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Potential of eDNA metabarcoding for the inventory of alpine plants and mammals in the Vallorcine catchment area

*ABRAN Camille¹, ALAMI Soufiane¹, BERTRAND Océane¹, BESSONNIER Trystan¹, DROUET Arthur¹, KOVACS Marion¹, LOPES Opaline¹, TORO ULLOA Mael¹.

*Alphabetical Order

Supervised by : CRUAUD Perrine^{1,2}, FRANÇOIS Clémentine^{1,2}, HAUDRY Annabelle^{1,3}, LEFEBURE Tristan^{1,2}, PANSU Johan^{1,2}.

¹Université Claude Bernard Lyon 1, 69 000 Villeurbanne, France

²Laboratoire d'Ecologie des Hydrosystèmes Naturels et Anthropisés, CNRS UMR 5023, Université Claude Bernard Lyon 1, 69 000 Villeurbanne, France

³Laboratoire de Biométrie et Biologie Evolutive, CNRS UMR 5558, Université Claude Bernard Lyon 1, 69 000 Villeurbanne, France

Abstract :

Environmental DNA (eDNA) is increasingly used in ecology to detect biodiversity, at relatively low cost. In this study, we evaluate how effectively eDNA reflects mammal and plant diversity in a mountain watershed, using the Nant de Loriaz catchment as a case study. We compared eDNA results with traditional methods such as vegetation plots and camera traps for mammals, tested differences between water and sediment samples, and inspected how eDNA signals change along an upstream-downstream gradient. Our results show that eDNA reflects only part of the diversity detected by traditional surveys, with some species detected only by eDNA and others only by traditional methods. This comparison highlights the complementarity of both approaches, with eDNA improving detection of elusive or hard-to-observe species, while traditional surveys provide more reliable species-level identification for mammals. Sediment samples mostly reflect local communities and may retain DNA for longer periods, providing a snapshot of species that persist in a given location. In contrast, water samples integrate eDNA from both local aquatic sources and upstream inputs, combining signals from nearby and more distant communities. Along the upstream-downstream gradient, we found no clear nestedness pattern; instead, high species turnover indicates that eDNA primarily reflects local communities shaped by environmental conditions rather than passive downstream transport. Our results show that eDNA can improve the precision and resolution of biodiversity identification, but its performance depends on environmental conditions and context. These findings highlight both the potential and the limitations of eDNA for informing conservation decisions.

Keywords : Alpine biodiversity, Environmental DNA, Metabarcoding, Molecular biology, NGS, Bioinformatic



1. Introduction

Environmental DNA (eDNA) is genetic material originating from the hair, skin, urine, feces, gametes, or carcasses of organisms that is present, in a more or less degraded form, in water, soil, or sediment (Beng & Corlett, 2020). DNA is, in fact, continuously released by organisms and it can persist in the environment for several years before being degraded. The rate of DNA degradation depends on the biological, physical, and chemical properties of the microenvironment (Pedersen et al., 2015).

DNA metabarcoding designates high-throughput identification using the total and typically degraded DNA extracted from an environmental sample (Taberlet et al., 2012). With the invention of PCR-based amplification of DNA and the design of universal primers, species identification moved towards being based on direct sequencing or hybridization of PCR products (Taberlet et al., 2012). Metabarcoding uses universal primers to simultaneously detect millions of DNA fragments from the widest possible range of species from multiple trophic levels and domains of life (Beng & Corlett, 2020). eDNA metabarcoding enables the detection of genetic material from various environmental samples, including water and sediments (Tuhřinová et al., 2026). This approach allows for the identification of taxa by comparing recovered DNA sequences with reference barcode databases (Tuhřinová et al., 2026). eDNA presents an alternative and potentially more integrative source of biodiversity information, with genetic material preserved for extended periods, from months to centuries (Tuhřinová et al., 2026). The advantage of this would be to enable an inventory of ecosystem biodiversity to be made, with the advantage of being non-intrusive and providing a comprehensive representation of ecosystems.

Mountains are highly sensitive ecosystems, particularly vulnerable (Tuhřinová et al., 2026). Species inhabiting these locations have limited refugia, making them susceptible to warming temperatures and environmental alterations (Tuhřinová et al., 2026). Therefore, metabarcoding in alpine environments, which are difficult-to-access ecosystems, could compensate for the limitations of traditional identification techniques, such as observation and photo captures.

The main objective of this study is to assess the ability of eDNA metabarcoding to characterize mammal and plant diversity within a mountain watershed. Using the Nant de Loriaz catchment (Vallorcine, France) as a case study, we address the following research questions:

- (I) Do molecular and traditional methods of studying biodiversity (plants and mammals) provide similar results? Using data collected by CREA Mont-Blanc, a scientific NGO, we will compare our eDNA results with their field observations.
- (II) Do water and sediment matrices provide a similar eDNA signal? This question aims to assess whether the matrix from which environmental DNA is collected significantly influences the detected biodiversity and its relative abundance.
- (III) How does river eDNA integrate biodiversity signals along an upstream-downstream gradient? We test two hypotheses: one in which the eDNA signal accumulates downstream, and another in which community composition is progressively renewed.

The study area spans an altitudinal gradient from 1 287 to 2 320 m (Figure 1), with sampling sites distributed from alpine to forested zones. This catchment-scale design allows the investigation of altitudinal patterns in species detection as well as the potential downstream transport of eDNA.

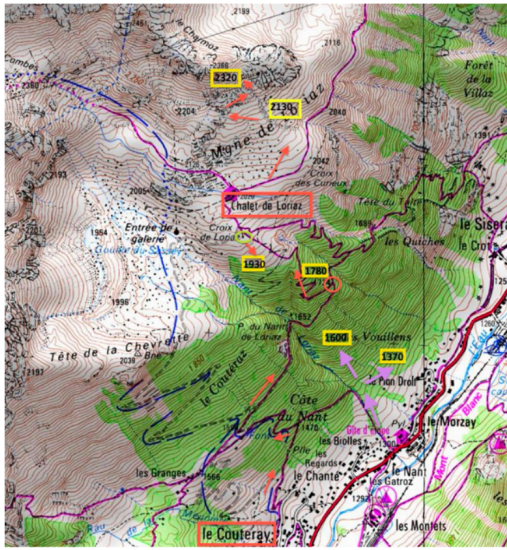


Figure 1 : Emplacement des placettes du gradient de Loriaz

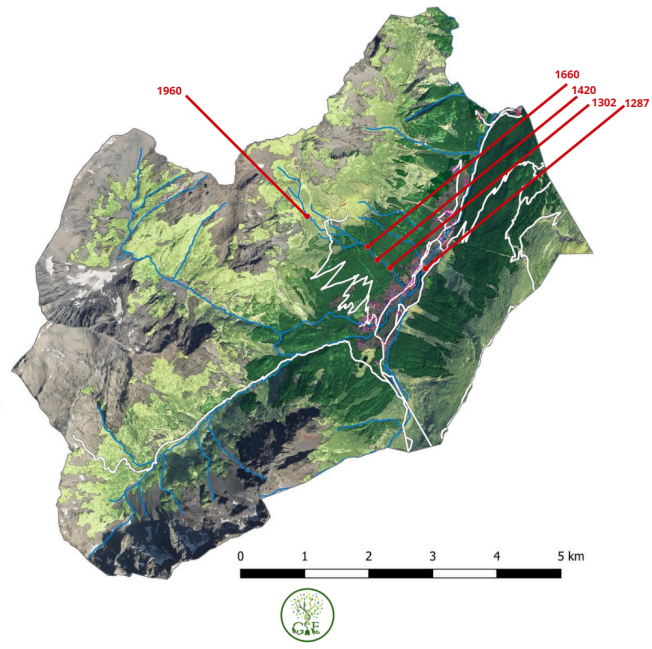


Figure 1 : Location of sampling sites along the altitudinal gradient of Nant de Loriaz (Vallorcine, France). Left: topographic map showing the location of the Loriaz gradient plots between 1,287 m and 2,320 m altitude, including the CREA Mont-Blanc monitoring sites. Right: boundaries of the Nant de Loriaz watershed (white line), hydrographic network (blue lines) and location of the main eDNA sampling sites (red dots), with indication of their altitude.

2. Material and methods

2.1. Study site and sampling

Fieldwork took place on September 4 and 5, 2024, during which time all samples were collected along the Nant de Loriaz river in Haute-Savoie (Figure 1), at different locations at different altitudes (site 1 - 1,960 m, site 2 - 1660 m, site 3 - 1420 m, site 4 - 1302 m), with the exception of samples taken at site 5 (1287 m), which came from the Eau Noire, another watercourse into which the Nant de Loriaz flows. For each sampling site, the temperature, conductivity, and pH of the water were measured using a multiparameter probe. The environmental context is presented in the form of a map in Figure S1.

A total of 15 sediment samples were collected from the river, including three replicates per site. All samples were collected using a sterile spoon and syringe. Before being placed in 15 mL Falcon tubes, the sediments were decanted to remove as much water as possible. The samples were stored in a cooler at 4°C during the trip and then in freezers at -20°C once in the laboratory.

For water samples, three replicates were taken for sites 1 to 4, one replicate for site 5 and one filtration control, for a total of 14 samples. 20L of water were collected and filtered for each sample using one of the two available pumps, both of which contained WATERRA 0.45µL capsule filters. After filtration, 35 mL of Longmire preservation buffer was added to the capsules, which were then shaken manually for 10 minutes before being stored in airtight plastic bags at 4°C during the trip and then in freezers at -20°C once in the laboratory, after being transferred to Falcon tubes. Filtration control was performed with pure sterile water, collected and stored using the same tools and method as the other water samples.

2.2. DNA extraction, amplification and sequencing

The protocols and results of this part are available on the Git repository (Figure S2)

The DNA from water samples was extracted using the Nucleospin eDNA water kit from Macherey Nagel (ref 740402) with the following modifications. Falcon tubes containing the

filtered water samples with Longmire lysis buffer were centrifuged at maximum speed (3,354 g) for 45 minutes at 10°C. The supernatant was removed and 1.5 mL of lysis solution was added along with 25 µL of proteinase K, vortexed for 10 seconds, and incubated with agitation at 250 rpm for 10 minutes.

The lysate was transferred to a new 2 mL tube. At the inhibitor removal step, 270 µL of ACID Buffer was added instead of 360 µL, followed by centrifugation at 10,000 g for 2 minutes, and the supernatant was removed. The tube was inverted once and the pellet was resuspended by pipetting. Following centrifugation at 10,000 g for 3 minutes, 25 µL of NucleoTrap suspension was added and mixed by pipetting. After incubation at ambient temperature for 5 minutes, samples were centrifuged at 450 g for 3 minutes.

At the binding step, 38 µL of PREC3 buffer was added to the pellet. The suspension was transferred to a NucleoSpin eDNA XS column (pre-labeled with sample name) and centrifuged at 10,000 g. The flow-through was discarded. Washing steps were performed with 400 µL of Resuspension Buffer RESU (vortexed for 1 minute at 10,000 g, supernatant discarded), followed by 400 µL of Wash Buffer WB (vortexed for 1 minute at 10,000 g, supernatant discarded without approaching the pellet). After drying centrifugation at 10,000 g, 100 µL of Elution Buffer BE was added. Samples were vortexed for 5-10 seconds until all material was resuspended, incubated at ambient temperature for 1 minute, centrifuged at 10,000 g, and the eluate was collected. A negative control was included at the lysate step. Samples were stored at 4°C.

The DNA from sediment samples was extracted using the Nucleospin Soil kit from Macherey Nagel (ref 740780) with the following modifications. Fifteen grams of defrosted sediment were transferred to a 50 mL Falcon tube and mixed with an equivalent volume of phosphate buffer (~15 mL). This mixture was agitated for 15 minutes on an orbital/rotary shaker to allow desorption of eDNA from sediment particles. Two 2 mL aliquots from each sample were transferred to fresh 2 mL tubes and centrifuged at 10,000 g for 10 minutes to pellet sediment debris and particles.

The supernatants were carefully transferred to fresh 2 mL tubes, and 400 µL of SB Buffer was added to each aliquot to adjust binding conditions. The mixture was then loaded onto a NucleoSpin Soil column pre-labeled with the sample name. The SB/supernatant mixture was loaded in 550 µL aliquots, with the column centrifuged at 11,000 g for 1 minute after each load, and the flow-through discarded. This process was repeated 4-5 times to fully load both aliquots. Washing steps followed: the first wash used 500 µL of SB Buffer (centrifuged at 11,000 g for 30 seconds), the second wash used 550 µL of SW1 Buffer (centrifuged at 11,000 g for 30 seconds), and the third and fourth washes each used 650 µL of SW2 Buffer with 2 seconds vortexing before centrifugation at 11,000 g for 30 seconds. After all washes, the column was centrifuged at maximum speed for 2 minutes to dry the membrane completely. DNA was eluted by placing the column into a pre-labeled 1.5 mL Lobiind tube and adding 50 µL of Elution Buffer SE directly onto the membrane without touching the membrane. After incubation at ambient temperature for 1 minute, the column was centrifuged at 11,000 g for 30 seconds, and the eluted DNA was recovered. A negative control was included at the lysis step. Samples were stored at 4°C.

PCR1 amplifications were performed targeting seed plants and mammals, with all mammalian amplifications including human-blocking primers to reduce human DNA contamination. Triplicates of each sample were prepared for both plant and mammal amplification. Each PCR1 reaction contained 23 µL of Multiplex PCR Plus Master Mix from Qiagen (ref 206152) at a final concentration of 1X (in a final reaction volume of 25 µL) and 2 µL of template DNA. Negative controls consisted of 23 µL of master mix and 2 µL of ultrapure water. PCR reactions were set up in PCR plate strips.

