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Short Communication

Environmental DNA provides information on sediment sources: A study in catchments affected by Fukushima radioactive fallout

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HIGHLIGHTS

- Excessive sedimentation has deleterious impacts on riverine environments.
- We need detailed information on the land use sources supplying such sediment.
- We test the potential of eDNA to identify the plant types found in the source areas.
- eDNA discriminated sediment collected in rivers draining forests vs. cropland.
- eDNA has significant sediment source fingerprinting potential.

GRAPHICAL ABSTRACT

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ABSTRACT

An excessive supply of sediment is observed in numerous rivers across the world where it leads to deleterious impacts. Information on the sources delivering this material to waterbodies is required to design effective management measures, and sediment tracing or fingerprinting techniques are increasingly used to quantify the amount of sediment derived from different sources. However, the current methods used to identify the land use contributions to sediment have a limited discrimination power. Here, we investigated the potential of environmental DNA (eDNA) to provide more detailed information on the plant species found in sediment source areas as a next generation fingerprint. To this end, flood sediment deposits ($n = 12$) were collected in 2017 in two catchments impacted by the Fukushima radioactive fallout along differing river sections draining forests, cropland or a mix of both land uses. Conventional fingerprints (i.e. fallout radionuclides and organic matter properties) were also measured in these samples. The conventional fingerprint model results showed that most sediment samples contained a dominant proportion of subsoil material. Nevertheless, the eDNA information effectively discriminated the three above-mentioned groups of sediment, with the dominance of tree, shrub and fern species in sediment sampled in rivers draining forests versus a majority of grass, algae and cultivated

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plant species in sediment collected in rivers draining cropland. Based on these encouraging results, future research should examine the potential of eDNA in mixed land use catchments where the contribution of topsoil to sediment dominates and where the cultivation of land has not been abandoned in order to better characterize the memory effect of eDNA in soils and sediment.

1. Introduction

Soil erosion is often exacerbated in agricultural catchments where erosion rates may be an order of magnitude greater on cropland relative to grassland and forested landscapes (Cerdan et al., 2010; Montgomery, 2007). Although particle detachment processes are generally reduced in areas with dense vegetation or an abundant litter layer, soil erosion regularly occurs on these natural landscapes (Fukuyama et al., 2010; Laceby et al., 2016b). Soil erosion rates are accelerating worldwide (Amundson et al., 2015), resulting in deleterious on-site and off-site consequences (Evrard et al., 2007).

One major off-site consequence of soil erosion are elevated suspended sediment loads that reduce fish spawning habitats (Owens et al., 2005), decrease reservoir life spans (Foucher et al., 2014) and degrade water quality (Gateuille et al., 2014). Accordingly, it is important to quantify the relative contribution of the major sources supplying suspended sediment to river networks in order to develop effective best management practices that mitigate deleterious downstream effects of accelerated soil erosion.

Sediment source fingerprinting is increasingly used (Walling, 2013) to estimate the relative source contributions of detached particles through the analysis of physical or biogeochemical properties, or 'fingerprints,' in source soils and their downstream sediments (Walling et al., 1979). A variety of fingerprinting properties can be used to estimate sediment source contributions, including fallout radionuclides (e.g. ^{137}Cs, $^{210}Pb_{xs}$, ^{7}Be) (Evrard et al., 2013), element geochemistry (Douglas et al., 2003), mineral magnetics (Walden et al., 1997), colour (Legout et al., 2013) or organic parameters (TN, TOC) and their isotopes (Gourdin et al., 2015), amongst others.

There are three fundamental approaches to sediment source fingerprinting. First, researchers can investigate the dominant erosion processes (e.g. surface or subsurface erosion) contributing material to the river network (Wallbrink et al., 1998). Second, researchers can examine temporal dynamics, to verify whether sediment transiting the river network consists of recently or previously eroded material (Evrard et al., 2016a). Third, researchers can determine the dominant spatial source areas contributing particulate material. For this latter approach, the dominant spatial areas often investigated include lithological regions (e.g. bedrock or surficial geology) (Le Gall et al., 2017), soil types (Lepage et al., 2016), and land use or land cover (Tiecher et al., 2017).

To investigate sediment derived from different land uses or land covers, researchers are increasingly tracing the vegetation signatures from sources areas with carbon and nitrogen elemental concentrations (TOC and TN) and their stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) (Gourdin et al., 2015; Huon et al., 2013; Laceby et al., 2016b). As the discrimination power of these properties is limited, particularly in areas with a mixture of C_3 and C_4 plants (Evrard et al., 2013), researchers are expanding their toolbox, for example, by tracing the fatty acids in sediments to their source soils with Compound-Specific Stable Isotope (CSSI) analysis (Gibbs, 2007). These approaches provide detailed information regarding the vegetative source signature of the sediments, including potentially different land uses (e.g., permanent pasture, woodland, cultivated land) or even agricultural practices (e.g. maize and stubble) (Blake et al., 2012). Nevertheless, the discrimination potential of these organic tracers is typically limited to the broad land uses (Bravo-Linares et al., 2018) and debate remains regarding the conservative behavior of these organic tracers during their transport across the landscape (Koiter et al., 2013; Reiffarth et al., 2016). Therefore, other fingerprints are required that provide more specific and potentially more conservative source information.

