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# Organic Carbon Quality Impacts Benthic Microbial Nitrate Reduction

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1 **Title:** Organic carbon quality impacts benthic microbial nitrate reduction

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12  
13 **Abstract:**

14 Microbial nitrate reduction in wetland soils and sediments plays an important role mitigating the excess  
15 of nitrogen released into the environment. The reduction of nitrate to its reduced gaseous form, i.e. denitrification,  
16 is tightly linked to the oxidation of organic carbon. In intertidal wetlands, the organic carbon derived from aquatic  
17 or terrestrial primary producers strongly differs in composition, which in turn might affect nitrate reduction rates.  
18 In the current study the impact of carbon quality on benthic nitrate reduction in a temperate intertidal wetland  
19 sediment was determined. To this end, nitrate reduction rates were determined over a three-week period using an  
20 anoxic controlled flow-through reactor approach using sediments amended with carbon from different vegetation  
21 types. Organic carbon from algal or plants was mixed with the sediment at the same absolute quantity of carbon.  
22 A significant initial increase in nitrate reduction rates was observed in the presence of algal (microphytobenthos)  
23 derived organic carbon, most likely due to its labile structure; however, rates decreased rapidly, most likely due to  
24 a depletion of the labile source. This indicates the dependence of denitrifiers to the microalgae turnover in non-  
25 vegetated sediment. Despite a delay, the addition of plant derived organic carbon, increased nitrate reduction rates  
26 with a higher amount of total nitrate reduced over the duration of experiment compared to the microphytobenthos  
27 addition. These results highlight that presence of both labile and recalcitrant carbon sources in sediment is essential  
28 to support an efficient nitrogen biogeochemical cycle.

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30  
31 **Keywords:** Denitrifying microbes; Microphytobenthos; Nitrate reduction rates; Plants; Wetlands

32  
33 **Declarations:**

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37 + LA/P/0094/2020)

38  
39 **Conflicts of interest/Competing interests**

40 The authors have no conflicts of interest to declare that are relevant to the content of this article.

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**Availability of data and material**

All relevant data are within the manuscript or in supplementary material

**Code availability**

Not Applicable

**Authors' contributions**

JM performed conception and design of this study, material preparation, sample collection, analyzes, interpretation of data, and wrote the first draft of the manuscript. LJ performed analyzes, interpretation of data, and contributed to the manuscript. AML performed conception and design of this study, material preparation, analysis, interpretation of data and contributed to the manuscript. All authors read and approved the final manuscript.

**Ethics approval**

The prefectural decree n ° ME/2020/09 was established to authorize the samplings within the national reserve carried out for this study (Project FEREE). No further authorization was required, and this field study did not involve endangered or protected species.

**Consent to participate**

All authors agreed to participate in this research study

**Consent for publication**

All authors agreed to published this research study

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74 **1. Introduction**

75 In aquatic ecosystems, nitrogen pollution is a major ecological threat that can lead to environmental  
76 degradations and notably eutrophication (Granéli et al. 2008; Sardans et al. 2012). Eutrophication events have  
77 increased worldwide over the last three decades and are a major environmental, health and economic concern  
78 (Sanseverino et al. 2016). The sources of nitrogen pollution and its consequences including the dynamics of  
79 eutrophication have been studied extensively (Anderson et al. 2002; Galloway et al. 2003). Whereas, reducing  
80 anthropogenic nitrogen pollution at the source is crucial, mitigation of nitrogen pollution can also be achieved by  
81 optimizing and stimulating biological nitrate elimination.

82 The biological nitrate reduction processes performed by bacteria and archaea plays a crucial role in nitrogen  
83 elimination. These processes occur in anoxic environments in presence of nitrates. Among them, the dissimilatory  
84 nitrate reduction to ammonium: DNRA (Tiedje 1988; Decleyre et al. 2015) transforms nitrate into ammonium but  
85 retains inorganic nitrogen in the system. In comparison, denitrification (Tiedje et al. 1983) and anaerobic  
86 ammonium oxidation: anammox (Van de Graaf et al. 1995; Strous et al. 1999; Kuenen 2008) allow the complete  
87 reduction of nitrate into inert dinitrogen (N<sub>2</sub>) gas, eliminating nitrogen from the system. Denitrification has been  
88 shown to be dominant over anammox in coastal sediments (Zhou et al. 2014). Whereas anammox is an autotrophic  
89 process, DNRA and denitrification are heterotrophic processes for which the carbon present in sediment plays a  
90 key role. In addition to carbon quantity (Starr and Gillham 1993), carbon quality was also shown to be crucial  
91 regarding nitrate reduction capacities (Hume et al. 2002; Sirivedhin and Gray 2006; Dodla et al. 2008; Stelzer et  
92 al. 2014). Indeed, the carbon quality influences the major parameters of nitrate reduction such as the reduction  
93 rates, the dissolved organic carbon demand, and the bacterial community structure (Lee and Welander 1996; Obaja  
94 et al. 2005).

95 Intertidal wetlands represent ecosystems with all conditions required for intense microbial nitrate reduction.  
96 Due to the sediment submersion, anoxic conditions prevail. Furthermore, nitrate concentrations are often elevated  
97 due to intensive agricultural and industrial activities in upstream watersheds (McLusky and Elliott 2004; Barbier  
98 and Hacker 2011). Large amounts of allochthonous organic carbon with aquatic and terrestrial origin settle on  
99 wetland soils, this includes carbon transported by rivers, tides, and the leaching of the upstream lateral zone during  
100 rain events (Bouillon and Boschker 2006). In addition, there is a large variability in autochthonous organic carbon  
101 quality since the mosaic of habitats due to several gradients related to tidal working (salinity, immersion) result in  
102 different plant communities in soils (Ehrenfeld et al. 2005). The wetlands soil organic carbon thus covers a large  
103 variability in composition and degradability, ranging from labile (e.g. microalgae) to less degradable sources (e.g.  
104 aquatic and terrestrial plants). These variability in wetland's soil organic carbon quality is likely to alter the  
105 microbial community structure (Phillips et al. 2002; Waldrop and Firestone 2004; Bastian et al. 2009) and  
106 consequently the decomposition activity (Fontaine et al. 2011; Wang et al. 2014), as specific microbial groups  
107 control the decomposition of labile and recalcitrant carbon pools (Waldrop and Firestone 2004; Fontaine and Barot  
108 2005). Through its availability and characteristics, the organic carbon related to vegetation type can thus influence  
109 wetland's nitrate reduction capacities. The plant species variation was shown to play an important role in  
110 determining nitrate reduction potential (Caffrey and Kemp 1990; Ehrenfeld 2003; Windham and Ehrenfeld 2003;  
111 Alldred and Baines 2016) and denitrification capacity (Bastviken et al. 2005, 2007; Fernandes et al. 2016) in  
112 sediments. Previous studies highlight that presence of microalgae resulted in significantly higher microbial nitrate  
113 reduction (Morelle et al. 2022) and that the carbon from plants increased the denitrification potential by showing

114 a negative correlation with their cellulose, hemicellulose and lignin contents (Hume et al. 2002; Chen et al. 2011).  
115 A recent study put forward that presence of microalgae appears to be an important driver of nitrate reduction rates  
116 in riparian sediments although plant communities predominate (Laverman et al. 2021). It therefore appears that  
117 the quality of the carbon supply in a sediment will define its nitrate reduction potential, which would be negatively  
118 correlated with its degradability. However, this assumption is based on studies that were conducted separately,  
119 with different methods, and with different plant-specific sediment types, making it unclear that only carbon quality  
120 is involved and leaving the extent of its impact still unknown.

121 The aim of this study was to determine how carbon quality affects the nitrate reduction rates in a temperate  
122 intertidal wetland sediment. To this end, four subsamples of a non-vegetated sediment were supplied with the same  
123 amount of carbon from different autochthonous carbon sources presenting different C:N ratios which is accepted  
124 as an index of organic matter quality (Nicolardot et al. 2001). We hypothesize that in the presence of the same  
125 absolute amount of organic carbon, the microalgae-derived organic carbon (lower C:N ratio), easier to degrade  
126 and more available than plants-derived organic carbon (higher C:N ratios), will result in a higher nitrate reducing  
127 activity. The potential nitrate reduction rates were monitored for 18 days using an experimental approach with  
128 flow through reactors. The difference in carbon quality between plants was assessed through their elemental  
129 contents (carbon - C<sub>org</sub>, nitrogen - N, C:N ratio) and composition (i.e. lipids, polysaccharides, lignin and tannin).  
130 To establish the impact of the carbon additions on the degradation activity, external enzymatic activities were also  
131 determined at the end of the experiment. This study shows how the difference in carbon quality affects the  
132 microbial nitrate reduction rates with time and highlights the importance of both microalgae and plants as organic  
133 matter sources for this ecological functionality of growing interest.

