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**Sedimentary context controls the influence of ecosystem engineering by bioturbators on
microbial processes in river sediments**

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1 By modifying the physical environment, ecosystem engineers can have inordinately large
2 effects on surrounding communities and ecosystem functioning. However, the significance of
3 engineering in ecosystems greatly depends on the physical characteristics of the engineered
4 habitats. Mechanisms underlying such context-dependent impact of engineers remain poorly
5 understood whereas they are crucial to establish general predictions concerning the
6 contribution of engineers to ecosystem structure and function.

7 The present study aimed to decrypt such mechanisms by determining how the environmental
8 context modulates the effects of ecosystem engineers (bioturbators) on micro-organisms in
9 river sediments. To test the effects of environmental context on the role of bioturbators in
10 sediments, we used microcosms and recreated two sedimentary contexts at the laboratory by
11 adding a layer of either fine (low permeability) or coarse (high permeability) sand particles at
12 the top of a gravel-sand matrix. For each sediment context, we examined how the sediment
13 reworking activity of a bioturbating tubificid worm (*Tubifex tubifex*) generated changes of the
14 physical (sediment structure and permeability) and abiotic environments (hydraulic discharge,
15 water chemistry) for micro-organisms. The biotic influences of the bioturbation process were
16 measured on microbial characteristics (abundances, activities) and leaf litter decomposition as
17 a major microbe-mediated ecological process.

18 Results showed that *T. tubifex* significantly increased hydraulic discharge (by about 6-fold)
19 and restore aerobic conditions in O₂-limited sedimentary habitats covered by fine sand (low
20 permeability treatment). Consequently, worms stimulated microbial communities developed
21 on buried leaves and leaf litter breakdown increased (+30%). In contrast *T. tubifex* had a low
22 influence on water exchanges and O₂ availability in highly permeable sediments. As
23 bioturbation did not modify the abiotic environment in sedimentary habitats covered by
24 coarse sand, *T. tubifex* did not influence the abundances and activities of microorganisms
25 developed on leaves.

1 Our study demonstrated that the significance of ecosystem engineering on the functioning of
2 aquatic ecosystems cannot be quantified without assessing the complex interactions between
3 bioturbation activities and sedimentary characteristics. We strongly suggest that context
4 dependency mainly modulates the effects of ecosystem engineers on other biota by
5 controlling the ability of engineers to modulate the availability of limiting factors for other
6 organisms.

7

8 **Introduction**

9 Habitat modification by engineer organisms has been recognized as a major ecological
10 process with large consequences for biodiversity and ecosystem functions in a broad range of
11 ecosystems (e.g. see Lavelle et al 1997, Crooks 2002, Mermillod-Blondin and Rosenberg
12 2006, Wright and Jones 2006, Wright et al. 2006, Badano and Marquet 2008, Gutiérrez et al.
13 in press). Ecosystem engineers can have inordinately large effects on communities by
14 modifying their surrounding physical environment (Jones et al. 1994, see Fig.1 for detailed
15 engineering sequence). The beaver (*Castor canadensis*) is a classical example of ecosystem
16 engineer. Its activities (essentially dam-building) can increase (i) the proportion of flooded
17 soils (water and wetlands) in the landscape (Johnston and Naiman 1990) and (2) the retention
18 of sediment, organic material (Naiman et al. 1986) and nutrients (Naiman and Melillo 1984)
19 in the channel by decreasing water velocity, ultimately affecting the structure of animal and
20 plant communities in the landscape (Naiman et al. 1988, Hägglund and Sjöberg 1999, Wright
21 et al. 2002, Anderson and Rosemond 2007). Beside this emblematic example of beavers, it
22 exists a wide diversity of engineer organisms and then engineering mechanisms (Berke 2010)
23 that significantly impact structure and functions. For example, the sediment reworking
24 activities of bioturbators can have marked effects on microbial communities developed on

1 sediments by affecting hydrological fluxes and biogeochemistry at water-sediment interface
2 (Mermillod-Blondin and Rosenberg, 2006; Mermillod-Blondin et al. 2004, Nogaro et al.
3 2009). Whatever the engineering mechanisms (e.g. through bioturbation, physical engineering
4 ...), a change in resource availability relative to the unmodified state may suffice to observe
5 positive or adverse engineering effects on some biotic variables (e.g. absolute and relative
6 abundance and richness of species in the surrounding communities) describing the structure of
7 communities (Bertness 1984a,b, Jones et al. 1997, Menge 2000). The theory of ecosystem
8 engineers also suggests that the highest effects of engineers on physical habitat produced the
9 highest changes of biotic variables (Jones et al. 1994). However, the influence of an
10 ecosystem engineering activity also depends on the environmental context in which it happens
11 (Crain and Bertness 2006, Wright and Jones 2006). Because ecosystem engineers affect
12 community through environmentally mediated interactions, a given engineering process
13 (similar density of a given engineer species) can have contrasted effects on some biotic
14 variables across environmental gradients and thus can appear as idiosyncratic (Jones et al.
15 2004, Moore 2006). However, most of studies dealing with ecosystem engineering only
16 examine the relationships between the engineer (e.g. number of individuals) and (i) the
17 physical characteristics of the environment or (ii) the other species, in a unique environmental
18 context. To establish general principles and predictions (for future changes) about the effects
19 of engineers on communities, there is a need to fully understand mechanisms underlying
20 context dependency and thus to examine the whole ‘cause-effect relationships’ sequence (see
21 Fig. 1) in contrasted environments.

22 In the present study, we aimed to tackle this context dependency in river sediments. The
23 functioning of lotic ecosystems partly depends on the microbially-mediated biogeochemical
24 processes (nutrient cycling, organic matter (hereafter OM) processing) realized in the
25 hyporheic zone (hereafter HZ, sedimentary interface between surface water and groundwater)

1 (Grimm and Fisher 1984, Findlay 1995, Naegeli and Uehlinger 1997, Boulton et al. 1998,
2 Fellows et al. 2001). By controlling hydrological exchanges between the surface and the
3 sediments, and thus chemical conditions (e.g. availability of dissolved oxygen DO) in the HZ,
4 sediment characteristics such as permeability appears as crucial factors controlling the
5 structure and activities of microbial communities developed on river sediments (e.g. Valett et
6 al. 1990, Brunke and Gonser 1997, Mermillod-Blondin and Rosenberg 2006). In this context,
7 bioturbators can have major influence on the HZ (and *a fortiori* on the whole-stream)
8 ecological functioning through modification of sediment structure and permeability (Nogaro
9 et al. 2009, Nogaro & Mermillod-Blondin 2009). However, the magnitude of bioturbation-
10 driven change in hydraulic conductivity is expected to depend on physical hydraulic
11 conditions which are more or less constraining for micro-organisms living in HZ (Hakenkamp
12 and Palmer 2000, Mermillod-Blondin, in press). As physical hydraulic conditions are
13 essentially linked with sediment characteristics, the effects of bioturbators as ecosystem
14 engineers in river sediment are expected to be sedimentary-context dependent. By applying
15 the conceptual framework of ecosystem engineering in rivers, bioturbators are expected to
16 major influence on hyporheic ecological processes in sedimentary systems where: (1) they are
17 able to drastically modify habitat physical characteristics by actively reworking sediment, (2)
18 these physical changes result in alteration of the hydraulic conductivity in the sedimentary
19 habitat and finally (3) the change in hydraulic conductivity modifies resource availability for
20 interstitial microorganisms involved in studied processes (Fig.1). In riverbeds covered by
21 excessive deposition of fine sedimentary particles, permeability, hydrological exchanges and
22 associated input of resources from the surface for interstitial organisms are reported to be low
23 (Beschta and Jackson 1979, Schächli 1992, Wood and Armitage 1997). In this context,
24 bioturbation would have more influence on water exchanges between surface and hyporheic
25 zone than in highly permeable sediments.