Environmental DNA (eDNA) is the DNA obtained from environmental samples such as water and sediment. The DNA of plants and animals living in soil and above the soil is transferred to the river network by erosion processes, and was shown to be preserved in deposited particulate matter (Giguet-Covex et al., 2014; Parducci et al., 2017; Pedersen et al., 2015). The preservation of eDNA is much higher in sediment – including in material dating back to the Holocene or the Pleistocene (Willerslev et al., 2003) – than in freshwater ecosystems (Dejean et al., 2011; Thomsen et al., 2012). With recent advances in High Throughput Sequencing, eDNA offers a novel approach that could provide far more detailed sediment source information than CSSI tracers. In other fields, eDNA has already provided highly detailed information regarding specific plant types and/or land management practices with the analysis of eDNA in ancient sediment in lacustrine sediment cores (Ficetola et al., 2018; Pansu et al., 2015; Sjogren et al., 2017). However, to the best of our knowledge, eDNA has never been analysed in contemporary sediments with the objective of determining the relative contribution of different sediment sources through the improved discrimination of different vegetation species.

In catchments of Northeastern Japan impacted by radioactive fallout from the Fukushima Dai-ichi Nuclear Power Plant (FDNPP) accident in March 2011 (Evrard et al., 2015), the quantification of the land use contributions to sediment transiting the rivers to the Pacific Ocean is imperative in order to prioritize remediation works. Previous fingerprinting research based on the measurement of carbon and nitrogen elemental concentrations and their stable isotope ratios showed that subsoils were the dominant sources (45 ± 26%) of particulate matter to the rivers, followed by cultivated land (38 ± 19%) and forests (17 ± 10%) including evergreen and deciduous species (Laceby et al., 2016b). However, the identification of the land use contributions was provided with limited resolution so far. Although areas cultivated with rice (*Oryza sativa* L.) were shown to supply a disproportionate amount of radiocesium contaminated sediment to the region's waterbodies (Yoshimura et al., 2016), uncertainties remain regarding the specific contribution of paddies compared to that of other cultivated fields or that of forested areas to sediment transiting these rivers.

Accordingly, the objective of this study is to explore the potential of eDNA as a next generation sediment source fingerprint. As this is, to our knowledge, the first application of eDNA in a sediment source fingerprinting context, our goal is to compare the discrimination potential of the source information provided by eDNA to that achieved with carbon and nitrogen properties (TOC, TN, $\delta^{13}C$ and $\delta^{15}N$) and fallout radionuclides (^{137}Cs, ^{134}Cs). Our main objective is to demonstrate the potential utility of eDNA as a sediment source fingerprint, through highlighting its higher source discrimination potential relative to conventional sediment fingerprinting approaches. Our secondary objective is to overview the challenges and opportunities of developing eDNA as a sediment fingerprinting parameter of the future.

2. Materials and methods

2.1. Study area

This research was conducted in the Niida (275 km^2) and Ota (77 km^2) catchments (Fig. 1), draining the main radioactive contamination plume of the Fukushima Prefecture, Northeastern Japan. This region is exposed to a very erosive climate (mean annual rainfall of ~1400 mm), with the occurrence of spring floods and typhoons being the main hydro-sedimentary events that may lead to significant erosion and sediment transfer in rivers (Chartin et al., 2017; Laceby et al., 2016a). The Niida and Ota River catchments are