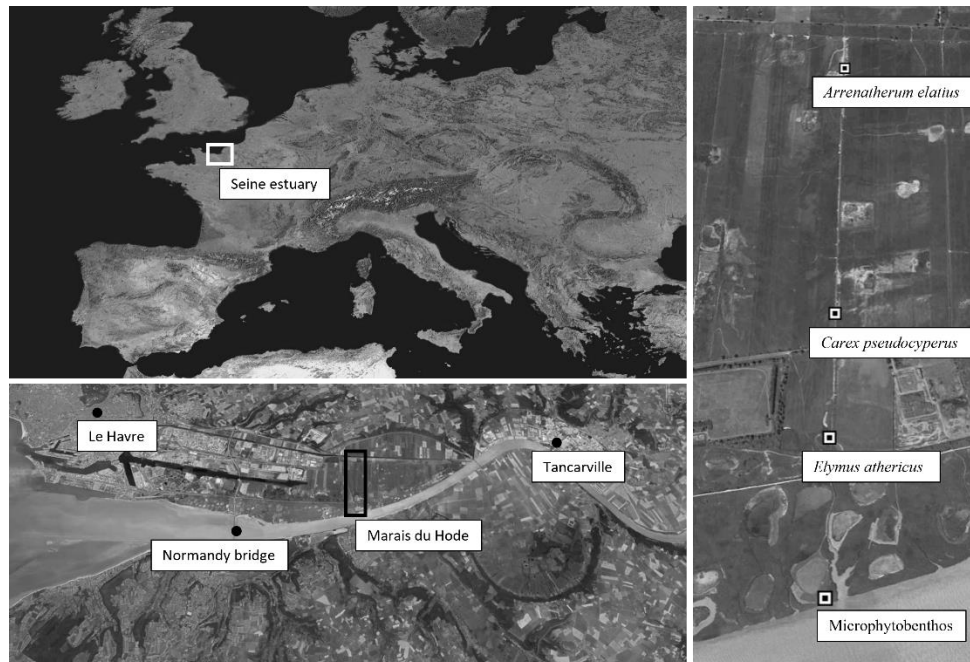
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## 135 2. Material and methods

### 136 2.1. Sampling

137 The sediment and the different carbon sources used for this experimental study were sampled in the Northern  
138 intertidal area of the downstream Seine estuary and its associated wetland (“Marais du Hode”, fig. 1). Four areas  
139 located at different lateral distances from the river which presented different dominant plant species were selected  
140 and sampled in September 2020. At the riverbank (0 m), biofilms of microphytobenthos were collected while  
141 individuals of *Elymus athericus* were collected at 150 m, *Carex pseudocyperus* at 700 m, and *Arrenatherum elatius*  
142 at 2000 m further into the wetland. Microphytobenthos was obtained by gently taking the top 2 mm of the sediment  
143 surface covered with a natural microphytobenthic biofilm and on average ten individuals (stems, leaves) of each  
144 plant species were collected from the areas corresponding to their dominance (fig. 1). For each of the three plant  
145 species selected, stems and leaves were conserved together, freeze-dried (24 h) and ground using a planetary mill  
146 (model PM100, Retsch®) to obtain a dry powder. For microphytobenthic biofilms, samples were totally freeze-  
147 dried (24 h) before being ground using a ball mill (model MM400, Retsch®). Thereby, the used form of  
148 microphytobenthos corresponds to a biofilm/sediment mix originating from the top of a colonized sediment  
149 surface. The sediment used for the experiment was sampled in March 2021 by taking the top 2 cm of a non-  
150 colonized sediment surface from the intertidal mudflat allowing to avoid presence of roots. The fresh sediment  
151 was conserved at 4°C before experiment. After homogenization, an aliquot of fresh sediment (5 ml) was weighted  
152 and freeze-dried (24-h) for initial sediment characterization. The water content ( $\omega$ ; %) was subsequently  
153 determined as a percentage of water relative to the total dry weight. One replicate of freeze-dried sediment was

154 conserved for particle grain size determination, and another was ground using a ball mill (model MM400,  
155 Retsch®). The ground samples of the 4 organic carbon sources and of the sediment were characterized in terms of  
156 elemental concentrations ( $C_{org}$  and N), and molecular composition (markers of lipids, polysaccharides, lignin and  
157 tannin) before the experiment.  
158



159  
160 **Figure 1** Map of the sampling sites, located in the wetland “Marais du Hode” (Seine estuary, Normandy, France:  
161 49°27’01.6’’N - 0°12’20.0’’E, WGS84). According to the dominant species along the transect from river to saline meadow,  
162 the microphytobenthos biofilms were sampled at the riverbank (0 m from the river), *Elymus athericus* at 150 m, *Carex*  
163 *pseudocyperus* at 700 m, and *Arrenatherum elatius* at 2000 m further from the riverbank.  
164

## 165 2.2. Structural Characterization analyses

166 The particle grain size distribution was determined by laser diffraction using a Malvern particle size analyzer  
167 in combination with a liquid sample autosampler. The mud sediment fraction was estimated as the percentage of  
168 silt particles < 63  $\mu\text{m}$  (% of fine particles).

169 Elemental concentrations of organic carbon ( $C_{org}$ ) and nitrogen (N) were measured in sediment and in the  
170 different dry powders using an elemental analyzer (model Vario Pyro Cube, Elementar®). Before  $C_{org}$   
171 determination, samples were acid-treated with 2N HCl to remove the carbonate, rinsed with deionized water, dried  
172 at 60 °C, and ground.

173 Polysaccharides were extracted from 0.5g for microphytobenthos and from 0.01g for plants dry powders in 5  
174 ml of distilled water (MilliQ) after 1 hour of incubation at 35°C under continuous mixing (30 rpm). After  
175 centrifugation (4 °C, 3000 rpm, 10 minutes), supernatants were collected for determination of the carbohydrates  
176 and protein contents. Total carbohydrates content was determined using the phenol–sulfuric acid assay with  
177 glucose as a standard (Dubois et al. 1956) and proteins content was determined using the Bradford assay reagent  
178 (Bio-Rad) with bovine serum albumin (BSA) from Sigma-Aldrich as standard (Bradford 1976). For carbohydrates,  
179 absorbance was read, upon incubation of the samples with reagent for 40 minutes at room temperature, using a  
180 Uvikon XL© spectrophotometer (Secomam, Aqualabo, France) at 485 nm. For proteins, absorbance was read,

181 after that assayed samples have been incubated with reagent during 5 minutes at room temperature, using a  
182 microplate reader SAFAS Xenius XL© (SAFAS Monaco) at 590 nm.

183 The molecular composition of the different carbon sources was assessed by performing thermochemolysis  
184 using tetramethylammonium hydroxide coupled to gas chromatography and mass spectrometry (THM-GC-MS)  
185 according to Jeanneau et al. (2014). Briefly, approximately 1mg of freeze-dried microphytobenthos or plants was  
186 introduced into an 80  $\mu$ L aluminum reactor with an excess of solid tetramethylammonium hydroxide (ca. 3 mg).  
187 The THM reaction was performed on-line using a vertical micro-furnace pyrolyser PZ-2020D (Frontier  
188 Laboratories, Japan) operating at 400 °C. The products of this reaction were injected into a gas chromatograph  
189 GC-2010 (Shimadzu, Japan) equipped with a SLB 5MS capillary column in the split mode (60 m  $\times$  0.25 mm ID,  
190 0.25  $\mu$ m film thickness). The temperature of the transfer line was 321°C and the temperature of the injection port  
191 was 310 °C. The oven was programmed to maintain an initial temperature of 50 °C for 2 minutes, following an  
192 increase to 150 °C at 15°C min<sup>-1</sup>, a subsequent increase to 310 °C at 3 °C min<sup>-1</sup> where it remained for 14 minutes.  
193 Helium was used as the carrier gas, with a flow rate of 1.0 ml min<sup>-1</sup>. Compounds were detected using a QP2010+  
194 mass spectrometer (Shimadzu, Japan) operating in the full scan mode. The temperature of the transfer line was set  
195 at 280 °C, the ionization source at 200 °C, and molecules were ionized by electron impact using an energy of 70  
196 eV. The list of analyzed compounds and mass/charge (m/z) ratios used for their integration are given in the  
197 supplementary materials (table S1). Compounds were identified based on their full-scan mass spectra by  
198 comparison with the NIST library and with published data (Nierop and Verstraten 2004; Nierop et al. 2005).

