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Identifying the sources of sediment using plant-based eDNA – a proof of principle analysis

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Abstract

Purpose Understanding sediment origins is crucial for effective catchment management, especially given the strong influence of vegetation on geomorphological processes. This study presents a proof of principle analysis investigating plant-based environmental DNA (eDNA) as a tool for tracing sediment sources. Two contrasting catchments were selected: a lowland agricultural area with rotating crop cycles and a high mountain environment with semi-natural vegetation. The approach aims to link sediment production to vegetation types to improve land degradation assessment and management strategies.

Methods eDNA was analysed using an amplicon sequencing approach targeting the plant species in soil samples from representative land cover types and river sediment from four flood events. Data analysis included quantifying the eDNA concentration, identifying indicator species of plant communities, determining relative abundances and visualizing differences in community composition.

Results The findings confirm that soils carry a distinct eDNA signature reflective of plant communities, even in degraded or eroded conditions. Our results demonstrate that eDNA in eroded sediment from *Solanum tuberosum* L. (potato) fields in a lowland catchment was correctly identified and that at a high mountain environment, vegetation from heath and forest dominated the eDNA signal in sediment after flood events. However, the study also highlights important limitations associated mostly to sampling.

Conclusion Plant-based eDNA shows promise for identifying sediment sources and providing ecological context. However, its effectiveness depends on factors such as eDNA persistence, source sampling, and sediment connectivity, rendering the method semi-quantitative. Further research is needed to improve consistency and broader applicability.

Keywords Amplicon sequencing · Paleoecology · SedDNA · Soil erosion · TrnL · Sediment fingerprinting

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1 Introduction

Identifying the sources of sediment is essential for understanding sediment dynamics and addressing critical land degradation issues (Collins et al. 2017). This process involves tracing sediment back to its origins by examining physical and chemical properties that remain intact during transport. Indicators such as geochemical signatures or spectro-colour characteristics, often linked to catchment lithology or soil type, can be used to infer sediment provenance. Among the various tools available, fallout radionuclides are particularly useful for distinguishing between sediment derived from surface erosion and that originating from deeper processes such as landslides, gully formation, or subsurface erosion (Evrard et al. 2020). Advances in this field have led to a growing array of tracers and an expanding scope of applications for sediment source identification (Owens et al. 2016; Collins et al. 2020).

Environmental DNA (eDNA) offers promising potential for improving the identification of sediment sources, particularly in catchments with complex vegetation patterns (Frankl et al. 2022). Its high taxonomic resolution allows for accurate detection of plant species and differentiation between vegetation types, providing insights into the plant-soil associations contributing to sediment production (Yoccoz et al. 2012; Alsos et al. 2018; Edwards et al. 2018). This can be especially useful in environments where vegetation significantly influences slope stability and erosion processes (Ghestem et al. 2014; Kim et al. 2017; Eichel et al. 2023; Ohler et al. 2023). In such settings, conventional tracers used in sediment fingerprinting, such as stable isotopes or elemental concentrations, may offer limited resolution, particularly when multiple vegetation types are involved (Evrard et al. 2013). In contrast, eDNA enables more precise discrimination among sediment sources, enhancing our ability to link sediment to specific landscape units. Incorporating vegetation-sensitive indicators like eDNA could therefore improve catchment management strategies by offering a clearer understanding of how different areas contribute to sediment loads (Evrard et al. 2011).

However, the use of eDNA also comes with important limitations. While eDNA can persist in soils and sediments over ecologically relevant timescales (Pietramellara et al. 2008), it is subject to degradation from processes such as microbial activity, UV radiation and oxidation during the hydrological transport (Zulkefli et al. 2019; Nevers et al. 2020; Saito and Doi 2021). To preserve eDNA signatures, sampling is ideally conducted during or soon after flood events, focusing on fine sediment fractions that better retain eDNA (Frankl et al. 2022). Still, unlike geochemical or mineral tracers, there is currently no standardized method to assess or correct for eDNA loss in transit, limiting the ability to validate the conservative behaviour of eDNA (Holman et al. 2021). Additionally, the high spatial and temporal variability of eDNA, which is shaped by vegetation type, biomass, and soil conditions, restricts its use in quantitative mixing models (Johnson et al. 2023). Despite these challenges, the potential to link sediment sources to vegetation makes eDNA a compelling avenue for further research and application, echoing its successful use in reconstructing environmental histories from lake sediments (often referred to as (lake) sedDNA or "ancient" aDNA) (Pietramellara et al. 2008). Furthermore, understanding the performance of eDNA to identify sources of sediment under varying erosion regimes holds broad scientific significance, especially in assessing the interpretive power allocated to it in palaeoecological reconstructions (Morlock et al. 2023).

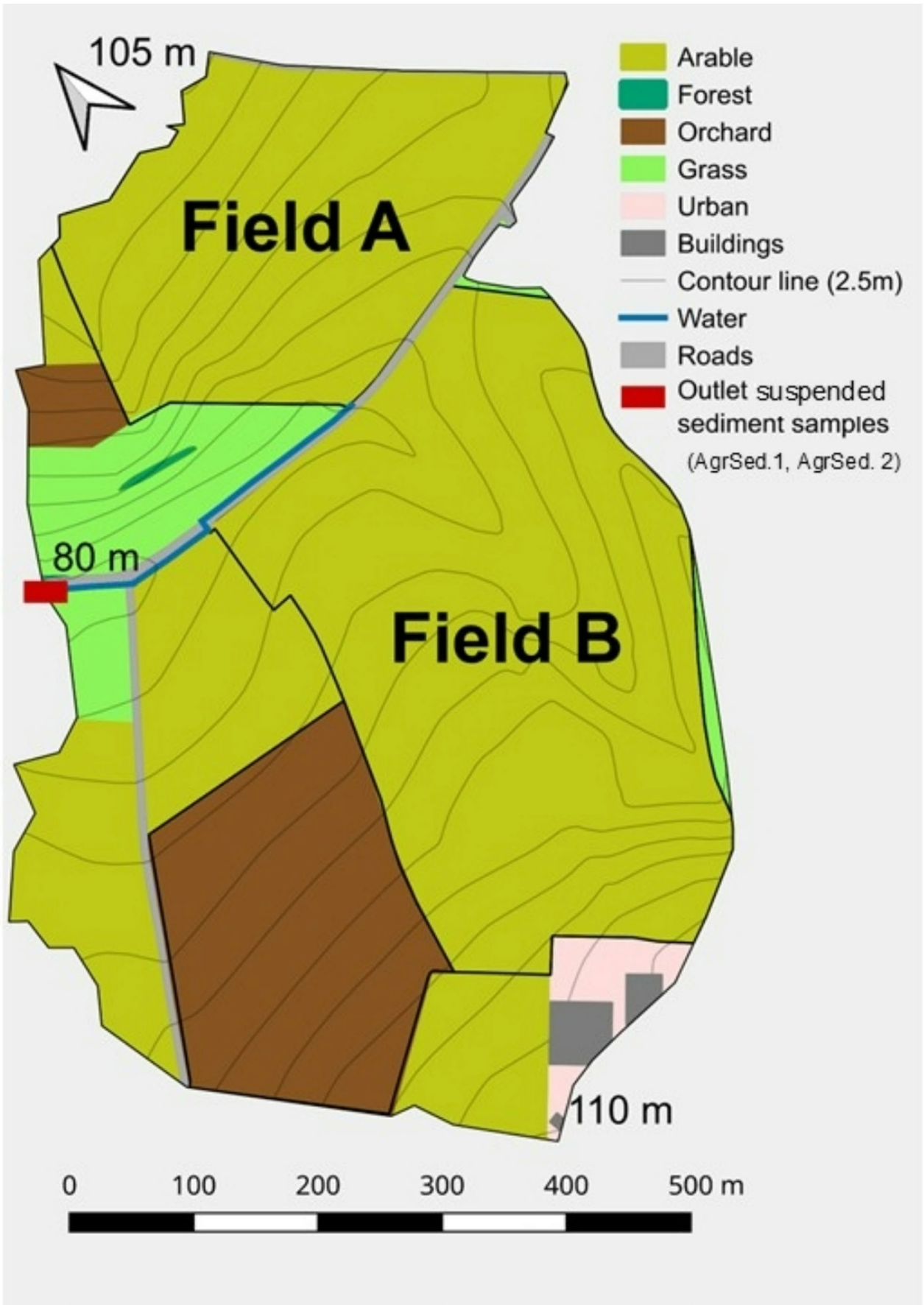
This study explores the potential of plant-based environmental DNA (eDNA) to aid in the identification of sediment sources through a proof of principle analysis. To test the hypothesis that sediment retains an eDNA signal reflective of its source area, even in highly degraded contexts, this study examines two contrasting catchments: a lowland agricultural area with rotating crop cycles and a high mountain environment featuring semi-natural vegetation transitioning from dense to degraded land. We propose that combining plant-derived eDNA signals with vegetation maps could enhance the spatial resolution of source attribution, offering a more targeted understanding of sediment contributions. Such an approach holds promise for informing strategies to mitigate land degradation and manage natural hazards more effectively. We examined this hypothesis by investigating the eDNA concentration, site-specific plant associations and their indicator species, as well as relative abundance and community composition, to gain insights into plant–soil–sediment interactions.