1 The aim of our study was therefore to examine how the environmental context (sediment
2 characteristics) modulates the effects of an active bioturbator as ecosystem engineer in river
3 sediments. We tested the effects of the bioturbator *Tubifex tubifex* Müller (Oligochaeta,
4 Tubificidae) on the characteristics and activities of microbial communities in two sedimentary
5 habitats with contrasted textures (topped by coarse permeable sand *versus* topped by fine sand
6 with low permeability) by using slow filtration columns. For each habitat, we broke down and
7 examine the ecosystem-engineering process into detailed intermediate steps (see Fig.1)
8 including sediment reworking, physical (hydraulic conductivity) and abiotic (availability of
9 nutrients and electron acceptors used for OM mineralization) changes on the sedimentary
10 habitat. The ecosystem-engineering effects (biotic changes) were measured on the
11 characteristics (bacterial and fungal abundances) and activities (potential aerobic respiration,
12 potential denitrification, hydrolytic exoenzymatic activities) of the microbial community
13 developed on leaves buried in sediments. Decomposition of leaf litter (measurement of
14 breakdown rates) -a crucial microbially-mediated ecological process in river sediment- was
15 used as a final step in the engineering process. Bioturbators were expected to have the most
16 significant influence in sedimentary systems covered by fine particles, where restricted
17 hydrological exchanges (low hydraulic conductivity and associated sharp decrease in O₂ and
18 following electron acceptors) are supposed to constrain microbial communities.

19

20 **Methods**

21

22 *Experimental design*

23 To address how sediment context modulates the effects of a bioturbator at the water-
24 sediment interface of rivers, we employed a factorial experimental approach in which the
25 occurrence of *T. tubifex* and sediment texture were manipulated. Experiments were carried out

1 in slow filtration columns (n = 16 experimental units, height = 35 cm and inside diameter = 10
2 cm; Mermillod-Blondin et al. 2005, Navel et al. 2010) filled with sediment, at constant
3 temperature (15 ± 0.5 °C) under a 12 h light / 12 h dark cycle.
4 These mesocosms were filled by successively adding gravel (2-4 mm diameter, 300 g) and
5 then sand (100-1000 μm , 170 g), 8 times. We manipulated the surface sediment texture by
6 adding a 2 cm thick layer of either fine sand (90% of particles < 150 μm diameter, low
7 permeability: “fine sand treatment”, n = 8 columns) or coarse sand (90% of particles > 300
8 μm diameter, n = 8 columns) at the top of the gravel-sand base. The thickness of the top
9 sediment layer was in accordance with observations reported from riverbeds impacted by fine
10 sediment deposits (Wood & Armitage 1997). Analyses performed before the start of the
11 experiment indicated that the specific area and the amounts of total organic carbon (TOC),
12 nitrogen (TN) and phosphorus (TP) in the sediment were higher in the fine than in the coarse
13 sand (Table 1). All the sedimentary material was collected from the Rhône River, elutriated
14 and cleaned with deionised water to eliminate fauna and coarse particulate organic matter
15 (CPOM). Moreover, the whole-sediment layer was kept in the dark to suppress possible
16 photoautotrophic processes.

17 During sediment installation, a set of 35 leaf discs (diameter: 12 mm) of alder (*Alnus*
18 *glutinosa* (L.) Gaertner), a common species along rivers characterized by fast leaf degradation
19 (Abelho 2001), was inserted between two circular sieves (3 mm mesh) at a depth of 9 cm
20 below the sediment surface in each column. Discs were cut avoiding central veins of leaves
21 collected from the riparian zone of the Rhône River during abscission (October 2008). Leaves
22 were conditioned in small-mesh bags immersed in a nearby river (located on the campus of
23 the University Claude Bernard Lyon 1, Lyon, France) for 10 days, i.e. a time sufficient to
24 allow microbial colonization (Suberkropp and Chauvet 1995). After installation of sediment
25 and leaf litter, aerated artificial river water ($96 \text{ mg.L}^{-1} \text{ NaHCO}_3$, $39.4 \text{ mg.L}^{-1} \text{ CaSO}_4 \cdot 2\text{H}_2\text{O}$,

1 60 mg.L⁻¹ MgSO₄ · 7H₂O, 4 mg.L⁻¹ KCl, 19 mg.L⁻¹ Ca(NO₃)₂ · 4H₂O and 1.6 mg.L⁻¹
2 (CH₃CO₂)₂CaH₂O; pH = 7.5; US EPA 1991) was added at a constant hydraulic head (ΔH = 3
3 cm), to a depth of 10cm above the sediment surface of each mesocosm. Openings in each
4 mesocosm allowed sampling water at different times during the experiment.

5 Seven days after sediment installation (T7) (time necessary to obtain a physico-chemical
6 stabilization of the system), we added a set of 100 individuals of *T. tubifex* to half of the
7 experimental units (n = 4 per treatment). The density of tubificid worms in the experimental
8 units (around 12,800 individual.m⁻²) was in accordance with densities reported in field studies
9 (Fruget 1989, Martinet 1993). *T. tubifex* is a common deposit feeder that inhabits sandy and
10 muddy habitats, which can actively rework sedimentary particles (McCall and Fisher 1980)
11 and increase sediment permeability (Nogaro and Mermillod-Blondin 2009, Nogaro et al.
12 2009). The potential influence of *T. tubifex* on leaf litter degradation was expected to result
13 from the influence of *T. tubifex* as ecosystem engineers rather than a direct feeding on leaf
14 litter. To verify that *T. tubifex* do not feed on leaves, we conducted a preliminary experiment
15 using aerated aquatic microcosms in which 35 alder leaf discs were deposited at the surface of
16 a fine layer of sediment for 59 days. We measured that the occurrence of 100 individuals *T.*
17 *tubifex* did not significantly influence the leaf litter breakdown rate nor the microbial
18 abundances and activities associated with leaf litter (unpublished data).