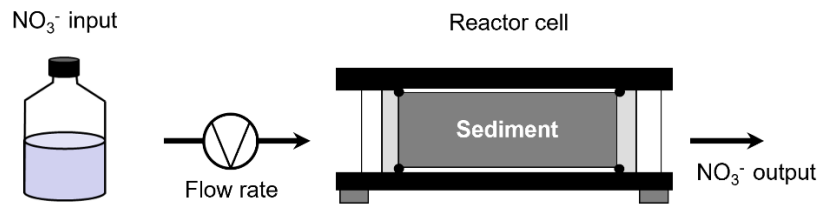
199 Target compounds (151 compounds) were classified into three categories: lipids including fatty acids with a  
200 number of C atoms from 6 to 32 and fatty alcohols including phytol; phenolic compounds including lignin and  
201 tannin markers; and the markers of carbohydrates. The peak area of the selected m/z for each compound was  
202 integrated and corrected by a mass spectra factor calculated as the reciprocal of the proportion of the fragment  
203 used for the integration and the entire fragmentogram provided by the NIST library (table S1). The proportion of  
204 each compound class was calculated by dividing the sum of the areas of the compounds in this class by the sum  
205 of the peak areas of all analyzed compounds expressed as a percentage. The analytical uncertainty for this  
206 analytical method, expressed as a relative standard deviation, ranged from 10 to 20% depending on the samples  
207 and the target compounds. Each sample was analyzed once. For each sample, the results of ten theoretical analyses  
208 were calculated by randomly drawing, for each molecule, ten theoretical area values in the 20% range around the  
209 measured value. The function RANDBETWEEN of Excel was applied.

210

### 211 **2.3. Flow through reactor (FTR) experiment**

212 To evaluate the impact of the different organic carbon sources on the bacterial activity in the sediment, an  
213 experiment in flow through reactors was conducted (fig. 2). Five distinct conditions including the control were  
214 performed in triplicate resulting in 15 reactors. To this end, each organic carbon source was added in the form of  
215 a freeze-dried powder to a subsample of homogenized fresh sediment and well mixed. Differences between  
216 conditions resulted from the different quality of the added carbon while the control corresponded to the natural  
217 sediment. The weight of dry powder inoculated into the sediment corresponded to a 4% addition of organic carbon  
218 per reactor and was calculated using the results of the previously described elemental analyses. This corresponded  
219 to 4.4g of dry powder added to sediment for the microphytobenthos condition, 0.82g for *Elymus athericus*  
220 (*Elymus*), 0.87g for *Carex pseudocyperus* (*Carex*), and 0.9g for *Arrhenatherum elatius* (*Arr.*); for a total amount of

221 150g inoculated sediment per condition. For each condition, the homogenized inoculated sediment was equally  
222 distributed into three Plexiglass® rings (inner diameter: 4.2 cm; height: 2 cm). A 0.2 µm pore size PVDF  
223 (Durapore®) membrane filter and a glass fiber backing filter (1.2 mm thick, 4.7 cm diameter, PALL Corp., NY)  
224 were then placed over the sediment surface at both sides of each Plexiglas® ring. The resulting sediment reactor  
225 cells were then enclosed by POM (poly-oxy methylene) Delrin® caps with inflow and outflow channels. A detailed  
226 description of the FTR design and the determination of potential rates can be found in (Laverman et al. 2006,  
227 2012).  
228



229 **Figure 2 Schematic overview of the flow through reactors (FTR) allowing the determination of nitrate reduction rates.**  
230 The 2 cm reactor cell contains sediment of known volume ( $V = 27.7 \text{ cm}^3$ ) and is supplied with an anoxic inflow solution  
231 containing nitrate ( $\text{NO}_3^-$  input), at a known flow rate. The dried and ground plant and algal materials were separately mixed  
232 within different sub-samples of the same sediment and placed in the reactor cells  
233  
234

235 To ensure nitrate availability all along the experiment, each condition was supplied by an inflow solution  
236 containing potassium nitrate ( $\text{KNO}_3^-$ ) at a concentration of 5 mM. The salinity of the inflow solutions was adjusted  
237 at 5 with NaCl to match the salinity measured in water near the sampling sites. In addition, the inflow pH was  
238 adjusted to match the ambient conditions ( $\text{pH} = 8$ ) using  $\text{NaHCO}_3$  ( $0.2 \text{ g L}^{-1}$ ). All input solutions were kept anoxic  
239 by purging them with  $\text{O}_2$ -free  $\text{N}_2$  during at least 5 minutes before the start of the experiment. All the experiments  
240 were run at constant temperature ( $21 \pm 2 \text{ }^\circ\text{C}$ ) and protected from light to avoid photosynthesis and consequent  
241 oxygen production during the experiment.

242 The 15 reactors were supplied by the input solutions at a constant flow rate ( $Q = 2 \pm 0.15 \text{ mL h}^{-1}$ ) using a  
243 peristaltic pump (Gilson minipuls) allowing the sediments to be saturated with the saline anoxic nitrate solution  
244 during the entire duration of the experiment (18 days). The reactor outflow samples were collected at 4 hours  
245 intervals and stored at  $4^\circ\text{C}$  until analysis. The samples collected each day were analyzed for ammonium ( $\text{NH}_4^+$ ),  
246 nitrite ( $\text{NO}_2^-$ ), and nitrate ( $\text{NO}_3^-$ ) concentrations using a Gallery™ Discrete Analyzer (ThermoFisher scientific,  
247 Waltham, Massachusetts, USA) within 2 days of collection.

248 At the end of the experiment, a sub-sample of  $2.75 \pm 0.02 \text{ g}$  of fresh sediment from each reactor was used for  
249 the determination of the potential external enzymatic activities.

250 The nitrate reduction rates and the nitrite and ammonium production rates in the flow-through reactors ( $\text{nmol}$   
251  $\text{cm}^{-3} \text{ h}^{-1}$ ) were calculated using the equation  $R = (\Delta C \times Q) / V$  where  $Q$  is the volumetric flow rate,  $V$  the reactor  
252 volume ( $27.7 \text{ cm}^3$ ) and  $\Delta C$  the absolute difference in concentration of the dissolved nitrogen between the inflow  
253 solution and the outflow sample. Thereby, nitrate reduction rates were calculated by substituting the nitrate  
254 concentration from the outflow to the inflow solution. As ammonium and nitrite were not supplied throughout the  
255 inflow solutions, their production rates were determined using the outflow concentrations.

256



## 2.4. External Enzymatic Activities

The potential external enzymatic activities of four soil enzymes were measured based on the method described by Maxwell et al. (2020). The enzymes measured were those which catalyze the degradation of organic carbon that convert cellulose into cellobiose (1,4- $\beta$ -D-cellobiohydrolase) and cellobiose into glucose ( $\beta$ -1, 4-glucosidase), that hydrolyze the N-terminal leucyl groups (L-leucine aminopeptidase), and that hydrolyze the organic phosphates (acid phosphatase). Briefly, each  $2.75 \pm 0.02$  g of sediment was homogenized in 91 mL of 50 mM sodium acetate buffer. Then, 120  $\mu$ L of the resulting sediment solution (SS) was inserted in a black flat-bottomed 96-well plate in two technical replicates per reactor (6 replicates per conditions). The wells were incubated with fluorometric substrates for 3 h at 25 °C with a resuspension of the mixture within the wells performed every 30 min using an eight-channel pipette. Additional quench control replicates of SS, 4-methylumbelliferone or 7-amino-4-methylcoumarin standard curves (60  $\mu$ L of respectively 0–100  $\mu$ M and 0–10  $\mu$ M concentrations) and controls without substrate addition (120  $\mu$ L SS + 60  $\mu$ L Milli-Q water), were included for each sediment sample. The fluorescence was measured by a microplate reader (SAFAS Xenius XM, SAFAS Monaco) using an excitation wavelength at 365 nm and emission wavelength at 450 nm. From the fluorescent values and standard calibration curves, each enzyme activity was calculated and converted in  $\text{nmol g}^{-1}$  dry sediment  $\text{h}^{-1}$ .

## 2.5. Statistical analyses.

The potential significant variability of measured parameters between the conditions was tested by one-way analysis of variance (AOV) using the software Sigma-Plot v12 or R v4.0.2. Shapiro-Wilk normality tests and Bartlett equal variance tests were used, and in the non-parametric cases, AOV on ranks or Kruskal Wallis tests were performed. Each test was followed by a Pairwise Multiple Comparison Procedures (Dunn's Method) or a Tukey test to discriminate differences between conditions.