This PCR used either trnL primers (P1) targeting the P6 loop from the chloroplast intron to amplify 10-200 bp long barcodes of plants (Taberlet et al., 2007) at a final concentration of 0.1 µM, or MamP007 primers (P2) targeting the mitochondrial 16S gene to amplify 53-84 bp barcodes of mammals (Giguet-Covex et al., 2014) at a final concentration of 0.1 µM.

Blocking primers (P3) targeting human 16S mitochondrial DNA were used when amplifying mammals' barcodes at a final concentration of 2 μM to reduce the amplification of human DNA. The PCR underwent an initial denaturation at 95°C for 5 minutes to activate the Taq HotStart, followed by 45 cycles of PCR, each composed of 30 seconds of DNA denaturation at 95°C, 30 seconds of hybridization at 50°C and 30 seconds of elongation at 72°C. The PCR1 ended with 10 minutes of final elongation at 68°C.

PCR1 products were analyzed by agarose gel electrophoresis on 1% agarose gels with 0.5X TAE buffer stained with ethidium bromide (0.5 $\mu\text{g}/\text{mL}$). Five microliters of each PCR product were mixed with 2 μL of gel loading buffer and loaded into gel wells. Electrophoresis was performed at 100 Volts for 30 minutes. The triplicates were then pooled together into a single 96-well PCR plate, and a quality control was performed on the pooled products using 5 μL of each pool on a 1% agarose gel under identical conditions.

The pooled PCR1 products were then processed at the [Symbiotron platform](#) for purification, indexing, and final purification. Prior to PCR2, the pooled PCR1 products were purified using the NucleoMag NGS Clean-up and Size Select kit from Macherey Nagel (ref 744970) with a 1.8X bead-to-sample ratio to avoid size selection and retain small amplified fragments. Purification was automated using the Kingfisher Apex instrument. The purified PCR1 products then underwent PCR2, which used the Illumina tail as primers to add "tags" and sequencing adapters. Each PCR2 reaction contained 25 μL of 2x KAPA HiFi HotStart ReadyMix, 5 μL of IDT indices (pre-plated), 10 μL of PCR Grade Water, and 10 μL of purified PCR1 product, for a total volume of 50 μL . The PCR2 was composed of an initial denaturation at 95°C for 3 minutes to activate the Taq HotStart. It was followed by 10 PCR cycles, each with 30 seconds denaturation at 95°C, 30 seconds hybridization at 55°C and 30 seconds elongation at 72°C. It ended with 5 minutes of final elongation at 72°C. The PCR2 products were then purified using the same kit and automated procedure (Kingfisher Apex) with a 1.8X bead-to-sample ratio to avoid size selection and retain small amplified fragments.

Quality controls were performed by Quantifluor measurement. All samples (n=68) were then normalized to 4 ng/ μL using a Beckman Coulter Biomek 4000 liquid handling workstation. Subsequently, 10 μL of each normalized sample were pooled together in a single Eppendorf Safelock tube. A final quality control using Qubit quantification confirmed a pooled library concentration of 4.74 ng/ μL . Finally, the amplicons sequencing was outsourced and performed on an Illumina NovaSeq platform by Novogene (Munich, GmbH) using paired-end reads (2 x 150 bp).

P1 : trnl primers used to amplify plants barcodes:

Trnl-g-F: 5'-GGGCAATCCTGAGCCAA-3'

Trnl-h-R: 5'-CCATTGAGTCTCTGCACCTATC-3'

P2 : MamP007 primers used to amplify mammals barcodes :

MamP007F:

5'-CGAGAAGACCCTATGGAGCT-3'

MamP007R: 5'-CCGAGGTCRCCCAACC-3'

P3 : Specific Human blocking primers used to reduce the amplification of Human DNA :

MamP007_B_Hum1:

5'-GGAGCTTTAATTTATTAATGCAAACAGTAC
C-C3-3'

2.3. Bioinformatics

A common pipeline (Figure S3) was created for analyses on mammals and plants. Each step was split into different scripts. All scripts used during the various analyses are available in the project's Git repository (Figure S2)

2.3.1. Reads preprocessing and quality control

Raw sequencing reads were demultiplexed by the sequencing facility.

The first steps of bioinformatic analyses were performed on the virtual research environment of the IFB Biosphere of Lyon1. Ubuntu 22.04 was used as the operating system. A quality control of the reads was then done using FastQC and MultiQC (v.1.33), to evaluate the quality of the sequenced samples. Primers were then removed from the reads using Cutadapt (v.4.4). Finally, reads pairs (R1 and R2) where one of the two or each of the reads was shorter

than 7 nucleotides were discarded, using Cutadapt (v.4.4), to avoid downstream artefacts linked to empty or too short reads.

2.3.2. Denoising and ASVs processing

Denoising was performed on R (v.4.3.3) using the DADA2 package (v.1.30.0). The data were filtered and cleaned to remove reads containing ambiguous bases, or with more than 2 errors or too short in length (less than 7pb). The sequences were then dereplicated and denoised to retain only a single sequence with its abundance. Read pairs R1 and R2 were merged using the default parameters, i.e., an overlap of 12pb. Finally, a contingency table of all ASVs found with their abundance per sample was generated, as well as a FASTA file of all ASV sequences

2.3.3. Creation of a reference database & taxonomic assignment

Two taxonomic databases (mammals and plants) were generated for this project using OBITools4 tools (version 4.4.0). The plant database was constructed using in silico PCR with trnL primers based on the PhyloAlpes regional molecular database (<https://phyloalps.osug.fr/>), which contains more than 3,000 plant taxa found in the Alps. The mammal database was produced using in silico PCR with MamP007 primers from the NCBI global molecular database (<https://www.ncbi.nlm.nih.gov/>). Once the sequences from the in silico PCRs had been obtained, they were filtered to retain only those with a complete taxonomic annotation (Kingdom, Phylum, Class, Order, Family, Genus, Species). A second filtering step was subsequently applied based on sequence length, retaining only sequences ≥ 7 nucleotides for plants and ≥ 50 nucleotides for mammals, corresponding to the expected size of the target barcode region. Finally, the resulting databases were saved in VSEARCH format to facilitate their formatting and use in the taxonomic assignment algorithm.

Taxonomic assignment was performed using the DTAXA algorithm (Package DECIPHER version 2.30.0 in R), based on sequence composition. To ensure optimal assignment with the IDTAXA algorithm, the databases underwent additional filtering steps and were reformatted to the

required format: Only taxa composed exclusively of nucleotides labeled “A”, “T”, “C”, or “G” were retained. The number of variants was limited to a maximum of 50 per species in order to prevent the overrepresentation of certain taxa (particularly human sequences or domesticated taxa). For the NCBI mammalian reference database, additional filtering was applied to retain only the orders Artiodactyla, Carnivora, Erinaceomorpha, Eulipotyphla, Lagomorpha, Rodentia, and Homo sapiens, corresponding to the groups of alpine mammals likely to be detected in our samples. Ultimately, 3,449 sequences were retained for plant ASV assignment, and 5,125 sequences were used for mammal ASV assignment. Details of the composition of the filtered reference databases used for assignment are available in Figure S4 and S5.

The cleaned databases were then formatted according to the structure expected by IDTAXA and trained on themselves to generate a taxonomic assignment model. This model was then used to classify our sequences. The IDTAXA algorithm assigns each sequence a taxonomic classification accompanied by a bootstrap score for each hierarchical level. If a taxonomic rank is assigned a bootstrap score of less than 50/100, the assignment stops at that level, reducing the risk of false positives during analysis.

2.3.4. Decontamination et samples filtering

PCR amplifications were found in the filtration, extraction and PCR controls. An ordination of the samples (Figures 2-3) shows that some controls are similar to the other samples : the extraction controls in the plants' samples, and all of the controls in the mammals' samples. Indeed, the contaminated controls contain ASVs with a similar sequencing depth than that of the other samples, and each contaminated control is different in terms of reads abundance and composition (Figures 2-3). As a consequence, we suspect that different cross-contamination events took place between the samples. As a result, we used the decontamination tool :

Contaslayer , from the Metabar package (version 1.0.0) of R. Contaslayer compares the abundance of each ASV inside the samples and controls. If an ASV is found in a higher number in a control, it is considered to be a contaminant

We then filter the dataset so as to remove the ASV not assigned to Streptophyta for the Plants samples, which correspond to 58% of the ASV (Figure S6 c) but only 14% of the reads in all of the ASVs in these samples (Figure S6 d). We chose to filter out all of the ASVs not assigned to Streptophyta Phylum because it was the largest Plantae group in which the ASVs were assigned.

For the mammals samples, all of the ASV not assigned to mammals were filtered out, as well as all of the ASV assigned to Homo genera, which in total correspond to 90% of the ASVs (Figure S6 a) but only 42% of the reads in all of the ASVs in these samples (Figure S6 b). The ASVs assigned to the Homo genera were removed because of their high abundance in all of the samples, most likely due to contamination

and will be deleted from the dataset : in total, 96 out of 3713 mammals ASVs, as well as 83 out of 191, 9 plant ASVs were taken out of the samples.

of the samples during the sampling and the biomolecular treatment by the experimenter, as well as the presence of Homo sapiens in the studied mountain watershed. This high ASV abundance in all samples can potentially lead us to underestimate the difference between the samples.

The decontaminated ASVs will be used to answer the first question aiming to compare molecular and traditional methods of studying biodiversity. The decontaminated and filtered ASVs will be normalized so as to be used to answer the questions 2 and 3, aiming to compare the difference of DNA signal depending on the matrix (water or sediment) and the altitude of the sampling point will use more specifically the decontaminated and filtered ASV.

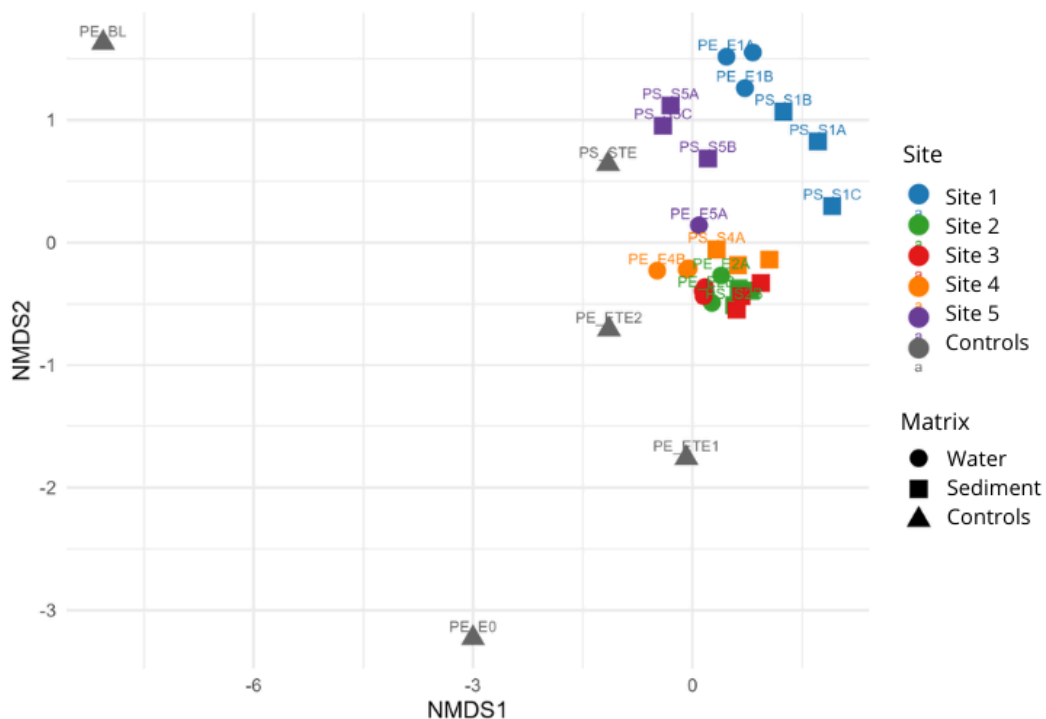


Figure 2 : NMDS of plants community composition based on the repartition of the ASVs in the Plants samples along the Nant de Loriaz altitudinal gradient and the sampled matrix (water or sediment). Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity using the repartition of the ASVs of Plants genera in the Plants samples before decontamination.

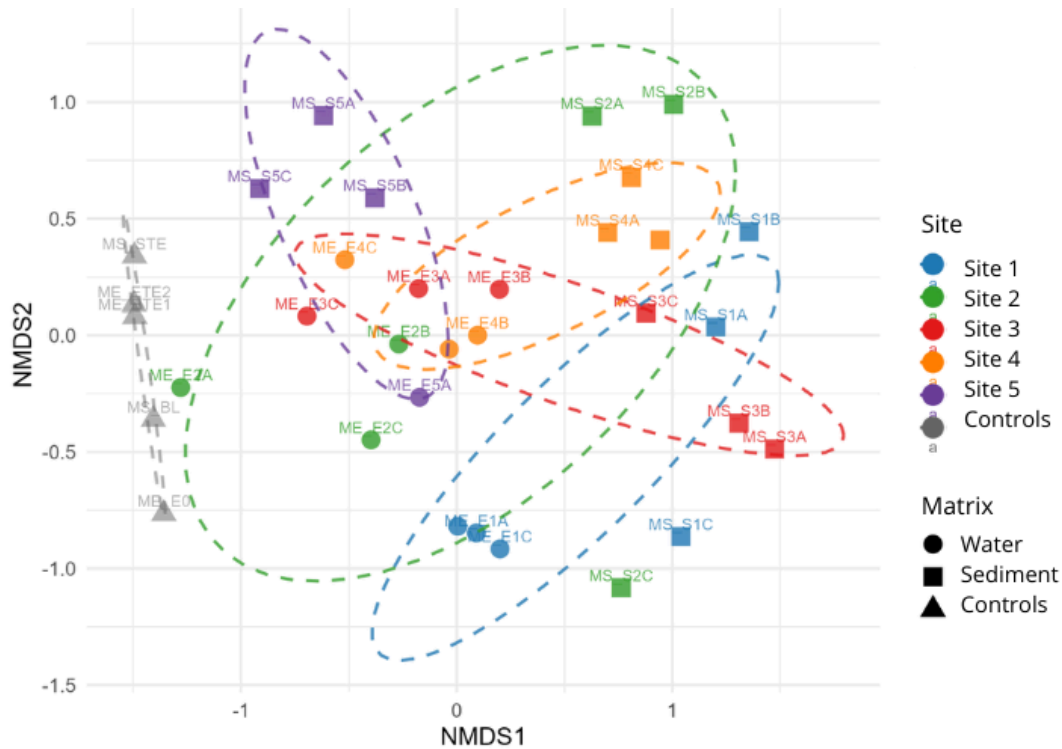


Figure 3 : NMDS of mammals community composition based on the repartition of the ASVs in the Mammals samples along the Nant de Loriaz altitudinal gradient and the sampled matrix (water or sediment). Non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis dissimilarity using the repartition of the ASVs of mammals genera in the Mammals samples before decontamination.