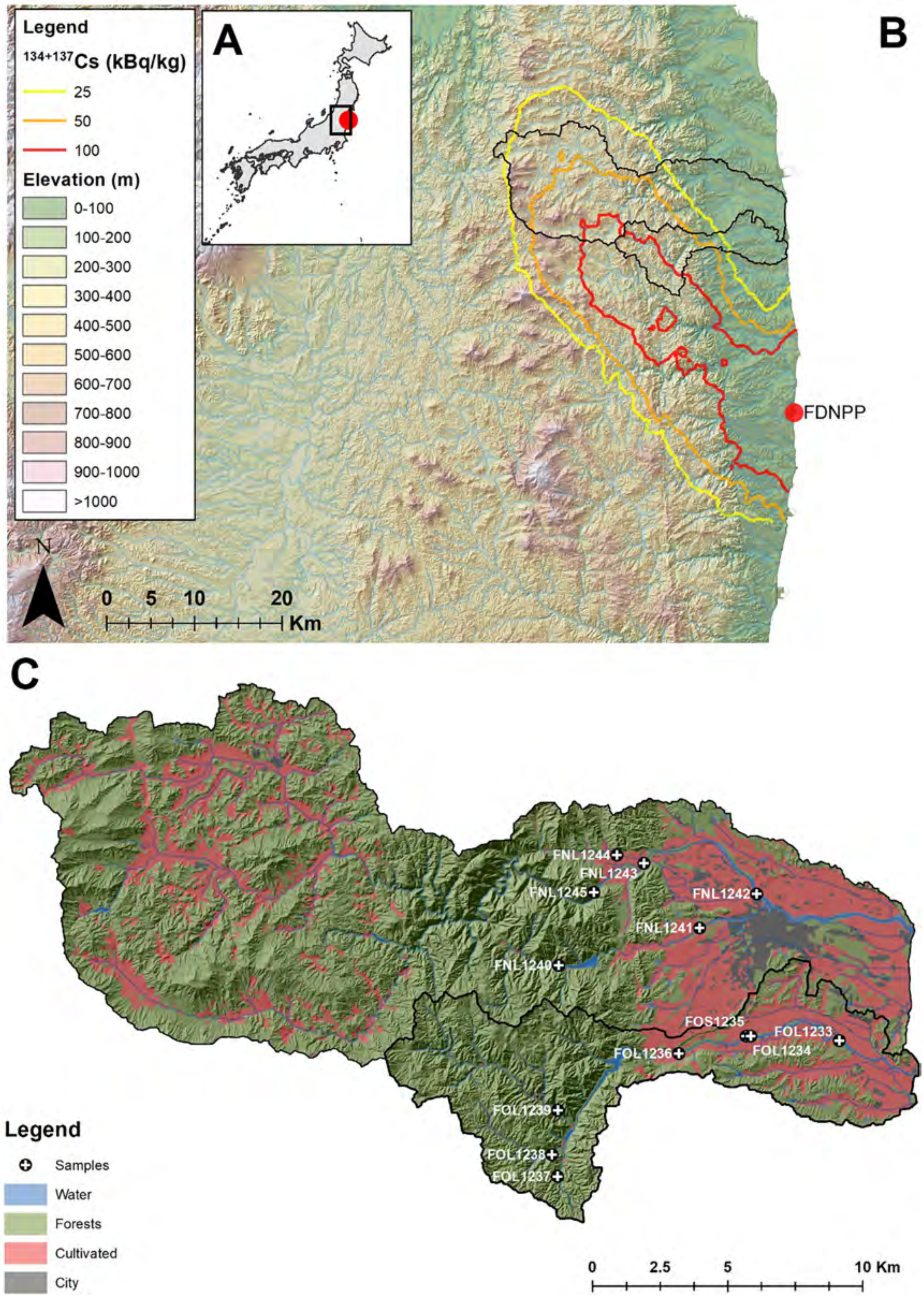

Fig. 1. A) Location of the radioactive contamination plume and the selected Niida and Ota catchments in the Fukushima Prefecture, Japan. B) The background map corresponds to the total radiocesium initial contamination of the soils (5-cm topsoil layer) decay-corrected to the 14 March 2011 (Chartin et al., 2013). C) Sampling design in the Niida and Ota catchment along with land use.

characterized by land uses that are representative of those found in the fallout impacted region, with a dominance of forests (75%) and cropland (21%).

The main catchment features include an upstream coastal mountain range (<900 m) and a broad, more densely inhabited, coastal plain (i.e. <100 m). Dams are installed at the transition between both zones on the Ota River (Yokokawa Dam) and on the main Niida River tributary (Takanokura Dam) in the coastal plain (i.e., Mizunashi River) to provide water for drinking, and irrigation to the local communities. These dams create sediment disconnectivities between upper and lower catchment areas, with dams storing sediment (Chartin et al., 2017). The Niida River mainstream is devoid of dam.

2.2. Sediment sampling

Sediment samples were collected in June 2017 at sites draining only forests ($n = 5$), a dominance of cropland ($n = 4$), or a mix of both land uses ($n = 3$; Fig. 1). These samples comprise particulate material that settled on channel banks, inset benches and floodplains during the falling limb of the last significant hydro-sedimentary event. One soil sample (0–5 cm surface layer) was also collected in a paddy field located near the Ota River for comparison (Table 1). For fallout radionuclide and carbon/nitrogen measurements, 5 subsamples (~5 g per subsample) of recently deposited material at each site were taken with a plastic spatula over a 5 m reach and composited into one sample. For the eDNA analyses, 5 subsamples (~5 g per subsample) were collected at each site with a trowel, sterilized with a blowtorch, mixed and homogenized. Approximately 15 g of material were stored in a sealed container with silica gel bags for in-situ drying.

2.3. Fallout radionuclide analysis and modelling

Prior to analyses, samples were dried at 40 °C for ~48 h, sieved to 2 mm, ground to a fine powder in an agate mortar, and pressed into 15 ml polyethylene containers for analysis. Fallout radionuclide activities were determined with gamma spectrometry using coaxial N- and P-type HPGe detectors (Canberra/Ortec). ^{137}Cs activities were measured at the 662 keV emission peak. ^{134}Cs activities were calculated as the mean of the counts found at the 605 and 796 keV emission peaks. All radionuclide activities were decay-corrected to March 14, 2011, the date of the main radionuclide fallout deposition (Kinoshita et al., 2011).

Based on the ^{137}Cs analyses, the relative contributions of contaminated (high ^{137}Cs) versus depleted (low ^{137}Cs) sources of radiocesium contaminated sediment were modelled in the Niida catchment (Table 2). The model was run with the source sample data from Evrard et al. (2016b) which included 118 samples of contaminated source soil and 42 samples of depleted source material sampled in the Niida catchment. A distribution modelling approach estimated the relative contribution of these two end-members (Laceby and Olley, 2015). The objective was to estimate whether the sediment samples in the Niida catchment were derived from upstream contaminated sources (e.g. forested reaches) versus cropland, or other areas in the coastal plains that did not receive significant fallout. Although the source dataset has been published in Evrard et al. (2016b), the sediment dataset is original and has not been published elsewhere.

Table 2
Dilution model results for the Niida catchment including the ^{137}Cs activities and modelled contribution from upstream areas that were contaminated by fallout by the FDNPP accident.

Sample ID	Basin	Sub-basin	Type	^{137}Cs Bq kg^{-1}	Error Bq kg^{-1}	Contam. source (%)	Error (%)
FNL1240	Niida	Mizunashi	Forest	7104	25	14.0%	1.2%
FNL1241	Niida	Mizunashi	Cultivated	3435	18	4.6%	1.0%
FNL1242	Niida	Niida	Mix	1893	15	1.4%	1.0%
FNL1243	Niida	Niida	Mix	977	9	0.0%	1.0%
FNL1244	Niida	Niida	Cultivated	957	9	0.0%	1.0%
FNL1245	Niida	Niida	Mix	6843	41	13.7%	1.2%

2.4. Carbon and nitrogen analysis and modelling

A subsample of the material analysed for fallout radionuclides was analysed for TOC, TN, δ^{13}C and δ^{15}N with a continuous flow Elementar® VarioPyro cube analyzer coupled to a Micromass® Isoprime Isotope Ratio Mass Spectrometer (EA-IRMS) at the Institute of Ecology and Environmental Science (iEES) in Paris. First, TOC and δ^{13}C were measured with a set of tyrosine standards (Coplen et al., 1983; Girardin and Mariotti, 1991). Second, TN and δ^{15}N were analysed with sample weights optimized after the carbon analyses. Oxygen for combustion was injected during 70s (30 ml min^{-1}) and temperatures were set at 850 °C and 1120 °C for the reduction and combustion furnaces, respectively (Agnihotri et al., 2014).