Fig. 1 Lowland agricultural catchment in South Limburg (the Netherlands)

2 Materials and methods

2.1 Study area

The lowland agricultural catchment, covering 0.38 km², is located in South Limburg, the Netherlands, within the loess belt known for its rolling topography and suitability for farming (Fig. 1). The area was gradually transformed into an open agricultural landscape, particularly after World War II, when industrial farming practices were introduced. This shift has resulted in increased soil erosion and more frequent muddy floods (Winteraeken and Spaan 2010). The catchment stretches from 80 to 110 m above sea level (a.s.l.), with an average slope of 0.063 m m^{-1} (Commelin et


105 m
Field A
Field B
80 m
110 m
Arable
Forest
Orchard
Grass
Urban
Buildings
Contour line (2.5m)
Water
Roads
Outlet suspended sediment samples
(AgrSed.1, AgrSed. 2)
0
100
200
300
400
500 m

al. 2022). The average annual precipitation is 757 mm, with the most critical period for soil erosion occurring in spring, when intense rainfall coincides with exposed soils during the early cropping season (Commelin et al. 2022). To mitigate soil erosion, carbon farming practices, including no-till farming, have been introduced in the catchment. Additionally, measures such as planting winter cover crops are now legally required to further reduce soil loss.

The high mountain catchment covers a surface area of 61 km², with an outlet at the village of Barèges (42°57'46" N 0°03'50" W) in the Central Pyrenees, France (Fig. 2). The catchment is situated in the axal zone of the mountain range and is built up of Hercynian metamorphic and plutonic rocks, which form a patchwork of lithological units (InfoTerre 2024). The steep-sloped topography bears an imprint of the Pleistocene glaciations. Loose deposits (of mixed lithology) such as moraines and talus slopes, therefore, form a large part of the terrain. With surface elevation ranging from 1162 to 2877 m a.s.l., the study area stretches from forested montane to the nearly bare nival vegetation zones. The higher catchment is predominantly characterized by pastoral land use, which has a long history in the region (Galop et al. 2011), while a ski station is located at the mountain pass, the Col de Tourmalet. The area receives abundant precipitation, with an approximate annual average of 1235 mm (Météo-France 2024). Winter precipitation is dominated by snowfall, and rain on snow events cause the most severe floods, such as the events studied in this work. Over the course of the past two centuries, the study area has experienced significant transformations in land use and cover as a result of socio-economic developments and natural hazards (Blanpied et al. 2020).

2.2 Studied hydro-meteorological events and sampling

Four hydro-meteorological flood events were recorded for this study, two in the lowland agricultural catchment and two in the high mountain catchment, each yielding a single (composite) sediment sample (Table 1). To prevent the gradual degradation of eDNA after deposition and the risk of contamination from compromising result reliability (Harrison et al. 2019; Nevers et al. 2020), samples were collected during or promptly after the floods.

In the lowland catchment, the two flood events were triggered by precipitation depths of 7.4 and 9.0 mm, resulting

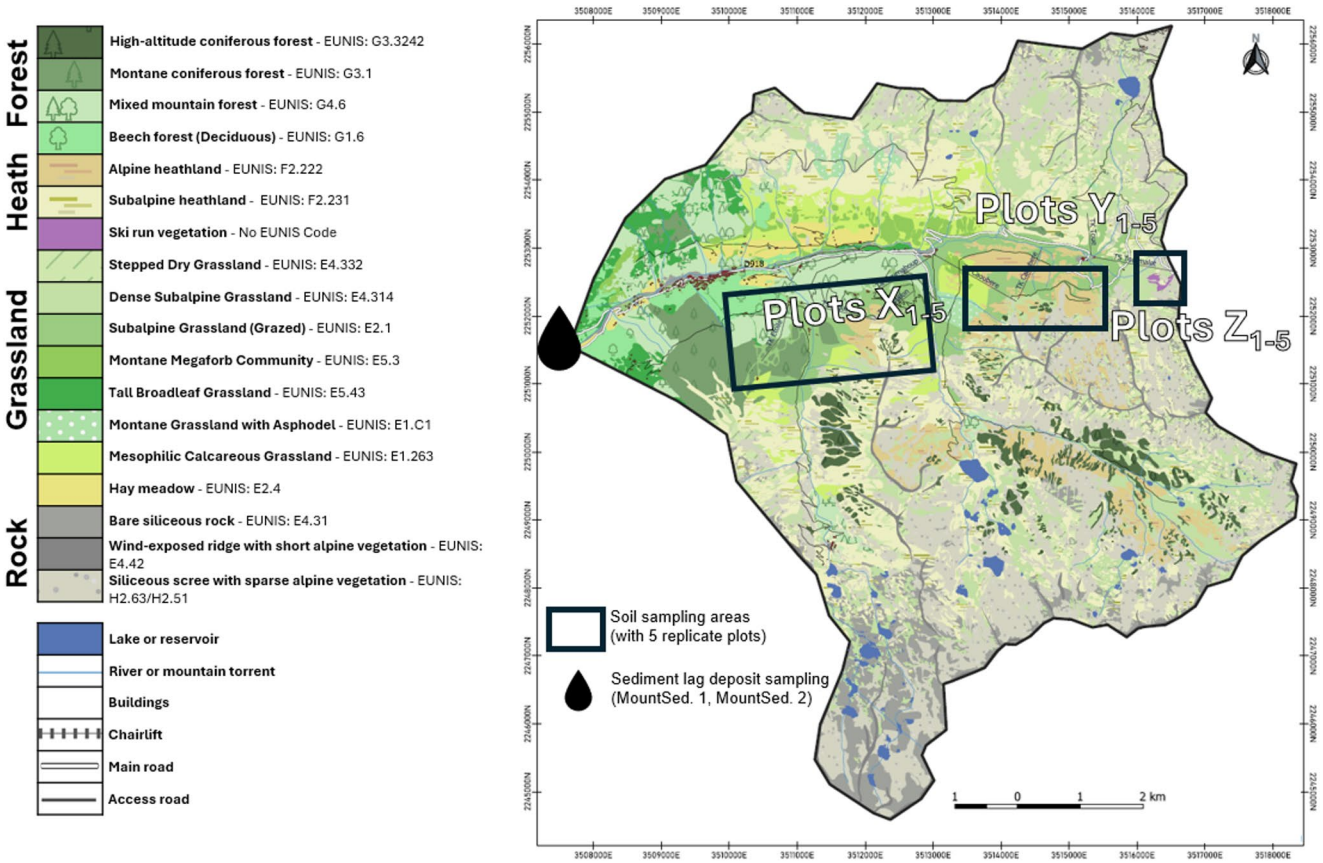


Fig. 2 Semi-natural vegetation dominated the high mountain catchment, which was mapped during fieldwork and classified according to UNIS codes, including dominant species per land cover type. Details on the methodology used for mapping the vegetation can be found in the Supplementary information S1