19 During the main experiment, hydraulic discharge rate was measured and water was
20 sampled every 10 days at 4 depths to determine O₂, NH₄⁺, NO₃⁻, NO₂⁻, PO₄³⁻, SO₄²⁻ and
21 dissolved organic carbon (DOC) concentrations, for all columns. At the end of the
22 experiment, columns were dismantled and sediment was cut into slices to quantify sediment
23 reworking and vertical distribution of invertebrates. Fungal biomass, total bacterial
24 abundance, abundance of active eubacteria, potential aerobic and anaerobic activities and

1 enzymatic activities involved in C and N cycles were determined on leaf discs, as described
2 below. Leaf discs were then dried and weighed to quantify mass loss during the experiment.

3

4 ***Physico-chemical analyses***

5 Every 10 days starting with day 6, a day before fauna addition (T6, T16, T26, T36, T46 and
6 T56), the outlet of each column was closed and water was shunted and sampled at +2 cm
7 above (H1) and -3 cm (H2), -8 cm (H3) and -13 cm (H4) below water-sediment interface
8 under similar hydraulic pressure conditions. An oxygen micro-sensor probe fitted in a glass
9 tube (OX 500, Unisense, Aarhus, Denmark) was used to determine O₂ concentration without
10 contact with atmospheric oxygen during sampling. NH₄⁺, NO₃⁻, NO₂⁻, PO₄³⁻ and SO₄²⁻
11 concentrations were determined following standard colorimetric methods (Grashoff et al.
12 1983) after filtration through Whatman GF/F filters (pore size: 0.7 µm; Millipore, Billerica,
13 MA, U.S.A.) using an automatic analyzer (Easychem Plus, Systea, Anagni, Italia). For DOC
14 measurements, water samples were filtered through Whatman HAWP filters (pore size: 0.45
15 µm; Millipore, Billerica, MA, U.S.A.) and acidified with 3 drops of HCl (35%). The DOC
16 concentration in water samples was measured with a total carbon analyzer (multi N/C 3100,
17 Analytik Jena, Jena, Germany) based on combustion at 900 °C after removal of DOC with
18 HCl and CO₂ stripping under O₂ flow.

19

20 ***Sediment reworking analyses***

21 Particle redistribution induced by worms in the sedimentary matrix was estimated by the
22 luminophore tracer technique (Gérino 1990). In each column, natural sediment particles (150-
23 300 µm) dyed with yellow luminescent paint were deposited uniformly at the top of the
24 sedimentary matrix a few hours after the introduction of *T. tubifex* (at T7). During column

1 dismantling (T59), the top 4 cm of sediment were cut into 0.5 cm thick slices, dried at 40 °C
2 (48 h) and homogenized before counting luminophores on 500 mg subsamples under U.V.
3 light (3 replicates per sampled slice). Vertical distribution of luminophores in the sediment
4 was obtained by expressing the density of particles (number.g⁻¹ dry sediment) obtained for
5 each slice as percentage of the total amount of luminophores obtained for the whole 4 cm top
6 sediment layer.

7

8 *Vertical distribution of tubificid worms*

9 After collecting subsamples on the top sediment for luminophore counting, sediment was
10 pooled into 5 cm thick sediment slices that were sieved (using a 500 µm–diameter sieve) to
11 collect living tubificids. Individuals recovered in each slice were preserved in 96% ethanol
12 and counted under a dissecting microscope. For each column, the vertical distribution of
13 tubificid worms in the sediment was determined by reporting the abundance of worms in each
14 slice to the total amount of worms retrieved in the overall sedimentary column (results for
15 each slice were expressed as percent).

16

17 *Microbial analyses*

18 *Fungal biomass*

19 For each column, 5 leaf discs collected at the end of the experiment were stored at -80 °C and
20 freeze-dried for 12 h before analysis. Fungal biomass was estimated with the ergosterol
21 quantification method in which saponified products were obtained by methanol refluxing
22 prior to saponification reaction using KOH/methanol (Gessner et al. 2003), following the
23 protocol detailed in Navel et al. (2010). Ergosterol was isolated from saponified products by

1 using Oasis HLB 3cc extracting columns (Waters Corporation, Milford, MA, U.S.A) and
2 elution with isopropanol. Mass of ergosterol in the sample was then calculated by using
3 HPLC system (HPLC 360/442, Kontron, Eching, Germany). Fungal biomass was estimated
4 from ergosterol amounts using a 182 conversion factor determined for aquatic hyphomycetes,
5 which are known to dominate fungal assemblages on decomposing litter (Gessner and
6 Chauvet 1993). Results were expressed in mg fungi.g⁻¹ dry mass of leaf litter.

7

8 *Bacterial abundances*

9 During column dismantling (at T59), 2 leaf discs were immediately collected and fixed in 4%
10 paraformaldehyde in phosphate-buffered saline (PBS; 0.13M NaCl, 7mM NaHPO₄, 3mM
11 NaH₂PO₄; pH=7.2) for 10 h. Fixed samples were subsequently washed twice in PBS and were
12 stored in ethanol and PBS (50:50) at 20 °C. After storage (2 weeks), leaf discs were
13 homogenized in 20 mL of 0.1% pyrophosphate in PBS using a sonicator with a 2 mm-
14 diameter probe at 50 W for two periods of 60 s. All homogenized samples were finally
15 supplemented with the detergent NP-40 (Flucka, Buchs, Switzerland) to a final concentration
16 of 0.01 %. Aliquots (10 µL) of homogenized samples were spotted onto gelatine-coated slides
17 and were hybridized with Cy3-labelled oligonucleotide probe (mix of EUB 338, EUB 338 II
18 and EUB 338 III, eubacteria) and concomitantly stained with the DNA intercalating dye
19 DAPI (200 ng.µL⁻¹, Sigma, Buchs, Switzerland) according to Navel et al. (2010). Numbers of
20 DAPI- and Cy3-bacteria were expressed as numbers of bacteria and numbers of active
21 eubacteria (hybridized with EUB 338, Karner & Fuhrmann 1997) per g dry leaf.

22

23 *Microbial activities*

1 All microbial activities were measured within the 24 h following columns dismantling, with
2 leaf discs stored at 4 °C before analysis.

3 Enzymatic activities: β –glucosidase (EC: 3.2.1.21), β –xylosidase (EC: 3.2.1.37) and leucine
4 aminopeptidase (EC: 3.4.11.1) activities were measured on 2 discs (2 times) by fluorimetry
5 using constant volume of substrate analogs: 4-methylumbelliferyl- β -D-glucoside (MUF-glu;
6 750 μ M, 2 mL), 4-methylumbelliferyl-xylosidase (MUF-xyl; 1000 μ M, 2 mL) and L-Leucine-
7 4-Methyl Coumarinyl-7-amideHCl (MCA-leu; 1000 μ M, 2 mL), respectively. Incubation at
8 20 °C (40 min) was stopped by transferring into boiling water before centrifugation (5000 G;
9 4851 rpm, 3 min). Fluorimetry measurements were realised on a mix of supernatant (300 μ L)
10 and buffer (30 μ L, pH 10.4) using a microplate reader (SAFIRE, TECAN Group Ltd,
11 Switzerland) with excitation wavelength of 363 nm and emission wavelengths of 441 nm for
12 MUF-glu and MUF-xyl. Wavelengths were set at 343 nm (excitation) and 436 nm (emission)
13 for MCA-leu. Litter dry mass (drying at 70 °C for 48h) was determined at the end of analyses
14 to express results as nmol of hydrolysed compound.h⁻¹.g⁻¹ dry leaf litter. For each sample,
15 values were corrected by the fluorimetric signal obtained with a formaldehyde-killed control
16 (measurements realised in similar conditions on 2 discs previously treated 30 min with a 39 %
17 formaldehyde solution).