To evaluate the significance of rate variabilities along the experimental time and the correlations between the measured parameters, Pearson correlation coefficients were calculated, and the corresponding  $P$  assessed. Variables with a significant positive correlation ( $P < .05$ ) tend to increase together while with a significant negative correlation they tend to decrease together. In case of non-significance ( $P > .05$ ), the correlation coefficient was not considered (NS) and the exact  $P$  was given.

The statistical analyses performed on the molecular composition of the organic carbon sources were performed using R v4.1.1. First the list of target compounds was reduced by keeping the molecules with a mean relative proportion higher than 0.5 %. The set of molecules was reduced from 112 to 55. Then a first principal component analysis (PCA) was performed using the relative proportions of the 55 target compounds as variables. The variables with a correlation lower than 0.5 with the two first factors were removed, resulting in a new set of 52 variables. This final list was used to calculate a new PCA and to perform a clustering. PCA and clustering were calculated with the package FactoMineR.

## 3. Results

### 3.1. Initial characterization of the sediment and the organic carbon sources

The natural sediment used in the experiment consisted of 55.8% of fine particles ( $< 63\mu\text{m}$ ), and a water content of 42.5%. The initial sediment contained  $62.2 \text{ mgC g}^{-1}$  and  $2.02 \text{ mgN g}^{-1}$  leading to a C:N ratio of 36.3. The analyses of elemental concentrations showed that microphytobenthic biofilms presented significantly lower total

297 contents of carbon and nitrogen ( $77.8 \pm 0.9 \text{ mgC g}^{-1}$  and  $5.9 \text{ mgN g}^{-1}$  respectively) on a weight basis than the three  
 298 plants (table 1; AOV –  $P < .001$ ). The plants showed similar contents of total carbon but significantly different  
 299 contents of total nitrogen (table 1; AOV –  $P < .001$ ) led to significantly distinct C:N ratios ranging from 15.5 for  
 300 microphytobenthos to 73.5 for *Carex* (table 1; AOV –  $P < .001$ ).

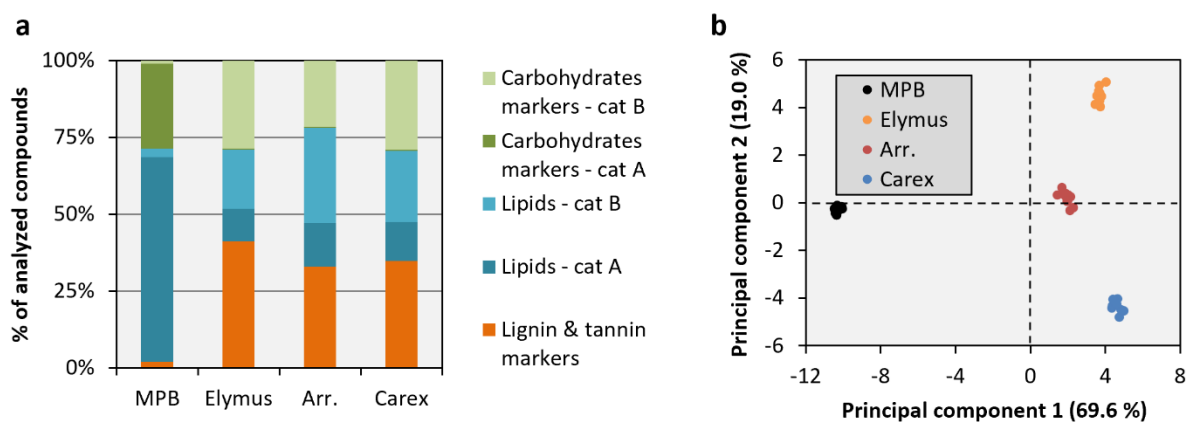
301 The polysaccharide analyses showed significant differences in carbohydrate contents (table 1; AOV –  
 302  $P < .001$ ) with the lowest values for microphytobenthos ( $4.53 \pm 0.04 \text{ mg g}^{-1}$ ) and the highest for *Carex* ( $53.71 \pm$   
 303  $1.63 \text{ mg g}^{-1}$ ). Regarding the protein contents, the microphytobenthos showed significantly lower contents than  
 304 plants (table 1; AOV –  $P < .001$ ) while no significant differences were recorded between plants (table 1; AOV –  
 305  $P > .6$ ).

306  
 307 **Table 1 Characterization of the organic carbon sources added in the different reactors.** Values are presented  
 308 in average (+SD) (n=5 for microphytobenthos and n=3 for plants) for total organic carbon ( $C_{\text{org}}$ ) and nitrogen (N)  
 309 concentrations ( $\text{mg g}$  of dry sediment $^{-1}$ ), C:N ratios (molar:molar), and polysaccharides contents for carbohydrates  
 310 and proteins ( $\text{mg g}$  of dry sediment $^{-1}$ ). For each parameter, the associated letters illustrate the statistical differences  
 311 between conditions after AOV and pairwise comparison tests

| Organic carbon source       | Elemental content ( $\text{mg g}^{-1}$ ) |              | C:N ratio    | Polysaccharides content ( $\text{mg g}^{-1}$ ) |             |
|-----------------------------|--|--------------|--------------|--|-------------|
|                             | $C_{\text{org}}$                         | N            |              | Carbohydrates                                  | Proteins    |
| Microphytobenthos           | 77.8 (0.9) a                             | 5.9 (0.1) a  | 15.5 (0.2) a | 4.5 (0.0) a                                    | 0.1 (0.0) a |
| <i>Arrenatherum elatius</i> | 380.7 (0.1) b                            | 12.5 (0.1) d | 35.5 (0.3) b | 35.4 (0.6) b                                   | 1.3 (0.1) b |
| <i>Elymus athericus</i>     | 416.7 (4.8) c                            | 9.1 (0.2) c  | 53.7 (0.5) c | 41.0 (0.2) c                                   | 1.1 (0.1) b |
| <i>Carex pseudocyperus</i>  | 391.8 (7.0) b                            | 6.2 (0.1) b  | 73.5 (0.7) d | 53.7 (1.6) d                                   | 1.2 (0.2) b |

312  
 313 The analysis of the molecular composition of organic carbon sources by THM-GC-MS showed differences  
 314 between the four sources (fig. 3). The largest difference was between microphytobenthos and plants (fig. 3a) that  
 315 was quantified by the first PCA component which represented 69.6% of the variance (fig. 3b). The analysis of  
 316 microphytobenthos mainly produced lipids with less than 20 C atoms in their hydrocarbon chain (70% of the  
 317 analyzed compounds) and particularly  $nC_{16:0}$  (29%) and  $nC_{16:1}$  (27%). Polysaccharides represented 28% of the  
 318 analyzed markers and were mainly desoxy aldonic acids produced by hexose (27%). The analysis of the three  
 319 plants produced similar distributions of target compounds with important proportions of the markers of lignins and  
 320 tannins (from 33 to 41%) and of lipids (from 30 to 45%). The distributions of lipids were characterized by the  
 321 occurrence of fatty acids with more than 20 C atoms in their hydrocarbon chain,  $\omega$ -hydroxy acids (from  $nC_{20}$  to  
 322  $nC_{28}$ ), polyhydroxy acids (10,16-diOHC $_{16}$  and 9,10,18-triOHC $_{18}$ ), fatty alcohols (from  $nC_{26}$  to  $nC_{32}$ ) and the  
 323 absence of  $\alpha,\omega$ -diacids. This distribution is typically of cutin origin (Del Río and Hatcher 1998). The markers of  
 324 polysaccharides represented from 22 to 29% of the analyzed compounds and were dominated by two isomers of  
 325 permethylated hexapyranose (from 6 to 12%) and methylcyclopentanedione (4%). Unlike the analysis of  
 326 microphytobenthos, desoxy aldonic acids, produced by the analysis of free and terminal monosaccharides (Fabbri  
 327 and Helleur 1999), were almost absent. Moreover, this type of distribution of polysaccharides markers has already  
 328 been described in wood analysis (Challinor 1995). This could mean that the terminal monosaccharides of  
 329 polysaccharides were not available to react with the tetramethylammonium hydroxide during the  
 330 thermochemolysis and that they were implied in a chemical bound in larger biomolecules such as lignocelluloses.