2.3.5. Normalisation

In order to compare the composition of samples with each other, a normalization step is essential. In this study, we applied rarefaction to the decontaminated and filtered files using the `rarefy_even_depth()` function from the `Phyloseq` package in R. For plant samples, the rarefaction depth was set at 144,916 reads, a satisfactory value given the total number of reads. This threshold allows all samples to be retained. We expect the rarefied plant samples to accurately reflect the composition of the communities, including rare taxa. However, rarefying the mammal samples proved to be more complex. A large number of taxa were lost due to filtering, which retained only sequences assigned to mammals.

Many ASVs initially assigned to non-mammalian taxa were thus excluded, resulting in a significant reduction in the number of available

reads, particularly in sediment samples. Normalization was therefore performed at a lower depth, i.e., 29,796 reads, the minimum threshold allowing 11 water samples (ME_E1A, ME_E1B, ME_E2B, ME_E2C, ME_E3A, ME_E3B, ME_E3C, ME_E4A, ME_E4B, and ME_E5A) and four sediment samples (MS_S5A, MS_S5B, and MS_S5C) out of a total of 28 initial samples. Given this shallow depth of rarefaction for mammals, the results should be interpreted with caution, as losses of diversity and the underrepresentation of certain taxa may bias the assessment of the actual composition of the communities.

The normalized ASVs will be used to answer all of the questions said to be 2 and 3 aiming to compare the difference of DNA signal depending on the matrix samples (water or sediment) and the altitude of the sampling point.

2.4. Statistical analyses

Most of the analyses were performed at the genus level, as this taxonomic level offers the best compromise between resolution and reliability of assignment, with taxonomic assignment tests indicating better resolution at the genus level than at the species level, without any major loss of information compared to the family level (Figure S7).

2.4.1. Do molecular and traditional methods of detecting biodiversity (plant and mammal) provide similar results ?

All analyses were performed with R (version 4.3.3). The data were imported and filtered with tidyverse (version 2.0.0), notably with dplyr (version 1.1.4) and readr (version 2.1.5) to remove the plots > 2000 m and unclassified taxa. The missing Latin names have been completed thanks to the taxize package (version 0.10.0), which queries the NCBI databases to retrieve the taxonomic classification. To quantify the dissimilarity of composition between the CREA data and our metabarcoding data, we calculated the Sørensen index via vegdist, method = "bray", binary = TRUE from the vegan package (version 2.7.1). We then explored the differences in composition using an NMDS analysis, with metaMDS() and vegan. The results were visualized with ggplot2 (version 3.5.2), made interactive with plotly (version 4.11.0) and formatted with ggpubr (version 0.6.2). The comparison of genera and families detected by the classical approach and metabarcoding was carried out with Venn diagrams generated by eulerr (version 7.0.2).

2.4.2. Do water and sediment matrices provide a similar eDNA signal ?

All statistical analyses for this question use the decontaminated, filtered and normalized reads and are performed at the genus level using R (version 4.3.3). Differences in eDNA signal between water and sediment matrices were first visualized using Non-metric Multidimensional Scaling (NMDS) based on Bray-Curtis dissimilarities. Differences in taxonomic composition were then assessed using a PERMANOVA based on Bray-Curtis dissimilarities, a beta-diversity index allowing to compare the composition of communities

depending on the matrix sampled at different altitude, here by using a proxy : the ASVs. Observed genus richness was then compared between matrices using a Wilcoxon signed-rank test to account for non-normal distribution. Venn diagrams were used to compare the number of genus found in both matrices and those only found in one of them (either water or sediment), for plants and mammals independently. Finally, Alluvial plots allowed visualization of taxonomic shifts across sites depending on the sampled matrix for the plants.

2.4.3. How does river eDNA integrate the biodiversity signal along an upstream-downstream gradient ?

All statistical analyses were performed using R (version 4.3.3). After decontamination, taxonomic filtering, and rarefaction, the datasets were organized into a phyloseq object (phyloseq package, version 1.46.0) and aggregated at the genus level using the tax_glom() function. Most analyses were conducted on plant data, as mammal datasets showed insufficient statistical power to draw robust conclusions. Alpha diversity, estimated using observed richness, was visualized with bar plots to compare local community structure among sites and between matrices. Differences in taxonomic composition were explored using bar plots and alluvial diagrams generated with the ggalluvial package (version 0.12.5) and ggplot2 (version 4.0.1). Beta diversity was quantified using Bray-Curtis (relative abundance) and Jaccard (presence/absence) dissimilarity indices calculated with phyloseq, then analyzed by non-metric multidimensional scaling (NMDS) using the phyloseq, vegan, and ggplot2 packages. Differences in community composition among sites along the altitudinal gradient were tested using PERMANOVA. Homogeneity of multivariate dispersion among sites was assessed using the betadisper function from the vegan package (version 2.6-10). Turnover and nestedness components were estimated by partitioning Sørensen and Jaccard indices derived from presence/absence matrices using the betapart package (version 1.6.1). In addition, the presence-absence signal of *Bos taurus* was examined across sites for both matrices.

3. Results

3.1. Do molecular and traditional methods of detecting biodiversity (plant and mammal) provide similar results ?

Long-term biodiversity monitoring is essential for assessing ecological risks and predicting future trends (Tuhřinová et al., 2026). CREA Mont-Blanc is a scientific NGO whose objective is to explore the impact of climate change on biodiversity and share this knowledge with decision-makers and citizens (<https://creamontblanc.org/fr/>).

Having carried out monitoring for several years, they have information obtained using several traditional techniques. For mammals, they used camera traps located at the same altitudes as us (at two sites located at over 2,000 metres above sea level). Cameras are used to capture still and moving images of wildlife (Stephenson, 2020). To identify plants, they laid out ropes, which also enabled them to determine their abundance as well as their diversity.

The CREA sampling sites can be seen in Figure 1. It appears that this laboratory takes samples at altitudes above 200 metres. While traditional methods have provided valuable insights, advances in molecular techniques, such as metabarcoding, now offer powerful tools for rapid and comprehensive biodiversity assessments (Tuhřinová et al., 2026). Identification typically relies on easily observable morphological characteristics, but various DNA-based strategies have been developed for those in cases where morphology-based identification proved problematic (Taberlet et al., 2012).

For non-metric multidimensional scaling (NMDS) based on Sørensen's dissimilarity (Figure 4) using presence/absence data for mammal genera recorded by CREA (A) and

plant genera recorded by CREA (B), the points represent sampling sites, colored according to altitude. Sørensen's dissimilarity was chosen because the dataset consisted of presence-absence data. NMDS was used as it is well suited for visualizing patterns in community composition based on non-Euclidean distance measures. For mammals, the separation between high and low-altitude sites is weak, with significant overlap. In contrast, plants show a clear separation of sites above 2,000 m, indicating a strong effect of altitude on community composition.

The detection of mammal species differed between traditional camera-trap conducted by CREA and eDNA metabarcoding. A total of 26 mammal taxa were detected across both approaches. 8 genera were detected exclusively by CREA, 5 exclusively by eDNA, and 13 genera were shared between both methods. Camera traps detected several burrowing and arboreal mammals, including *Marmota*, *Meles*, *Lepus*, and *Eliomys* (Figure 5, A). In contrast, eDNA metabarcoding detected genera such as *Neomys*, *Microtus*, *Mus*, *Oryctolagus* and *Muscadinus* which were not recorded by camera traps (Figure 5, B).

For the plant families, a total of 77 families were detected across both approaches. 40 families were detected exclusively by eDNA, 3 exclusively by CREA, and 34 families were shared between both methods (Figure 5, C). *Asteraceae*, *Thesiaceae* and *Blechnaceae* are the 3 families only detected by CREA (Figure 5, D). We also performed this analysis at the genus level for plants. A total of 178 genera were detected across both approaches: 32 genera were detected only by CREA, 107 by eDNA, and 39 by both approaches. We did not include the plot for genera because it is unreadable, but you can see the data and plot in the Git repository (Figure S2).

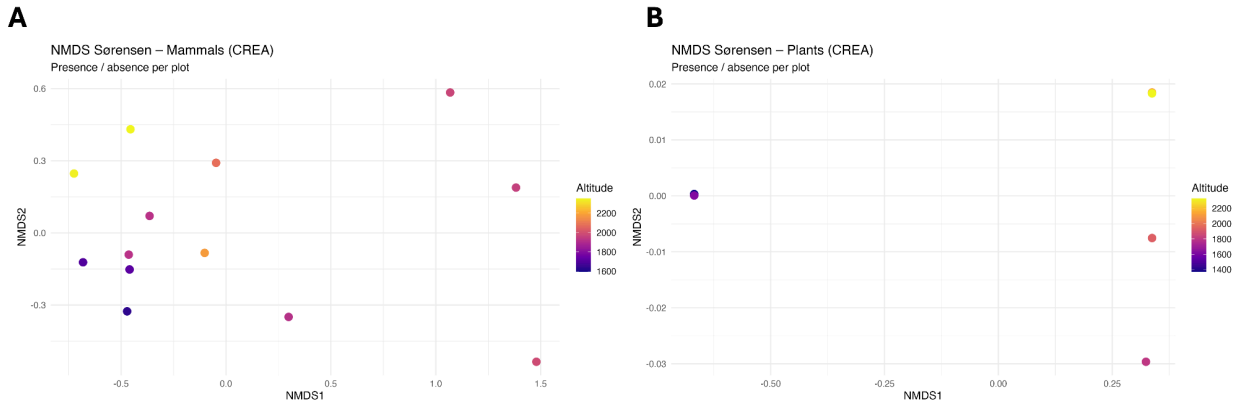


Figure 4 : NMDS of mammals (A) and plants (B) community composition based on CREA presence/absence data along the Nant de Loriaz altitudinal gradient.

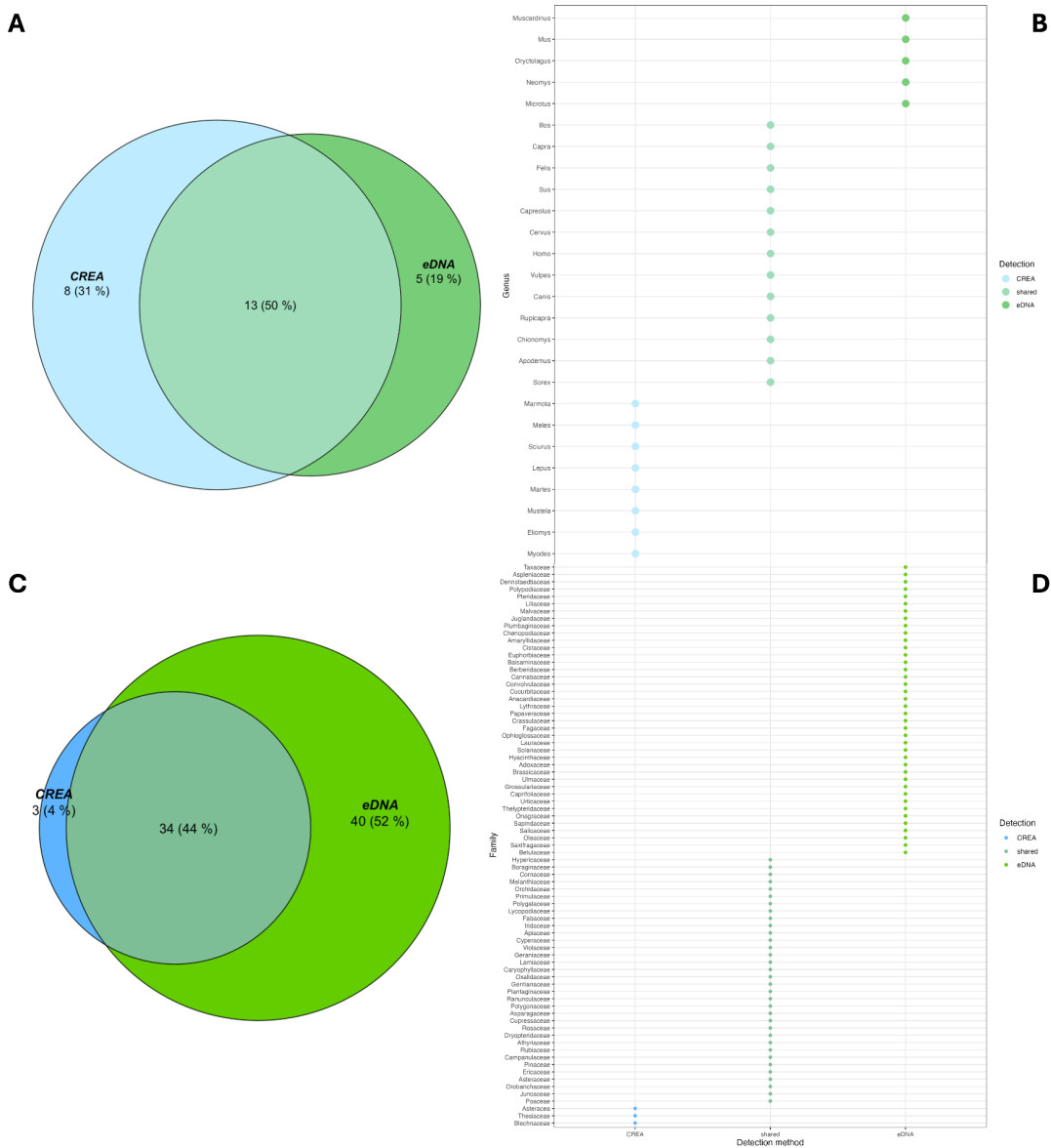


Figure 5 : Venn diagram representing the genera of mammals (A) as well as the families of plants (C) detected by traditional method and by eDNA metabarcoding. The dots plots show in detail the genera of mammals detected (B) for each method as well as the families of plants (D).