18 Potential aerobic respiration and anaerobic denitrification activities were measured on leaf
19 discs following the slurry technique (Furutani et al. 1984). Leaf discs (n = 4 for respiration
20 and n = 6 for denitrification) were placed in 150 mL flasks supplemented with feeding
21 solutions to optimize microbial activity. For the measurements of CO₂ production
22 (respiration), the incubation was conducted under aerobic conditions with 5 mL of a feeding
23 solution of glucose (7.5 g.L⁻¹) and glutamic acid (7.3 g.L⁻¹). For the measurements of N₂O
24 production (denitrification), the incubation was under anaerobic conditions with a N₂
25 atmosphere. The feeding solution was a mixture of 5 mL of a KNO₃ (2.2 g.L⁻¹), glucose (7.5

1 g.L⁻¹) and glutamic acid (7.3 g.L⁻¹) solution. Acetylene (10% v/v) was introduced in N₂
2 saturated atmosphere to stop N₂O-reductase activity. CO₂ and N₂O productions were
3 calculated from measurements of concentrations at 2 h and 6 h incubations by using gas
4 chromatography on a microcatharometer (M200 micro gas chromatograph, MTI Analytical
5 Instruments, Richmond, CA, U.S.A.). After the drying of leaf discs (70 °C for 48h), results
6 were expressed in µg of C or N.h⁻¹.g⁻¹ dry leaf litter.

7

8 *Leaf litter degradation*

9 For each column, the total dry mass of leaf litter after 59 days was calculated as the sum of
10 the dry masses of samples used in microbial analyses and that measured for the remaining leaf
11 material (common drying method: 70 °C for 48 h), with correction for the set of 5 discs that
12 were freeze-dried for fungal biomass assessment. Results were compared to the initial dry
13 mass determined on 5 additional sets of 35 alder discs (228.8 ± 6.25 mg) at the start of the
14 experiment.

15

16 *Data treatment*

17 Repeated measures of hydraulic conductivity were analyzed using mixed model analysis of
18 variance with “sediment” (“coarse sand” vs. “fine sand”), “worms” (“with” vs. “without”) and
19 “time” as fixed factors, and experimental unit (“column”) as random factor. Repeated
20 measures of vertical profiles in O₂, DOC, NH₄⁺, NO₃⁻, NO₂⁻, SO₄²⁻ and PO₄³⁻ were analysed
21 similarly, with “depth” as additive fixed factor. Vertical distribution of tubificid worms was
22 studied by using two-way analysis of variance (ANOVA) with “sediment”, and “depth” as
23 main factor. Vertical distribution of luminophores was studied by using similar procedure
24 with “sediment”, “depth” and “worms” as main factor. Influences of sediment permeability

1 and tubificid worms on data obtained on buried leaf litter (daily dry mass loss, fungal
2 biomass, total abundance of bacteria, abundance of active bacteria, % active bacteria,
3 enzymatic activities, potential aerobic respiration and potential denitrification) were examined
4 using two-way ANOVAs with “sediment” and “worms” as main factors. The method of
5 contrast was used to determine significant differences between treatments (Crawley 2002).
6 Hydraulic conductivity and microbial activities on leaves (glucosidase, leucine
7 aminopeptidase activity and potential denitrification activities) were log-transformed before
8 statistical analysis in order to fit the assumption of homoscedasticity. Abundances of
9 luminophores and worms retrieved at the end of experiment for each layer within a same
10 column were expressed as percent of the total abundance for the whole column, and were
11 arcsin-transformed before analyses. Statistical analyses were performed using JMP 8.0 (SAS
12 Institute, Cary, NC, U.S.A.), version 8.0.1. Significance for all statistical tests was accepted at
13 $\alpha < 0.05$.

14

15 **Results**

16

17 *Influence of sediment physical characteristics on sediment reworking activity.*

18 The physical structure of the habitat influenced the vertical distribution of tubificid worms in
19 sediment (Fig. 2; “sediment-by-depth interaction effect”: $F_{(3,24)} = 83.44$, $P < 10^{-4}$). While the
20 major part of individuals were retrieved in the top sediment layer when covered by fine sand
21 (93% in the first 5 cm), most of worms were retrieved deeper in the sediment of columns
22 covered with coarse sand (around 65% were found after 10 cm depth). The presence of worms
23 in systems increased the transport of luminophores from the sediment surface to the
24 sedimentary column (Fig. 2; “worms-by-depth interaction effect”: $F_{(7,96)} = 29.78$, $P < 10^{-4}$).

1 This effect of worms on luminophore profiles was strongly influenced by the physical
2 characteristics of the sedimentary habitat ($F_{(7,96)} = 9.61$, $P < 10^{-4}$). The percentage of
3 luminophores buried at depth was less than 5% in the “coarse sand” treatment whereas it was
4 more than 20% in the “fine sand” treatment” (Fig. 2).

5

6 *Influence of the sediment physical characteristics on hydraulic exchanges and microbial* 7 *processes involved in CPOM processing*

8 Mean (\pm S.D.) hydraulic conductivity measured for the “fine sand” treatment was about 8-fold
9 lower than for columns topped with “coarse sand” (Fig. 3; 2.02 ± 0.67 and $15.95 \pm$
10 4.81 cm.h^{-1} , respectively). The decreases with depth of O_2 and NO_3^- concentrations (Fig. 4;
11 $F_{(3,276)} = 933.73$ and 134.90 , respectively, $P < 10^{-4}$ for both) in the interstitial water were
12 higher in “fine sand” than in “coarse sand” treatment (“sediment-by-depth interaction effect”:
13 $F_{(3,276)} = 200.85$ and 159.26 for O_2 and NO_3^- respectively, $P < 10^{-4}$ for both). This difference
14 was particularly marked in the top sediment layer (O_2 concentrations reduced by about 87%
15 and 13% in “fine sand” and “coarse sand” treatments, respectively), and led to lower O_2 and
16 NO_3^- concentrations in the sedimentary habitat covered by fine sand deposits ($F_{(1,12)} = 631.70$
17 and 409.18 for O_2 and NO_3^- respectively, $P < 10^{-4}$ for both). Peaks of DOC, NH_4^+ , NO_2^- and
18 PO_4^{3-} concentrations were only recorded in the “fine sand” treatment, leading to higher
19 concentrations of these solutes in “fine sand” than in “coarse sand” treatment (Fig. 4; $F_{(1,12)} =$
20 93.02 , 665.57 , 193.27 , 16.13 , for DOC, NH_4^+ , NO_2^- and PO_4^{3-} respectively, $P < 10^{-4}$ for all).