The carbon and nitrogen parameters were used to trace land use/land cover sources with a source data set from Laceby et al. (2016b). This included TOC, TN, δ^{13}C and δ^{15}N data for 46 forest, 28 cultivated and 25 subsoil source samples analysed with identical methods at iEES. A concentration dependent distribution modelling approach estimated the relative contribution of these two end-members (Laceby et al., 2015) and provide the foundation for comparing the eDNA analyses with modelled source contributions that differentiate agricultural sources from those anticipated from forests and subsoil/decontaminated sources in the Fukushima coastal region that was impacted by the FDNPP accident (Table 3). Although the source dataset is published in Laceby et al. (2016b), the sediment dataset is original and has not been published elsewhere.

Table 1
Location and characteristics of the sediment sampling sites, concentrations in fallout radionuclides (^{137}Cs, ^{134}Cs) and associated ^{134}Cs/^{137}Cs activity ratio, organic matter properties in the soil/sediment samples.

Sample ID	Basin	Sub-basin	Type	Y	X	Sampling date	^{137}Cs (Bq kg^{-1})	^{134}Cs (Bq kg^{-1})	^{134}Cs/^{137}Cs	TN (%)	TOC (%)	TOC/TN	δ^{13}C (‰)	δ^{15}N (‰)
FOL1233	Ota	Ota	Cultivated	37.60223	140.992928	28/06/2017	875	860	0.98	–	0.6	–	−26.3	–
FOS1235	Ota	n/a	Paddy soil	37.603681	140.955704	28/06/2017	1385	1360	0.98	0.26	3.2	12.2	−23.8	5.4
FOL1234	Ota	Ota	Cultivated	37.603668	140.953856	28/06/2017	3210	3265	1.02	0.14	1.62	11.6	−25.9	4.4
FOL1236	Ota	Ota	Cultivated	37.597836	140.925567	28/06/2017	6335	6260	0.99	0.09	1.22	13.6	−27.4	3.9
FOL1237	Ota	Ota	Forest	37.556682	140.874831	29/06/2017	17,380	17,080	0.98	0.05	0.75	15.0	−27.6	3.4
FOL1238	Ota	Ota	Forest	37.564072	140.872601	29/06/2017	20,530	20,560	1.00	0.06	0.91	15.2	−28.0	2.4
FOL1239	Ota	Ota	Forest	37.578823	140.874961	29/06/2017	48,730	48,855	1.00	1.10	19.29	17.5	−29.3	−0.4
FNL1240	Niida	Mizunashi	Forest	37.627235	140.875257	29/06/2017	7105	7050	0.99	0.08	1.2	15.3	−28.8	1.7
FNL1241	Niida	Mizunashi	Cultivated	37.639792	140.934335	29/06/2017	3435	3465	1.01	0.11	1.2	11.3	−28.1	4.2
FNL1242	Niida	Niida	Mix	37.651152	140.958309	29/06/2017	1895	1870	0.99	0.06	0.7	11.3	−26.1	6.3
FNL1243	Niida	Niida	Mix	37.661327	140.911026	29/06/2017	980	965	0.98	–	0.3	–	−25.1	–
FNL1244	Niida	Niida	Cultivated	37.664088	140.899675	29/06/2017	960	935	0.97	0.06	0.6	10.7	−27.0	4.5
FNL1245	Niida	Niida	Mix	37.651603	140.889993	29/06/2017	6845	6535	0.95	0.35	4.5	12.7	−26.8	3.3

Table 3
Results from the Carbon and nitrogen parameter model for the Niida and the Ota catchments including the ^{137}Cs activities and modelled contribution from upstream areas that were contaminated by fallout from the FDNPP accident. ME corresponds to the Model Error.

Sample ID	Basin	Sub-basin	Type	Forest (%)	ME (%)	Crop (%)	ME (%)	Subsoil (%)	ME (%)
FOS1235	Ota	n/a	Paddy soil	4%	1%	64%	2%	32%	3%
FOL1234	Ota	Ota	Cultivated	2%	2%	21%	3%	76%	10%
FOL1236	Ota	Ota	Cultivated	3%	2%	8%	12%	89%	20%
FOL1237	Ota	Ota	Forest	0%	1%	0%	5%	100%	15%
FOL1238	Ota	Ota	Forest	1%	3%	0%	5%	98%	17%
FOL1239	Ota	Ota	Forest	100%	1%	0%	1%	0%	1%
FNL1240	Niida	Mizunashi	Forest	6%	3%	1%	3%	93%	15%
FNL1241	Niida	Mizunashi	Cultivated	1%	2%	16%	3%	82%	16%
FNL1242	Niida	Niida	Mix	0%	1%	0%	4%	100%	9%
FNL1244	Niida	Niida	Cultivated	0%	1%	4%	2%	96%	15%
FNL1245	Niida	Niida	Mix	18%	1%	44%	3%	38%	16%