3.2. Do water and sediment matrix provide a similar eDNA signal ?

The matrix from which the eDNA is sampled can have a strong impact on the observed biodiversity and on each species' relative abundance (Zhan et al., 2025). These variations of observed community composition can come from differences in the experimental design, as sampling method and biomolecular treatment need to be tailored to each matrix because of their specificity, for example the presence of matrix-specific inhibitors. Moreover, the eDNA from each matrix will undergo different degradation, can come from different sources, be more or less diluted, as well as provide a signal from a community that is more or less recent and spatially restricted. As a result, we explored the eDNA signal provided by water and sediment matrix so as to assess whether the environmental matrix sampled significantly influenced the observed Alpine watershed communities composition.

We first used NMDS ordinations of the water and sediment samples, based on a beta-diversity metric : the Bray-Curtis dissimilarity index. It was followed by PERMANOVA tests.

For mammals (Figure S8), the permanova revealed no significant difference between communities detected in water and in sediments (permanova, p-value = 0.267), with the matrix type, explaining only a small proportion of the total variance ($R^2 = 0.085$). The multivariate dispersion test showed no significant differences between groups, indicating that the absence of a detected effect was not driven by heterogeneity in dispersion. These results indicate that for mammals, the effect of matrix type on the communities detected is limited. It is however necessary to consider these observations carefully, when taking into account the low number of ASV and samples after the decontamination, filtering and normalisation of

the Mammals samples.

On the other hand, for plants (Figure S9), the permanova revealed a significant difference between communities detected in water and sediments matrices (permanova, p-value = 0.001), still the matrix type only explained a minor proportion of the total variance ($R^2 = 0.09$). The multivariate dispersion test also showed no significant differences between groups, confirming the effect of matrix type on the Plants communities detected.

The genus richness of the observed communities sampled from water and sediment were then compared using wilcoxon test and visualised using boxplots (Figure 6 A,C). Venn diagrams were also used to better look at the variation in mammals' and plants' genus richness depending on the sampled environmental matrix (Figure 6 B,D).

The water matrix shows a clearly higher species richness compared to the sediment, for both plants (Fig. 1A) and mammals (Fig. 1C), with statistically significant differences ($p = 0.0402$ and $p = 0.0315$, respectively). Water samples exhibit high signal dispersion for plants, whereas the sediment matrix shows the opposite trend, with high signal dispersion for mammals. Conversely, the water matrix for plants and the sediment matrix for mammals appear more homogeneous.

The Venn diagrams reveal that both matrices share the majority of the information, with approximately 60% of genera common to both plants (Fig. 1B) and mammals (Fig. 1D). However, each matrix provides a unique signal. For plants, 26% of genera are water-specific, which could correspond to pollen or light suspended debris. For mammals, 30% of the genera are unique to the water, likely reflecting a recent signal from transient. Conversely, the genera unique to the sediment (15% for plants and 9% for mammals) support its role as an organic matter reservoir.

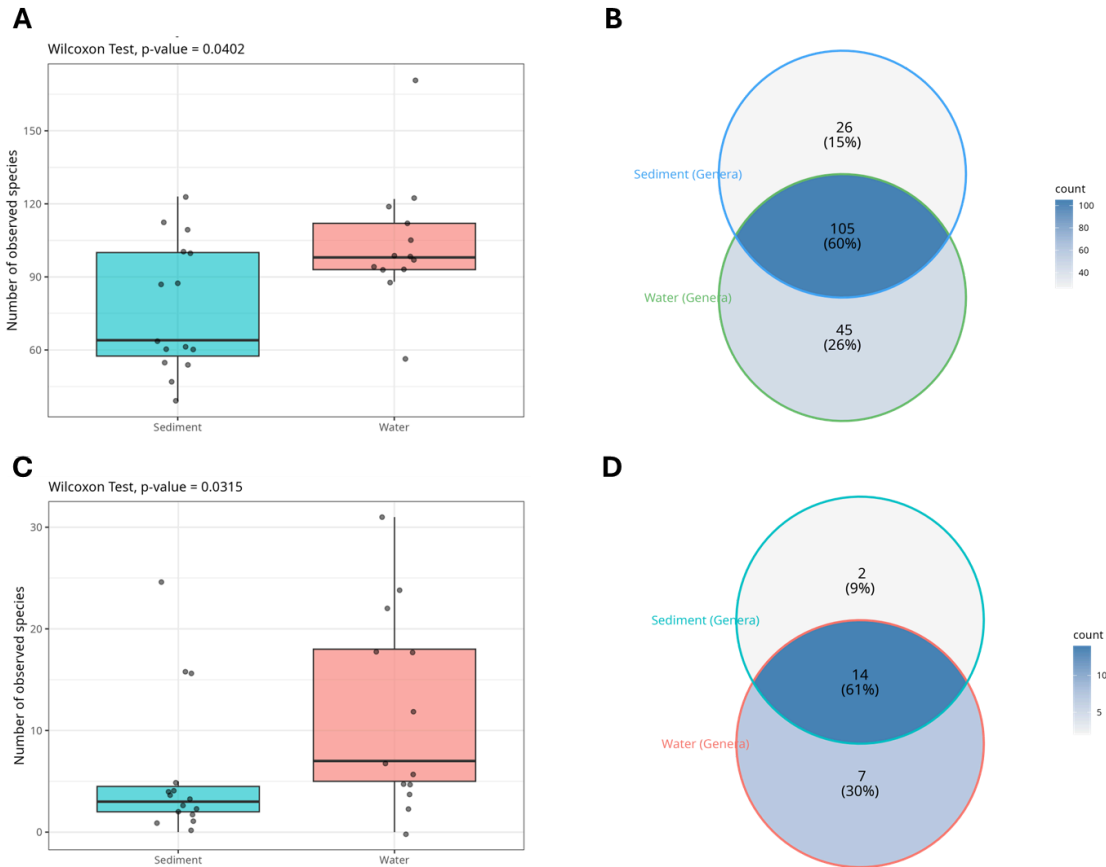


Figure 6: Comparison of eDNA signals between water and sediment. (A, C) Species richness for plants and mammals (Wilcoxon test). (B, D) Shared and unique genera for plants and mammals. (A, B) for Plants and (C, D) for Mammals

Finally, the variation in eDNA signals due to the sampled environmental matrices were studied at a site level using Alluvial plot and Bray-Curtis distances for the plants (Figure 7). We refrained from doing this analysis for the Mammals' samples considering their low number of AVS and samples after their normalisation.

The comparison between the two matrices reveals a visually distinct difference at Site 1, characterized by the dominance of *Athyrium* in the sediment compartment, which is confirmed by a high Bray-Curtis distance index of 0.704 (Figure 7). Conversely, Site 2 is characterized by high similarity between the two matrices (0.349); this congruence may be explained by the intermediate current intensity at this site, as indicated by the metadata. Site 3 shows moderate similarity (0.453), while Sites 4 and 5 are more divergent, with Bray-Curtis distances

of between 0.523 and 0.567. This divergence is explained by the role of water as a transport vector for allochthonous signals (pollen, hillslope debris), providing an instantaneous and diverse snapshot of the valley, whereas the sediment archives a local and persistent signal (reservoir)

The genus *Larix* appears dominant in the water at Sites 2, 3, 4, and 5, supporting our hypothesis of allochthonous input from pollen and needle-like leaves captured by the water surface before slowly settling in areas of low current intensity. In contrast, the genus *Athyrium* (lady-fern) is preferentially found in the sediments across all sites (Figure 7). This genus has a cosmopolitan distribution; each individual bears millions of sporangia that release fine, resistant spores that settle into the sediments and can persist there for several years or even decades (Dyer & Lindsay, 1992).

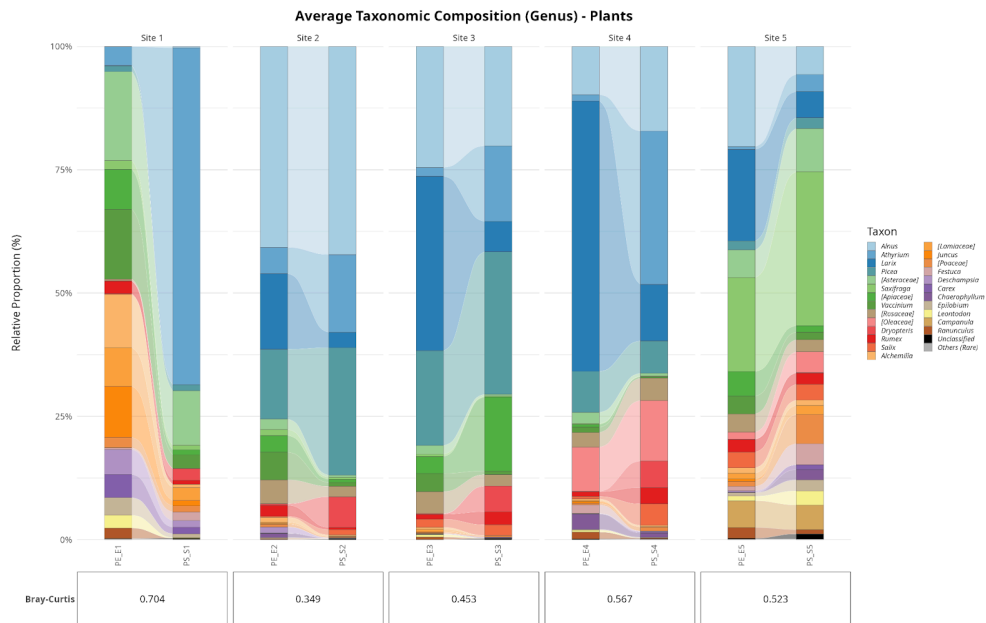


Figure 7: Taxonomic distribution of plant genera within water and sediment matrices, and summary table of Bray-Curtis distances.

3.3. How does river eDNA integrate the biodiversity signal along an upstream-downstream gradient ?

First, the richness of detected plant genera varies among sites and according to the type of matrix analyzed (Figure 8a). Overall, water samples exhibit higher genus richness than sediment samples, regardless of the site considered. A marked spatial heterogeneity is observed along the studied gradient. Sites 2 and 3 consistently show lower plant genus richness than sites 1, 4, and 5, independently of the matrix. In contrast, sites 4 and 5 are characterized by the highest richness values, particularly in water samples.

Taxonomic composition also varies among sites along the altitudinal gradient for both matrices (Figure 8b; Figure S10). In water samples, sites 2 to 4 are characterized by a strong dominance of the genera *Alnus*, *Saxifraga*, and especially *Larix*, with a maximum observed at site 4. In contrast, site 1 shows a more homogeneous

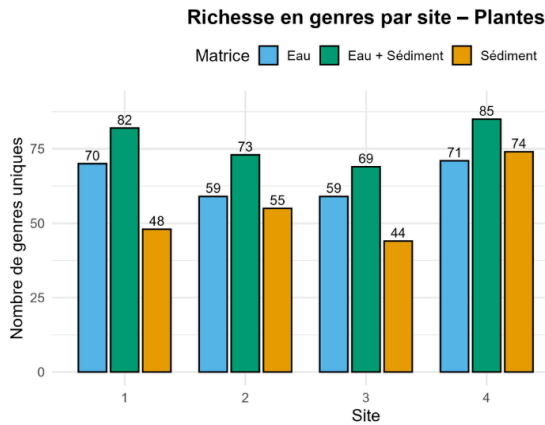
composition, with a high relative abundance of *Vaccinium*, *Salix*, and *Juncus*, whereas site 5 is distinguished by a diversification of dominant genera, notably *Saxifraga*, and a marked presence of *Campanula*. In sediment samples, a progressive decrease in the relative contribution of *Athyrium* (from site 1 to site 5) and *Alnus* (from site 2 to site 5) is observed downstream. Conversely, an increase in genera such as *Carex*, *Saxifraga*, *Ranunculus*, and *Rumex* is observed at site 5. Together, these results indicate a gradual shift in plant community composition along the studied gradient.

NMDS ordination based on Bray–Curtis dissimilarity highlights marked differences in plant community composition both among sites and between sampling matrices (water and sediment) (Figure 9a). The first ordination axis primarily discriminates samples according to site, whereas the second axis mainly separates the two matrices. Samples from sites 2, 3 and 4 show higher compositional similarity, whereas sites 1 and 5 display more distinct community compositions.