21 Determinations of the dry mass of leaf litter retrieved at the end of the experiment (Fig. 5A)
22 showed that the daily mass loss rate was 31% lower in the “fine sand” treatment than in the
23 “coarse sand” treatment ($F_{(1,12)} = 14.39$, $P = 0.043$). In parallel, the total abundance of bacteria
24 (Fig. 5B), the abundance of active bacteria (Fig. 5C), the fungal biomass (Fig. 5D), the

1 glucosidase activity (Fig. 5H) and the leucine aminopeptidase activity (Fig. 5I) were
2 significantly lower in “fine sand” than in “coarse sand” treatment (contrasts: comparisons
3 without *T. tubifex*: $|t|_{12} = 2.57, 2.87, 2.50, 5.18$ and 7.14 , respectively, $P < 0.028$ for all).
4 Potential aerobic respiration (Fig. 5E), potential denitrification (Fig. 5F) and xylosidase
5 activities measured on leaves were not significantly influenced by sedimentary conditions
6 (contrasts: $|t|_{12} = 1.43, 0.99$ and 0.76 , respectively, $P > 0.176$ for all).

7

8 ***Context-dependent influence of tubificid worms on hydrologic exchanges, biogeochemical*** 9 ***processes and CPOM processing***

10 The influence of tubificid worms on hydraulic conductivity (Fig. 3) and water chemistry (Fig.
11 4) was dependant on the physical characteristics of the top sediment (“sediment-by-worms
12 interaction effect”: $F_{(1,12)} = 24.67, 48.41, 147.73, 13.76, 96.24$ and 4.04 for hydraulic
13 conductivity, O_2 , NO_3^- , SO_4^{2-} , NH_4^+ , and PO_4^{3-} concentrations, respectively, $P < 0.044$ for
14 all). While worms had low influence on hydraulic conductivity and concentrations of solutes
15 in “coarse sand” treatment, they increased by more than 6 fold the hydraulic conductivity in
16 “fine sand” treatment. Consequently, the presence of tubificid worms increased O_2 and NO_3^-
17 concentrations and strongly reduced the peaks of solutes (DOC, NH_4^+ , NO_2^- and PO_4^{3-})
18 released in the sedimentary columns with “fine sand” treatment.

19 Similarly, the influence of *T. tubifex* on microbial characteristics and associated processing of
20 buried leaf litter was dependant on the physical structure of the sedimentary habitat (Fig. 4).
21 We did not observe any influence of *T. tubifex* on microbial characteristics measured on
22 leaves buried in “coarse sand” systems (Fig. 5; contrasts: $|t|_{12} = 0.24, 0.83, 0.02, 1.32, 0.97$
23 and 1.05 for total abundance of bacteria, abundance of active bacteria, fungal biomass,
24 xylosidase, glucosidase and leucine aminopeptidase, respectively, $P > 0.207$ for all) except for
25 potential aerobic respiratory activity ($|t|_{12} = 2.77$, $P < 0.018$). In contrast, *T. tubifex* had a

1 positive influence on most microbial variables in “fine sand” treatment (contrasts: $|t|_{12} = 4.63$,
2 4.42, 3.05, 8.04 and 4.46 for total abundance of bacteria, abundance of active bacteria,
3 xylosidase and leucine aminopeptidase respectively, $P < 0.009$ for all; and $|t|_{12} = 1.98$, $P =$
4 0.071 for glucosidase activity) with the exception of potential denitrification ($|t|_{12} = 0.21$, $P <$
5 0.835).

6 In parallel, *T. tubifex* increased the daily loss rate of leaf litter mass in the “fine sand”
7 treatment by about 30% (Fig. 4A; $|t|_{12} = 3.40$, $P < 0.006$) where they counteracted the
8 negative influence of fine sediment deposition on leaf litter degradation ($F_{(1,12)} = 5.61$, $P =$
9 0.036). Such an effect was not observed in the “coarse sand” treatment (Fig. 5A, $|t|_{12} = 0.17$, P
10 = 0.867).

11

12

13 **Discussion**

14

15 *Contrasted biogeochemical processes induced by sediment characteristics.*

16 Our study confirmed the expectation that the biogeochemical functioning of the hyporheic
17 zone is strongly influenced by the sediment context. The high hydraulic discharge rates in
18 systems topped by a 2-cm thick layer of coarse sand (highly permeable systems) generated
19 aerobic conditions (O_2 concentration $> 2 \text{ mg.L}^{-1}$) throughout the sedimentary matrix (to a
20 depth of 13 cm). As a consequence of the O_2 availability, NO_3^- and SO_4^{2-} -as less energetically
21 favourable electron acceptors for OM mineralization (Hedin et al. 1998)- were not consumed
22 for OM mineralization. In these conditions, we did not observe any significant production of
23 solutes linked to anaerobic OM degradation in sediments (i.e. NH_4^+ , PO_4^{3-} and DOC) (Nogaro
24 et al. 2007). Hydraulic conductivity and discharge rates were 85% lower in sediment covered
25 by a 2-cm thick layer of fine sand, in comparison with systems topped by coarse sand. As a

1 consequence of the reduced hydraulic discharge rates, we observed sharp decreases in O₂ and
2 NO₃⁻ concentrations along depth in these systems. The rapid along-depth succession of
3 metabolic pathways was in accordance with predictable thermodynamic sequences (based on
4 free energy yields): O₂ being consumed first during OM mineralization in the oxic zone,
5 followed by the consumption of NO₃⁻ (denitrification), manganese and iron oxides, SO₄²⁻ and
6 carbon dioxide (Hedin et al. 1998, Baker et al. 2000, Kristensen 2000). Since O₂ was limiting
7 in the first centimeters of sediments, most of the sedimentary matrix (and thus buried leaf
8 litter) was under anaerobic conditions, leading to the release of NH₄⁺, PO₄³⁻ and DOC. In such
9 O₂-limited system, microbial abundances (total abundance of bacteria, abundance of active
10 bacteria and fungal biomass) and activities (glucosidase and leucine aminopeptidase
11 activities) were altered, and rates for microbial-mediated ecological processes occurring in the
12 HZ were reduced (leaf litter decomposition was 30% lower than in not-stressed coarse-sand
13 treatment).