Table 1 Sediment sample metadata for the four sampled flood events. Coordinates for the sediment samples in the lowland agricultural area are not available due to a non-disclosure agreement with the farmer

Sample location on Figs. 1 and 2	AgrSed. 1	AgrSed. 2	MountSed. 1	MountSed. 2
Environment	Lowland agriculture	Lowland agriculture	High mountain	High mountain
Catchment area (km²)	0.38	0.38	61	61
Location (Latitude, Longitude, decimal degrees)	n.a.	n.a.	42.894, 0.055	42.894, 0.055
Elevation (m above sea level, a.s.l.)	80	80	1162	1162
Date	28/05/2019	18/08/2019	17/12/2021	20/01/2022
Type	Suspended Sediment	Suspended Sediment	Composite Lag Deposit	Composite Lag Deposit

in peak discharges of 0.251 and 0.071 $m^3 s^{-1}$, respectively. Field observations suggest that sediment export for both events can largely be attributed to erosion on Field B, where *Solanum tuberosum* L. (potato) was grown. During the first event in May 2019 (AgrSed. 1), the potato crop in Field B was still in early growth stages, while Field A's winter wheat (*Triticum aestivum* L.) was well developed. The second event in August 2019 (AgrSed. 2) corresponded with the late cropping season, when all crops had reached the maturation stage. For each event, a single suspended sediment sample was collected using an ISCO 3700 automatic sampler (Teledyne Isco, Inc., United States), which pumps runoff via tubes into containers when (peak) discharge occurs. We hypothesized that flushing the equipment prior to sampling minimizes contamination from remnant eDNA in the tubes, leading to very low eDNA reads compared to those from the sampled suspended sediment. Additionally, given the data processing threshold (above 100 reads, Sect. 2.4), any residual eDNA is unlikely to influence the results. Upon arrival at the laboratory, the suspended sediment samples were stored in a freezer at -18 °C.

In the high mountain catchment, two intense precipitation events were recorded in December 2021 and January 2022, both of which triggered severe floods. The torrential rains on December 9 and 10, 2021, were preceded by an exceptionally moist period, which led to saturated soils and a significant snowpack at higher elevations. For instance, just before the event, a snow depth of 1.80 m was recorded in Cauterets at 1920 m a.s.l. The rainfall on January 9–10, 2022, was particularly remarkable, with a cumulative precipitation reaching 200 mm on some high mountain slopes (Météo-France 2022). One notable aspect of the events was the fluctuating temperatures, which caused the 0 °C isotherm to drop to altitudes of 1000–1200 m a.s.l. Consequently, the occurrence of rain on snow further intensified the hydrological response. Field observations have indicated that the ski runs were highly degraded by soil erosion, while numerous shallow landslides occurred in heathlands. Both are considered important sediment sources. For each event, a single composite lag deposit sample (15 g) was collected shortly after sediment was exposed in the riverbed (Table 1). While lag deposits typically reflect hydrological conditions during the falling limb of a flood, the sampling height above the channel bed may indicate contributions from different sources (Battista et al. 2020). Sampling sediments from the highest locations in the riverbed supports the assumption that our high mountain samples also represent peak flow conditions. Each sample is a composite of sediment collected at 10 locations within a 5 m² area. In order to prevent (cross-)contamination, sediment sampling for eDNA analyses occurred with sterilised (using 70% ethanol) nitrile gloves, a facial mask, and using a sterilised plastic spatula, which was replaced before each sampling event. The sediment samples that served for eDNA analyses were frozen at -20 °C upon arrival in the laboratory.

2.3 Benchmarking the eDNA soil signals

A critical step in identifying sediment sources is establishing a reliable reference for the source material. In the lowland agricultural area, standing crops provide an accurate representation of species distribution across the catchment. However, given the persistence of eDNA in soil (Foucher et al. 2020) and the presence of crop residues from previous seasons on the surface in a no-till cropping system, it is important to consider both present and past crop rotations when analysing soil samples for the lowland agricultural area (Fig. 3). This consideration ensures a more accurate understanding of the soil eDNA signal in relationship to land cover change. Given the dominance of recent crop rotations in soil eDNA signatures (Foucher et al. 2020), we restricted the recording of land cover to the past 2.5 years prior to the suspended sediment sampling.

In the high mountain catchment, we aimed to validate the assumption that specific eDNA signatures are associated with distinct plant communities and to corroborate previous research (Edwards et al. 2018; Hiiesalu et al. 2012; Ariza et al. 2022; Johnson et al. 2023). To this end, we selected three dominant vegetation types that captured the area's spatial heterogeneity: montane, mixed forest (approx. 1450 m a.s.l.), subalpine heathland extensively used for grazing (approx. 1800 m a.s.l.), and subalpine ski runs characterized by sparse, herbaceous vegetation (approx. 2000 m a.s.l.) (Fig. 4). For each vegetation type, five plots of 5 × 5 m were

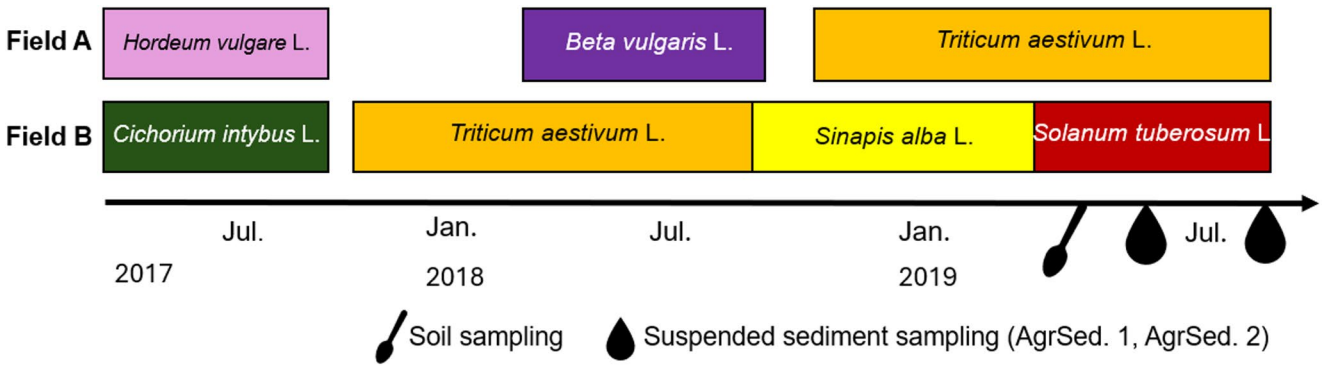


Fig. 3 The recent crop rotations for the agricultural Fields **A** and **B**, along with the timing of the runoff events during which suspended sediment was collected at the outlet of the lowland agricultural catchment

Fig. 4 In the high mountain catchment, samples were taken in (**a**) mixed forest, (**b**) heathland and (**c**) on ski runs. The yellow ribbon delineates the 5 × 5 m plots that were examined to relate plant cover with soil eDNA

selected, respecting a distance of at least 100 m between them. Per plot, a complete recording of the plant species present was done, together with a field-assessment of their ground cover in percentage (Supplementary information S2). Soil samples were collected from the top 10 cm beneath the litter layer by combining 16 subsamples taken at the junctions of a 1 m grid within the plot. The protocol for eDNA sampling, described in Sect. 2.2., was respected.

