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#### Human-induced river runoff overlapping natural climate variability over the last 150 years: Palynological evidence (Bay of Brest, NW France)

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#### Abstract :

For the first time a very high resolution palynological study (mean resolution of 1 to 5 years) was carried out over the last 150 years in a French estuarine environment (Bay of Brest: NW France), allowing direct comparison between the evolution of landscapes, surface water, and human practices on Bay of Brest watersheds, through continental (especially pollen grains) and marine (phytoplanktonic microalgae: cysts of dinoflagellates or dinocysts) microfossils. Thanks to the small size of the watersheds and the close proximity of the depositional environment to the mainland, the Bay of Brest represents an ideal case study for palynological investigations. Palynological data were then compared to published palaeo-genetic analyses conducted on the same core and to various available instrumental data, allowing us to better characterize past environmental variability since the second half of the 19th century in Western Brittany. We provide evidence of some clues of recent eutrophication and/or pollution that affected phytoplankton communities and which appears linked with increased runoff (higher precipitations, higher percentages of riparian forest pollen, decline of salt marsh-type indicators, and higher values of the XRF Ti/Ca signal), mainly explained by the evolution of agricultural practices since 1945 superimposed on the warming climate trend. We assume that the significant relay observed between dinocyst taxa: Lingulodinium machaerophorum and Spiniferites bentorii around 1965 then followed by Spiniferites membranaceus after 1985, attests to a strong and recent eutrophication of Bay of Brest surface waters induced by high river runoff combined with abnormally elevated air temperatures, especially obvious in the data from 1990. The structure of the dinocyst community has thus been deeply altered, accompanied by an unprecedented increase of Alexandrium minutum toxic form at the same period, as confirmed by the genetic quantification. Despite this recent major anthropogenic forcing, the fossil pollen sequence also records natural climate variability. We highlight, for the first time, a possible connection between climate (AMO modes) and fossil pollen records (especially tree pollination rates) in coastal sediments using tree percentage fluctuations as an indirect

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#### Highlights

▶ High resolution palynological study (last 150 yrs) in a French estuary ▶ Increased runoff on mainland since 1945 ▶ Increased eutrophication indices since 1980 ▶ Changes in phytoplankton communities and increased toxic algal blooms ▶ Connection between AMO modes and fossil pollen records (tree pollination rates)

Keywords : Pollen grains, Dinoflagellate cysts, Estuarine dynamics, River runoff, Climate variability

#### Introduction

During the past few decades, coastal environments have been increasingly studied worldwide due to natural fluctuations (e.g. climate variability and geomorphological changes) and anthropogenic pressures (e.g. eutrophication, soil artificialisation, CO<sub>2</sub> rejects) in order to better evaluate human forcing and future climate trends (e.g. IPCC, 2014). Furthermore, the effects of soil fertilization on the composition and structure of microbial coastal communities is one of the major topics of present-day environmental sciences, taking into account that changes in pH, moisture, and nutrient rates are assumed to greatly affect biodiversity over many trophic levels (Guillaud and Bouriel, 2007; Diaz and Rosenberg, 2008).

North-western European coastal environments are interesting case studies because of their connection to the main Atlantic atmospheric (e.g. North Atlantic Oscillation, NAO; and East Atlantic pattern, EA) and oceanic (e.g. Atlantic Multidecadal Oscillation, AMO; and Atlantic Meridional Overturning Circulation, AMOC) patterns that govern regional and global climates over different timescales. In Brittany (NW France), weather regimes and North Atlantic Sea Surface Temperatures (SST), are the main mechanisms that drive precipitation patterns and related river discharges (Tréguer et al., 2014; Ruprich-Robert and Cassou, 2015), as well as storm surges (van Vliet-Lanoë et al., 2014). As a major consequence, this region is today characterized by intense soil weathering, leading to intensive nutrient discharges in the coastal waters (Meybeck et al., 2006; Tréguer and De La Rocha, 2013).

In this context, palynological studies can provide essential information about past environmental conditions through combined analyses of phytoplanktonic microalgae (especially dinoflagellate cysts or dinocysts) and pollen grains (Turon, 1984; Sanchez-Goñi et al., 2000; Turon et al., 2003; Mudie et al., 2007; García-Moreiras et al., 2015). However,

these studies, that are numerous for the Holocene, rarely cover the last century for which environmental changes can also be approached by instrumental data allowing comparison with palynological proxies.

Concerning pollen analysis, a well-known bias exists (i.e. over or under-representation) regarding the continental surface covered by vegetation and fossil pollen percentages calculated from modern sediment archives (e.g. Gaillard et al., 1998; Mazier et al., 2015; Ganne et al., 2016), as recently discussed for Bay of Brest watersheds versus modern sediments retrieved within the bay (Lambert et al., 2017). Furthermore, some studies highlight a large influence of climate-driven processes on the pollination rates of some trees (van Vliet et al., 2002; García-Mozo et al., 2006; Besancenot and Thibaudon, 2012), as well as on the transport of pollen grains through temporal variability of precipitation and thus river runoffs. These fluvial discharge variations in water volume and nutrient load, according to natural and/or anthropogenic factors, may also be responsible for the recrudescence of toxic algal blooms over the past decades (Klouch et al., 2016 a,b). Dinocyst analysis then represents an indirect approach to the reconstruction of the palaeobiodiversity of dinoflagellate communities and thus of one of the major components of primary productivity known to be very sensitive to environmental changes. Both signals (marine for dinocysts and continental for pollen grains) are often studied together in palaeoenvironmental reconstructions (Combourieu-Nebout et al. 1998; Turon et al., 2003; Sangiorgi and Donders, 2004), constituting a land-sea continuum involving a large number of natural (oceanic and atmospheric) and human forcings. The comparison between the palynological data carried out along the last century and the instrumental dataset collected in the same area will allow us to thus precisely identify the different factors driving pollen and dinocyst variations, and to

better interpret these palynological tools for future Holocene palaeoecological reconstructions.

