC decomposition when soil particles were

295   mechanically disturbed and kept in suspension. A simple conceptual model, whereby the effect of mechanical disturbance is assumed to depend on the initial structural stability of soil aggregates can explain this difference in findings: mechanical disturbance is only important when soil aggregates are strong enough to withstand the disruptive forces imposed by immersion in water.

300 Our study also highlights the role of aquatic microbial organisms in SOC decomposition in river systems. Aquatic microbial organisms are capable of attacking old, stable SOC, leading to rapid SOC decomposition in river systems. Given the variability of aquatic microbial community composition along different aquatic systems, understanding the linkage between aquatic microbial community composition and abundance on the one hand and the resultant SOC mineralization rates on the other hand would be important to better understand CO_2 outgassing in aquatic systems.

Data availability

305 All data used and produced through this study are available upon request.

Author contribution

GG, SB, LJ and MZ involved in the design of the experiments, and MZ carried out the experiments. SB and MZ conducted the laboratory analysis. All authors offered advice on the data analysis and contributed to the paper preparation.

Declaration of Competing Interest

310 The authors declared that there is no conflict of interest.

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Table 1. Experimental setup for incubations under controlled laboratory conditions

Treatment number	Treatments	Number of repetitions	Ingredients
1	SOC, rotation	6	soil + 0.7 μm filtered river water+ inoculum
2	SOC, stationary	6	soil + 0.7 μm filtered river water+ inoculum
3	DOC control, rotation	6	0.7 μm filtered river water+ inoculum
4	DOC control, stationary	6	0.7 μm filtered river water+ inoculum
5	SOC without AMO, rotation	6	soil + 0.2 μm filtered river water
6	SOC without AMO, stationary	6	soil + 0.2 μm filtered river water

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Table 2. Characteristics (texture, OC content, C/N ratio and δ^{13}C) of the two soil samples

Soil samples	soil texture		OC content (%)	C/N (weight/weight)	δ^{13}C (‰)
arable land	Sand %	69	2.40 %	9.9	−29.4
	Loam %	29			
	Clay %	3			
forest	Sand %	56	5.20 %	17.6	−28.6
	Loam %	40			
	Clay %	4			

Table 3. Total amount of C mineralized and POC, DOC loss at the end of the incubation series calculated as the difference in
weight that was introduced and the weight recovered from the final sample.

Soil type	Treatments	Total amount of C mineralized (derived from DO, mg)	POC loss (mg)	Percentage loss of POC (%)	DOC loss (mg)	POC+DOC loss (mg)
arable land soil	SOC+AMO/rotation	0.31 ± 0.09	0.50	13	0.04	0.54
	SOC+AMO/stationary	0.27 ± 0.06	0.33	9	0.10	0.43
	DOC control/rotation	0.15 ± 0.07	\	\	0.14	\
	DOC control/stationary	0.16 ± 0.05	\	\	0.24	\
	SOC-AMO/rotation	0.13 ± 0.04	0.21	5	−0.01	0.20
	SOC-AMO/stationary	0.14 ± 0.04	0.24	6	−0.01	0.23
forest soil	SOC+AMO/rotation	0.20 ± 0.03	0.34	11	−0.10	0.24
	SOC+AMO/stationary	0.18 ± 0.04	0.22	7	−0.04	0.18
	DOC control/rotation	0.11 ± 0.04	\	\	0.12	0.12
	DOC control/stationary	0.13 ± 0.08	\	\	0.11	0.11
	SOC-AMO/rotation	0.09 ± 0.03	0.03	1	−0.11	−0.08
	SOC-AMO/stationary	0.08 ± 0.02	0.16	5	−0.09	0.07
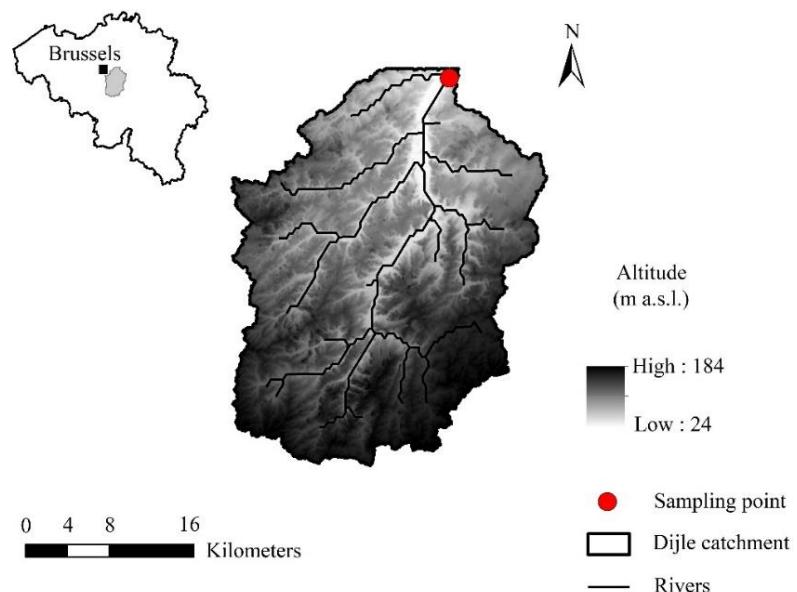
Figure 1. Location of Dijle catchment and the sampling point