Analysis of alpha diversity for plants

Description of alpha diversity in sediment and water samples across different sites

a) Genus richness per site



b) Sample composition per sample

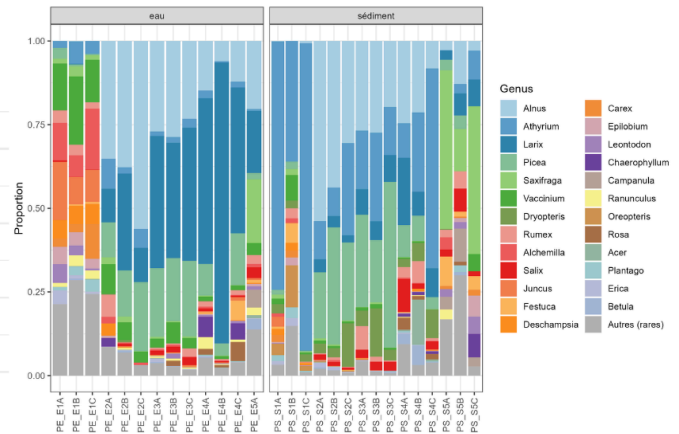
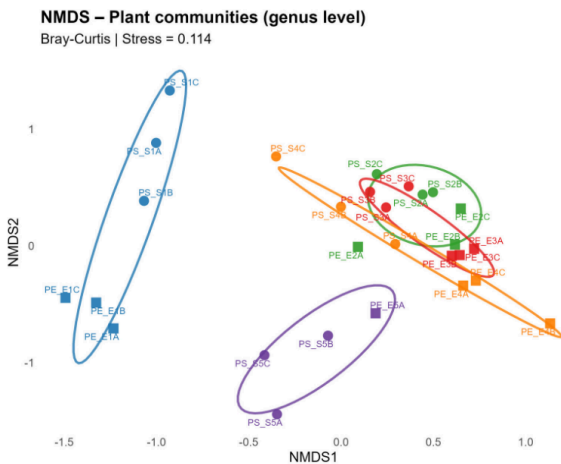


Figure 8: Spatial variation in plant genus richness per site (a) and taxonomic composition per sample (b) along the altitudinal gradient

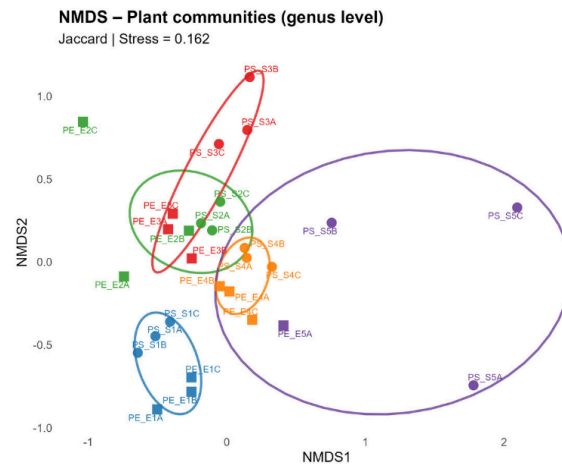
Analysis of beta diversity for plants

Description of beta diversity in sediment and water samples across different sites

a) NMDS with Bray-Curtis



b) NMDS with Jaccard



c) NMDS legend

- Sample type
- Eau
 - Sédiment
- Site
- S1
 - S2
 - S3
 - S4
 - S5

Figure 9: Beta diversity patterns of plant communities across sites and sample matrix revealed by NMDS ordination, based on Bray-Curtis dissimilarity index (a) and Jaccard Index (b).

These patterns are further supported by multivariate analyses of variance (PERMANOVA). The site factor explains a substantial proportion of the variance in community composition ($R^2 = 0.54$; $p = 0.001$), reflecting a strong spatial structuring of plant communities. Sample matrix (water vs sediment) also has a significant effect on

detected composition, although of lower magnitude ($R^2 = 0.15$; $p = 0.001$). The interaction between site and matrix explains an even larger proportion of the variance ($R^2 = 0.81$; $p = 0.001$), indicating that spatial patterns differ depending on the sampling substrate. Separate analyses performed for each matrix reveal significant spatial structuring in

both cases. For water samples, site explains a very large proportion of the variance in community composition ($R^2 = 0.86$; $p = 0.001$), indicating a strong differentiation of detected communities among sites. Sediment samples also show significant spatial structuring, although slightly less pronounced ($R^2 = 0.72$; $p = 0.001$).

NMDS ordination based on the Jaccard index (presence/absence) (Figure 9b), revealed patterns comparable to those obtained with the Bray–Curtis dissimilarity. The first axis primarily separates samples among sites, while the second axis distinguishes the two matrices.

The analysis of the homogeneity of the multivariate dispersion between sites follows the same pattern as the richness observed for plant samples across sites.

Beta-diversity decomposition analyses revealed a significant dominance of turnover processes in

plant samples, whether based on the Sørensen or Jaccard indices, with respective proportions of 65% ($R^2 = 0.49$, $p = 0.01$) and 72% ($R^2 = 0.49$, $p = 0.01$), compared to 35% and 28% attributed to nestedness-related processes. These results indicate that the compositional signal decreases with increasing distance and along the gradient, leading to a replacement of detected community composition across the altitudinal gradient. A strong turnover signal was also observed in mammal samples.

Analysis of the presence of *Bos taurus* confirms this trend (Figure 10). *Bos taurus* was detected at a high frequency at site 1 during sampling but was completely absent from the other sites. This signal is found in mammalian eDNA samples, both in water and sediments. A very weak presence was detected at site 3 in water samples, but this signal remained marginal. Overall, these observations confirm the predominance of renewal processes.

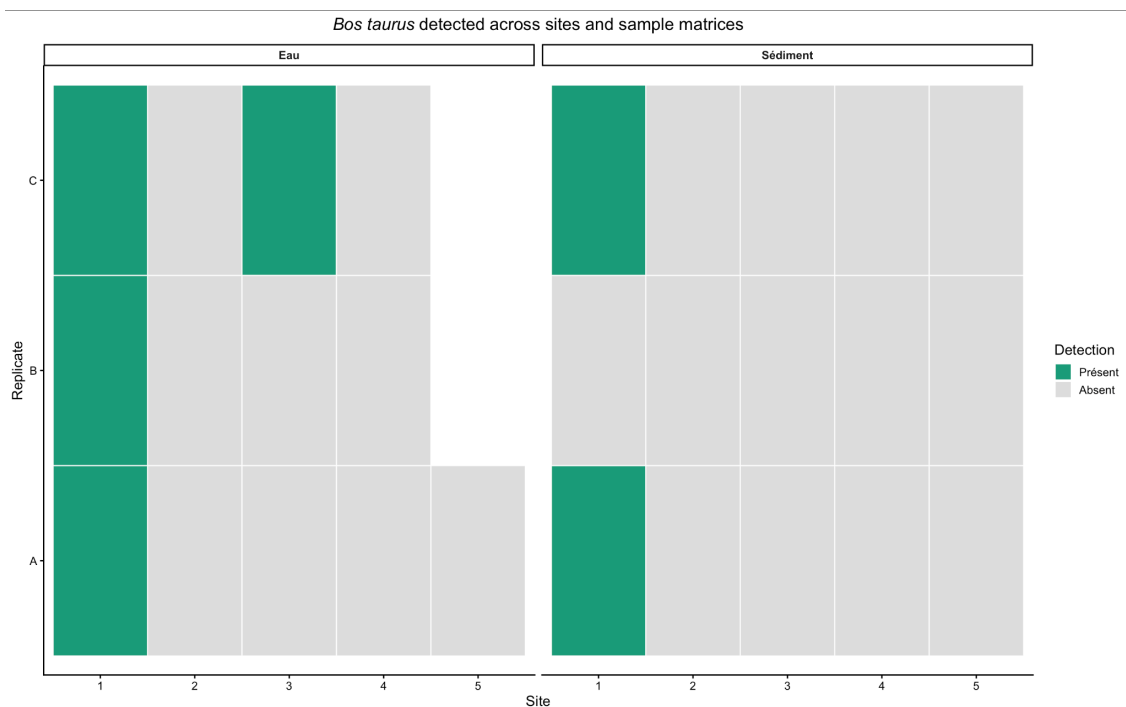


Figure 10: *Presence-absence pattern of Bos taurus eDNA across sampling sites and matrices*

4. Discussion

4.1 Question 1

This study aimed to compare environmental DNA (eDNA) metabarcoding with traditional biodiversity identification conducted by CREA Mont-Blanc. We compared eDNA results with vegetation surveys based on plot sampling and mammal surveys obtained from camera traps. Due to the low reliability of species-level taxonomic assignments by IDTAXA when using the markers employed in this study, particularly for mammals, we conducted our comparisons at the genus level for both mammals and plants (although the figures above are presented at the family level for improved readability) to ensure robust and reliable results.

4.1.1. Similarities with methods

Several typical alpine plant genera were detected by both approaches, indicating that eDNA metabarcoding is able to recover a representative fraction of local vegetation diversity. Moreover, some discrepancies between datasets disappeared after exclusion of non-comparable CREA sampling sites (sites above 2000 m altitude).

Site 5 is particularly noteworthy, as it does not belong to the same catchment and is located close to human habitations. At this site, eDNA revealed the presence of genera such as *Felis* and *Capra*, which is consistent with its proximity to inhabited areas where domestic species (domestic cats, goats, etc.) are common. Similarly, *Rupicapra* was detected only at site 5. The detection of *Rupicapra* at this site may represent a misassignment error given its phylogenetic proximity to domestic goats. Collectively, these results suggest that the taxa detected exclusively at site 5 likely represent either domestic or human-associated species, or alternatively, DNA transported downstream from higher altitudes in the catchment rather than true local alpine fauna.

Venn diagram comparison showed that CREA detected 8 unique mammal species, eDNA detected 5 unique species, and 13 species were

4.1.2. Differences for plants

NMDS plots based on Sørensen distances show that plant communities are strongly structured by altitude, reflecting their limited mobility. To ensure a fair comparison with eDNA data, we excluded sites above 2000 m, which are specific to high-altitude regions.

After this exclusion, several genus that had initially appeared shared between methods in the Venn diagram were no longer recorded by CREA but remained detectable in eDNA samples. Since these genus are unlikely to occur at such elevations, this pattern suggests that eDNA integrates signals from lower parts of the catchment. Overall, at a given altitude, eDNA appears to provide a more comprehensive representation of plant communities than CREA, which are limited to the sampled plots. This observation supports the hypothesis of downstream DNA transport, which will be discussed further in the next section. Therefore, eDNA does not only reflect strictly local communities but also provides a spatially integrated signal of the upstream landscape (Lyet et al., 2021). The detection of *Cardamine* may result from cross-contamination during DNA extraction, as this species was simultaneously processed for a separate genome assembly project. In addition, eDNA seems to have detected more plants than CREA observations. In fact, as the site 5 is located near human habitations, many ornamental garden plants were found using molecular methods, which is not the case with traditional methods, as these plants are not specific to alpine environments.

4.1.3. Differences for mammals

For mammals, which are more mobile, this pattern is less pronounced. Excessive removal of locations is not possible because observation points are more dispersed. Besides, camera traps used by CREA allow reliable species-level identification. In contrast, species-level assignment from eDNA remains challenging with the marker used in this study, resulting in frequent low-confidence taxonomic assignments and an increased risk of false positives. The

shared between both methods. The genera detected exclusively by eDNA (*Neomys*, *Microtus*, *Mus*, *Oryctolagus*, *Muscardinus*) may

exhibit ecological traits that reduce the efficiency of camera trapping. For instance, semi-aquatic lifestyles (e.g., *Neomys*) or small, cryptic rodent behavior could result in low triggering rates of camera traps, even when specialized rodent-targeted cameras are deployed, potentially explaining their detection only through eDNA methods.

Conversely, genera detected exclusively by CREA camera traps may have limited detection in eDNA due to their ecological characteristics. Many of these taxa are arboreal (*Sciurus*, *Eliomys*) or fossorial (*Marmota*, *Meles*) species that likely have infrequent contact with water, reducing their DNA shedding into aquatic environments. Additionally, their larger body size compared to micromammals makes them more readily detected by camera traps, which may explain their preferential detection through traditional photographic methods rather than eDNA metabarcoding.

However, the detection of genus with low assignment confidence (like *Felis*, *Capra*, *Rupicapra* and *Canis*) illustrates the limits of the approach and the importance of cautious interpretation of molecular data. Infrequent contact with water of terrestrial and semi-aquatic mammals can result in lower detection rates in eDNA samples compared to fully aquatic taxa (Goralczyk et al., 2025). This result is in agreement with previous studies showing that eDNA detection probability is strongly associated with species ecology, including diet, body size and frequency of contact with water (Lyet et al., 2021). Strict carnivores such as felids and some canids are consistently less detectable, likely because of their low densities and limited interaction with aquatic environments, whereas omnivorous and herbivorous species exhibit higher detection rates (Lyet et al., 2021).

4.1.3. Conclusion

In conclusion, for some taxa, eDNA performs better than traditional methods, for others it performs similarly, and for some it provides little additional benefit (Lankes et al., 2026). Besides, eDNA does not necessarily provide reliable information on abundance or biomass, although this may be achievable in some contexts (Lankes et al., 2026). eDNA should currently be interpreted primarily as an indicator of

occupancy rather than population size (Lyet et al., 2021). This variability highlights the complementarity rather than the substitutability of both approaches. That is why, combining traditional methods such as camera traps for terrestrial large mammals, and eDNA for aquatic species provides complementary insights into biodiversity (Stephenson, 2020).