14 The contrasts in biogeochemical conditions between the two sedimentary contexts were
15 consistent with field studies showing that reduced hydrologic exchanges due to clogging
16 favoured the occurrence of anaerobic processes such as denitrification, sulphato-reduction and
17 methanogenesis (Dahm et al. 1987, Brunke and Gonser 1997, Boulton et al. 1998, Lefebvre et
18 al. 2004). Our results are also in accordance with other studies showing that biogeochemical
19 conditions, in particular the availability of electron acceptors (mainly O₂ and NO₃⁻), strongly
20 affect (i) the fungal colonization of leaves (Medeiros et al. 2009) and microbial enzymatic
21 activities such as cellulase and peptidase activities (Montuelle and Volat 1997) and (ii) OM
22 degradation rates (Chauvet 1988, Claret et al. 1998, Dahm et al. 1998, Lefebvre et al. 2005). It
23 is therefore clear that sediment contexts that lead to low hydrological exchanges and O₂
24 concentration in sediments limit the growth and activity of microbial communities and
25 ultimately the rates of microbial-mediated ecological process occurring in the HZ. Finally, we

1 efficiently recreated the hydrological and biogeochemical functioning of 2 contrasted
2 sediment contexts and highlighted the key role played by O₂ as resource for microorganisms
3 developed in sedimentary habitats.

4

5 ***Modulation of bioturbator effects on biogeochemical processes by sediment context.***

6 The present study confirmed our hypothesis that the effects of bioturbators on hydraulic
7 conductivity and microbial-mediated processes depend on the sediment context.

8 As upward conveyors (feeding on sediment at depth and ejecting faecal pellets at the
9 sediment–water interface, Fisher et al. 1980, McCall and Fisher 1980), tubificid worms can
10 build networks of tubes and burrows that may extend as deep as 20 cm in sediments.
11 However, our results showed that both the bioturbation activity and the vertical distribution of
12 tubificid worms were modulated by the sedimentary characteristics. Using luminophore as
13 particle tracers, we noted that tubificid worms significantly reworked the top of the
14 sedimentary column with a fine sand layer whereas it was not the case with a coarse sand
15 layer. This contrast in bioturbation degree was linked to the vertical distribution of worms.
16 While worms used the whole sediment column in the systems topped with coarse sand, most
17 of tubificid worms were found in the top 0-5 cm in systems topped by a layer of fine sand.
18 Fine sand probably acted as preferential feeding zone for *T. tubifex* (Juget 1979, Rodriguez et
19 al. 2001), which strongly influenced the vertical distribution of worms in the sedimentary
20 column. The different bioturbation activities exhibited by *T. tubifex* in the two sediment
21 contexts could explain their contrasting effects on hydraulic conductivity (see Fig.1 for the
22 successive effects of bioturbators). By producing galleries through the fine sand layer, *T.*
23 *tubifex* create water pathways that counteracted the adverse effect of fine sediment deposition
24 on water exchanges. The 6-fold increase in hydraulic conductivity due to *T. tubifex* in the

1 fine-sand treatment stimulated the exchanges of water and O₂ from surface to deep sediment
2 layers, restoring aerobic conditions in the sedimentary column (Fig. 1). Modification of
3 aerobic-anaerobic conditions observed through the increase in O₂ and NO₃⁻ concentrations
4 was also associated with a lack of NH₄⁺, PO₄³⁻ and DOC accumulation in the sedimentary
5 habitat bioturbated by tubificid worms. The increase in electron acceptors (O₂ and NO₃⁻)
6 availability for micro-organisms with *T. tubifex* has stimulated microbial communities
7 (abundances and activities) associated to the buried OM, leading to an increase by 30% of
8 OM breakdown rate in sedimentary systems covered by fine sand (Fig. 1). In contrast,
9 tubificid worms did not affect hydraulic conductivity and the subsequent chemical conditions
10 (availability of electron acceptors) in sediments topped by coarse sand. Consequently,
11 bioturbators did not influence microorganisms and microbial-mediated processes in
12 sediments.

13 Our study clearly demonstrated that the contribution of bioturbating invertebrates on
14 ecosystem processes was negatively correlated with the hydrologic exchanges occurring at the
15 water-sediment interface of the studied system, supporting conclusions from other studies
16 (Hakenkamp and Palmer 2000, Boulton et al. 2002). Bioturbators are able to strongly
17 influence water fluxes (through biological decolmation) in sedimentary habitats characterized
18 by low hydrologic exchanges (affected by the deposition of fine sediment particles), whereas
19 they only slightly modulate existing water fluxes in habitats with high hydrologic exchanges.
20 If the low influence of bioturbators on hydraulic conductivity in systems covered with coarse
21 sand could be linked to their low sediment reworking activity (Fig. 2), it could also resulted
22 from the reduced ability of bioturbation to increase hydrologic exchanges in a system that is
23 already highly permeable.

24

25

1 ***What underlies context dependency in the impact of engineers on other biota?***

2 Most ecosystem engineering studies quantified the effects of engineers on some biotic
3 variables in a given habitat without taking into account the modulation of organism
4 engineering by environmental conditions (Crain & Bertness 2006). Our study clearly
5 demonstrates that the influences of bioturbators as engineers may vary across environmental
6 contexts. Few studies have already reported similar observations for various types of
7 ecosystems and various types of ecosystem engineers (e.g. Spooner and Vaughn 2006,
8 Nogaro et al 2009, de Moura Quierós A 2011) but they did not finely decrypt the complete
9 mechanisms by which ecosystem engineers and habitat characteristics interacted to shape
10 biological communities and/or ecosystem functions. More precisely, the environmental
11 context can influence the impacts of engineers on communities by modulating (i) the degree
12 with which its activity(ies) (or structure(s)) generates physical changes in the environment
13 and/or (ii) the degree with which physical changes can generate abiotic changes in the
14 environment (Fig. 1, see also Jones et al. 2010). As most of studies dealing with context
15 dependency examined only partially the engineering sequence, they could not provide
16 explanations about the mechanisms underlying context dependency. By examining the whole
17 engineering process as a detailed sequence of successive cause-effect relationships, our study
18 allowed to conclude that the influence of bioturbators on microbial-mediated processes was
19 mainly mediated by the organism ability to change abiotic factors that are limiting for micro-
20 organisms (i.e. O₂). This conclusion supports the general (and not tested before) idea that
21 ecosystem engineering is not an idiosyncratic process and that the magnitude of engineering
22 impacts on a biotic variable controlled by one or more limiting abiotic factors depends on the
23 degree to which the limiting abiotic factor(s) is/are modified relative to the unengineered state
24 (e.g., Gutiérrez et al. 2003, Gutiérrez and Jones 2006, Jones et al. 2010).

25

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25 **Tables**

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1 Table 1. Specific area, total organic carbon (TOC), total nitrogen (TN) and total phosphorus
2 (TP) of the sediment used as top-sediment layer (mean \pm SD, n= 4 for specific area, n=5 for
3 TOC, TN and TP).