2.5. eDNA analysis and interpretation

Sediment eDNA was extracted following the protocol described in Pansu et al. (2015), targeting extra-cellular eDNA. For each sample, ~15 g of sediment was mixed with 15 ml of saturated phosphate buffer (Na_2HPO_4; 0.12 M, pH ≈ 8) for 15 min. Two ml of the mixture was centrifuged (10 min at 10,000*g*); 400 μl of resulting supernatant was kept as starting material for extraction using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany), skipping the cell lysis step and following manufacturer's instructions (Taberlet et al., 2012). The extracted DNA was eluted in 100 μl of SE buffer and used as PCR template. Seven extraction controls were also conducted. The eDNA of vascular plants was amplified with the g-h primers, which amplify a short variable region on chloroplast DNA and have very limited mismatches with sequences of vascular plants (Taberlet et al., 2018). Additionally, six PCR controls containing PCR mix and no DNA template, along with six positive PCR controls were performed (Parducci et al., 2017). Each sediment sample and control was amplified in four PCR replicates (Ficetola et al., 2015). The sequencing was conducted by 2 × 125–base pair pair-end sequencing on an Illumina HiSeq 2500 platform. The sequences of DNA were filtered with OBITools software (Boyer et al., 2016). Plant sequences were assigned with a database of the vascular plants found in Japan. Sequences with a >97% match with a plant genus were kept, providing the table of the Molecular Taxonomic Units (MOTUs) to analyse. For each sample, the sum of the reads of the four replicates was calculated. Those MOTUs associated with a total number of reads lower than 1000 were removed from further analysis. The logarithm of the reads of those sequences kept for analysis +1 was then calculated as a measure of relative abundance (Yoccoz et al., 2012). A Principal Component Analysis (PCA) was conducted in order to discriminate various groups of sediment based on the significant MOTUs detected in the samples. A hierarchical classification of the retained MOTUs was also performed and, finally, a Permutational Multivariate Analysis of Variance Using Distance Matrices (PERMANOVA) was conducted to assess the amount of variation of the eDNA explained by the sources.

3. Results and discussion

3.1. Fallout radionuclides

Sediment in lag deposit samples draining forest soils had both significantly higher and more variable ^{137}Cs activity concentrations (mean (M): 23,436 Bq kg^{-1}; SD: 17,810 Bq kg^{-1}) compared to rivers draining mixed land uses (both forest and cropland) (M: 3238 Bq kg^{-1}; SD: 3156 Bq kg^{-1}) and cropland only (M: 2962 Bq kg^{-1}; SD: 2002 Bq kg^{-1}). The ^{137}Cs activity concentration measured in the paddy field soil was low (1384 ± 12 Bq kg^{-1}), although it remained in the range of values found in sediment collected in rivers draining cultivated land. $^{134}Cs/^{137}Cs$ activity ratios for all three sediment sample types were in a narrow range (0.95–1.02) consistent with the ratio expected from fallout from the FDNPP accident (Kobayashi et al., 2017).

Sediment ^{137}Cs activity concentrations were modelled in the Niida catchment to investigate whether they were derived primarily from contaminated regions in the catchments or those that have been decontaminated and/or not impacted by the initial fallout. On average, 6% (SD 7%) of sediment in the Niida catchment was modelled to be derived from sources contaminated by the initial fallout (e.g. upper catchment areas and surface soils). The forest sample from the Mizunashi reach had the highest contribution of fallout-contaminated landscapes (14%, Model Error (ME) 1.2%). The paddy field sample farther downstream on this reach had a 5% (ME 1%) contribution from contaminated areas. In contrast, on the main stem, three samples had a <2% contribution from contaminated areas. Interestingly, the sediment sample collected the farthest downstream in the Niida catchment had an increased contaminated area contribution of again ~14%. Overall, most of the sediment sampled was derived from sources that were not contaminated with fallout from the FDNPP (e.g. subsoils, decontaminated soils, or regions near the coast that did not receive fallout).

3.2. Carbon and nitrogen composition

The TN content was too low for accurate measurement in 2 of the 13 samples analysed (FNL1243 and FOL1233). These samples also had the lowest TOC (<0.6%) of all analysed samples indicating that they are mainly comprised of coarser material, most likely provided by subsoil sources. The sediment collected in the forested catchments had lower mean $\delta^{13}C$ (M −28.4‰, SD 0.8‰) and $\delta^{15}N$ (M 1.8‰, SD 1.6‰) compared to those sampled in cultivated catchments (M −27.1‰, SD 0.9‰ and M 4.3‰, SD 0.3‰ respectively). Three of the forested catchment sediment samples, and one of the cultivated catchment sediment sample (FNL1245) had low TOC (<1.2%) and low TN (<0.08%) suggesting higher contributions of subsoil sources that are known to be low in organic matter content. The three remaining cultivated catchment sediment samples had slightly higher TOC (M 1.4%, SD 0.2%) and TN (M 0.11%, SD 0.03%). The remaining forest catchment sample (FOL1239) had very high TOC (19.3%) and TN (1.1%). The carbon and nitrogen parameters suggest that the sediment collected in forested and cultivated catchments likely have significant subsoil source material contributing to the low TOC and TN content, with the exception of slightly elevated material derived from cultivated sources, and also one sample, owing to its very high organic contents, dominated by forest material.