2.4 eDNA analysis

To investigate how plant-based eDNA can support the identification of sediment sources, eDNA concentration, site-specific associations and their indicator species, relative abundance and community composition were investigated for soil and sediment samples. The eDNA analysis of soil and sediment samples was performed using an amplicon sequencing approach. The identification of eDNA reads was done from three replicate samples to ensure reproducibility. In brief, the extraction was followed by amplicon library preparation and consequent high-throughput sequencing to generate genetic data for taxonomic identification and ordination analyses. Additionally, a reference database was prepared to improve taxonomic assignment of the obtained sequences, in particular for the high mountain vegetation. Subsequent data processing yielded unique biologically relevant reads, i.e., zero-distance operational taxonomic units (zOTUs).

2.4.1 eDNA extraction and concentration

For the eDNA extraction, 15 g air-dried samples were mixed with 15 mL of saturated phosphate buffer (Na_2HPO_4; 0.12 M, pH ≈ 8) for 15 min followed by centrifugation for 10 min at 10,000 g, following Foucher et al. (2020). Next, 400 µL of supernatant was transferred to a clean 2 mL microcentrifuge tube, after which the actual DNA extraction was performed using the DNeasy PowerSoil Pro kit following the manufacturer's specifications. Following the extraction, a primary concern in validating the approach was to examine eDNA concentrations. To address this, eDNA concentration (ng/µL) was measured for all individual soil and sediment samples.

2.4.2 Amplicon library preparation and sequencing

Specific primers (A49425 (forward): 5'-GGG CAA TCC TGA GCC AA – 3'; B49466 (reverse): 5' – CCA TTG AGT CTC TGC ACC TAT C – 3' (Taberlet et al. 2007), were used to target the g-h regions of the *trnL* intron P6 loop, a chloroplast DNA marker commonly used for plant identification (Hollingsworth et al. 2011). The short g-h region allows for

a better recovery of ancient or fragmented eDNA and consequent plant identification, as larger regions are more likely to be degraded over time, making taxonomic assignments more difficult.

The Roche KAPA HiFi HotStart ReadyMix (2x) was used with 12.5 µL of Kapa HotStart mix, 2 µL of each primer (5 µM), 7.5 µL water, and 1 µL of the extracted DNA. The PCR program started by a denaturation step at 96 °C for 5 min, followed by 35 cycles of denaturation at 96 °C, annealing at 50 °C, and elongation at 72 °C, for 1 min each. PCR ended with a final elongation step of 20 min. PCRs were performed in duplicate, pooled and cleaned using AMPure XP beads (Beckman Coulter Inc.) following the manufacturer's protocol.

A second PCR was performed to add barcode tags to the amplicons. This PCR started with a denaturation at 95 °C for 3 min, followed by 12 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s and elongation at 72 °C for 30 s. PCR ended with 5 min at 72 °C. Illumina XT labels were used as primers. PCR products were again cleaned using AMPure XP beads (Beckman Coulter Inc.). Quality of the library prep was investigated using the High Sensitivity NGS Fragment Analysis Kit for the Agilent Fragment Analyzer. DNA quantification was done using a Qubit dsDNA Broad Range assay kit (Invitrogen), after which samples were diluted to equal concentrations and equimolarly pooled by taking 5 µL of the normalized samples. Samples were consequently sequenced at AZENTA Life Sciences (Germany) on the Illumina MiSeq v.2 (2 × 150 bp paired end) platform.

2.4.3 Reference database

To improve the identification of Pyrenean plant species in our sequencing dataset, 116 plant specimens from this high mountain area were collected and identified (Supplementary information S2), since many species are occurring only locally and are lacking from public databases. Using leaves, plant specimen DNA was extracted for all 199 species using the OmniPrep for Plant kit (G-Biosciences) following the manufacturer's protocol. Several regions of the P6 loop of trnL were targeted using either primers trnlD-trnlG or trnlC-trnlD or trnlG-trnlH (trnlD: 5'-GGG GAT AGA GGG ACT TGA AC-3'; trnlG: GGG CAA TCC TGA GCC AA; trnlC: 5'-CGA AAT CGG TAG ACG CTA CG-3'; trnlH: 5'-CCA TTG AGT CTC TGC ACC TAT C-3'). The PCR program started by a denaturation step at 96 °C for 5 min, followed by 35 cycles of denaturation at 96 °C, annealing at 55 °C, and elongation at 72 °C, for 1 min each. PCR ended with a final elongation step of 10 min. PCR products were cleaned using 1 µL per 5 µL of PCR product of a mixture containing 200 µL of FastAP (Thermo Fischer), 100 µL of Exonuclease I (Thermofisher), 30µL of ExoI buffer and 270 µL of aqua dest. Mixture and PCR products were incubated for 15 min at 37 °C, 15 min at 85 °C and brought back to 15 °C. This was consequently sequenced at Macrogen Europe (Amsterdam) via EZ SEQUENCING on a 3730xl DNA Analyzer. The g-h region was extracted bioinformatically and added to the taxonomy reference file for use with the mothur (v.1.42.1; Schloss 2009) *classify.seqs* command as described below.

2.4.4 Data processing and analysis

Quality of the amplicon sequencing data was checked using fastqc (Andrews 2010). Forward and reverse reads were merged using PEAR (Zhang et al. 2014), with minimum and maximum assembly length set to 10 and 300, respectively. Quality filtering was performed using USEARCH v.10.0.240 (Edgar 2013), using a quality threshold of Q30, a minimum length of 10 and a maximum expected error of 1. UNOISE3 (Edgar 2016) was applied for denoising amplicon reads, and chimera detection was performed with UCHIME (Edgar et al. 2011), retaining only unique biologically relevant reads as zero-distance OTUs (zOTUs). A taxonomy was assigned using the *classify.seqs* command in mothur v.1.42.1 (Schloss 2009), mapping reads with a cutoff of 0.80 to our custom made reference database using the identified plants. zOTUs were additionally BLASTed to the NCBI genbank database and manually checked to improve taxonomical assignment for unclassified reads.

Further data processing and analysis was performed with R v.4.2.3 (R-Core-Team 2023) in RStudio (Posit-Team 2023). To filter out noise and retain only the strongest signals, any zOTU with fewer than 100 reads was removed from the entire dataset, as well as unknown reads at the genus level, or classified as algae, bacteria, or moss. Consequently, the lowland and high mountain datasets were split and analysed separately, again retaining only zOTUs with at least 100 reads in either dataset. Within a dataset, samples were subset to the same number of reads with the *rrarefy* function in vegan v.2.6-4 (Oksanen et al. 2022). Finally, zOTUs with less than 100 reads in a sample were set to 0.

To address the hypothesis that sediment retains an eDNA signal reflective of their source areas, three analyses were performed: an Indicator Species Analysis of plant associations, relative abundances and community composition. The Indicator Species Analysis, often referred to as Multilevel Pattern Analysis in ecology, allows us to identify species that are strongly associated with specific land cover type or soil type. It was performed using the package *indicspecies* v.1.7.15 (De Cáceres et al. 2010). It evaluates the fidelity (frequency of occurrence) and specificity (uniqueness) of species in particular groups, and thus helps to determine which species best represent or "indicate" the predefined

plant associations (recent crop rotations or semi-natural vegetation types). More methodological information and details on the Indicator Species Analysis are available in the Supplementary information S3. Next, the relative abundance of indicator species was calculated per soil and sediment sample, allowing us to assess in a semi-quantitative way, the importance of plant-associated sediment sources in the catchment. Finally, Non-metric Multidimensional Scaling (NMDS) was used to visualize differences in community composition based on zOTU data. This ordination method facilitates comparison between soil and sediment samples, enabling preliminary evaluation of how closely sediment eDNA profiles align with those of potential source soils, conceptually analogous to assessing source contributions in conventional mixing models. NMDS allows for the effective representation of complex ecological relationships without assuming linearity, making it well-suited for the non-parametric nature of the dataset. NMDS was performed using the *metaMDS* function in vegan on Hellinger transformed data, using the default values.