In this study, a core from the outlet of the Daoulas Estuary in the Bay of Brest has provided a very high-resolution palynological record from 1870 AD to present. The Daoulas Bay records today the most intense toxic bloom of dinoflagellates *Alexandrium minutum* within the Bay of Brest (Klouch et al. 2016a). Furthermore, the palaeobiodiversity of dinoflagellate communities has been recently reported on the same sampled Daoulas core with molecular data (Klouch et al., 2016b), suggesting a great increase in bloom frequency of this toxic species *A. minutum* over the past decades. We then expect to find clues of recent eutrophication (i.e. nutrient enrichment of the aquatic system; Andersen et al., 2006) in the dinocyst assemblages study.

Main objectives of this study, connected to societal impacts for coastal ecosystems and North Atlantic climate knowledge, consist in:

- i) comparing palynological proxies and instrumental data available at a North Atlantic (SST, AMO modes), regional (atmospheric NAO/EA modes), and local scales (river flows, atmospheric temperatures),
- ii) deciphering natural climate variability from recent huge human impacts on Bay of Brest watersheds by cross-correlating the whole set of data including pollen, dinocysts, but also all other non-pollen palynomorphs such as freshwater microalgae.

#### 1. Bay of Brest environmental settings

#### 1.1. Geomorphological and geographical contexts

The Bay of Brest is located in north-western Brittany (NW France, W Europe; Fig. 1a,b) and is a shallow semi-enclosed basin of 180 km<sup>2</sup> surrounded by a 230 km long coastline. Its bedrock consists of Proterozoic igneous rocks in the north and Brioverian (Neoproterozoic, possibly Early Cambrian) to Palaeozoic (Ordovician, Devonian and locally Carboniferous) sediments in the south and east. Present-day low reliefs (few hills reach 330m high) are inherited from the peneplanation of the high Hercynian chain (Chauris and Plusquellec, 1980; Ballèvre et al., 2009). The study area has subsided since the Eocene and continues to slightly subside (0.02 to 0.04 mm/yr.) (Ziegler, 1992; Bonnet et al., 2000; Goslin, 2014), a value which can be considered as negligible at the timescale of the Holocene (40 mm/10,000 yrs.; Goslin, 2014). The river system has been established since the Tertiary (Hallegouet et al., 1994).

During the Quaternary, the Bay of Brest was submitted to alternations of glacial lowstands, river valleys being subsequently deepened by erosive processes, and interglacial highstands characterized by sediment infilling; the last transgressive episode thus corresponding to the Holocene (Gregoire et al., 2017). Bathymetry highlights submarine channels in the Bay of Brest that attest to palaeo-fluvial systems (Fig. 1a; Gregoire et al., 2016). Palaeo-channels of the two main current rivers, the Aulne and Elorn, are about 30 and 15 m deep respectively, and converge in the west at a trough of about 50 m deep (Troadec et al., 1997). Nevertheless, at present, the depth of the Bay of Brest does not exceed 10 m deep for approximately 60% of its surface (Monbet and Bassoulet, 1989).

Today, the Bay of Brest is characterized by a macrotidal influence with a maximum tidal amplitude of 8m (Troadec et al., 1997). Granulometry of modern sediments reflects specific hydrodynamic conditions mainly related to tidal currents (Gregoire et al., 2016). In its westernmost part, the Bay of Brest is connected to the Atlantic Ocean through the "Goulet", a strait of about 1.8 km large and 50 m deep. Marine waters flow into the Bay of Brest at each new tidal cycle with a current speed reaching 1 m/s, mainly oriented S-E. In the stream direction, sedimentation is mainly composed of sands and gravels (Gregoire et al., 2016). On either side of this axis, the current speed is strongly reduced and grain-size is characterized by fine to very fine sediments (silts and clays) in small bays (Troadec et al., 1997; Gregoire et al., 2016). The lack of interbedded sands and high energetic sedimentary structures in these bays argues for *in situ* deposits, an assumption reinforced by <sup>210</sup>Pb profiles carried out on modern sediments (Gregoire et al., 2016, 2017; Klouch et al., 2016b). In its easternmost part, the Bay of Brest receives its main freshwater supplies from the Aulne, the Elorn, and from the smaller Daoulas River (Fig. 1a). Bay of Brest watersheds (Fig. 2a) are characterized by 2,000 km of waterways and most of their runoff flows into the Bay of Brest through the Aulne River (114 km long; 1,224 km<sup>2</sup> of watershed and 20.40 m<sup>3</sup>/s. of annual debit; Troadec et al., 1997). The Aulne and Elorn River's outflows contribute to up to 85% of the total river discharges in the Bay of Brest (Delmas and Treguer, 1983). The salinity of the Bay of Brest is 34.5% and shows virtually no evolutionary trend over the last 20 years (Tréguer et al., 2014).

#### 1.2. Environmental settings of Bay of Brest watersheds

The department of Finistere is one of the leading agricultural regions of France, with a cultivated and/or grazed surface of about 60% (Quéré et al., 2008). At the scale of Bay of

Brest watersheds, continental surfaces are mostly occupied by farmlands, with crops and vegetables, and are often bordered by hedgerows (Corine Land Cover, i.e. biophysical land cover database from the European Environment Agency; Aune-Lundberg and Strand, 2010; Lambert et al., 2017). The department is indeed characterized by the highest expanse of hedgerow meadows recorded in Brittany (around 60,000 km). Agricultural areas account for more than 75.4% (temporary and permanent grasslands account for 30.5% and crops for 44.5%), forests and woodlands (including *Quercus, Corylus, Fagus, Pinus, ...*) for 11.6%, moors and grasslands (including Poaceae, Ericaceae, *Ulex, Cytisus*, Brassicaceae, Asteraceae, Ranunculaceae, Rosaceae, ...) for 6.1%, and coastal areas (cliffs with aerohalin grasslands, moors, and salt marshes: Chenopodiaceae, *Plantago* spp., Brassicaceae, *Ulex, and* Ericaceae, ...) for only 0.7% of Bay of Brest watersheds.