Sediment in three of the mainly forested catchments were modelled to be almost entirely derived from subsoil sources (M 97%, SD 3%), whereas the high TOC sample was modelled to be entirely from forested sources (100%). Importantly, there was a 0% contribution of cultivated land modelled for the forested sites, supporting that this model is providing relevant predictions as there are essentially no rice paddy fields

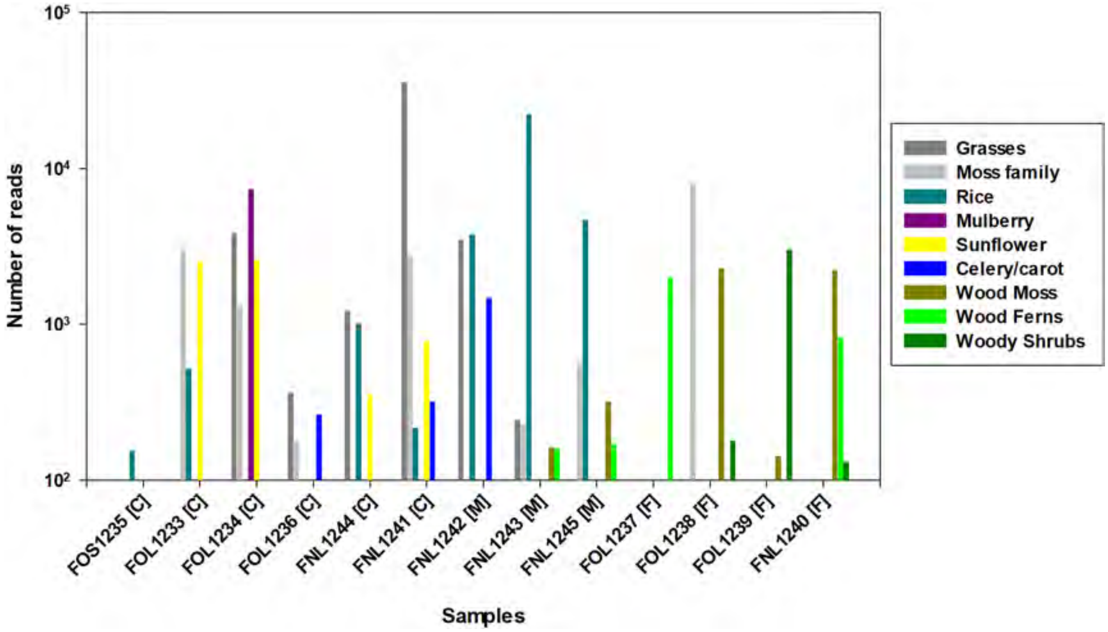

Fig. 2. Number of reads associated with a selection of plant types identified in the analysed samples collected in rivers draining cropland [C], forests [F] or a mix of cropland and forests [M].

in these forested regions. For the cultivated catchments, 86% (SD 8%) of sediment was modelled to be derived from subsoil sources, only 13% (SD 8%) from rice paddy fields or other agricultural sources, and with a mean of 2% (SD 1%) from forest sources. One of the two mixed catchment sediment samples (FNL1242) was modelled to be derived entirely from subsoil sources (100%) whereas the other (FNL1245), was modelled to be derived from 44% cultivated land, 18% forest and only 38% subsoil sources, the lowest subsoil source contribution modelled. Indeed, subsoil erosion processes dominate the supply of sediment across these catchments (M 77%, SD 33%). Furthermore, the sampling location FNL1245 behaved differently than the other samples again. The higher ^{137}Cs and contaminated source contribution in this sample relative to the others on this reach is likely due to the higher forest and cropland source contributions, which are diluted on the main stem of the Niida in downstream direction across the coastal plain.

3.3. eDNA

After filtering the data, 67 abundant MOTUs were kept for analyses (Appendix A). When examining those sequences individually (Fig. 2), woodland species including mosses, ferns and shrubs were almost exclusively found in sediment collected in rivers draining forested areas

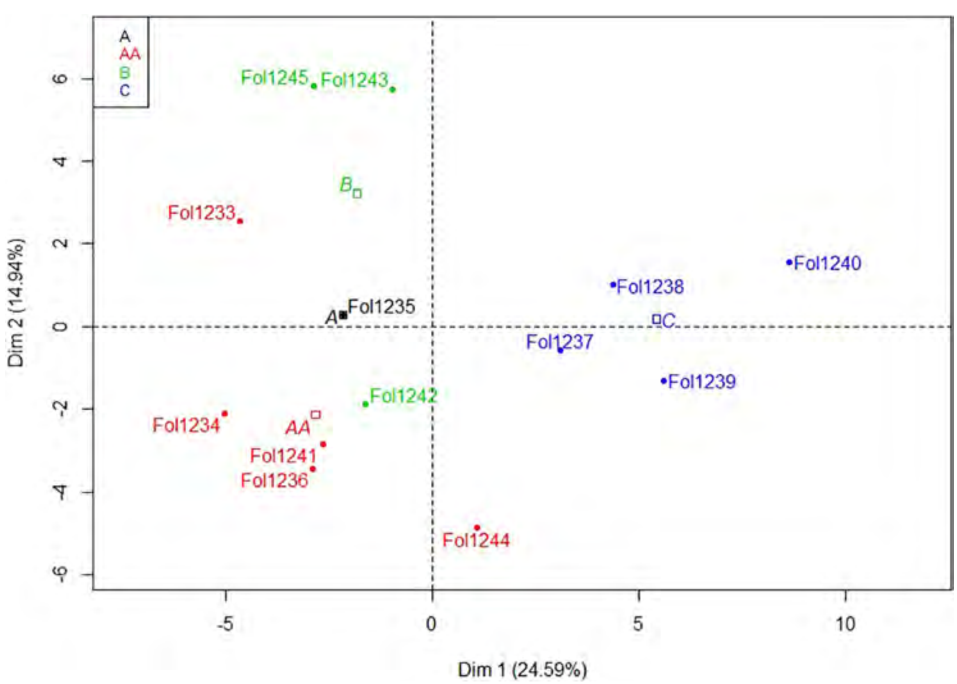

Fig. 3. Results of the Principal Component Analysis conducted to discriminate different groups of sediment samples based on the 67 significant MOTUs detected: group A – paddy field soil (in black); group AA – sediment collected in rivers draining cultivated land (in red); group B – sediment collected in rivers draining mixed land uses (in green); group C – sediment collected in rivers draining forests (in blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

