Supplementary Information (SI) for Environmental Science: Nano.

Digestive Biotransformation of Graphene Oxide and Reduced Graphene Oxide in the amphibian *Xenopus laevis*: Structural, Chemical, and Toxicological Changes

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Supplementary Data

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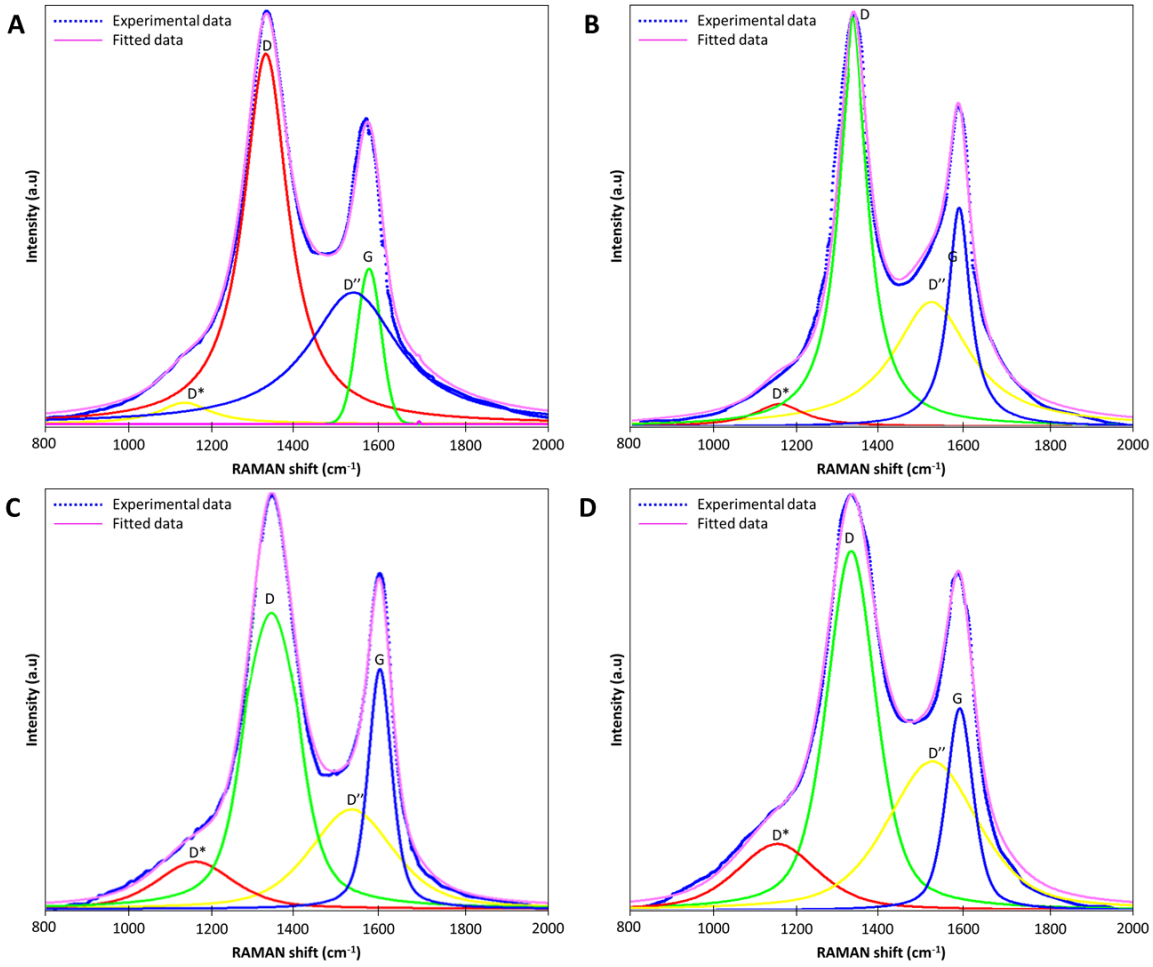
S.I 1: Deconvoluted Raman spectra for GO (A), rGO (B), dGO (C) and drGO (D).