In the 19<sup>th</sup> century, the rural landscape was essentially composed of cultivated parcels with relatively low vegetation and few trees. From the second half of the century, between 1850 and 1950, the network of hedgerow meadows grew considerably and saw the breaking up of agricultural land into many small cultivated plots delimited by slopes and hedges composed of bushes and shrubs. This new landscape mosaic dramatically wins over the heathlands area. Since the second half of the 20<sup>th</sup> century, the department of Finistere has increasingly recorded intensive agriculture practices and a mechanization of farming techniques; the department being placed today among the most mechanized in France (Flatrès, 1963). Recent studies showed that agriculture and particularly animal husbandries (intensive livestock of pig, poultry, and cattle farms, since the end of the Second World War ; Flatrès, 1963) are the main contributors (between 60 and 80%) to phosphorus flows (phosphates) and high nitrate concentrations (often largely exceeding the national standard of 50 mg/l) through soil leaching into Brittany rivers (Lemercier, 2003; Huet et al., 2005; Aurousseau et al., 2009).

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Despite the decreasing number of farms over the 20<sup>th</sup> century, total cultivated areas increased until the 1990s. More specifically, in the 1960-70s, the department held the French record of the use of fertilizers, especially of nitrogen and phosphoric acids (Flatrès, 1963). Then, between the 1960s and the 1980s, a new landscape, more open as compared to the previous high fragmentation of land ownerships, emerged in the countryside due to the grouping of agricultural lands. As a consequence, the sharp decline in the number of embankments, as well as the uprooting of trees that constituted hedgerow meadows, were responsible for active soil erosion and increasing runoff (Flatrès, 1979). The disappearance of hedgerow meadows, as well as fertilizer concentrations recorded in Bay of Brest catchment waters, then subsequently slowed down from the 1990s, after a peak of nitrate concentrations recorded in the early 1990s. Since 1996, nitrate concentrations in groundwater catchments decreased by 35% on average and by 26% in surface water catchments (www.bretagne.synagri.com). Between 1989 and 2014, agricultural areas decreased by 21,000 ha because of an increasing artificialisation of soils for housing, infrastructures, and economical activities. The level of pollution however still remains high in rivers; nitrogen fertilizers being still widely used in the region.

#### 1.3. Climatological context

#### **General overview**

The Bay of Brest and its watersheds are subjected to an oceanic climate characterized by mild temperatures (slight seasonal variations on the coastline and small daily temperature differences, with an average of 15°C in summer and around 7.5 °C in winter (Fig. 1c), and high regular rainfalls (with an average of 200 rainy days/yr. in Western Brittany and 800 to

1,000 mm/yr. measured in the Bay of Brest; Troadec et al., 1997 (Fig. 1c). Wind conditions, usually during autumn and winter, are mainly blowing from the southwest (Fig. 1d). Minor northeast and northwest wind components can also appear in the spring and summer (Troadec et al., 1997). Wind speeds in Brittany can exceed 100 km/h during 5 to 15 days/yr., generating major storms and related erosion/submersion impacts depending on the coastal exposure (van Vliet-Lanoë et al., 2014).

The climate in the Bay of Brest is due to the combined influences of atmospheric (i.e. North Atlantic Oscillation, NAO; East Atlantic pattern, EA) and oceanic (Atlantic Multidecadal Oscillation, AMO; Atlantic Meridional Overturning circulation, AMOC) configurations, with the superimposition of anthropogenic effects (e.g. eutrophication, soil artificialisation; Tréguer et al., 2014; Ruprich-Robert and Cassou, 2015). It is however unclear how these mechanisms interact with each other, one influencing the other in various and complex feedbacks (Tréguer et al., 2014; Ruprich-Robert and Cassou, 2015). In Brittany, the above-mentioned weather regimes, combined with Atlantic SST, mainly explain precipitation patterns and in turn river discharges (Tréguer et al., 2014), as well as storm surges (van Vliet-Lanoë et al., 2014).

#### North Atlantic climatic oscillations

Through atmospheric connections and air/ocean exchanges, SST variations in the North Atlantic Ocean (i.e. varying AMO modes with cycles of around 70 years) seem to control a large part of the multidecadal climate variability over the continents bordering the North Atlantic area (Knight et al., 2006; McCarthy et al., 2015; Ruprich-Robert and Cassou, 2015). Furthermore, the North Atlantic circulation (AMOC strength) appears to be a main precursor for SST variations (Msadek and Frankignoul, 2009) and then also for AMO oscillations.

Stronger AMOC will result in stronger heat transport from the tropics to northern latitudes of the North Atlantic Ocean (i.e. higher North Atlantic SST), thus implying positive modes of the AMO (McCarthy et al., 2015; Ruprich-Robert and Cassou, 2015).

Also, NAO modes must be considered on smaller time scales (i.e. pseudo-cycles of around 20 years; Hurrell, 1995). The NAO is the major natural atmospheric variability in the North Atlantic region, defined as differences of winter atmospheric pressures at sea level between the Iceland Low and the Azores High. Negative NAO modes are characterized by weakened mid-latitude westerlies as well as colder and drier winters above Brittany with less storminess, the opposite occurring during positive NAO modes (Cayan, 1992; Hurrell, 1995). The EA pattern is the second prominent mode of low-frequency atmospheric variability over the North Atlantic region, whatever the month considered, with a subtropical relationship. The EA is structurally analogous to the NAO mechanism and consists of a north-south dipole of anomaly centres spanning the North Atlantic Ocean from east to west (Barnston and Livezey, 1987; Ruprich-Robert and Cassou, 2015). Both phenomena (NAO and EA) may not be directly correlated and the dominant forcing acting on the study area is not yet understood. However, it is known that positive (/negative) modes of NAO and East Atlantic patterns are associated with above (/below)-average precipitations over north-western Europe (Tréguer et al., 2014).