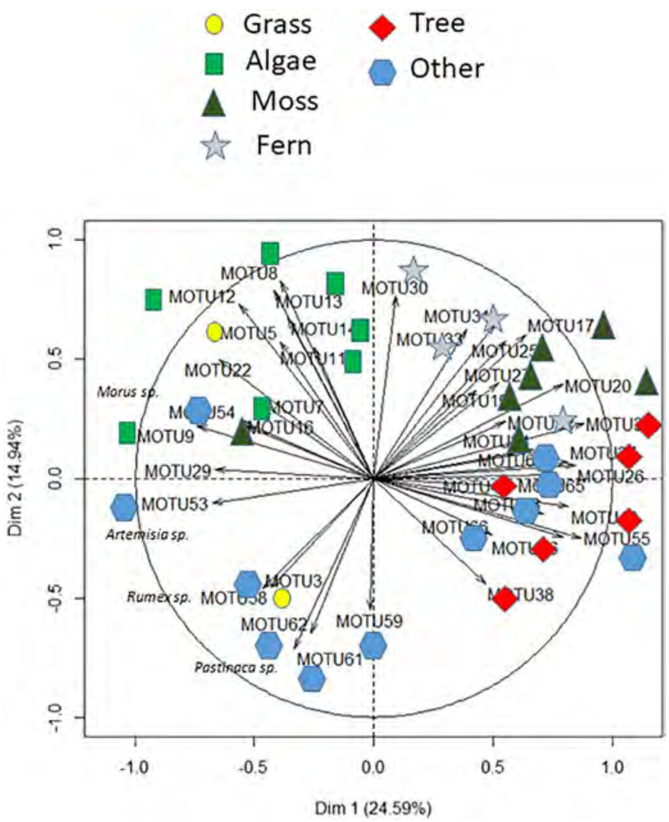

Fig. 4. Results of the hierarchical classification conducted on the 67 significant MOTUs found in the analysed soil and sediment samples. Only those variables with cos > 0.3 were shown on the graph.

or mixed land uses. In contrast, grasses and rice (*Oryza sativa* L.) dominated in the samples collected in rivers draining cropland and mixed land uses. In addition, species and/or families of cultivated plants were also found in these latter sediment samples, including Brassicaceae (e.g. cabbage), Fabaceae, Apiaceae (e.g., celery, carrot) or rice. These results demonstrate the potential of eDNA to provide a very detailed discrimination of those land use sources supplying sediment, with the identification of targeted crop types for instance.

To facilitate their analysis, the MOTUs were aggregated in 6 different groups: grasses, wetland grasses and cultivated plants (MOTUs 1 to 4); algae (MOTUs 5 to 14); mosses (MOTUs 15 to 29); ferns (MOTUs 30 to 35); trees and shrubs (MOTUs 36 to 50) and other plants including cultivated crops (MOTUs 51 to 67). The PCA (Fig. 3) using these 67 MOTUs showed a clear discrimination between samples corresponding to (i) the paddy field soil (group A) and (ii) those 3 classes of sediment identified during sampling: material collected in rivers draining cultivated land (group AA), in rivers draining mixed land uses (group B) and in watercourses draining forests (group C).

The hierarchical classification shows that the above-mentioned groups of MOTUs are well discriminated by the eDNA information (Fig. 4). Results from the PERMANOVA analysis showed that grouping explains a very large amount of variation of the eDNA composition ($R^2 = 0.47$), with a strongly significant effect ($p < 0.001$).

3.4. Combining the information provided by fallout radionuclides, organic matter composition and eDNA

For logistical reasons, the current research focused on the analysis of sediment deposits collected from different types of tributaries in catchments of the Fukushima region. The analysis of fallout radionuclides and organic matter properties of these samples, and the associated modelling showed that the majority of these samples contained significant proportions of subsoil material. Various sources may supply large quantities of subsoil material to the rivers in this region (Fig. 5). These