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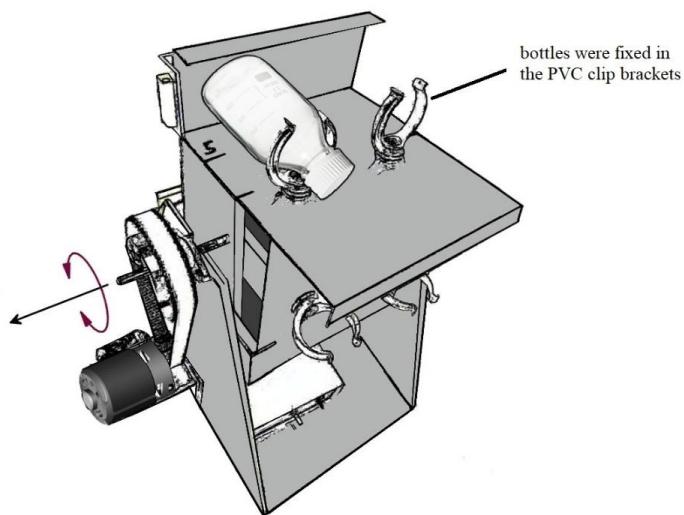
Figure 2. Schematic view of the swing system. The swing systems were placed in a temperature-controlled (20℃) incubation room

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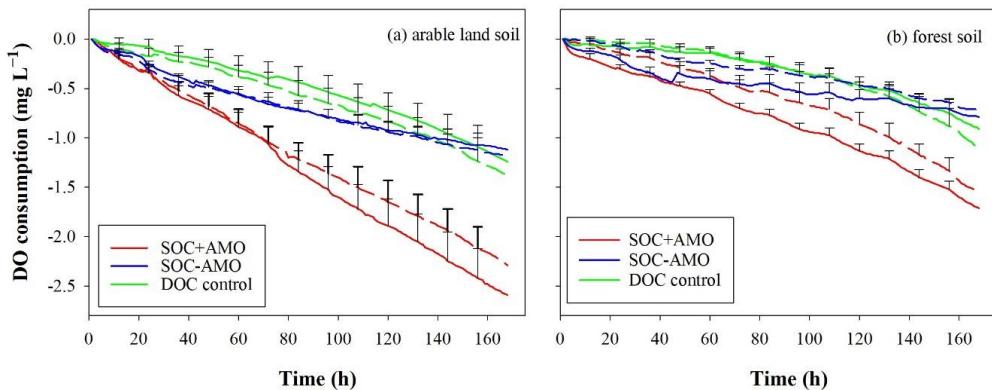
Figure 3. DO oxygen consumption of the SOC+AMO treatment (red), the SOC-AMO treatment (blue) and the DOC control treatment (green) during the 168h' incubations, under rotating (full lines) and standing (dashed lines) conditions: (a) incubation with soil from arable land (b) incubation with soil from forest Standard errors were corrected based upon 6 repetitions with different river water samples.

440
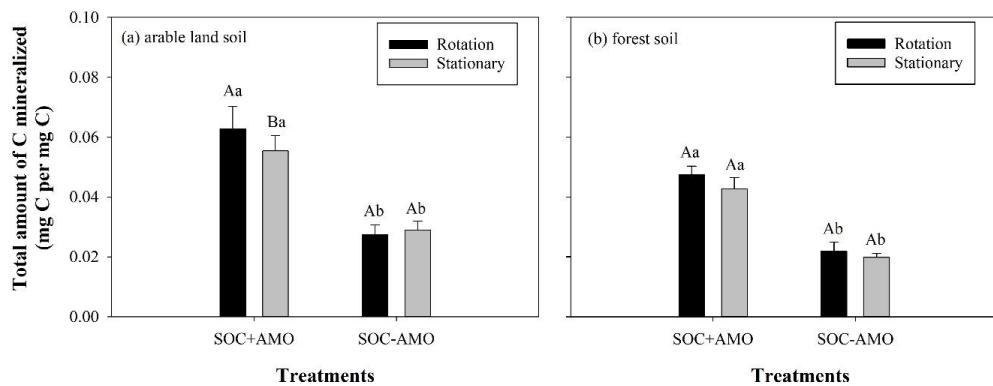
Figure 4. Total amount of C mineralized during the 168h' incubations with and without AMO under rotating and standing conditions with (a) soil from arable land (b) forest soils. Different capital letters indicate significant differences between treatments under rotation and stationary conditions at $p < 0.05$; different lowercase letters indicate significant differences between treatments with and without AMO at $p < 0.01$.