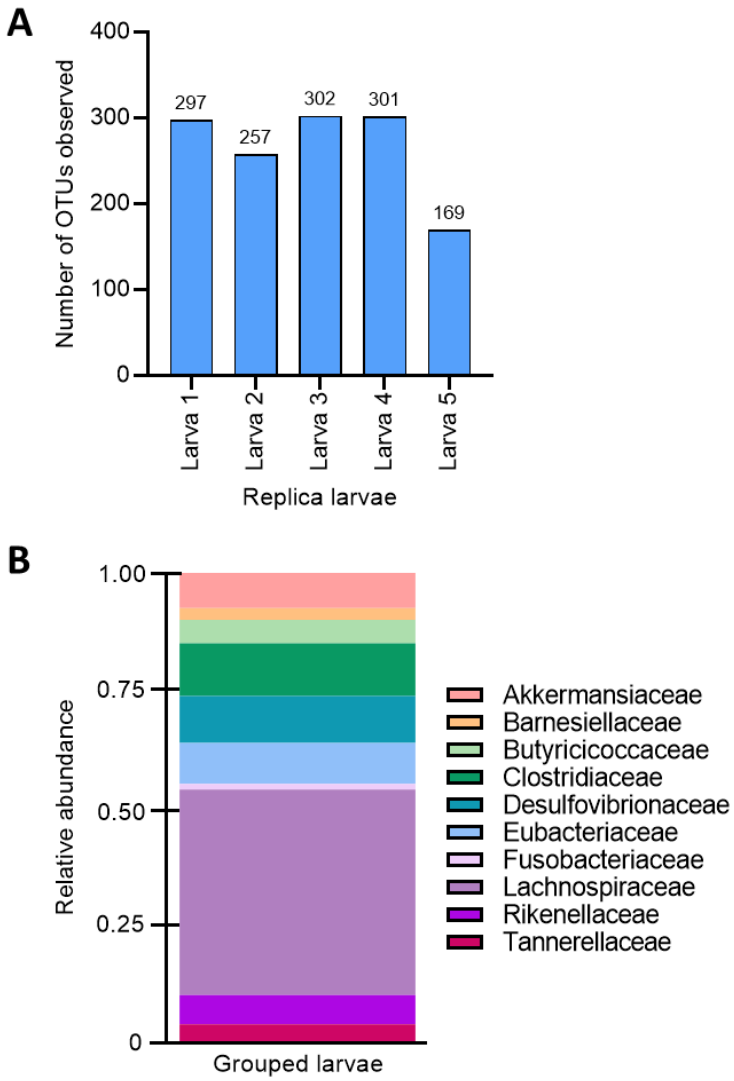
S.I 2: Intestinal microbiota analysis protocol

At the beginning of the experiment, the intestines of individuals from the same clutch as those used for exposure were collected (n = 5) and frozen at −20 °C until DNA extraction to characterize the microbiota of Xenopus larvae. Total DNA was extracted using a DNAeasy Power Soil kit Pro® (Qiagen, France) following the manufacturer's instructions excepted for a heating step at 65 °C for 10 min added after addition of the lysis solution to increase cell disruption. DNA concentration in extracts was measured using a spectrophotometer Nanodrop ND-1000® (Thermo Fisher Scientific®). DNA extracts were stored at −20 °C.

The V3–V4 region of the bacterial 16S rRNA gene was amplified using the universal primers 341F and 806R. PCR reactions were carried out using GoTaq® Rapid PCR Master Mix (Promega, Ref. CS3083A02) in 12.5 µL volumes, containing 10.5 µL of DNA extract (~10 ng/µL) and 1 µL of each primer. Cycling conditions consisted of initial denaturation at 95 °C for 1 min, followed by 38 cycles of denaturation/annealing/extension at 95/55/72 °C for 4/2/10 sec and a final elongation at 72 °C for 1 min. A second PCR was then performed to add sequencing adapters and dual indices compatible with Illumina MiSeq sequencing. The products were pooled in equimolar amounts according to band intensities, and fragments between 400–750 bp were size-selected and purified using a PippinHT system (Sage Science) with a 1.5% agarose cassette (Marker 15C). The purified library pool was quantified by fluorimetry (Qubit 4.0, Thermo Fisher Scientific) and quality-checked by capillary

electrophoresis (QIAxcel®, Qiagen). Sequencing was carried out on an Illumina MiSeq platform using a 2 × 251 bp paired-end run with the MiSeq Reagent Kit v2 (500 cycles; Illumina, Ref. MS-102-1003). PCR and sequencing were performed by Biomnigene company (www.biomnigene.fr, Besançon, France).

The raw sequences were processed using the FROGS pipeline (v4.1.0) which was implemented on the Galaxy online platform (v25.0.2.0). Initial quality control was performed with MultiQC, which confirmed good overall read quality and therefore did not require additional trimming. Paired-end reads were merged with a maximum mismatch rate of 0.1 using Vsearch (Rognes et al., 2016), and sequences outside the length range of 380–500 nucleotides were discarded. Operational Taxonomic Units (OTUs) were generated with Swarm (Mahé et al., 2014) using an aggregation distance of 1. Chimeric sequences were detected and removed with Vsearch. OTU filtering retained only those observed in at least two independent samples, and clusters with a minimum relative abundance below 0.0005 (proportion of all sequences) were discarded, following the recommendations of Bokulich *et al.* (Bokulich *et al.*, 2013). Taxonomic assignment was performed against the SILVA 16S database (release 138.1, Pintail100) using BLASTn+ (Camacho et al., 2009). Downstream analyses were carried out in R using the Phyloseq package (v1.46.0) (McMurdie and Holmes, 2013).



S.I 3: Data on the intestinal bacterial microbiota of the larvae used in the digestion experiment. Number of OTUs observed (A) and the top 10 most abundant bacterial families (n = 5) (B).

References

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