Furthermore, hydrological processes combined with decay dynamics can make it difficult to connect the eDNA signal with where it was produced (Goralczyk et al., 2025).

4.2. Question 2

This part of the study aimed to compare water's eDNA signal and sediment's eDNA signal, in order to assess the extent to which eDNA provides similar information depending on the sampled matrix. We compared decontaminated, filtered and normalised ASVs created from Mammals' and Plants' eDNA sampled from water or sediment of the Nant de Loriaz catchment (Vallorcine, France).

4.2.1. Alpha diversity variations

Significant differences were found between the matrices' eDNA signals when considering the communities alpha diversity, here genus-level richness, both for the plants and mammals samples. Moreover, the genus unique to the sediment matrix supports its role as an organic matter reservoir. Indeed, this eDNA may be more persistent in sediment thanks to particle settling and/or retarded degradation thanks to the adsorption of the DNA molecules in sediments, which could result in longer-lasting eDNA signals, meaning the observed biodiversity in these samples may represent current or past site occupancy (Turner et al., 2015) such as annual plants or mammals that previously frequented the site. However, while each matrix had a distinct eDNA signal, they still shared a majority of their genus. Moreover, the study of site by site variation of eDNA signal showed that the variation of eDNA signal between the environmental matrices also varies depending on the sampling site. These variations may be linked to the strength of the

current, where sites experiencing stronger current (such as site 2), seem to show higher similarity between the matrices. This homogeneity may be due to the resuspension and transport of sedimentary eDNA, helping sediment and water samples to stay similar (Turner et al., 2015), being positively linked to the strength of the current.

4.2.2. Beta and Alpha diversity differences in variations

While significant differences were found between the matrices' eDNA signals when considering the communities alpha diversity, Beta-diversity, calculated using the Bray-Curtis dissimilarity index, did not reveal significant differences between the water and sediment communities for the mammals. Significant differences were however observed between environmental matrices for the plants.

This difference could be a result of the experimental design and analysis strategy used. Indeed, preserving realistic relative taxon abundances is challenging in eDNA metabarcoding, due to extraction, PCR amplification and sequencing biases, as well as normalization steps. These treatments may differently impact the abundance-based metrics, such as the beta diversity, and richness-based metrics, such as the alpha diversity.

4.2.3. Limits of the analysis

While the plant datasets provided robust and interpretable signals, the mammalian eDNA analysis encountered significant technical bottlenecks. Rarefaction curves revealed an extreme heterogeneity in sequencing depth, with some water samples exceeding 250,000 reads while most sediment samples remained below critical thresholds. Furthermore, a high proportion of sequences were assigned to non-target taxa. This 'molecular noise' overwhelmed the signal of wild alpine fauna. Consequently, applying standardized statistical normalizations or calculating dissimilarity indices like Bray-Curtis would have resulted in biased ecological interpretations. For these reasons,

the mammalian comparative analysis between water and sediment need to be considered carefully and was excluded from the comparison of the matrices' site by site to maintain the scientific integrity of the inventory of the biodiversity in the Vallorcine catchment area.

4.3. Question 3

According to the niche-based environmental filtering theory of community assembly, biodiversity is distributed non-randomly in space as a result of environmental constraints that select species according to their ecological niches and functional traits (Keddy, 1992; Weiher & Keddy, 1995). Alpine ecosystems are subjected to strong multidimensional ecological gradients, integrating variations in temperature, atmospheric pressure, radiation, and resource availability (Doležal & Šrútek, 2002). These ecological gradients act as environmental filters, leading to pronounced spatial structuring of biological communities along the altitudinal gradient (Jacobsen, 2004; Rahbek, 2005). Consequently, numerous studies have reported a decline in species richness toward higher elevations. In this context, we investigate mammal and plant communities using environmental DNA (eDNA) from water and sediment samples collected along a mountain stream following an altitudinal gradient. We formulate two main hypotheses. First, we expect changes in community composition, with stronger structuring at higher elevations. Second, we test how the eDNA signal is distributed along the stream under two contrasting scenarios:

- the nestedness hypothesis, whereby the signal accumulates downstream with water flow, such that communities detected downstream represent a nested subset of upstream communities;
- the turnover hypothesis, whereby the signal degrades with time and distance, leading to a renewal of detected community composition along the altitudinal gradient.

4.3.1 H1 : Spatial patterns of biodiversity distribution

Our results indicate a marked spatial structuring of biodiversity along the Nant de Loriaz, particularly for plant communities. First, the distribution of observed richness and detected taxa highlights clear differences among sites along the altitudinal gradient. Contrary to expectations, observed richness does not follow a strictly monotonic pattern from upstream to downstream. The relatively high richness observed at site 1 may be related to a more open habitat, potentially more favourable to biodiversity and to the capture of environmental DNA, whereas sites 2, 3 and 4 correspond to more closed environments, characterized by steep slopes and strong water flow. Site 5 exhibits the highest richness, which may be explained by a larger catchment area, allowing the integration of environmental DNA originating from a broader upstream surface.

In terms of taxonomic composition, the observed patterns among sites appear ecologically consistent. Site 1, located in the subalpine zone, displays a community composition characteristic of this altitudinal level. In contrast, sites 2, 3 and 4, situated within a relatively homogeneous forest ecotype, show highly similar community compositions. Finally, site 5, located in a more anthropized ecotype, exhibits a distinct taxonomic composition. Overall, assemblages become progressively more distinct along the gradient, with an increasing contribution of taxa associated with milder climatic conditions and a higher representation of human-associated taxa, in accordance with known ecological gradients in mountain environments (Doležal & Šrůtek, 2002). Beta-diversity analyses further support these observations, with site emerging as the most explanatory factor for differences in community composition among samples. Together, these results support our initial hypothesis that community composition varies significantly along the altitudinal gradient.

4.3.2 H2 : Processes underlying community structuring

Beta-diversity analyses suggest that the spatial distribution of biodiversity detected by eDNA along the Nant de Loriaz is predominantly structured by turnover processes rather than by a nestedness pattern. Partitioning of beta diversity indicates that, for both mammals and plants, turnover accounts for the majority of the observed dissimilarity among sites, regardless of the index used (Sørensen or Jaccard), in accordance with the methodological framework proposed by (Baselga, 2010). For mammals, total dissimilarity is extremely high, reflecting a strong turnover signal and indicating an almost complete replacement of detected taxa among sites. However, when considered in light of the limited quality and quantity of the available data, this observation is likely biased and should be interpreted with caution.

In plant communities, overall dissimilarity is largely explained (>65%) by turnover processes, indicating a substantial renewal of communities detected by eDNA along the altitudinal gradient, whereas nestedness-related processes contribute less to the observed patterns. Contrary to expectations under a nestedness-driven scenario, no systematic increase in species richness was observed at downstream sites, and communities exhibit strong compositional dissimilarity among sites.

The case of *Bos taurus* provides a particularly illustrative example of this pattern. Detection of this species, whose local presence was confirmed at site 1, was strictly limited to this site and absent from immediately downstream sites. These results suggest that longitudinal transport of eDNA is strongly limited in this alpine system. Processes such as dilution, degradation, and sedimentation are known to substantially reduce the effective transport distance of eDNA in lotic environments (Deiner & Altermatt, 2014; Pont et al., 2018), and these constraints may be further amplified in alpine contexts by strong hydrological dynamics,

marked discharge variability, and high environmental heterogeneity (Deiner et al., 2016).

4.3.3 Limitations and perspectives

Several limitations should be considered when interpreting these results. First, environmental DNA (eDNA) detection can be strongly influenced by hydrological conditions, including discharge, turbidity, and sediment dynamics, which may affect both the persistence and transport of genetic material (Shogren et al., 2017). In addition, taxon-specific detection biases may arise from variable primer performance, uneven taxonomic resolution of reference databases, and intrinsic differences in DNA production and degradation rates among organisms. Contamination and poor primer specificity for mammals, which reduce the effective sequencing depth after rarefaction, strongly bias compositional analyses of these samples.

In contrast, analyses conducted on plant communities exhibited high statistical power after rarefaction, making compositional patterns more ecologically interpretable. Finally, the limited number of sampled sites and replicates, combined with the absence of temporal replication, restricts the generalization of these results to the entire hydrographic network and to other alpine contexts. Multi-season sampling, coupled with increased spatial coverage of sampling stations, would allow a more robust assessment of the temporal stability of observed trends and provide deeper insights into the mechanisms governing the spatial distribution of environmental DNA.

5. Conclusion

Our results from the Nant de Loriaz catchment show that eDNA reflects local biodiversity patterns across multiple taxonomic groups.

For plants, eDNA provided a more comprehensive representation of local communities than traditional plot-based surveys, detecting species that were missed in vegetation plots and capturing signals from upstream communities. In fact, eDNA integrates signals not only from local sources but also from upstream, creating a composite signal whose interpretation depends on hydrological processes, species ecology, and sampling design (Lyet et al., 2021).

For mammals, eDNA was less precise at the species level, but it successfully detected elusive taxa that are difficult to observe with camera traps, such as semi-aquatic or micro-mammal. Detection is influenced by the ecology of species, sampled matrix, hydrological processes, and methodological limitations such as non-target amplification or cross-contamination. Indeed, the quality of the signal is highly dependent on the sampling site and matrix, and the volume of filtered water, while the complex fate of eDNA complicates ecological interpretation (Lyet et al., 2021).

These patterns highlight the complexity of eDNA behavior in river networks (Goralczyk et al., 2025). To place these observations in a broader context, it should be noted that eDNA can improve the precision and resolution of biodiversity assessments, but its effectiveness is context-dependent (Lankes et al., 2026). This context-dependence highlights the importance of understanding both the capabilities and limitations of eDNA as a monitoring tool.

Effectively, environmental DNA has the potential to become a widely used tool in ecology and conservation, especially given that biodiversity conservation is currently limited by a lack of information on species distribution and ecology (Lankes et al., 2026). Thus, eDNA constitutes a powerful but imperfect tool that must be used in a complementary manner to other approaches to effectively support conservation decisions (Goralczyk et al., 2025).

In terms of future perspectives, improvements in bioinformatics pipelines could enhance data reliability. Initially, we explored processing our eDNA sequences with OBITools, but we ultimately used the DADA2 pipeline as it provided more reliable, reproducible, and

advanced results. Completing and standardizing an OBITools workflow remains a future objective to allow comparisons across different pipelines. Comparisons with regional reference databases for mammal assignment and the testing of tools such as Kraken2 for decontamination could further refine species detection. On the statistical side, exploring alternative normalization methods could reduce biases and improve the comparability of abundance estimates.

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Authors contribution

Sampling : Abran, Bally, Beaume, Bertrand, Bessonnier, Boizet, Drouet, Kovacs, Leonie, Lopes, Robin, Rondot, Said, Toro Ulloa

Molecular biology : Bally, Beaume, Bertrand, Bessonnier, Boizet, Drouet, Kovacs, Leonie, Lopes, Robin, Rondot, Said, Toro Ulloa

Bioinformatic pipeline : Abran, Alami, Bertrand, Bessonnier, Drouet, Kovacs, Lopes, Toro Ulloa

Writing :

Abstract : Abran

Introduction : Abran, Kovacs

Material and methods :

- Study site and sampling : Kovacs
- DNA extraction and sequencing : Bessonnier, Lopes
- Reads preprocessing and quality control : Lopes

- Denoising and ASVs processing : Bessonnier, Kovacs
- Creation of a reference database & taxonomic assignment : Drouet
- Decontamination et samples filtering : Abran, Lopes
- Normalisation : Drouet

Statistics analysis :

- Question 1 : Abran, Bessonnier, Kovacs
- Question 2 : Lopes
- Question 3 : Bertrand, Drouet

Results :

- Question 1 : Abran, Bessonnier, Kovacs
- Question 2 : Alami, Lopes, Toro Ulloa
- Question 3 : Bertrand, Drouet

Discussion :

- Question 1 : Abran, Bessonnier, Kovacs
- Question 2 : Alami, Lopes
- Question 3 : Bertrand, Drouet