4

	Specific area (cm ² g ⁻¹)	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	TP (mg kg ⁻¹)
Coarse sand	59.37 \pm 0.34	0.97 \pm 0.08	0.17 \pm 0.03	3.76 \pm 3.47
Fine sand	1465.00 \pm 33.17	16.6 \pm 1.6	1.35 \pm 0.06	6.62 \pm 1.36

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26 **Figure captions**

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2 Fig. 1. Successive steps for (i) a general case of ecosystem engineering sequence (in
3 grey) and (ii) the effects of *T. tubifex* on microorganisms in sedimentary habitats
4 (study case, in white). Note that feedbacks to the engineer and other relationships in the
5 ecosystem engineering can exist and are not represented here but can exist (for more
6 information, see Gutiérrez and Jones 2008, Jones et al. 2008).

7

8 Fig. 2. Vertical distribution of *T. tubifex* in sediment and their associated effect on vertical
9 profiles of luminophores in sediment columns covered by fine sand or coarse sand.

10

11 Fig. 3. Effect of *T. tubifex* on hydraulic conductivity measured every 10 days in sediment
12 columns covered by fine sand or coarse sand.

13

14 Fig. 4. Effect of *T. tubifex* on depth profiles for O₂, N-NO₃⁻, N-NH₄⁺, N-NO₂⁻, and DOC
15 concentrations determined at 4 depths (from H1: 2 cm above sediment interface, to H4: 13 cm
16 below sediment interface) after 36 days in sediment columns covered by fine sand or coarse
17 sand.

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19 Fig. 5. Effect of *T. tubifex* on leaf mass loss rate (A), and microbial characteristics measured
20 at the end of the experiment on leaves buried at 9 cm depth in sediment columns covered by
21 fine sand or coarse sand: total abundance of bacteria (B), abundance of active bacteria (C),
22 fungal biomass (D), potential respiratory activity (E), potential denitrification (F), xylosidase
23 (G), glucosidase (H), and leucine aminopeptidase activities (I).

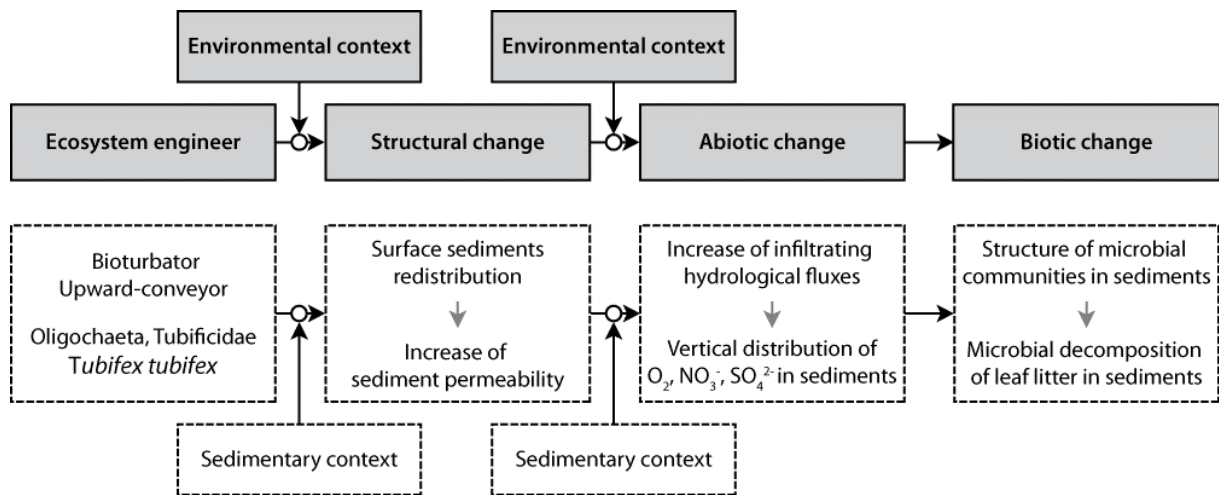
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3 **Figures**

4

5 Figure 1

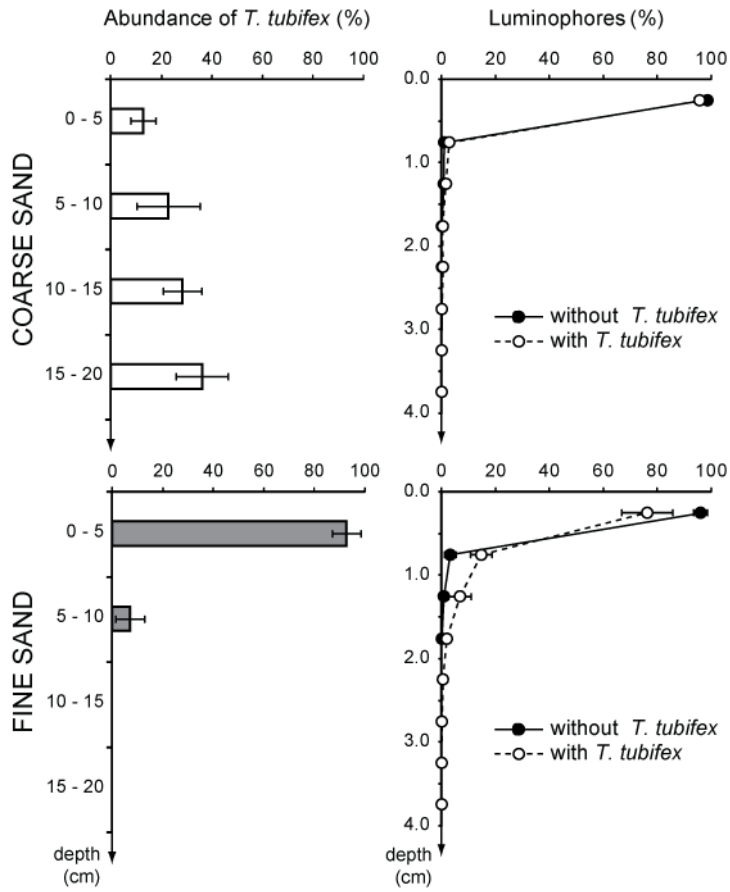


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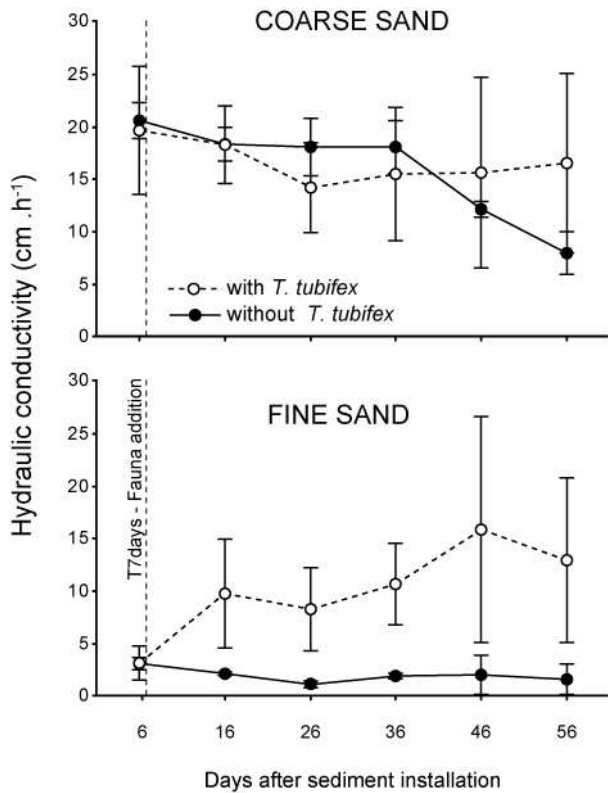
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9 Figure 2