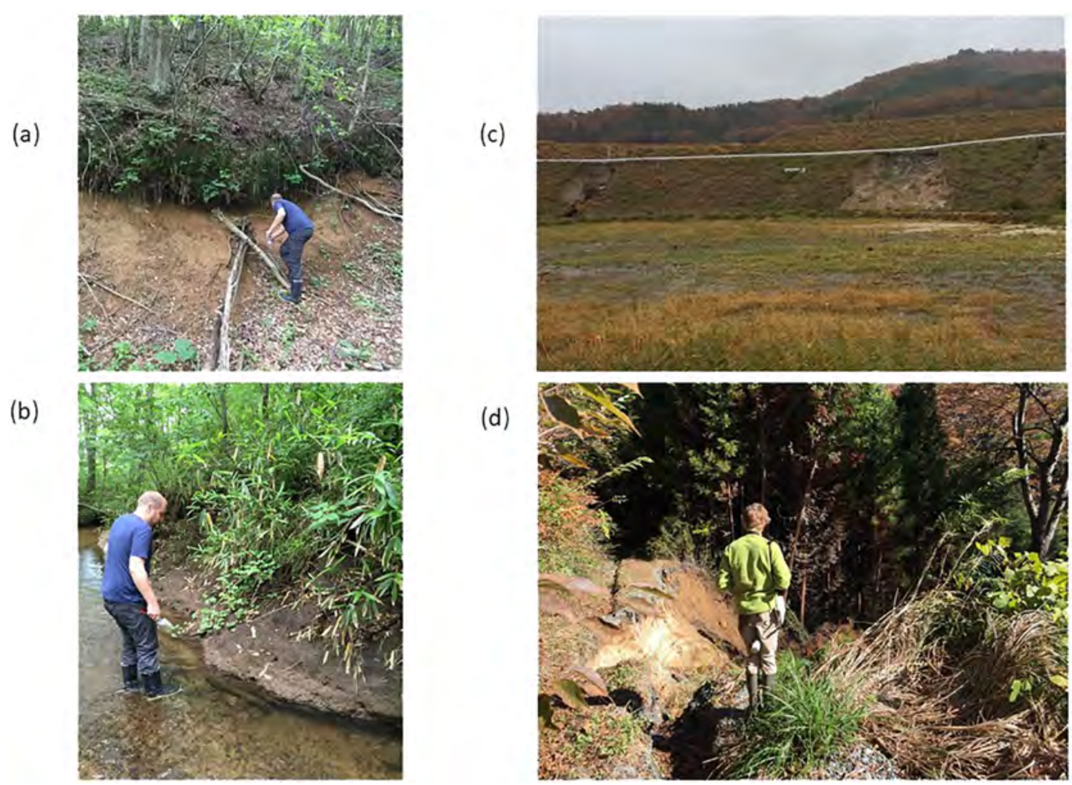

Fig. 5. Example of sources delivering subsoil material to the rivers in the study site: (a) subsoil erosion in forests; (b) exposed channel bank in an un-channeled river section; (c) shallow landsliding on a roadside nearby a paddy field; (d) deeper landslide on a forested hillslope.

include the mobilization of deep soil horizons under forests as a result of landsliding, gullying or road-side erosion (Mizugaki et al., 2008). Channel bank erosion may also provide a significant quantity of material to the rivers, in un-channeled reaches (Fig. 5b). Accordingly, soil particles are not only transferred by overland flow, they are also generated by erosion of deeper soil horizons. Nevertheless, this deeper soil material is still tagged with eDNA. A significant number of MOTUs (67) were identified in these samples containing high proportions of subsoil and the analysis of this eDNA provided the discrimination of the different sediment sample groups identified during fieldwork. As a result, a dominance of tree, fern and moss species were found in sediment collected in rivers draining forests, whereas a majority of grass and cultivated species were detected in sediment collected in watercourses draining cropland.

3.5. Implications for future research and concluding remarks

Although the Fukushima case study was expected to be a relatively straightforward approach for discriminating land use-derived sediment sources in different rivers draining either forested areas or cropland, the situation was more complex owing to the large proportion of subsoil material transiting these river systems (Fig. 5). This is also likely due to the fact that our sampling approach targeted sediment deposited in the river channel after flooding events, rather than suspended matter transiting rivers. However, this approach was guided by the precautions required to avoid the biological contamination of the samples during their collection in the field and the necessity to sterilize tools to collect the lag deposit samples rather than filter large volumes of river water containing lower quantities of suspended matter.

Furthermore, cultivated land was abandoned by farmers after the nuclear accident in March 2011. During the sampling campaign conducted in 2017, agricultural activities had not restarted in the study area. This situation likely explains the dominance of subsoil material transiting these rivers as a dense vegetation cover rapidly covered the abandoned fields and protected them against erosion, thereby limiting the contribution from the erosion of cultivated topsoils. Furthermore, this situation outlines the need to further investigate the memory effect of eDNA of cultivated plants in soils after 6 years of abandonment, although the presence of plants cultivated in the region before the nuclear accident (e.g. cabbage, rice and mulberry) was demonstrated in the current research.

Based on these preliminary, encouraging results, future applications should be preferentially conducted in mixed land use catchments where the contribution of surface material to sediment transiting rivers is dominant and where agricultural activities have not been abandoned for several years. The systematic collection of both soil and sediment samples should be conducted in order to examine the memory effect of eDNA in soils and the potential changes in signatures from the soil to the sediment, including samples collected in rivers and in deposition areas (e.g. ponds or lakes).

Although the design of un-mixing models to quantify sediment source contributions to sediment is the ultimate objective of this research, multiple important phases of research are required before we can model eDNA effectively. This has been recently demonstrated by many reviews in the sediment tracing literature (Koiter et al., 2013; Laceby et al., 2017; Owens et al., 2016). For example, the conservative behavior of eDNA must be thoroughly researched to evaluate its persistence on the particles during mobilization, transport and deposition processes. Indeed, prior to modelling, we need to understand if eDNA properties are maintained during these processes, or vary in a predictable way. Furthermore, we need to investigate whether there is an affinity for eDNA to be bound to different particle size fractions or even different soil and or mineral types. If eDNA indeed behaves conservatively during sediment generation and deposition processes, without significant particle size impacts, researchers will have to then investigate what is the most appropriate approach to using eDNA in end member mixing models to quantitatively apportion source contributions to material transiting river networks. The analysis of artificial mixtures – in which known proportions of the different sources are combined in the laboratory – will likely be necessary to develop the best approach to incorporate eDNA into end member mixing models. We believe that the quantitative use of eDNA in sediment source fingerprinting models will be extremely beneficial for managing the degradation of our riverine, lacustrine and potentially estuarine environments through providing a more detailed source information than many of the currently employed fingerprints.

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