Conclusion : Abran

Acknowledgment : Bessonnier

Page layout : Kovacs

Logo : Bessonnier

Git : Bessonnier

Review : Bessonnier, Lopes

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Supplementary material

Map of environmental eDNA sampling sites

Environmental setting of the municipality of Vallorcine

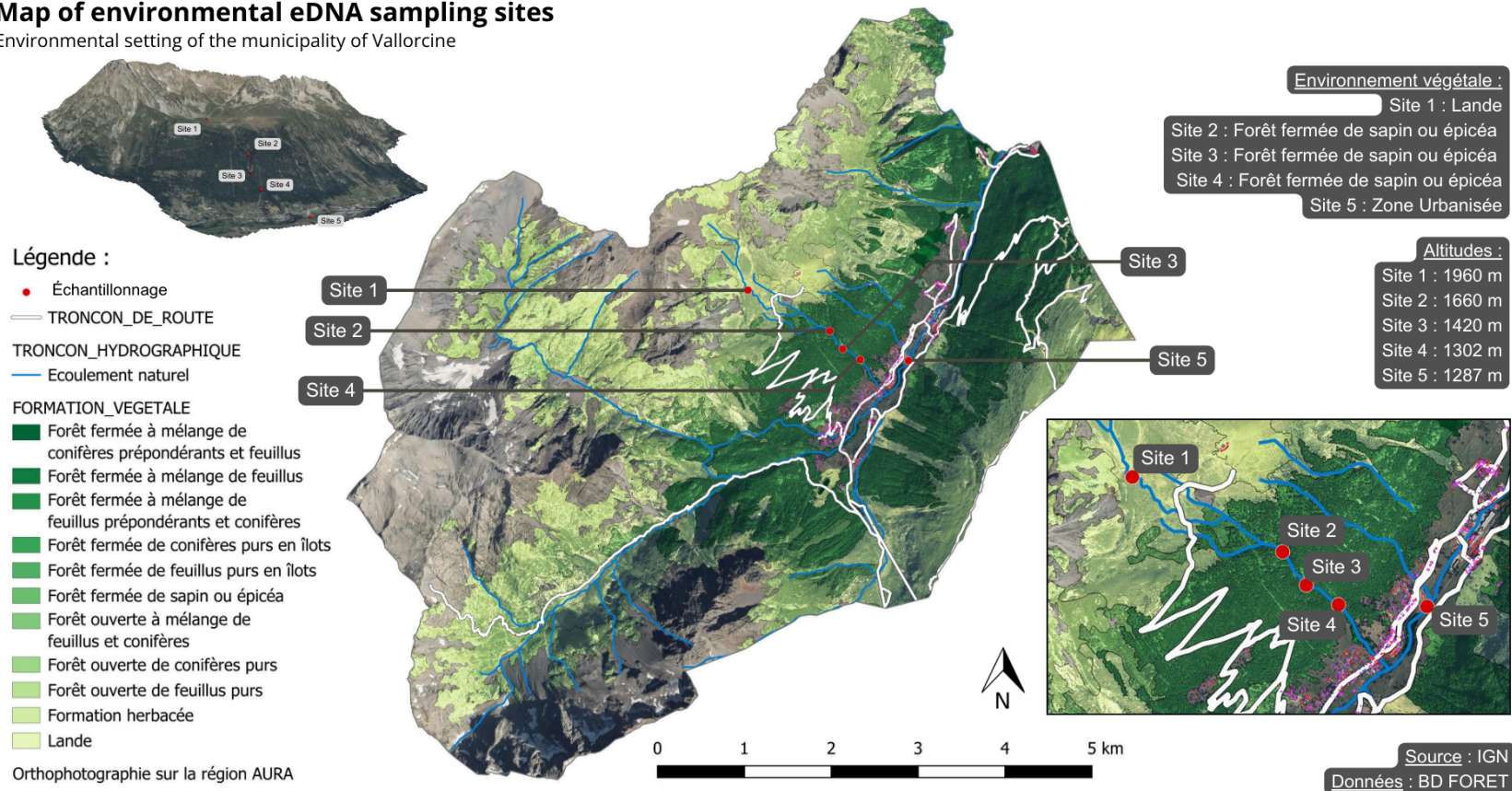


Figure S1. Environmental context and location of eDNA sampling sites across Vallorcine municipality (France). Central panel: Overview map of Vallorcine showing five eDNA sampling sites (red dots) on orthophotographic background, with vegetation formations classified per BD FORÉT (dense/clear coniferous, deciduous and mixed forests; herbaceous and shrubland formations). Right panel: Detailed map of Nant de Loriaz catchment highlighting hydrographic network (blue lines), road network (white lines), and sampling site positions along the stream gradient. Left panel: 3D topographic map of the Nant de Loriaz watershed. Data sources: IGN orthophotography, BD FORÉT

Figure S2: Git link : https://forge.univ-lyon1.fr/groups/ge_ateliers

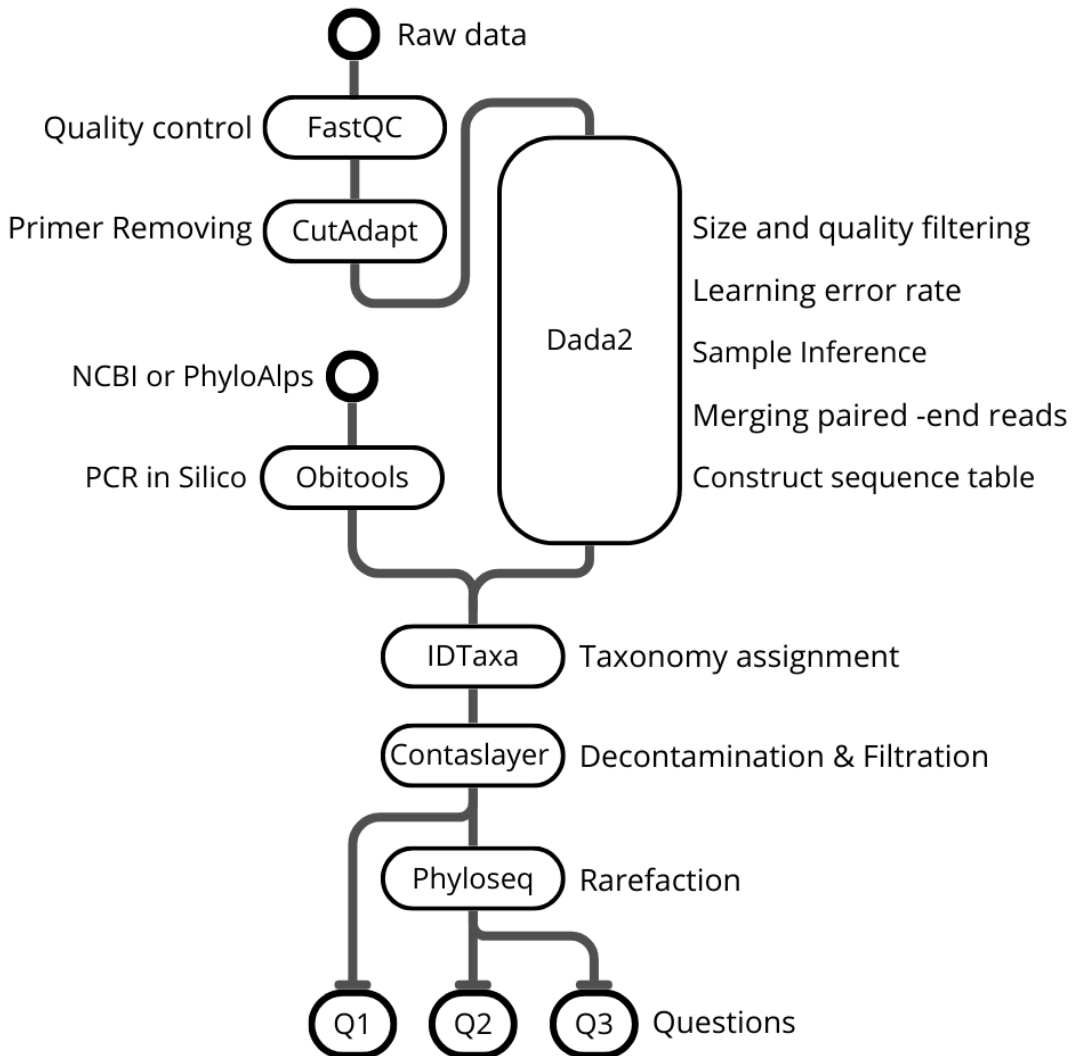


Figure S3: Bioinformatics pipeline for processing metabarcoding amplicon

Reference database composition at family level (all families)

Total sequences: 4103 | Total families: 74 | DB = Mammal

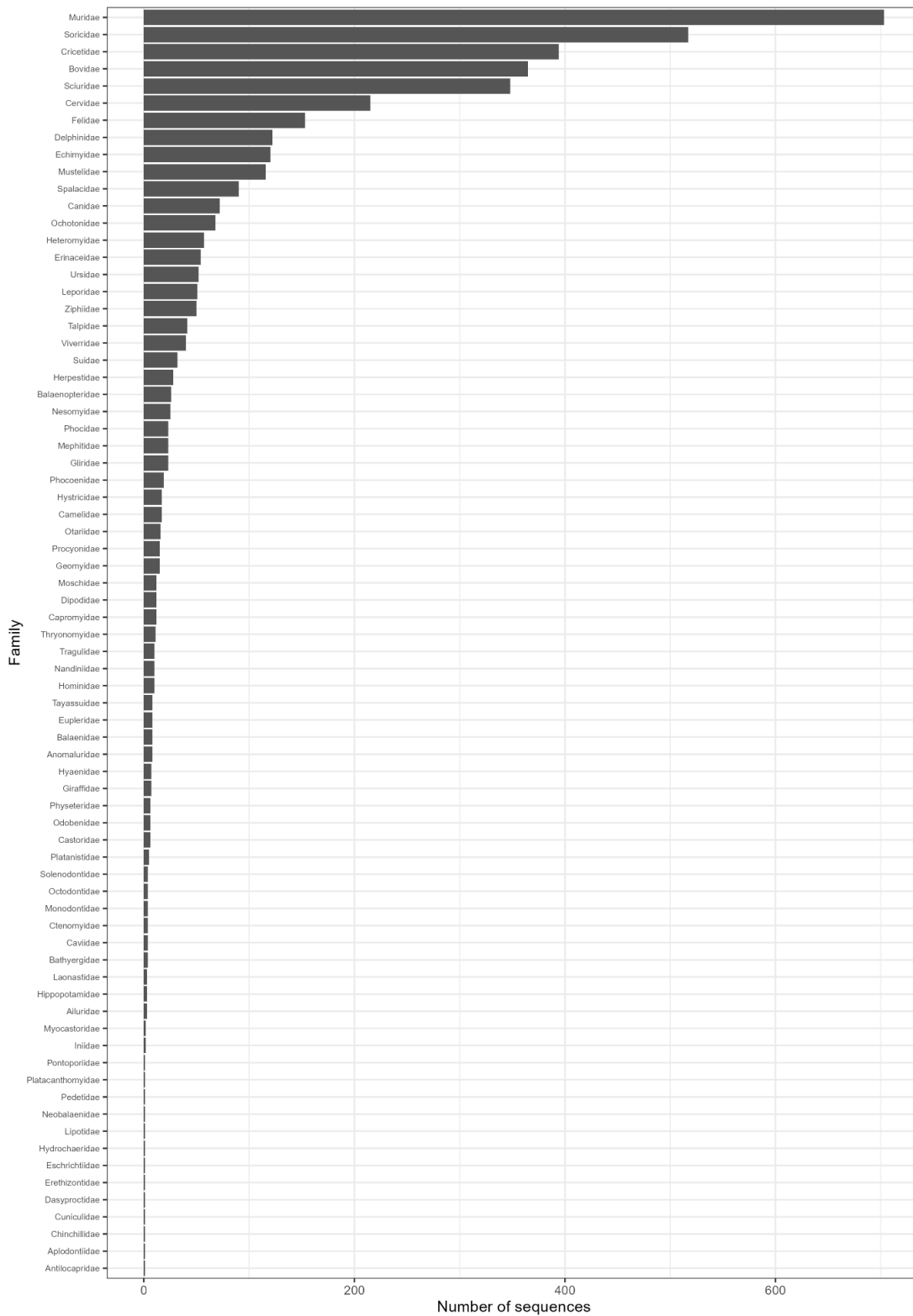


Figure S4: Details of the composition of the Mammal reference database used for taxonomic assignment

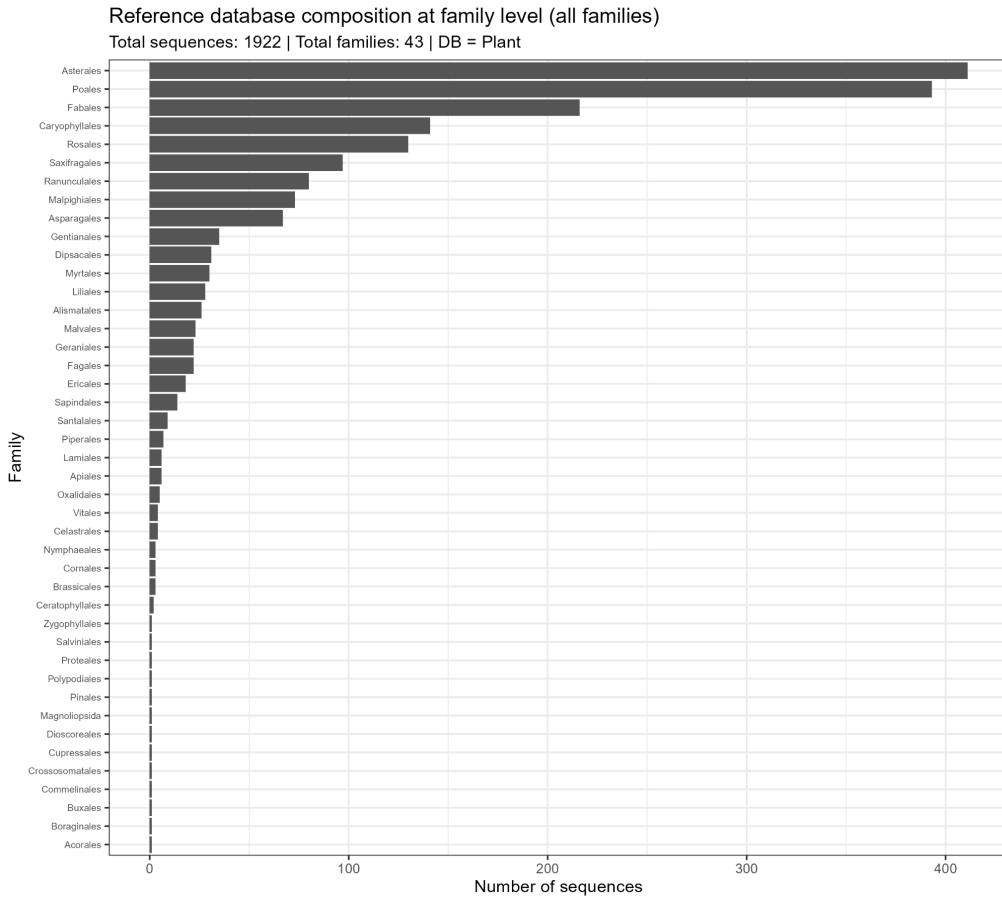


Figure S5: Details of the composition of the Plant reference database used for taxonomic assignment