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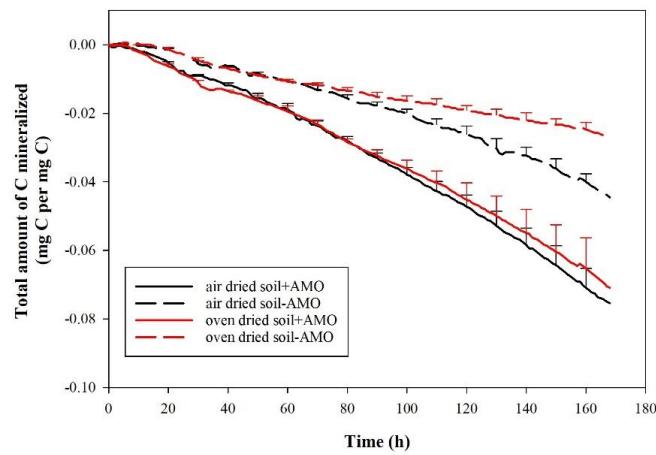
Figure 5. Cumulative amount of C mineralized during incubation with soil under two drying methods: a) oven dried soil (red); b) air dried soil (black), with AMO (full line) and without AMO (dashed line).
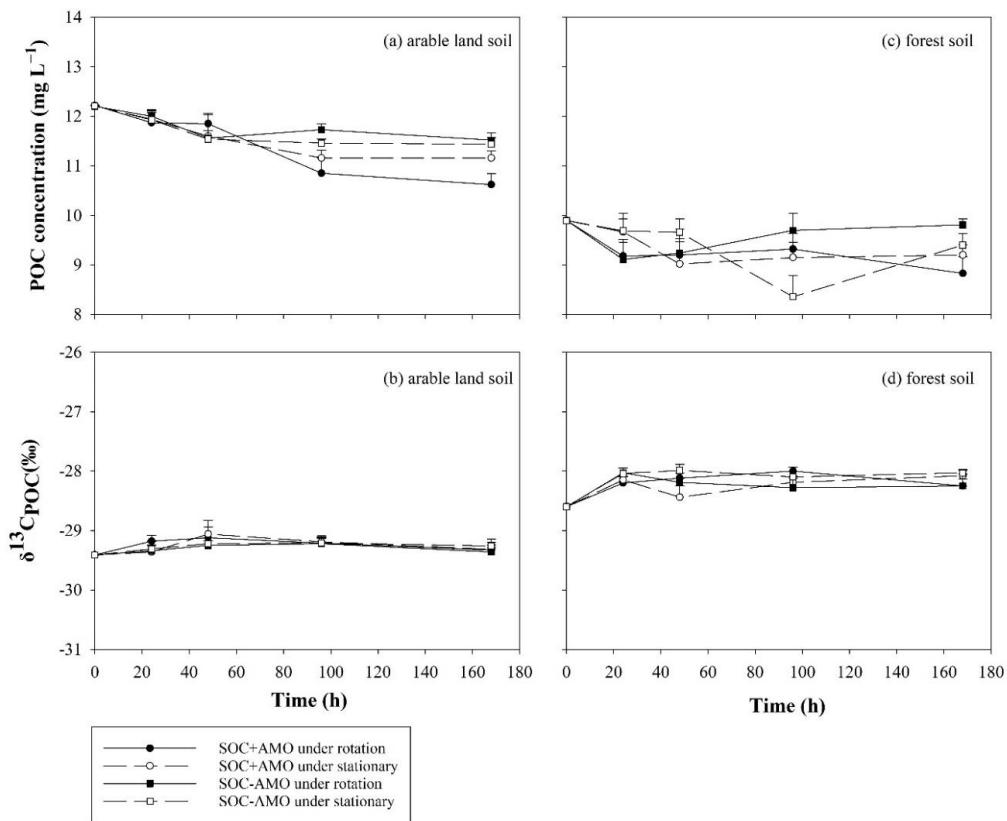
Figure 6. Evolution of POC concentrations and $\delta^{13}C_{POC}$ values during the incubations for the arable land soil (a and b respectively) and the forest

450   soil (c and d respectively).

Figure 7. Evolution of DOC concentrations and $\delta^{13}C_{DOC}$ values during the incubations for the arable land soil (a and b respectively) and the forest soil (c and d respectively).

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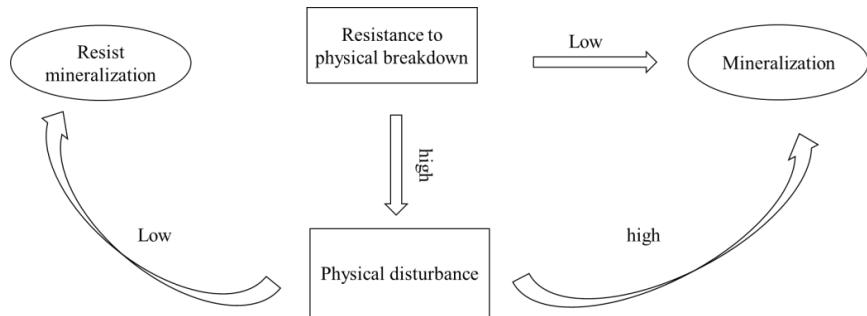
Figure 8. Conceptual figure representing the effect of physical disturbance on SOC decomposition process depending on the physical protection of SOC.