#### 2. Material and methods

#### 2.1. Study core: sedimentological characteristics and age model

Small bays of the Bay of Brest are characterized by low hydrodynamics and mean current sedimentation rates of around 0.15 cm/yr. (Gregoire et al., 2016). In this study, core I\_09 was collected in the Daoulas estuary (48°20'46.6''N; 4°17'41.208''W; 3 m depth; 58 cm long), using a manual PVC corer of 60 cm in length, during the SERABEQ I cruise (2014, Albert-Lucas vessel, IFREMER). Core I\_09, mainly characterized by clayey sediments (Fig. 2), was sampled immediately after coring in order to minimize oxidation for genetic analysis (detection of ancient DNA in sediments; Klouch et al., 2016b). The final age model (cf. Klouch et al., 2016b for the detailed methodology) was based on  $^{210}$ Pb<sub>xs</sub> (T<sub>1/2</sub> = 22.3 years) and  $^{137}$ Cs depth profiles (Schmidt and De Deckker, 2015). The core ranges from 1870 (+/-6.5) AD to 2013 (+/- 1.3) AD, with a mean sediment accumulation rate of about 0.4 cm/yr. (Klouch et al., 2016b). Sampling, each centimeter from 0 to 17 cm, then every 2 centimeters downwards, provides a study resolution of 1 to 5 years.

#### 2.2. Palynological study

#### Laboratory procedure

For palynological treatments, we sampled an aliquot (3-4 cc) of sediment among 40 residues kept refrigerated after DNA analyses (Klouch et al., 2016b). Samples were treated at the EPOC laboratory (University of Bordeaux, Pessac, France) following a standard protocol described by de Vernal et al. (1999) that includes cold 10-25-50% HCl and cold 45-70% HF to remove, respectively, carbonate and siliceous fractions (*http://www.epoc.u-*

*bordeaux.fr/index.php?lang=fr&page=eq\_paleo\_pollens*). Then, after sieving at 10μm, all final residues were mounted between slides and coverslips with glycerin. Palynomorph concentrations were calculated with the marker grain method consisting of adding one tablet containing 20,848 Lycopodium spores before palynological treatments; these exotic spores being then counted in parallel with studied palynomorphs (Stockmarr, 1971; de Vernal et al., 1999; Mertens et al., 2009).

#### **Palynological identification**

For each analyzed sample, a minimum of 300 pollen grains and 150 dinocysts have been systematically counted, using a Leica DM 2500 at magnifications of X630 and X1000, in order to provide robust assemblages from a statistical point of view (Fatela and Taborda, 2002). Taxa identification follows Beug (1961), Faegri and Iversen (1989), Moore et al., (1991), Reille (1995), for pollen grains and Rochon et al. (1999) for dinocysts.

Pollen and dinocyst percentages were calculated independently for each taxonomical group, on a main sum excluding non-identified taxa. Other Non-Pollen Palynomorphs (NPP) were only expressed in absolute concentrations (number of palynomorphs/cm<sup>3</sup> of dry sediments). The "continental" *versus* "marine" (C/M) ratio, that includes selected continental and freshwater (*Halodinium, Pediastrum, Concentricystes*, amoebae) *versus* selected marine (foraminiferal linings, *Radiosperma corbiferum*) NPP microrests, has been used in this study to discuss continental versus marine influences. Finally, we calculated the number of taxa per sample (S) using the "Past version 1.75b" software (Hammer et al., 2001), allowing us to discuss the pollen and dinocyst diversity through time.

#### 2.3. Foraminiferal analysis

In this study, a minimum of 300 benthic foraminiferal counts were performed on the fraction  $>150 \mu m$  (Leica M60 binocular, X60 magnification) so as to express in percentages the number of specimens exhibiting morphological anomalies (atrophied, hypertrophied or overlapped chambers). Indeed, shell deformations are known to represent excellent tracers of water pollution (Alve, 1995; Polovodova and Schönfeld, 2008; Martins, 2011).

#### 3. Palynological results from I\_09 Daoulas core

#### 3.1. Palynomorph diversity and concentrations

Considering pollen assemblages, 51 different taxa have been identified (Appendix 1) with an average diversity of 26 taxa per slide (Fig. 3). In parallel, 18 different dinocyst taxa have been identified (Appendix 1) with an average diversity of 7 taxa per slide. Finally, 19 other NPP have been determined and include different types of spores, freshwater and marine microalgae, amoebae, and copepod eggs (Fig. 3; Appendix 1).

From the base of the core to 35 cm, palynomorph concentrations are rather low (i.e. 4,263 spores/cm<sup>3</sup>; 7,285 cysts/cm<sup>3</sup>; 31,344 pollen/cm<sup>3</sup>), always below the mean value of each dataset (Fig. 3). Then, at 35 cm, significant increases in pollen, dinocyst, and spore concentrations are noticeable with values 3, 5, and 4 times higher, respectively. Also, this limit underlines a re-increase (after the slight occurrence noted between 53 and 47 cm) of freshwater microalgae (especially Concentricystes and Halodinium) and other continental markers (cf. C/M ratio; Fig. 3), while H. problematica (estuarine algae) and amoebae concentrations are characterized by a drastic drop. This leads us to consider this limit as a major transition identified as limit A in Fig. 3. From limit A, all palynomorph concentrations are characterized by strong amplitudes between high values (i.e. between 10,000 and 20,000 spores/cm<sup>3</sup>; 30,000 and 50,000 cysts/cm<sup>3</sup>; 80,000 and 100,000 pollen/cm<sup>3</sup>) and lower ones, typical of those recorded before 35 cm. However, a second major transition can be identified at 17 cm, referred to as limit B in Fig. 3. This limit evidences the highest freshwater fossil concentrations, up to 2,000 algae/cm<sup>3</sup>, and thus the highest C/M ratio recorded throughout the core. Amoebae concentrations sharply increase after 13 cm (up to 1,000 amoebae/cm<sup>3</sup>) while *R. corbiferum* concentrations strongly drop, reaching below average values for the entire core

(i.e. 111 algae/cm<sup>3</sup>). The same trend is observed for foraminiferal linings. Regarding the diversity of pollen (between 18 and 33 different taxa) and dinocyst (between 2 and 12 different taxa) assemblages (Fig. 3), an increase of pollen (-dinocyst) diversity is especially observed from limit B onwards (-limit A).