3 Results

3.1 eDNA presence

The analysis of eDNA concentrations (ng/µL) confirms that soils with abundant vegetation produce strong eDNA signals (Table 2). In contrast, ski runs, which have sparse vegetation cover, exhibited noticeably lower but still relatively high eDNA levels compared with the agricultural soils. The latter may point to an overall low preservation of the eDNA signatures in the agricultural land. Most sediment samples had low eDNA concentrations, with the exception of MountSed. 1 (mountain catchment).

3.2 Discrimination of soil and sediment samples: lowland agricultural setting

Raw reads number for the lowland dataset was 1,236,955 and 209 zOTUs, which was reduced to 414,130 (27,608.67 ± 252.25 per replicate) reads and 51 genera in 56 zOTUs (11.87 ± 7.58 per replicate) after quality filtering. Allowing us to identify which species are strongly associated with specific soil and sediment samples, the Indicator Species Analysis revealed that only two genera were retained for the soil samples, and were additionally only marginally significant, namely *Jasione* sp. for Field A ($p = 0.045$, stat = 0.994), and *Rubus* sp. for the combined group of Field A, Orchard and the August 2019 sediment sample ($p = 0.046$, stat = 0.999). Among the sediment samples, the August 2019 event (AgrSed. 2) displayed a marked eDNA signal for *Solanum tuberosum* L. (potato), accounting for up to 75% of the relative abundance, ranking third in overall abundance after *Festuca* and *Sagina*. *Brassicaceae* sp. also featured prominently, with up to 29% relative

Table 2 eDNA concentration for the sample types 1: soil with dense vegetation in the high mountain environment ($n = 10$), 2: soils with sparse vegetation in the high mountain environment ($n = 5$), 3: soils from agricultural land in the lowland area ($n = 3$), 4: sediment samples ($n = 4$, locations mentioned in Table 1). S.D. is standard deviation

1	(ng/µL)	2	(ng/µL)	3	(ng/µL)	4	(ng/µL)
Forest 1	79.17	Ski 1	6.43	Field A	3.81	AgrSed. 1	1.18
Forest 2	140.6	Ski 2	13.80	Field B	1.43	AgrSed. 2	3.77
Forest 3	96.60	Ski 3	15.53	Orchard	3.52	MountSed. 1	36.17
Forest 4	112.27	Ski 4	24.20			MountSed. 2	2.39
Forest 5	88.70	Ski 5	10.81				
Heath 1	74.13						
Heath 2	87.13						
Heath 3	91.20						
Heath 4	83.50						
Heath 5	80.20						
Mean	**93.35**	**Mean**	**14.2**	**Mean**	**2.92**	**Mean**	**10.88**
S.D.	19.74	S.D.	6.59	S.D.	1.30	S.D.	16.89

abundance in Field B and 7% in AgrSed. 2, placing it as the fifth most abundant taxon. These results are consistent with field observations suggesting that Field B had contributed significantly to the sediment composition during this event. In contrast, the May 2019 sediment sample (AgrSed. 1) lacked dominant crop-related taxa, exhibiting a more diffuse and less distinctive eDNA profile.

The NMDS plot (Fig. 5), allowing us to visualise patterns in community composition similarities, reveals clear distinctions in eDNA profiles across soil and sediment samples. Orchard replicates were tightly clustered, indicating consistent eDNA profiles within this group. Replicates from Field A also showed clustering, though with slightly more variability. The clustering, however, is not primarily driven by the standing crops. Instead, residues from previous crop rotations appear to play a significant role in shaping the observed clustering patterns. For instance, *Beta vulgaris* L. (beetroot) eDNA from past rotations contributes to the eDNA profile in Field A. In contrast, Field B replicate samples are more dispersed, suggesting greater variation in eDNA signatures during a soil sampling period when *Sinapis alba* L. (yellow mustard), typically used as a green manure, was only recently replaced with *Solanum tuberosum* L. (potato) (Fig. 3).

Sediment samples Sed.1 and Sed.2 largely occupy different sectors in the NMDS ordination (Fig. 5). Sed.1 replicates tend to cluster more closely with Field A, suggesting similar plant community composition. In contrast, Sed.2 replicates were more dispersed. Two replicate samples, however, clustered on the positive side of the NMDS1 axis, where Field B was also situated (which was most affected by erosion during the flood events). Overall, the dispersed patterns in replicate samples suggest that the eDNA vegetation signal was distorted by persistent eDNA of past vegetation, with widespread or weedy taxa not captured in source sampling, or inputs from unsampled microhabitats (e.g., road edges).

3.3 Discrimination of soil and sediment samples: high mountain system

The raw high mountain dataset had 4,046,163 reads and 252 zOTUs, of which 964,345 (20 515.98 ± 227.56 per replicate) reads and 78 zOTUs (9.19 ± 4.99 per replicate) from 67 genera remained after quality filtering. The Indicator Species Analysis identified 23 genera as significantly associated with 1 or more land cover groups: 11 genera were indicative for forest, particularly *Fagus sylvatica* L. ($p < 0.001$, stat = 0.82), 10 species were indicative for heathland,

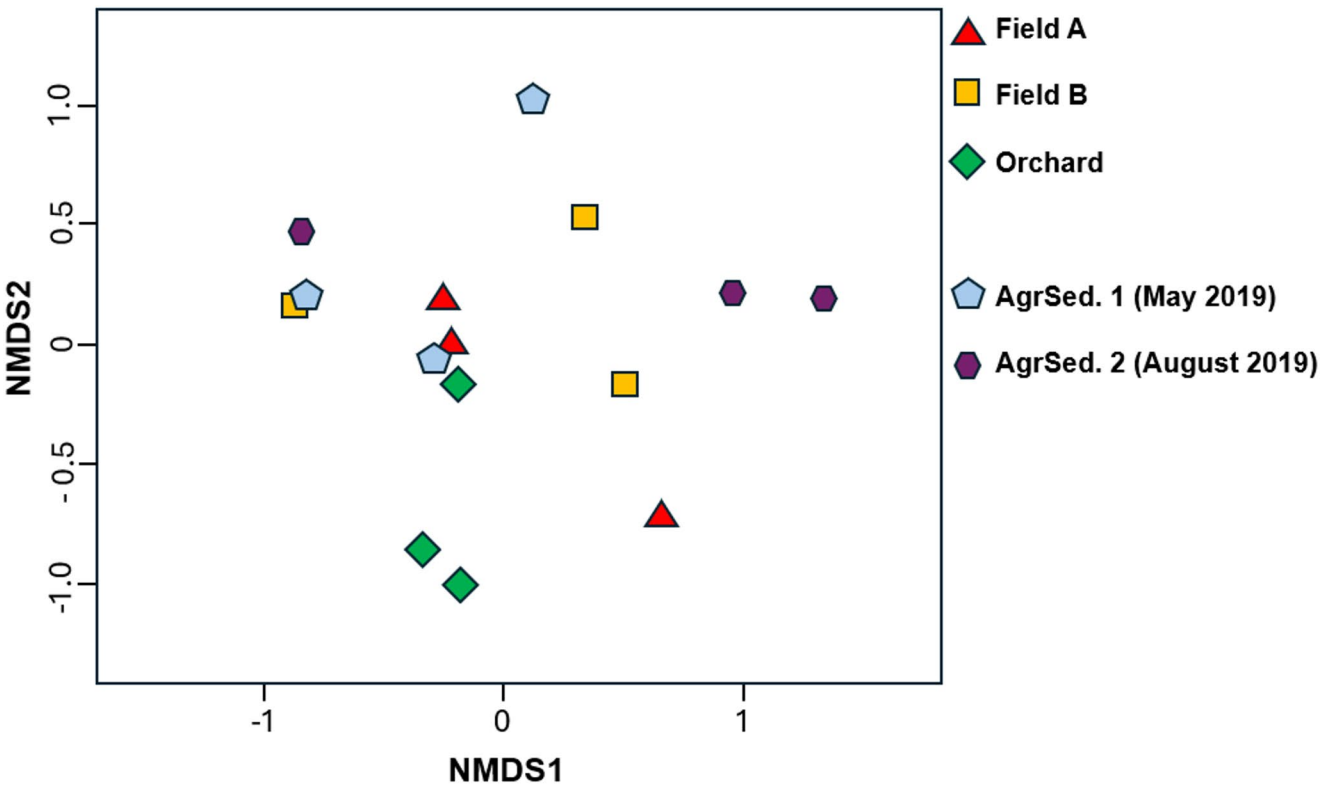


Fig. 5 Non-metric Multidimensional Scaling (NMDS) visualizing the community composition differences based on zOTU data (samples with three replicates) for both soil and sediment data in the lowland agricultural catchment (Netherlands). Each point represents one of three replicates from Field **A**, Field **B**, the Orchard, or flood sediment (AgrSed. 1 and AgrSed. 2). The NMDS stress value of 0.106 indicates a good ordination fit, suggesting that this two-dimensional plot reliably reflects the community dissimilarities for interpretative purposes