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2 Figure 3

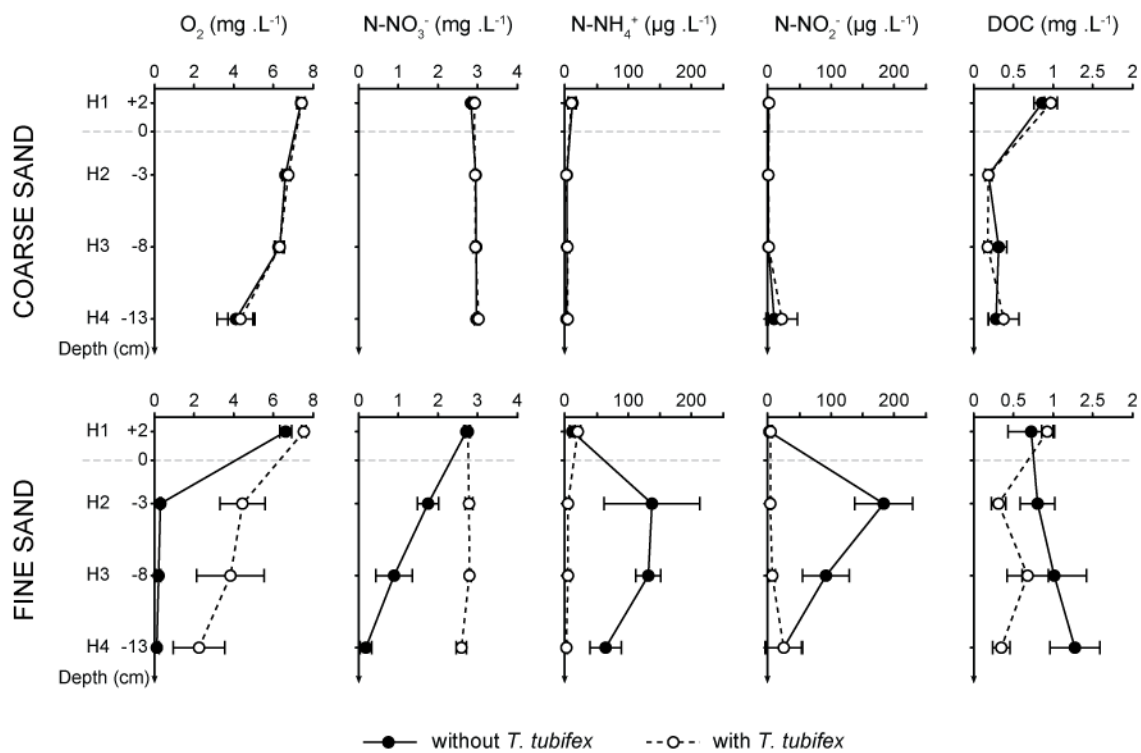


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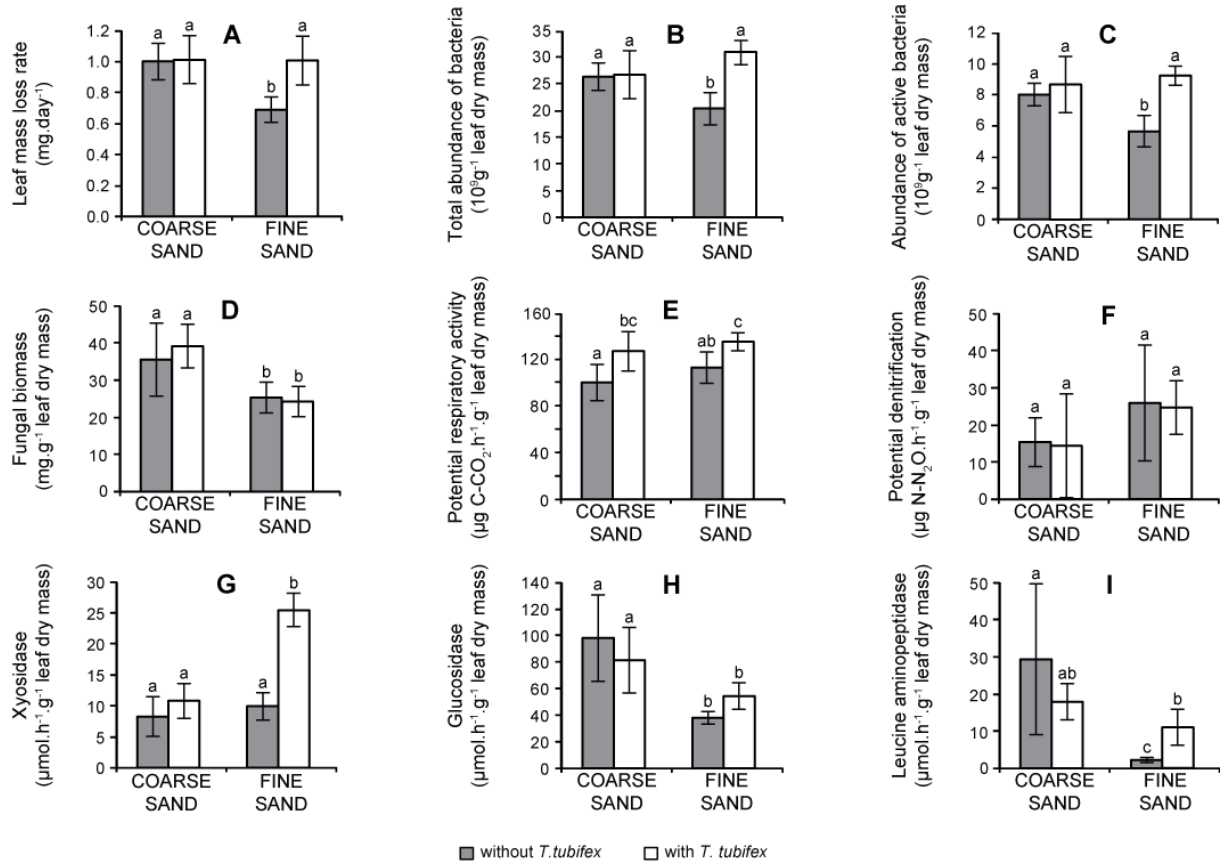
3 Figure 4



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5 Figure 5

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