331 Although the three plants have similar distributions, they differentiated by the clustering mainly along the second  
 332 PCA component that represented 19 % of the variance of the dataset (fig. 3b).  
 333



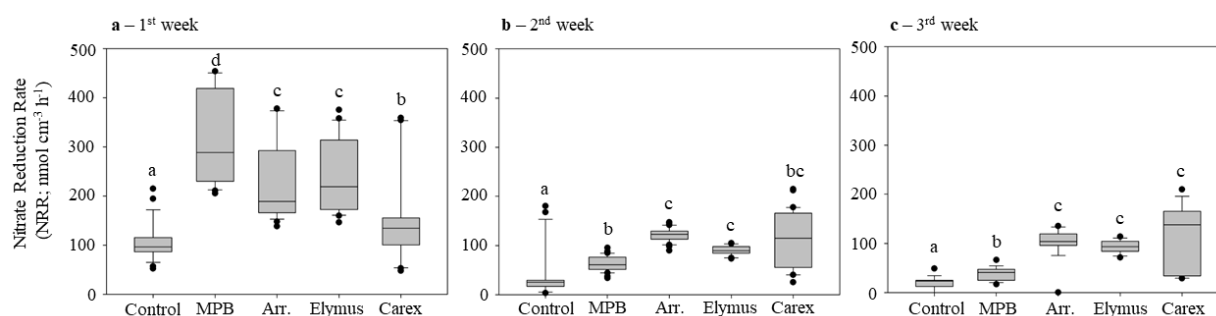
334  
 335 **Figure 3 Diversity of the molecular composition of organic carbon sources.** (a) Distribution of the target compounds in  
 336 five chemical classes: Lignin and tannin markers (orange), Lipids (blue) that are differentiated into category A: fatty acids  
 337 with less than 20 C atoms and category B: fatty acids with more than 20 C atoms, hydroxyacids and fatty alcohols and finally  
 338 Carbohydrates (green) that are differentiated into category A: desoxyaldonic acids and category B: all the other carbohydrates  
 339 markers. (b) Projection of organic carbon sources in the plane formed by the first two principal components calculated with  
 340 the distribution of the target molecules  
 341

### 342 3.2. Dynamics of the nitrate reduction rates

343 During the first week, nitrate reduction rates in the natural sediment showed little variation with on average  
 344  $105 \pm 25 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$  (fig. 4a). In comparison, the amendment of plant and algal biomass to the sediment  
 345 led to a significant increase of the nitrate reduction rates from the beginning. The nitrate reduction rates were  
 346 significantly higher in the presence of microphytobenthos with values up to  $311 \pm 78 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ , whereas  
 347 they were significantly lower with Carex up to  $151 \pm 66 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ . Elymus and Arr. Showed intermediate  
 348 values with nitrate reduction rates up to  $231 \pm 58$  and  $223 \pm 66 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$  respectively. Therefore, the  
 349 quantity of  $\text{N-NO}_3^-$  reduced during the first week was the highest upon microphytobenthos addition and lowest  
 350 after Carex addition (table 2). For each amended sediment, the highest nitrate reduction rates were recorded the  
 351 first day of the experiment and followed by a rapid and significant decrease over the first week (Supp. fig. 1a).

352 During the second week, the non-amended control still showed the lowest nitrate reduction rates with on  
 353 average  $41 \pm 35 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ . Unlike during the first week, microphytobenthos showed lower nitrate  
 354 reduction rates ( $63 \pm 14 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ ) than the other plant amended sediments (fig. 4b). Nitrate reduction  
 355 rates were higher with Carex and Arr. With rates up to  $121 \pm 11$  and  $113 \pm 49 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$  respectively and  
 356 intermediate with Elymus ( $87 \pm 11 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ ). Along this second week, values were significantly  
 357 decreasing for the control and for microphytobenthos, whereas constant for Elymus and Arr. and significantly  
 358 increasing for Carex (Supp. fig. 1a).

359 During the third week (fig. 4c), nitrate reduction rates remained the lowest in control ( $20 \pm 9 \text{ nmol NO}_3^- \text{ cm}^{-3}$   
 360  $\text{h}^{-1}$ ) and lower with microphytobenthos ( $38 \pm 11 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ ) than with plants which presented on average  
 361  $105 \pm 30 \text{ nmol NO}_3^- \text{ cm}^{-3} \text{ h}^{-1}$ . Along this period, rates were significantly decreasing for Carex and Arr. While  
 362 constant for the other conditions (Supp. fig. 1a).



364  
365 **Figure 4** Boxplots of the Nitrate Reduction Rates for each week of the experiment in the five tested condition. With  
366 the control (natural sediment), and the different conditions with an addition of a organic carbon source: Microphytobenthos  
367 (MPB), *Arrenatherum elatius*, *Elymus athericus*, and *Carex pseudocyperus*. For each week of the experiment, significant  
368 differences between conditions were obtained on each week using AOV and pairwise comparison tests; differences were  
369 indicated by the letters of the different statistical groups

370  
371 **Table 2** Quantity of nitrogen consumed through nitrate reduction. Values are given for each week of the  
372 experiment (1 to 3) and for the overall experiment (18 days). For each week, the letters illustrate the statistical  
373 differences between the conditions after a one-way analysis of variance followed by a pairwise comparison test

| Organic carbon source       | N-NO <sub>3</sub> reduced (mgN) |                |                |                 |
|-----------------------------|---------------------------------|----------------|----------------|-----------------|
|                             | Week1                           | Week2          | Week3          | Total           |
| Control                     | 3.69 (0.27) a                   | 3.44 (2.32) a  | 1.35 (0.30) a  | 8.49 (2.48) a   |
| Microphytobenthos           | 11.72 (0.24) d                  | 4.58 (0.24) ab | 2.28 (0.22) a  | 18.58 (0.59) b  |
| <i>Arrenatherum elatius</i> | 8.39 (0.08) c                   | 8.28 (0.33) b  | 6.52 (0.42) b  | 23.19 (0.80) bc |
| <i>Elymus athericus</i>     | 8.65 (0.16) c                   | 5.96 (0.01) ab | 5.62 (0.14) b  | 20.24 (0.17) bc |
| <i>Carex pseudocyperus</i>  | 7.06 (0.38) b                   | 8.48 (0.65) b  | 10.04 (1.03) c | 25.58 (2.05) c  |

374  
375 Overall, the addition of an organic carbon source, in the form of algal or plant material, led to significantly  
376 higher quantities of N-NO<sub>3</sub><sup>-</sup> reduced in amended sediment compared to the control (table 2). Considering that the  
377 absolute quantity of added carbon was the same for the amended sediments, the quality of the carbon led to  
378 different quantities of N-NO<sub>3</sub><sup>-</sup> reduced during the experiment which was the highest with Carex (25.6 mgN-NO<sub>3</sub><sup>-</sup>)  
379 and the lowest with microphytobenthos (18.6 mgN-NO<sub>3</sub><sup>-</sup> ; table 2).

380 For all conditions and throughout the whole experiment, the nitrite production rates showed a significant  
381 positive correlation with the nitrate reduction rates (Pearson correlation coefficients > 0.8 and  $P < .001$ ). By  
382 comparing the estimated nitrite produced (1 NO<sub>3</sub><sup>-</sup> reduced = 1 NO<sub>2</sub><sup>-</sup> produced) and the measured concentrations,  
383 the amounts of nitrites reduced were estimated. The ratio of reduced to produced nitrites was significantly lower  
384 (AOV,  $P < .001$ ) in the control with  $83.6 \pm 1.5\%$  than in amended sediments (on average  $93.8 \pm 0.5\%$ ) with no  
385 significant differences between them (AOV,  $P > .06$ ).