particularly *Avenella flexuosa* L. ($p<0.001$, stat = 0.98), *Vaccinium myrtillus* L. ($p<0.001$, stat = 0.89), *Trisetum flavescens* (L.) P.Beauv. ($p<0.001$, stat = 0.88) and *Nardus stricta* L. ($p<0.001$, stat = 0.82), while only *Phleum pratense* L. ($p<0.001$, stat = 0.96) typified the ski plots, and *Carduus carlinoides* Gouan was significant for the combined forest and ski group, albeit with a low value of 0.64 ($p=0.03$) (Supplementary Information S3).

The relative abundance analysis of indicator species on soil samples showed clear habitat-specific species distributions, with replicates generally confirming the reliability of the sampling (Fig. 6). Alpine species dominated the ski runs with sparse herbaceous vegetation, tree species dominated the forest habitat and meadow species were more prevalent in the heathland. However, there was variability between replicate samples, especially for heathland, that was characterised by species such as *Athyrium filix-femina* (L.) Roth, *N. stricta* and *T. flavescens*, reflecting the more heterogeneous environment. In contrast, the ski runs were dominated by fewer species (mostly *A. flexuosa* and *V. myrtillus*), reflecting the more homogeneous and degraded environment that they represent. *Abies pectinata* L., *F. sylvatica* and *Picea abies* L. had strong contributions in forest samples, consistent with their role as indicator species for montane or forested environments. The abundance of the other genera (i.e. non-significant genera resulting from the indicator species analysis; Fig. 6) varied quite substantially between plots and replicate samples and so they are grouped to simplify visualisation.

The relative abundance analysis of indicator species for the sediment samples highlights the variability in species composition across sediment samples and replicate samples (Fig. 7). Overall, species other than those identified as indicator species of soils dominated the eDNA signal. Furthermore, within each sediment sample, replicate samples varied significantly. For example, MountSed. 2 (A) and MountSed. 2 (B) differred greatly, with MountSed. 2 (A) showing more diverse species contributions compared to the dominance of *Corylus* in MountSed. 2 (B). Despite the dominance of the "other" category, several indicator species appeared in samples, such as a high abundance of the genera *Phleum* and *Trisetum* in the MountSed. 1 replicate samples, which are indicative for, respectively, ski runs and heathland.

The NMDS plot revealed clear clustering of soil samples according to plant communities (Fig. 8). Forest, heathland, and ski-run soil samples each formed distinct clusters in the ordination space. The ski-run samples clustered most tightly, reflecting very similar plant DNA assemblages

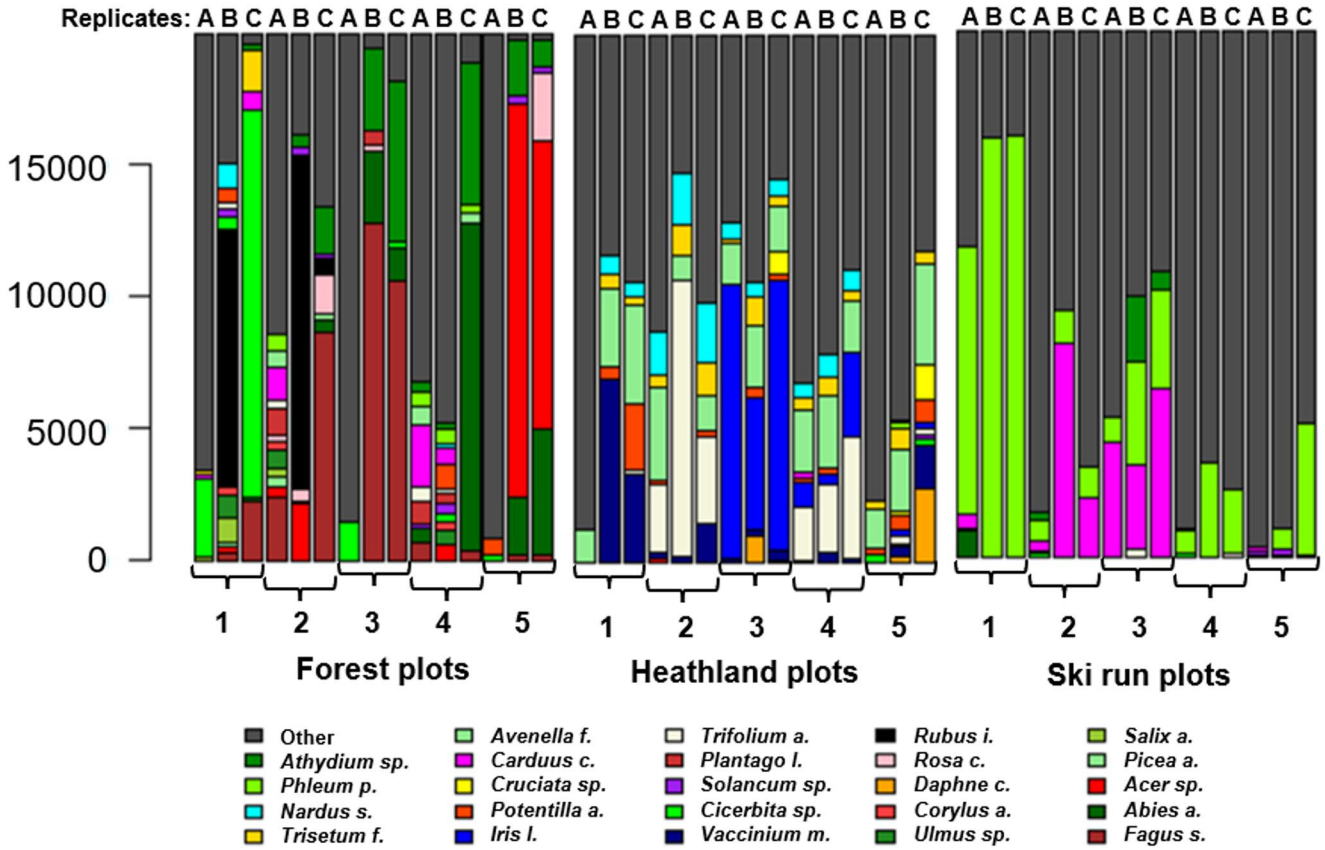


Fig. 6 Relative abundance of indicator plant species for replicates ($n=3$) per soil sample from montane mixed forest, subalpine heath, and ski runs. All other genera are grouped under "other" to accentuate the selected indicator species