#### 3.2. Pollen assemblages

Major taxa (those exceeding a threshold of 2% among the whole assemblage) are depicted in Fig. 4. Among trees, deciduous *Quercus* (mean value of 20% for the whole core), *Corylus* (mean value of 10%), *Alnus* (mean value of 12%), and *Pinus* (mean value of 10%) are the most represented, while herbaceous taxa are dominated by Poaceae (mean value of 16%), and to a lesser extent by Chenopodiaceae (mean value of 6%) and *Cerealia*-type (mean value of 4%). *Cerealia*-type pollen grains were detected according to the size criterion given by Joly et al. (2007) (i.e.  $47\mu$ m for the grain diameter and  $11\mu$ m for the annulus). These pollen percentage averages are generally similar to mean values calculated from Bay of Brest modern sediments (Lambert et al., 2017) in the Daoulas Bay (identified with red dots in Fig. 4). Also, for *Alnus*, Chenopodiaceae, and Poaceae, top values from the I\_09 core are obviously similar to mean modern values reconstructed in the area (Lambert et al., 2017).

Throughout the sequence, *Alnus* displays an obvious increasing trend, from around 5% until 27 cm to systematically above 10% after 27 cm, with peaks reaching 20-25% especially after limit B. A similar trend is observed for *Betula* at 27 cm (1.5 to 3%). This leads us to consider a third major transition in between the two limits described above, and referred to as limit C in Fig. 4. It is also worth noting the slight increasing percentages of *Fraxinus*, *Salix*, and *Hedera* after limit B (17cm), a period also characterized by the persistence of the highest

percentages of *Alnus* ever recorded in the core (i.e. systematically above 12%). Finally, *Quercus* does not present a linear homogeneous trend but rather exhibits several fluctuations of an amplitude of 10-15% around a mean value throughout the record. Among herbaceous taxa, Poaceae are clearly anti-correlated with trees (i.e. especially *Quercus*) and similarly marked by several fluctuations, ranging between 10 and 20%. Lower-average values are noted after limit B, while Chenopodiaceae and *Plantago maritima* are characterized by decreasing trends, especially obvious after limit A. Percentages of other herbaceous taxa are rather uniform all along the core even if characterized by weak amplitudes.

#### 3.3. Dinocyst assemblages

Dinocyst assemblages (Fig. 4) are largely dominated by *Spiniferites bentorii* (mean value of 32% for the whole core), *Spiniferites belerius* (mean value of 25%), and *Lingulodinium machaerophorum* (mean value of 21%). *Spiniferites membranaceus*, *Spiniferites ramosus*, cysts of *Pentapharsodinium dalei*, *Operculodinium centrocarpum*, and *Selenopemphix quanta* are characterized by percentages up to 20%, and minor species are composed of *Spiniferites delicatus*, *Spiniferites lazus*, and *Spiniferites mirabilis* with combined percentages lower than 10%. Previous studies conducted on current Brittany coastal sediments have documented a dominance of *S. bentorii*, *S. ramosus*, *O. centrocarpum*, and *L. machaerophorum* in dinocyst assemblages, with a particular prevalence of the latter species (over 80%) in estuarine domains and inland waterways (Morzadec-Kerfourn, 1976, 1979).

*L. machaerophorum* is characterized by above-average values (20 to 40%) until limit A. From this limit, and especially limit C, *L. machaerophorum* percentages significantly drop, reaching values below the average. It is worth noting that *L. machaerophorum* is a species that has

commonly been interpreted as a proxy for stratified waters in various contexts (estuaries and fjords: Morzadec-Kerfourn, 1976; Dale et al. 1999; Dale, 2009; Ganne et al., 2016, and even in marine environments associated with typical climate intervals of higher fluvial discharges: Zaragosi et al., 2001; Penaud et al., 2009). On the contrary, a generally increasing trend of S. bentorii percentages is noted in our study, with clear fluctuations anti-correlated with S. belerius ones. Among other characteristic features, S. membranaceus is continuously present with above-average values from limit B (17 cm). Today, S. bentorii is commonly associated to warm waters (Zonneveld et al., 2013) and anthropogenic-induced nutrient-enriched areas (Pospelova et al. 2002). It is also worth noting that the decreasing profile of L. *machaerophorum* is extremely close to the one depicted by Chenopodiaceae percentages in the pollen record (Fig. 4). Interestingly, Chenopodiaceae are typical herbaceous plants from tidal-influenced environments. This halophile vegetation commonly grows on salt marshes together with Poaceae and Asteraceae. Before limit A, the association of Poaceae, Chenopodiaceae, and L. machaerophorum is a clear signal of the dominance of salt marsh environments. After limit A, and especially after limit B, the salt marsh signature is less pronounced in favour of a palynological signal mainly characterized by the Alnus - S. membranaceus association.

#### 4. Discussion

#### 4.1. Climate forcing on tree pollination rates

Mean tree pollen percentages(i.e. AP, 58%; Figs. 4 and 5) recorded in core I\_09 are similar to the tree signature currently recorded in Bay of Brest modern sediments (57%; Lambert et al., 2017). However, since 1870, these values fluctuate throughout I\_09 core with amplitude of 10-20%, mainly explained by *Quercus* percentages (in addition to *Alnus* at the top of the core). More precisely, a significant increase (+/- 15%) is observed between 1890 and 1930 (cf. raw data in Fig. 5), as well as from 1975 upwards, the Poaceae signature being then anti-correlated as expected (particularly noteworthy for cereals). These variations can be partially explained by the agricultural practices (see section 2.1 this paper) with increasing hedgerows up to 1950 (mosaic landscape) and their drop during the 1970's (grouping of agricultural surfaces). However, this is not consistent with the increase of pollen trees percentages from 1985 to now and raises question regarding *Quercus (Alnus* in a lesser extend) variations. Therefore, natural modes of North Atlantic climate changes may be involved.