### 386 387 3.3. Dynamics of the ammonium production rates

388 In the control sediment, relative low values NH<sub>4</sub><sup>+</sup> production rates were measured with a maximum of  
389  $2.4 \pm 0.6$  nmol NH<sub>4</sub><sup>+</sup> cm<sup>-3</sup> h<sup>-1</sup>, and no significant variation recorded over the duration of the experiment  
390 (Supp. fig. 1b). Significant higher NH<sub>4</sub><sup>+</sup> production rates were recorded in the presence of microphytobenthos  
391 during the three first days of the experiment (up to  $25.5 \pm 2.2$  nmol NH<sub>4</sub><sup>+</sup> cm<sup>-3</sup> h<sup>-1</sup>). These rates slowly decreased

392 over time down to values close to  $4 \text{ nmol NH}_4^+ \text{ cm}^{-3} \text{ h}^{-1}$  at the end of the experiment (Supp. fig. 1b). High  $\text{NH}_4^+$   
 393 production rates were also recorded in the first days of the experiment with Arr. (up to  $33.9 \pm 4.1 \text{ nmol NH}_4^+ \text{ cm}^{-3} \text{ h}^{-1}$ )  
 394 while lower values were observed with Elymus, and Carex (up to  $8.9 \pm 2.1$  and  $2.4 \pm 2.1 \text{ nmol NH}_4^+ \text{ cm}^{-3} \text{ h}^{-1}$  respectively). However, in comparison with the microphytobenthos, the  $\text{NH}_4^+$  production rates values in  
 395 sediments amended with plant-derived organic matter decreased rapidly to values close to zero at the end of the  
 396 first week. However, during the second or third week of the experiment, the  $\text{NH}_4^+$  production rates significantly  
 397 increased again up to  $3.9 \pm 0.4$  for Elymus,  $5.2 \pm 0.8$  for Arr., and  $3.3 \pm 0.3 \text{ nmol NH}_4^+ \text{ cm}^{-3} \text{ h}^{-1}$  for Carex (Supp.  
 398 fig. 1b).  
 399

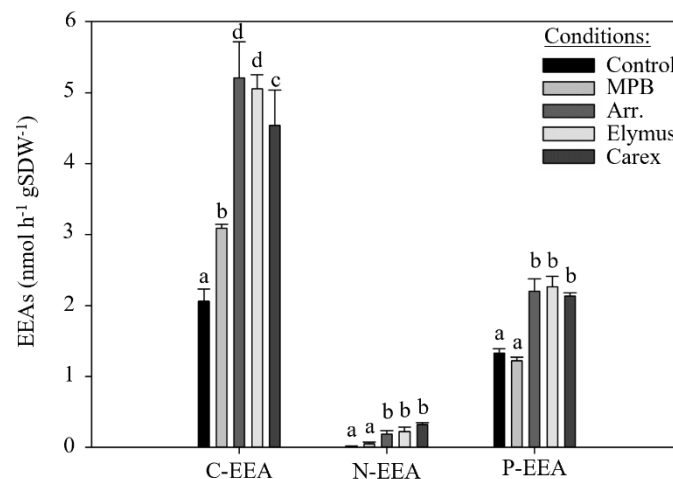
400

### 401 3.4. Potential External Enzymatic activities

402 The carbon-related external enzymatic activities, with the two enzymes catalyzing the degradation of organic  
 403 carbon ( $\beta$ -1,4-glucosidase and 1,4- $\beta$ -D-cellobiohydrolase), showed significant differences between the conditions  
 404 (fig. 5). The lowest values were recorded for the control ( $2.1 \pm 0.2 \text{ nmol h}^{-1} \text{ g}^{-1}$ ) while the highest were observed  
 405 in sediments amended with Elymus and Arr. (on average  $5.1 \pm 0.4 \text{ nmol h}^{-1} \text{ g}^{-1}$ ). High values were also observed  
 406 with Carex ( $4.5 \pm 0.5 \text{ nmol h}^{-1} \text{ g}^{-1}$ ) while intermediate values were observed with microphytobenthos  
 407 ( $3.1 \pm 0.1 \text{ nmol h}^{-1} \text{ g}^{-1}$ ).

408 The nitrogen-related external enzymatic activities (L-leucine aminopeptidase) showed no significant  
 409 difference between the control and microphytobenthos (AOV;  $P = .66$ ) which showed mean values of  $0.01 \pm 0.01$   
 410 and  $0.05 \pm 0.03 \text{ nmol h}^{-1} \text{ g}^{-1}$  respectively (fig. 5). No significant differences were recorded between sediments  
 411 amended with plant residues (AOV;  $P > .4$ ) with a mean value of  $0.22 \pm 0.07 \text{ nmol h}^{-1} \text{ g}^{-1}$ . However, a significant  
 412 difference was recorded between the two statistical groups: control and microphytobenthos *versus* plants (AOV;  
 413  $P < .05$ ).

414 Likewise, the phosphorus-related external enzymatic activities (acid phosphatase; fig. 5) showed significant  
 415 higher values (AOV;  $P < .001$ ) for conditions with plant residues with on average  $2.2 \pm 0.1 \text{ nmol h}^{-1} \text{ g}^{-1}$  than the  
 416 control and microphytobenthos which showed on average  $1.3 \pm 0.1 \text{ nmol h}^{-1} \text{ g}^{-1}$ .



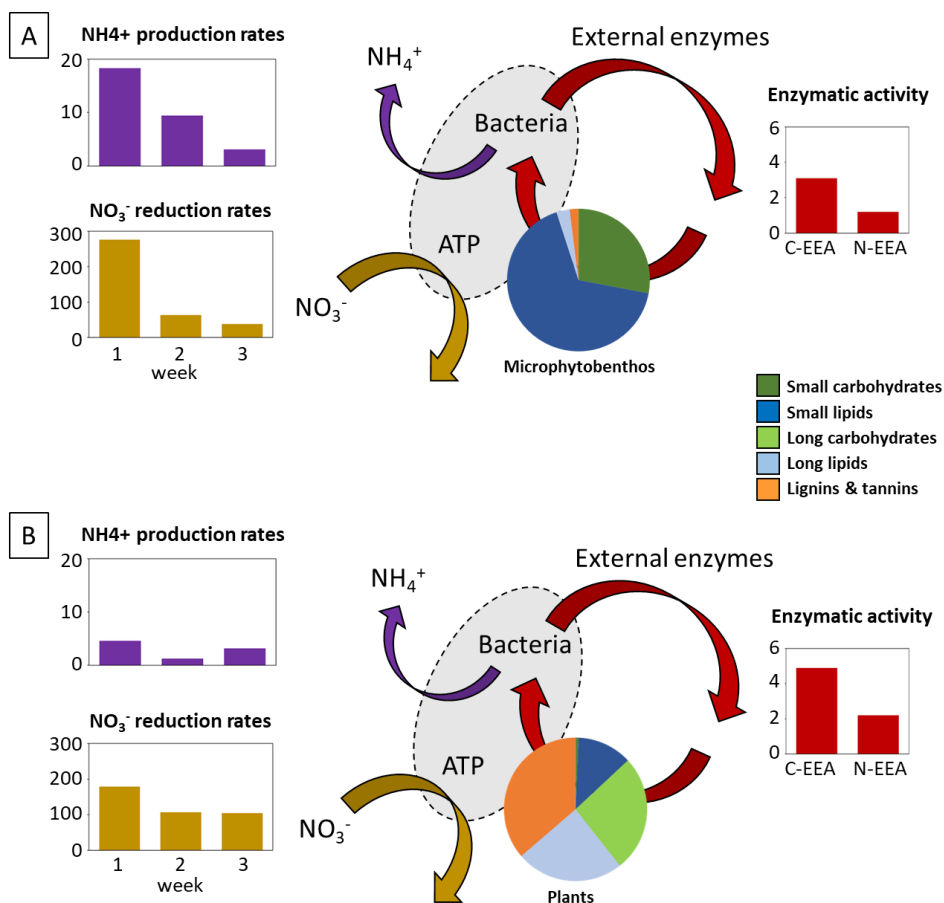
417

418 **Figure 5 Average External Enzymatic Activities ( $\text{nmol h}^{-1} \text{ gSDW}^{-1}$ ) at the end of the experimental time (after 18 days**  
 419 **in reactors) for the different treatments.** The error bar indicates the standard deviation  $n=6$ . With the C-related external  
 420 enzymatic activities (C-EEA) which represent the  $\beta$ -1,4-glucosidase and the 1,4- $\beta$ -D-cellobiohydrolase, the N-related (N-  
 421 EEA) which represent the L-leucine aminopeptidase, and the P-related (P-EEA) which represent the acid phosphatase.  
 422

422

423 **4. Discussion**

424 The results of this study show that the addition of organic carbon originating from different vegetation types  
 425 increases microbial nitrate reduction rates in an intertidal sediment. Our results show that, during the first week,  
 426 the microalgal-derived organic matter has significantly higher impact on these rates than the plant-derived organic  
 427 matter. This is in line with our hypothesis that microphytobenthos, with a low C:N ratio, represents a more easily  
 428 and rapidly utilizable organic carbon source. However, the microphytobenthos was rapidly exhausted. The plant-  
 429 derived organic matter, originating from three different species, also led to increased nitrate reduction rates,  
 430 albeit initially lower than in the presence of microphytobenthos. However, after 2-3 weeks, the presence of plant-derived  
 431 organic matter resulted in higher nitrate reduction rates than in the presence of microphytobenthos (fig. 6).  
 432 Integrated over the whole duration of the experiment, the amount of nitrate reduced was higher in sediments  
 433 amended with plants than with microphytobenthos. The plant-derived organic carbon sources are thus essential to  
 434 complement the depletion of easily degradable carbon sources like microphytobenthos and allow the microbial  
 435 nitrate elimination to be even more efficient than in non-vegetated sediments.  
 436