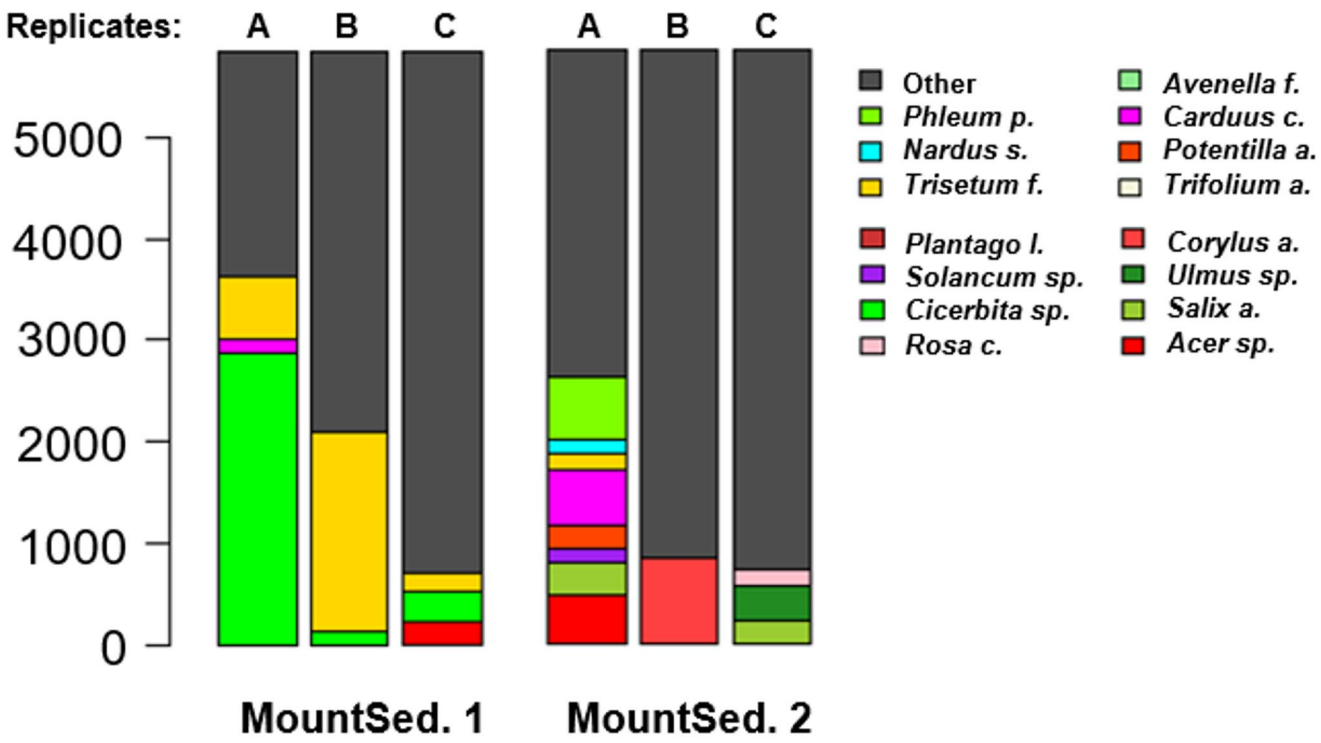


Fig. 7 Relative abundance of indicator species recorded in the sediment samples of the flooding events in the mountain catchment

across those sites. Forest soil samples also grouped together but were more widely dispersed in the NMDS plot, suggesting greater variability in plant species composition among the forest locations. Heathland soils formed a comparatively compact cluster, indicating a more uniform vegetation eDNA signature across the heath sites.

The relative positions of MountSed. 1 and MountSed. 2 samples imply source contributions from forest and heath (Fig. 8). The proximity of MountSed. 2 to the forest cluster suggests that its eDNA is dominated by forest-derived taxa, consistent with inputs of litter or soil from nearby trees; the lower part of the catchment is forested. MountSed. 1 tends to cluster closer to heath, although that the ordination implies a more mixed vegetation signature. For example, this sample may contain eDNA from multiple source areas or plant taxa (from river bank erosion) not well represented in our reference soil samples. These NMDS results underscore that while the eDNA composition of the MountSed. 1 and MountSed. 2 samples matches specific upland vegetation sources (improving confidence in its provenance inference), their particular composition highlighted the challenges of sediment source attribution when eDNA signals are mixed or widely dispersed.

4 Discussion: lessons for provenance studies

We showed that soils carry an eDNA signal reflective of their dominant vegetation cover, even in highly degraded contexts such as eroded soils with sparse vegetation. This relationship was clearly demonstrated for semi-natural vegetation in the high mountain environment, where vegetation communities change only gradually over time. Our findings, therefore, align with pioneering studies such as that of Yoccoz et al. (2012), which demonstrated that plant-based eDNA in soils reflects the semi-natural local vegetation. While contributing to the rapidly evolving field of soil eDNA research (Edwards et al. 2018; Evrard et al. 2019; Lennartz et al. 2021; Morlock et al. 2023) this study expands the validity of eDNA analysis to environments heavily impacted by landslides and soil erosion. By demonstrating that plant-based eDNA remains a reliable indicator of vegetation cover even in disturbed landscapes, this research reinforces its potential as a valuable tool for identifying sediment sources in dynamic and erosion-prone settings. In dynamic agricultural settings where crop rotations shape vegetation cover, soils do not consistently reflect the current vegetation. Therefore, crop rotations must be taken into account. Similarly, Foucher et al. (2020) demonstrated that soil eDNA can reveal crop rotation patterns. Our findings support this and further suggest that crop rotations can