In our study, the observed fluctuating arboreal pollen percentages appears closely related to the oceanic AMO index (Enfield et al., 2001) as well as to the atmospheric seasonality parameter (i.e. mean difference between summer and winter temperature) (Fig. 5).

The multidecadal variability characteristic of the AMO reflects changes in North Atlantic SST (Kerr, 2000); warmer SSTs being recorded across the entire North Atlantic basin during AMO positive modes (AMO +), and conversely during AMO negative modes. Basin wide Atlantic SST variations therefore play an important role in multidecadal climate changes observed in the northern hemisphere as recorded through averaged air temperatures (Schlesinger et al.,

1994) with milder climate conditions recorded over Western Europe during AMO + (McCarthy et al., 2015), or tree-ring data (Delworth and Mann, 2000; Gray et al., 2004). Furthermore, at the studied timescale, fluctuations observed in core I\_09 cannot be strictly attributed to the growth or decline of pollen-producing young plants (National Botanical Conservatory of Brest, personal communication). Since weather conditions are known to greatly influence current tree pollination rates (van Vliet et al., 2002; Garcia-Mozo et al., 2006; Besancenot and Thibaudon, 2012), we therefore suggest that an increase of North Atlantic SST (AMO +), by favoring milder springs and summers in Western Europe, may have contributed to the boost of tree pollination rates, and may then be responsible for the significant increases observed in arboreal percentages.

#### 4.2. Major runoff increase over the last 60 years

In parallel with the natural climate variability described above, higher percentages of *Alnus*, together with increasing freshwater palynomorph, pollen, and dinocyst concentrations, as well as the decreasing trend of *R. corbiferum* (marine algae), are observed from about 1945 (limit A; Fig. 6), and even more obviously since 1965 (limit C; Fig. 6). This trend is correlated with the XRF Ti/Ca signal, commonly interpreted as a detrital terrigenous advection signal through runoff and soil leaching (e.g. Bahr et al., 2005; Mollier-Vogel et al., 2013; Steinke et al., 2014). A recent study carried out on Bay of Brest modern sediments leads us to consider *Alnus* as a fluvial discharge indicator, based on its present-day distribution, which is correlated to river mouths (Lambert et al., 2017). Its fluvial discharge significance is thus confirmed, especially since this taxon has not been found to increase on the corresponding watersheds over the last decades (National Botanical Conservatory of Brest, personal

communication). In parallel with the *Alnus* trend, the combined increase of other trees typical from the riparian forest (especially *Salix*, *Betula* and *Fraxinus*; Fig. 6) suggests an increase in fluvial discharge and/or in precipitation over the past 60 years.

In parallel with these observations, the meteorological station of Rennes evidences a clear increase of precipitation in Brittany, well correlated with the XRF Ti/Ca ratio, probably arguing for higher runoffs on Bay of Brest watersheds and then increased Aulne River flows (Brittany DREAL; Fig. 6). For example, when looking more into details, lower Ti/Ca values recorded at around 1972-1976, 1982-1987, and 2003-2008, are well correlated with lower values of river flows and precipitations, giving strength to our Ti/Ca signal as an indirect proxy for runoff changes through time. It is also worth noting that higher precipitations, river flows, and riparian forest percentages are recorded after 1985, synchronously with the highest atmospheric temperatures (mean annual temperatures of about 12.5°C since 1985) recorded at the meteorological station of Rennes (dataset available since 1930; Fig. 7). Interestingly, it has been demonstrated that the recent rise in global air temperatures can promote a significant increase in runoff worldwide, caused by an acceleration of the hydrological cycle (Labat et al., 2004). One may advance with good reason that what is valid worldwide might not be true locally. However, the EA pattern is known to affect the rainfall budget in Western Europe (Ruprich-Robert and Cassou, 2015) and is also well correlated to the XRF Ti/Ca signal (Fig. 6).

From these sedimentological data (XRF Ti/Ca signal) and palynological evidences (riparian forest, freshwater fossils, palynomorph concentrations), combined with recent instrumental data (EA pattern, as well as air temperatures, precipitations, and river flows across Brittany), three major periods (i.e. pluri-decadal trends) of increased runoff and therefore fluvial inputs can be highlighted, first after the Second World War (limit A; Fig. 6), then after 1965 (limit C;

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Fig. 6), and finally after 1985 (limit B; Fig. 6). However, the warming context and the increasing rainfall pattern previously described are not the only parameters to take into account. Superimposed on these climate trends, other mechanisms may be involved from 1945 onwards. Indeed, higher river flows have been accentuated by increasing artificial agricultural surfaces in Bay of Brest watersheds (Fig. 7). After the Second World War, the implementation of a new agricultural policy led to the reunification of agricultural holdings (i.e. gathering of cultivated plots). The destruction of hedgerows then modified the erodability of soils and significantly contributed to runoff increases in Bay of Brest watersheds due to the absence of embankments (Flatrès, 1976; Merot et al., 1999). This limit is also clearly noticeable on the XRF Ti/Ca signature that obviously increases around 1945. From this period onwards, Chenopodiaceae and *Plantago maritima* percentages noticeably drop as a consequence of the local loss of the salt marsh signature in favor of a fluvial-type signal dominated by the riparian forest (Fig. 6).