437 **Figure 6. Schematic representation of the different carbon and nitrogen processes investigated in this study**  
 438 **as a function of the organic matter added to the sediment (A - Microphytobenthos or B - plants).** The  
 439 molecular compositions of organic carbon sources are given as relative percentages in the pie chart for  
 440 microphytobenthos and plants (average for the 3 species investigated). The external enzymatic activities (nmol h<sup>-1</sup>  
 441 g<sup>-1</sup>) related to degradation of organic carbon (C-EEA) and organic nitrogen (N-EEA) as measured at the end of  
 442 the 3-week incubation. The average nitrate reduction rates (nmol NO<sub>3</sub><sup>-</sup> cm<sup>-3</sup> h<sup>-1</sup>) and ammonium production rates  
 443 (nmol NH<sub>4</sub><sup>+</sup> cm<sup>-3</sup> h<sup>-1</sup>) are indicated per week.  
 444

#### 4.1. Importance of the easily available organic carbon for nitrate reduction at the early stage.

The positive effects of algal and plant organic matter addition on nitrate reduction rates occurred immediately, right from the start of the experiment. The presence of energy-rich, labile carbon present in the added organic sources must have allowed microorganisms to rapidly increase their energy via exoenzyme activity (Fontaine et al. 2003; Schimel and Weintraub 2003). This is in good agreement with the significantly higher exoenzyme activities recorded in the plant and algal amended sediments at the end of the experiment. The significantly higher initial nitrate reduction rates in the presence of microphytobenthos compared to plant derived organic matter are most likely related to the differences in their composition. In comparison to plants, the high proportions of low molecular weight lipids (< 20 C atoms in their hydrocarbon chain) and polysaccharides which were mainly desoxyaldonic acids (fig. 3a), concomitant with low amounts of lignin and tannins (< 2%) in microphytobenthos seem to jump start nitrate reduction. This is consistent with previous results showing that lipid degradation occurs over short timescales (days) and that the degradation rates of microalgae-derived fatty acids are higher than those derived from vascular plants (Canuel and Martens 1996). Moreover, it confirms the assumption that microphytobenthos is an easily degradable and rapidly used organic carbon source by bacteria, supplying labile carbon in the form of easily degradable lipids (structural fatty acids of cell membranes: phospholipids) and carbohydrates with relatively low amounts of recalcitrant compounds (Hardison et al. 2013; Kawaida et al. 2019). Albeit less pronounced; early-stage high nitrate reduction rates were also recorded upon addition of plant residues. This supports the assumption that the carbon from plants, despite being considered overall as difficult to degrade, also activates rapid microbial synthesis of extracellular enzymes (Marinari et al. 2000). The use of the aerial parts of the plants must also have played a role in our results since it was shown that the decomposition rates were fastest for leaves compared to ground parts where the structure limits the initial decomposition processes (Vichkovitten and Holmer 2004). The rapid mobilization of extracellular enzymes was already observed while studying the microbial community changes over time during decomposition of ryegrass (McMahon et al. 2005). A similar activation may be expected in the species *Elymus athericus* and *Arrenatherum elatius* used in the present study, belonging to the same order of plant as ryegrass (Poales). Despite a large fraction of complex molecules such as lignins and tannins (between 30 and 40%), the plant residues also contained between 60 to 70% of carbohydrates and lipids with a higher proportion of polysaccharides-carbohydrates than microphytobenthos (table 1). A part of them, also present in microphytobenthos (fig. 3a) must have a high degradation rate and has been rapidly used to enhance nitrate reduction at the beginning of the experiment. The differences in proportion of cat A lipids and polysaccharides between microphytobenthos and plants-derived organic matter, consistent with the extent of the observed stimulation of nitrate reduction rates suggest that those compounds are most likely the ones used at the early stages of the experiment.

Thereby, it appears that the extent to which nitrate reduction was stimulated is dependent on the quantity of easily degradable molecules which is assumed to be negatively correlated with C:N ratio. While low C:N ratio are usually associated to microbial and aquatic organic carbon and high C:N ratio to terrestrial plants (Leng and Lewis 2017), it was also shown that low C:N ratios usually indicates high decomposition rates of the organic material (Hedges and Weliky 1989; Dick and Osunkoya 2000). By comparing the C:N ratio of the different organic carbon sources and the microbial nitrate reduction rates measured during the first week, a significant negative correlation was observed (Pearson correlation coefficients between -0.60 and -0.99;  $P < .05$ ). This result confirms our hypothesis that on this short-time scale, the addition of a microalgae-derived organic matter (low C:N ratio) results

485 in higher nitrate reduction rates than the addition of a plant-derived organic matter (high C:N ratio). However,  
486 sediments supplied with *E. athericus* and *A. elatius* showed similar results despite dissimilar C:N ratio  
487 (respectively 35.5 and 57.3). This result suggests that these two plants had similar amounts of easily degradable  
488 molecules, which led to similar stimulation of nitrate reduction rates the first week; but a different amount of more  
489 complex molecules, which may explain their different C:N ratio. Therefore, the C:N ratio of the organic matter  
490 appears to be insufficient to accurately predict nitrate reduction rates. Determining the amount of easily degradable  
491 molecules present in the organic matter would be required, which is to date not possible and a future challenge.

492 The intertidal sediment used in this study most likely harbored a microbial community adapted to use carbon  
493 from microphytobenthos present on the surface of the sediment, rather than from plant residues. The increasing  
494 nitrate reduction rates over the course and differences in external enzymatic activities at the end of the experiment  
495 strongly indicate that the microbial community adapted upon the addition of plant-derived organic carbon. An  
496 adaptation of the microbial community because of competition for readily available carbon during the first days  
497 after carbon supply was shown in soil and rhizosphere (Baumann et al. 2009; Marschner et al. 2011). It can thus  
498 be hypothesized that the microbial community composition changed during the early stages of residue  
499 decomposition (i.e. 1-week), and consequently on nitrate reduction rates, in relation with the different qualities of  
500 the easily degradable molecules added. This is in agreement with previous studies that showed differences in  
501 respiration activity of different microbial communities related to composition, complexity, and degradability of  
502 plant-based carbon inputs caused, in the first days after addition (De Nobili et al. 2001; Mondini et al. 2006). This  
503 was attributed to low molecular weight compounds. Future, in-depth analysis of the microbial community structure  
504 (i.e. microbial diversity and functioning) would be required to test this hypothesis.

505

#### 506 **4.2. Importance of the more complex organic carbon to support efficient nitrate reduction.**

507 The positive effect of organic carbon addition on nitrate reduction rates at the early stages of the experiment  
508 was followed in all sediments by a strong decrease in nitrate reduction rates. The nitrate reduction rates in the  
509 sediments amended with microphytobenthos remained higher than in the control, however, became lower than in  
510 sediments with addition of plant residues. This must be related to the mainly labile form of the microphytobenthos  
511 leading to a rapid depletion of this resource not allowing a high nitrate reducing activity over the long-term. This  
512 highlights the dependence of nitrate reducing bacteria to a rapid microalgae turnover in benthic biofilm to sustain  
513 a strong bacterial activity in terms of nitrate reduction. Even more since in mudflat sediments, the  
514 microphytobenthos represent the main organic carbon source preferentially used by heterotrophic bacteria  
515 (Bouillon and Boschker 2006). However, it was shown that microalgae have a turnover (biomass to production  
516 ratio) between 1 and 10 days depending on the sediment nature, much lower at muddy than at sandy sites, mostly  
517 related to grazing pressure (Herman et al. 2001). This turnover appears too low to support the high range of  
518 bacterial activity (mineralization, nitrate reduction, etc.) at long-term and this suggests that sedimentary bacteria  
519 must depend on carbon from various origins. Thus, if certain carbon sources are preferentially mineralized such  
520 as microphytobenthos (Nielsen et al. 2004; Bouillon and Boschker 2006), this result implies that presence of more  
521 complex carbon sources in sediment such as macroalgae or terrestrial carbon (Burdige 2007) are necessary to  
522 support the biogeochemical cycling, particularly of nitrogen. Our results support this assumption since after one  
523 week of experiment, the nitrate reduction rates were higher in sediments amended with plant residues than  
524 microphytobenthos with up to a twofold higher amount of nitrate reduced the second week and a fivefold higher



525 amount reduced the third week (table 2). Altogether, the addition of the same quantity of carbon, resulted in a  
526 higher number of nitrate molecules reduced in the three weeks experiment; indicating the importance of the carbon  
527 quality on the total amount of nitrates reduced. This suggests a significant contribution of plant-derived organic  
528 matter to nitrate reduction in intertidal mudflats and especially the less readily available carbon compounds which  
529 could be related to the lignin and tannin molecules, the high molecular weight lipids related to the cutin,  
530 hydrophobic polymers from leaf surface, and the large carbohydrates such as lignocelluloses.