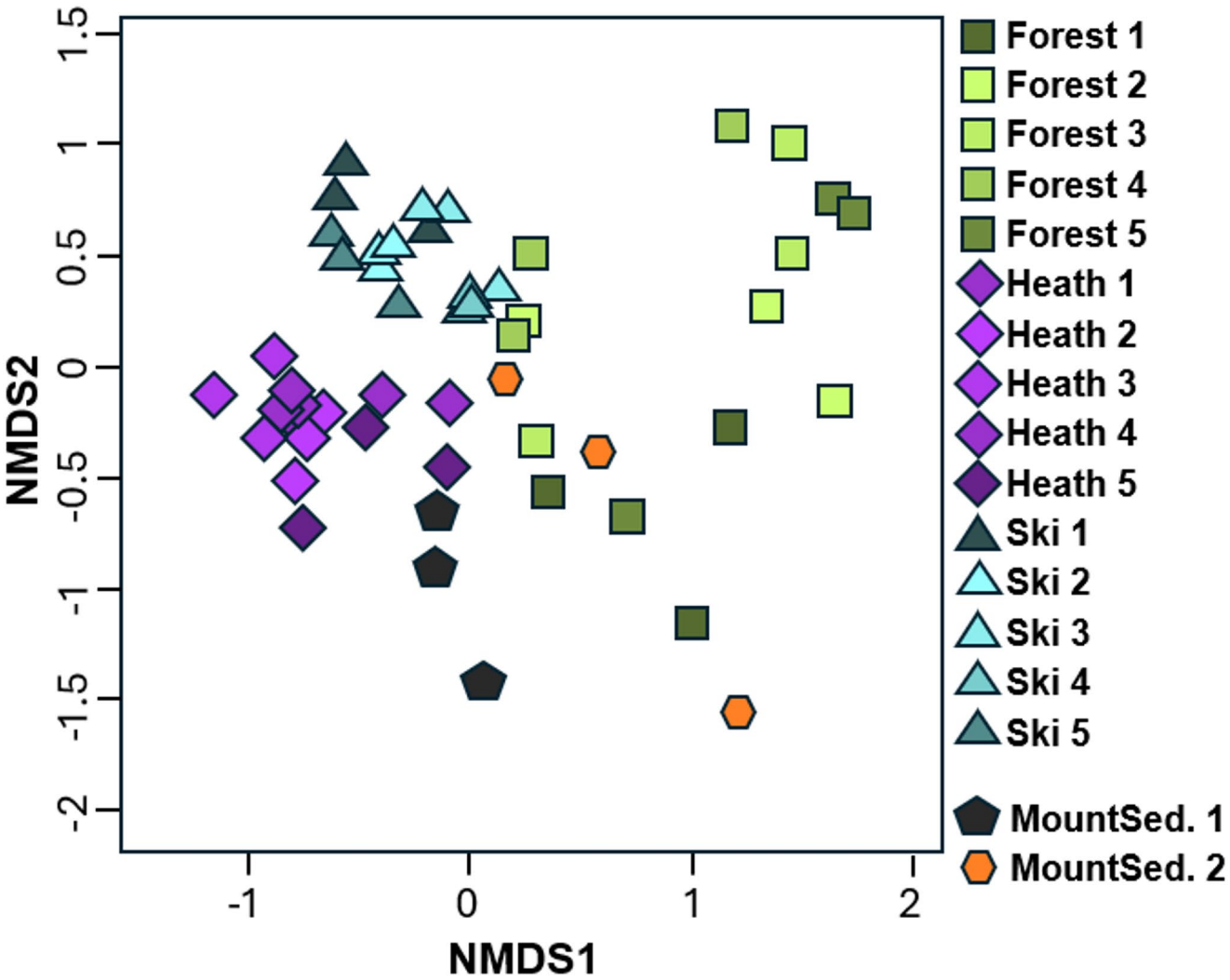


Fig. 8 Non-metric Multidimensional Scaling (NMDS) ordination of plant eDNA community composition for source soils and flood sediment samples in the high mountain catchment (Pyrenees). Each point represents one of three replicate samples per sample from forest, heathland, ski run habitats, or flood sediment (MountSed. 1 and MountSed. 2). The NMDS stress value is 0.177, indicating a moderate fit, with acceptable representation of community dissimilarities in two-dimensional space

help differentiate sediment sources when there is no recent overlap in planting history.

Sediment eDNA results largely confirm the hypothesis that sediment retains an eDNA signal reflective of its source area. In one lowland flood (AgrSed. 2, August 2019), the sediment carried a dominant *Solanum tuberosum* signal with ~ 75% of reads, and a *Brassicaceae* sp. (mustard) signal was also prominent. This result aligned with Field B being affected severely by soil erosion. Similar findings, albeit less clear, could be obtained from the species composition captured in the NMDS plot (Fig. 5). The earlier flood (AgrSed. 1, May 2019) produced sediment with a much more diffuse community and no single crop dominating, a period during which crops were in early growth stages. In the mountain catchment, one flood sample (MountSed. 1) clustered near the forest replicates in the NMDS space, indicative of the important contribution of nearby sources (forest occurred near the sediment sampling area in the lower parts of the catchment). While MountSed. 2 largely occupied the same region, it clustered closer to heath, indicating mixed source contributions.

As a proof of principle, our results highlight several limitations. In particular, substantial variability was observed among sediment replicates, highlighting the importance of adequate subsampling within each site. Certain replicates appeared as outliers, potentially due to the presence of coarse plant fragments or spatial heterogeneity in eDNA distribution. Additionally, dilution effects and mixing with eDNA from other sources, such as upstream transport or contamination, may have contributed to skewed community profiles in these samples. As emphasized by Pansu et al. (2015), increasing the number of replicates and applying

consistency checks, such as ordination-based clustering, are advisable practices for minimizing such effects. In the present study, the use of only three replicates per site in the lowland catchment likely limited the capacity to capture full community diversity, contributing to the low number of indicator taxa detected. Moreover, the common practice in eDNA studies of pooling replicates without prior evaluation of their coherence may obscure biologically meaningful discrepancies. Future work should therefore prioritize more robust replication schemes and implement analytical approaches capable of identifying and excluding anomalous subsamples.

It should be acknowledged that eDNA-based methods are inherently semi-quantitative and, as such, do not strictly conform to conventional definitions of sediment fingerprinting. As outlined in the introduction, conventional mixing models are not appropriate for eDNA datasets. While it is possible to infer, for instance, that the AgrSed. 2 sample was influenced by Field B, or that the MountSed. 2 composition reflects material from forests, there remains no reliable framework for quantifying the proportional contributions of each, albeit recent advancements in this domain (Yates et al. 2022). This limitation stems not only from variable eDNA persistence and degradation but also from issues of sediment connectivity and sampling completeness. Thus, the interpretive power of eDNA lies primarily in its capacity to identify contributing environments, rather than in enabling quantitative apportionment. The relative abundances are thus presented as a semi-quantitative indicator of sediment provenance.

An important limitation is that the principle of tracer conservativeness remains a fundamental assumption in sediment fingerprinting and must be carefully considered in the context of eDNA (Sherriff et al. 2015). For a tracer to support robust source attribution, it must remain stable during erosion, transport, and deposition. eDNA, however, is inherently labile and prone to degradation and fragmentation, especially in herbaceous species that are physically more fragile and decomposable than woody species. While short travel times and the known affinity of eDNA for fine mineral particles (e.g. clays) likely support some degree of signal preservation, sequence loss between source and sink is probable. In this study, short chloroplast trnL fragments were used to increase the likelihood of detecting degraded eDNA. Nonetheless, eDNA cannot be considered a fully conservative tracer. In the absence of empirical estimates for eDNA decay under natural flow conditions, source attributions based on eDNA must be treated as provisional. Addressing this limitation will require future research to quantify eDNA degradation rates in situ, potentially through controlled flume experiments or sequential flood monitoring, and to benchmark eDNA performance against conventional, inert tracers.

Landscape connectivity plays a significant role in shaping the sediment eDNA signal, as exemplified by the overlap of MountSed. 1 with the eDNA profile of the nearby forest. In addition, instances were noted where sediment samples contained taxa that were not recorded in the mapped vegetation surveys, suggesting contributions from habitats outside the primary sampling frame. Moreover, the taxonomic resolution can vary within and between taxonomic groups, meaning that not all sequences can be unambiguously assigned to a species or genus. There is also an inherent dependence on the reference database, which is, by definition, limited and may not encompass the full regional biodiversity. For example, the detection of *Avenella flexuosa* (a grass commonly associated with thin, upland soils) in stream sediments, despite its absence in the surveyed heathland plots, indicates inputs from more spatially dispersed or topographically connected areas. Similarly, *Trisetum flavescens* was identified in sediment samples although not observed locally, underscoring that runoff integrates eDNA across the broader catchment. These findings reflect the influence of both geomorphic and hydrological connectivity, wherein upstream or marginal habitats may contribute genetic material. Moving forward, research should expand the number of sampled events, increase replication within source categories, and incorporate additional tracers, such as geochemical markers or radionuclides, to improve the resolution and reliability of sediment provenance assessments. Furthermore, better understanding of how eDNA decays under different environmental and hydrodynamic conditions will be critical to evaluating its behaviour as a semi-conservative tracer.

Finally, the broader relevance of these findings extends beyond applied catchment science. Plant-derived eDNA in soils and fluvial sediments shares fundamental properties with sedimentary DNA (sedDNA) used in paleoecology. Demonstrating that contemporary flood sediments retain habitat-specific eDNA signatures strengthens the conceptual link between present-day erosion processes and the long-term deposition of ecological signals (Morlock et al. 2023). This underscores the value of modern eDNA studies as calibration points for reconstructing past environments from lake sediments, floodplains, or buried soil horizons. In this context, sediment eDNA tracing not only informs land degradation and restoration strategies, but also contributes to the broader effort to interpret environmental DNA across temporal and spatial scales.

5 Conclusions and future work

This proof of concept analysis explored the use of plant-based eDNA to identify sediment sources by examining two contrasting catchments: a lowland agricultural area with rotating crop cycles and a high mountain environment characterized by semi-natural vegetation. Our results support the hypothesis that the relationship between vegetation cover and soil eDNA enables eroded soils to carry a distinct eDNA signature. The results indicate that plant eDNA holds promise as a complementary, semi-quantitative tool alongside conventional sediment fingerprinting methods, particularly for areas where vegetation surveys or maps are available and for which capturing ecological context is relevant. However, the study also highlights important limitations. The reliability of eDNA for sediment source identification depends on the persistence of eDNA during transport, the completeness of source sampling, and the strength of sediment connectivity. Consequently, further research is required to evaluate the consistency and transferability of eDNA across varying hydrological, geomorphological, and ecological settings.

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Declarations

Competing interests The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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