#### 4.3. Major trophic disturbances since 1990

After 1965 (limit C), and especially 1985 (limit B), an atypical palynological signature is observed with several indicators (Fig. 7). Pollen assemblages have already been discussed as characterized by the highest riparian forest signature from 1965, with high percentages observed around 1970, 1995, 2005, and at the top of I\_09 core (Fig. 5). Regarding dinocyst assemblages (Fig. 4), a non-ambiguous relationship can be highlighted between *L. machaerophorum* and *S. bentorii* across limit C (1965) and then between *S. bentorii* and *S. membranaceus* across limit B (1985). In 1965, no particular climate shifts may explain this connection. In addition, it has been demonstrated a close relationship between

Chenopodiaceae and L. machaerophorum trends (Figs. 4 and 6), arguing for a common origin of their signature. Both palynological taxa may thus indicate salt marsh-like environments, and their decline argues for a change in fluvial dynamics probably also responsible for a trophic change in the dinoflagellate community. From 1965 onwards, it is notable that each recent peak of Alnus is associated with an even more pronounced drop of L. machaerophorum percentages, confirming the relationships between both fluvial and salt marsh signals, respectively. Despite its ecological coastal and estuarine preferences (Morzadec-Kerfourn, 1977; Ganne et al., 2016), L. machaerophorum is known to be very sensitive to nitrate and phosphate inputs, while these inputs can also benefit other species, such as S. bentorii (Zonneveld et al., 2013). A sharp increase of *L. machaerophorum* was observed in Norwegian fjords in response to the increased watershed eutrophication (Dale et al., 1999; Dale, 2009), while Pospelova et al. (2002) showed that, in some American anthropized estuaries, L. machaerophorum rather negatively responds to anthropogenic nutrient enrichment, in favor of other species such as S. bentorii. No consensus thus exists on a fossil dinocyst assemblagetype that would reflect nutrient enrichment in disturbed estuarine domains (Pospelova et al., 2002). There is no particular species that can be used to characterize high nutrient loading in NW Atlantic estuaries. Indeed, the different responses according to the studied estuaries can be explained by the different physico-chemical configurations (e.g. salinity, pH) (Price et al. 2017). Our results appear similar to those obtained by Pospelova et al. (2002), who concluded that L. machaerophorum does not respond positively to human inputs in shallow estuaries, having fewer consequences on water mass stratification than in deep Norwegian fjords (Dale et al., 1999; Dale, 2009).

Moreover, from 1985 onwards, other non-pollen palynomorph, micropaleontological, and genetic signals argue for atypical environmental conditions when compared with the whole 24

record (Fig. 7). We indeed observe a significant increase in amoebae concentrations (i.e. continental markers; Mitchell et al., 2008; Lambert et al., 2017). In addition, benthic foraminifera are known to strongly react to anthropogenic pollution. Shell deformations may indicate an immediate reaction to a human-induced environmental stress (Alve, 1995; Polovodova and Schönfeld, 2008; Martins, 2011). Here, despite the low proportion and diversity of benthic foraminifera in core I\_09, an increasing trend is observed with up to 6% of deformed shells, particularly since 1990. Finally, the increase in toxic algal blooms has previously been detected and quantified in the same core (Klouch et al., 2016b; cf. Figs. 2 and 7), especially from 1990, by the presence of fossilized DNA (qPCR method) of a particular dinoflagellate taxon, *Alexandrium minutum*, known to produce virulent toxins poisoning shellfish (Aylagas et al., 2014).

Interestingly, the main factor controlling phytoplankton community developments in the Bay of Brest can be directly related to river-induced fertilization (Quéguiner and Tréguer, 1984). Nitrate fluxes, respectively recorded in the Elorn and Mignonne rivers since 1970 and 2000 (source: *SAGE Elorn*), allow us to compare the evolution of agricultural practices (i.e. fertilizer uses) on Bay of Brest watersheds with our fossil data (Fig. 7). This indirect eutrophication indicator sharply increases from 1970 to 1990, then reaching a plateau of abnormally high values approaching "standard" thresholds at that time. Various remediation policies were then successfully applied to lower nitrate fluxes in Bay of Brest watersheds from 1990 to present. Nitrogen fertilizers are indeed widely used in Brittany and particularly in the Daoulas watershed, which is characterized by the largest nitrogen introductions in Brittany rivers then often exceed the national standard of 50 mg/l (Joanny et al., 1993), and largely dominate at the North Atlantic scale (Howarth et al., 1996), mainly coming from 25

agriculture (98% : use of fertilizers, extension of intensive farming; Aurousseau et al., 2009). Nitrate inputs in the Bay of Brest are well correlated with river flows (Delmas and Treguer, 1983) as evidenced in Figs 6 and 7. We then suggest that the increases in fluvial discharges since the late 1980s may have brought huge amounts of fertilizers that promoted an enhancement of the dinocyst diversity and total dinocyst concentrations (Fig. 3), probably promoting dinoflagellate productivity as also recorded by Harland et al. (2004) in Sweden. This may be also responsible for the growth of toxic dinoflagellate species (A. minutum; Klouch et al., 2016b) in Bay of Brest surface waters. Consequently, in addition to the obvious increased runoff for half a century, an increase in nitrogen fluxes, and probably of eutrophication since 1985-1990 (i.e. nutrient enrichment of the aquatic system; Andersen et al., 2006), in parallel with an increase in atmospheric temperatures has certainly contributed to the drastic alteration of plankton communities, as evidenced by the dinocyst relay and deformed benthic foraminiferal shells. It is also interesting to note that the Daoulas Bay, where the maximum occurrences of toxic algal blooms are observed today within the Bay of Brest (Klouch et al., 2016a), also corresponds to the site where maximal percentages of agriculture-type pollen markers are observed in Bay of Brest modern sediments (Lambert et al., 2017), confirming the link between increased runoff on agricultural soils and the disturbance of the phytoplankton community. This may be related to the small size of the watershed and the isolation of the Daoulas estuary from main tidal currents.

#### 5. Conclusion

The high resolution palynological study conducted over the past 150 years in the Bay of Brest (NW France) has highlighted the relationship between natural climate variability, human pressures, and fossil palynomorphs preserved in sediments (especially pollen grains and dinoflagellate cysts). Varying SST in the North Atlantic Ocean, by driving atmospheric temperatures across Europe, may have influenced tree pollination rates deduced from arboreal pollen percentage fluctuations. Superimposed on natural climate modes, increased human influence on watersheds is perceptible since 1945. Changes in agricultural practices, particularly the regrouping of cultivated areas and the reduction of hedgerows, led to a runoff increase, recorded through our palynological (increase of riparian forest as well as reduction of Chenopodiaceae and L. machaerophorum percentages) and sedimentological (XRF Ti/ Ca increase) markers. Also, changes in the structure of the dinocyst community (major trophic relay between L. machaerophorum and S. bentorii around 1965 followed then by S. membranaceus around 1985), and the intensification of toxic algal blooms (A. minutum) detected by genetic analyses, appear directly correlated to the increase of fertilizer use for agriculture over the past decades. Such changes caused strong environmental and health impacts. Here, for the very first time, a palynological study conducted in parallel with a genetic study provides clear driving mechanisms regarding the major causes responsible for recent phytoplankton (dinoflagellates and their cysts) changes affecting European coastal ecosystems. It demonstrates the strong potential of such micropaleontological approaches which allow drawing a comprehensive view of modern human induced environmental alterations in a retrospective frame.