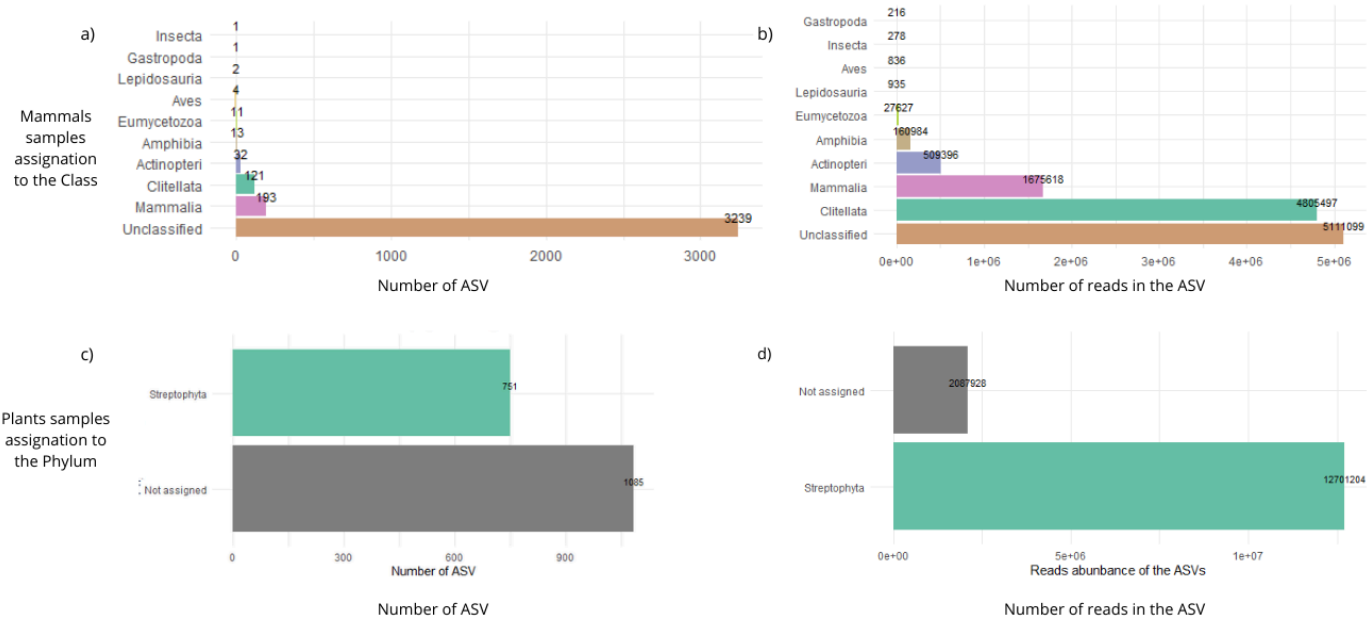


Figure S6: Barplot aiming to show the repartion of the ASVs and reads in the decontaminated samples to each assigned taxonomic group : a-b) Number of ASVs and of their reads assigned to the Class found in the Mammals samples ; c-d) Number of ASVs and of their reads assigned to the Phylum in the Plants samples.

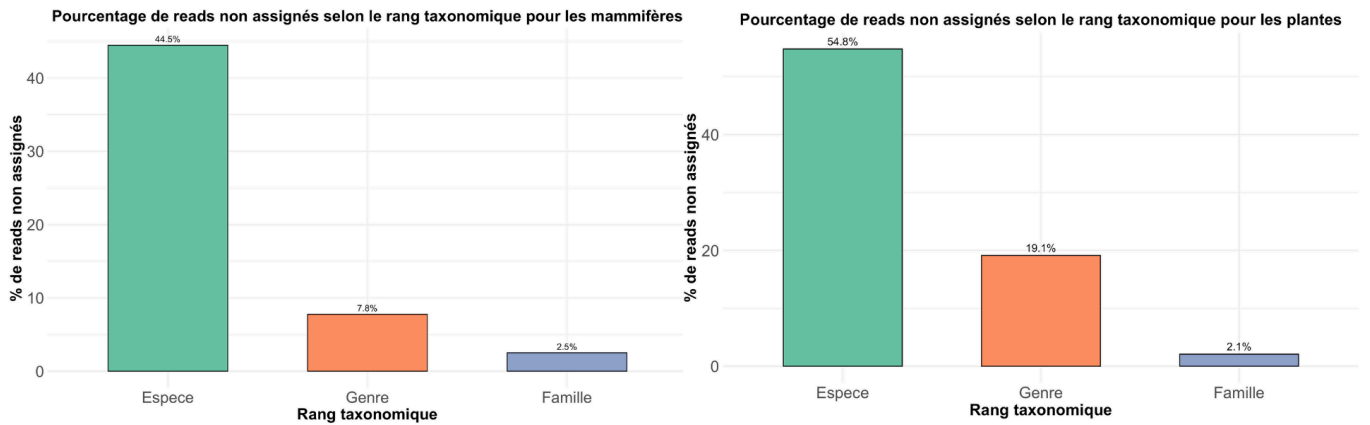


Figure S7: "Distribution of Unassigned Sequencing Reads by Taxonomic Rank in Mammal and Plant Datasets"

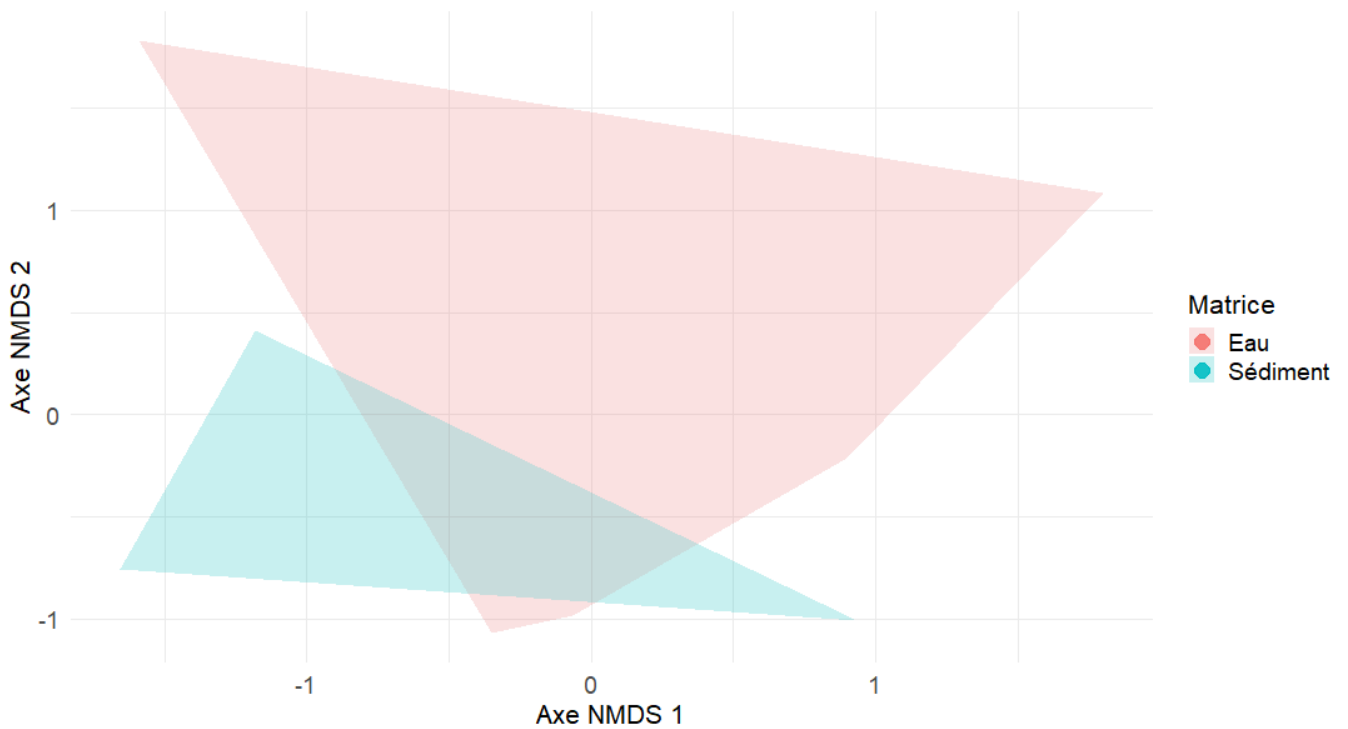


Figure S8: NMDS ordination of mammal communities Bray-Curtis dissimilarity (presence/absence)

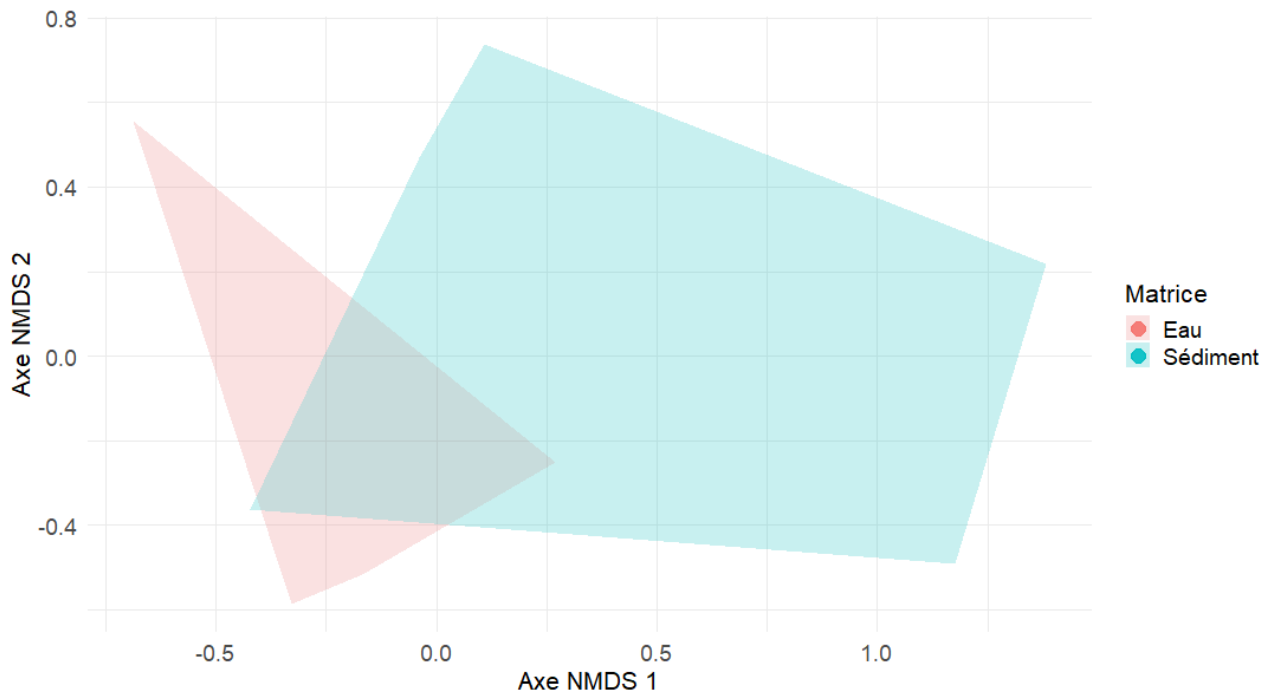


Figure S9: *NMDS ordination of plant communities* Bray-Curtis dissimilarity (presence/absence)

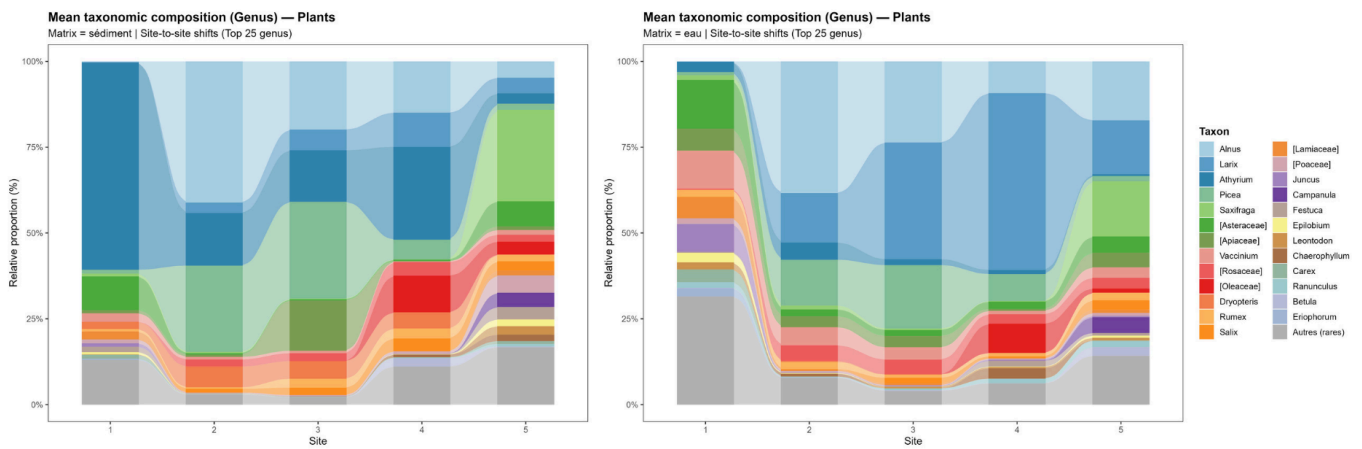


Figure S10: *Alluvial plot illustrating differences in community composition from upstream to downstream sites for both water and sediment matrices.*