531 The delay upon plant organic carbon addition before it supports higher nitrate reduction rates than in  
532 microphytobenthos is most likely due to the time required to degrade these less readily available carbon  
533 compounds. This assumption is in line with the C:N ratios since the delay was 2-weeks for *Elymus* and *Arr.* while  
534 it was 3-weeks for *Carex* and confirmed by the recorded  $\text{NH}_4^+$  production rates and enzymatic activities. The  
535 degradation of organic matter produces nitrogen in the form of  $\text{NH}_4^+$ ; i.e. nitrogen mineralization (Lemley et al.  
536 2014). Thereby, the significant increase in  $\text{NH}_4^+$  production rates during the second week for *Elymus* and *Arr.*,  
537 and during the third week for the *Carex* amended sediments suggest an increase in mineralization of organic carbon  
538 compounds. Moreover, after depletion of the readily available organic carbon, microorganisms are known to  
539 produce extracellular enzymes to hydrolyze the chemical bonds in the more complex molecules, and thus release  
540 assimilable molecules such as soluble carbohydrates for their metabolic reactions (Hoffman and Decho 1999).  
541 Thereby, the overall significant higher external enzymatic activity related to carbon, nitrogen, and phosphorous  
542 recorded in sediments amended with plant-derived organic matter indicates that their addition induced these  
543 activities and thus the overall degradability of organic matter. The significant differences observed between the C-  
544 related external enzymatic activities support the assumption that vegetation type is an important factor influencing  
545 them in coastal wetlands (Shao et al. 2015). However, our results suggest that this is not related to the C:N ratio  
546 since the C-related external enzymatic activities were lower in sediment amended with *Carex* than with *Arr.* and  
547 *Elymus*. Moreover, as our experiment was controlled, this result suggests that the environmental factors like  
548 temperature, pH but also the carbon quantity were not involved in the observed variation of the external enzymatic  
549 activities. Therefore, this result supports the assumption that the vegetation type influences the C-related external  
550 enzymatic activities through its chemical composition (Yin et al. 2014). The influence of the organic carbon quality  
551 could be due to the adaptation of the microbial community since it was shown that the quality of plant species  
552 influenced the microorganism's community whose composition determines the potential for enzyme synthesis and  
553 activity (Marinari et al. 2014). Based on the effects on the external enzymatic activities, we hypothesize that the  
554 microbial community changed upon the addition of the different plant derived organic matter. It should be noted  
555 that the number of nitrogen reducing gene copies, which were enumerated (qPCR) at the end of the experiment  
556 (data not shown) showed no significant differences among the different treatments. Therefore, it seems that the  
557 addition of the different organic carbon sources rather affected the microbial communities involved in the carbon  
558 decomposition process. This is in line with previous results showing that different carbon sources added to soil  
559 alter the microbial community structure (based on phospholipid analysis) responsible for decomposition (Waldrop  
560 and Firestone 2004). This is also in agreement with observations that changes in organic matter degradation rates  
561 are due to shifts in enzymatic capacity related to modifications in the microbial community (Wall and Moore  
562 1999).

563 Overall, although plant derived organic matter is structurally more complex and not as easy to use by bacteria  
564 than the more readily available carbon sources such as microphytobenthos, once the microbial community is able

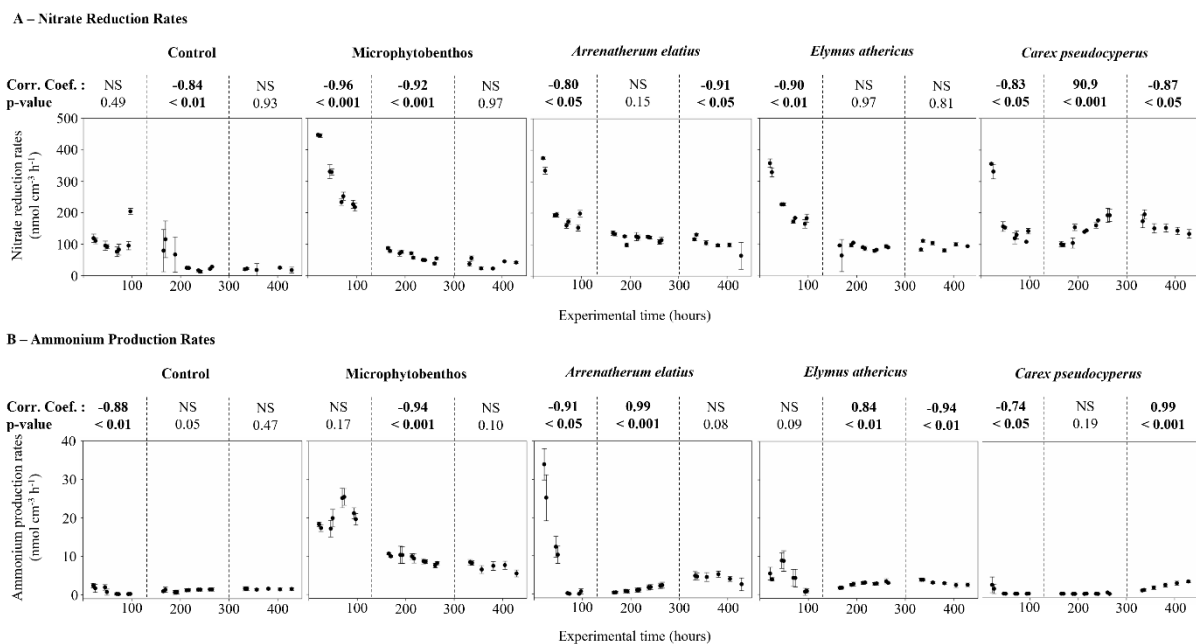
565 to use it, the plant-derived organic matter seems equally or more effective to support the nitrate reduction rates. It  
 566 can be hypothesized that this is due that easily degradable organic matter is mainly composed of small soluble  
 567 carbon molecules. These soluble molecules may not have been completely assimilated by the bacteria and flushed  
 568 out of the system after microphytobenthos addition. In comparison, the more complex molecules are immobilized  
 569 in the sediment until their degradation releasing soluble carbons in a slower and continuous way which could allow  
 570 a more complete assimilation of the organic carbon and thus a stronger support to the microbial nitrate reduction  
 571 process with plants. This is especially important regarding intertidal ecosystems such as mudflats and wetlands  
 572 that are regularly covered by tides which can also flush out such small soluble molecules.

573

## 574 5. Conclusion

575 This study experimentally demonstrated that both microphytobenthos and plants play a crucial role in benthic  
 576 nitrogen fluxes via nitrate reduction in intertidal sediments. This being made through the use, by denitrifiers, of  
 577 the readily available molecules over a short time span (i.e. < 1 week) and after degradation by using the less  
 578 available structure of carbon from plants residues over a longer period (2-3 weeks). The microphytobenthos and  
 579 plant sources are likely complementary in supporting this ecological functionality in wetlands and mudflats. This  
 580 is even more important in the context of climate change since the increase in extreme climatic events and sea level  
 581 rise represent a serious threat to the presence of many intertidal mudflats, adjacent wetlands, and their associated  
 582 ecological values.

583



584

585 **Supplementary figure 1 Dynamics of (A) Nitrate Reduction rates and (B) Ammonium Production Rates**  
 586 **along the 18 days (430 hours) of the experiment for the five different conditions tested.** With the control and  
 587 the conditions with organic carbon addition (Microphytobenthos; *Arrenatherum elatius*; *Elymus athericus*; *Carex*  
 588 *pseudocyperus*). For each week, the decrease or increase of values were illustrated with a Pearson correlation.  
 589 Week with a significant increase of the values showed a positive coefficient and  $P < .05$  while a significant  
 590 decrease showed a negative coefficient with a  $P < .05$ . For the non-significant  $P > .05$ , the correlation coefficients  
 591 were not considered (NS) and the exact  $P$  were given.

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