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activites

#### 8. Figure caption

**Figure 1:** Locations of : a) the Bay of Brest in north-western France, as well as of : b) I\_09 study core (orange star) in the Daoulas Estuary, and of the hydrological stations (yellow points A and B) from which river discharge data are discussed in the manuscript. c) Present-day climate data with an ombrothermic diagram (temperatures and precipitations). d) wind rose showing prevailing mean annual wind directions averaged over 10 years (source : Météo France).

**Figure 2:** I\_09 core photograph and X-Ray radiography, with the sampling procedure carried out for palynological and micropaleontological analyses (this study) and genetic analyses (Klouch et al., 2016b).

**Figure 3:** Depth chart representing dinocysts, pollen grains, spores and major non-pollen palynomorphs (NPP) in concentrations. From left to right, NPP comprise foraminiferal linings, *Radiosperma corbiferum* (marine algae), freshwater fossils (*Halodinium, Concentricystes, Pediastrum*), amoebae, and *Hexasteria problematica* (estuarine algae; Milzer et al. 2014). The C/M index is a ratio between selected continental (*Concentricystes, Pediastrum, Halodinium, and amoebae*) and marine markers (*R. corbiferum* and foraminiferal linings). Grey bands highlight intervals characterized by total dinocyst and pollen concentrations higher than the average of the series.

**Figure 4:** Depth chart diagram representing major pollen taxa and dinocyst species (above 2%) in percentages, along I\_09 core (Daoulas Estuary, Bay of Brest). Grey bands highlight intervals characterized by total dinocyst and pollen concentrations higher than the average of the series (cf. Fig.3).

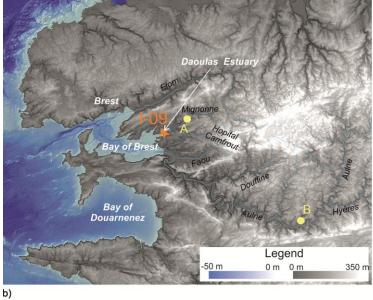
**Figure 5:** Comparison between selected pollen percentages (cereals, *Quercus*, sum of *Alnus*, *Salix, Betula*, and *Fraxinus*, and sum of trees), with XRF Ti/Ca ratio, the measured AMO index for this time period (Enfield et al., 2001), as well as atmospheric temperature data and seasonality (difference between summer and winter mean temperatures) from the Rennes meteorological station (the most complete meteorological record of Brittany for the study time period ; infoclimat.fr).

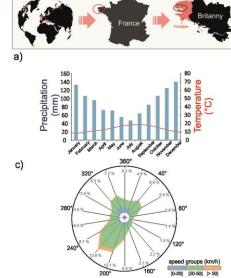
**Figure 6:** Comparison between palynological data from I\_09 core, data from literature, and instrumental data from *in situ* measurements in watersheds. From left to right: precipitations from the Rennes meteorological station (infoclimat.fr), EA pattern index (NOAA), XRF Ti/Ca ratio from the study core, selected pollen percentages (*Plantago maritima*, Chenopodiaceae, *Betula*, *Alnus* and the sum *Fraxinus* + *Salix*), Aulne River flows (from DREAL Bretagne (i.e. regional directorate for the environment) and SOMLIT-Brest (i.e. French marine monitoring network, http://somlit.epoc.u-bordeaux1.fr), concentrations of freshwater fossils (sum of *Halodinium, Pediastrum* and *Concentricystes*), as well as marine algae *R. corbiferum*, pollen grains and dinocysts. Grey bands highlight intervals characterized by total dinocyst and pollen concentrations higher than the average of the series (cf. Fig.3).

**Figure 7:** Comparison between selected palynological data from I\_09 core, data from literature, and instrumental data from *in situ* measurements in watersheds. From left to right: XRF Ti/Ca ratio from the study core, dinocyst percentages (*L. machaerophorum, S. bentorii*, cysts of *P. dalei* and *S. membranaceus*), amoebae concentrations, percentages of deformed foraminiferal shells, detection curve of *A. minutum* species in the same core (from Klouch et al., 2016b), measured nitrate concentrations in the Elorn and Mignonne rivers (SAGE Elorn) and in the Aulne river (ECOFLUX: river monitoring network that collects samples at the mouth of rivers of Western Brittany on a weekly basis, wwwiuem.univ-brest.fr/ecoflux) and measured atmospheric temperatures (Rennes meteorological station; infoclimat.fr). Grey band highlights the interval characterized by pollution indicators (deformed benthic foraminifera, *A. minutum*, nitrate concentrations).

#### 8. Appendix caption

Appendix 1: Exhaustive table of palynological results (individual counts).

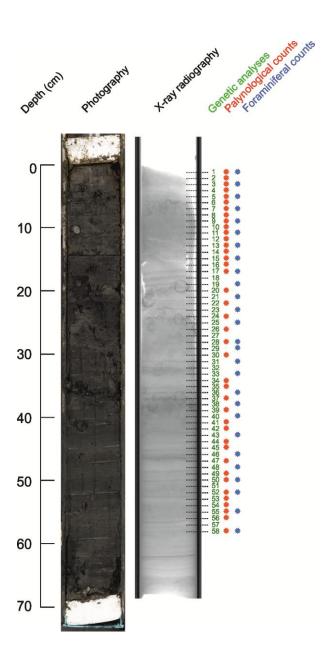




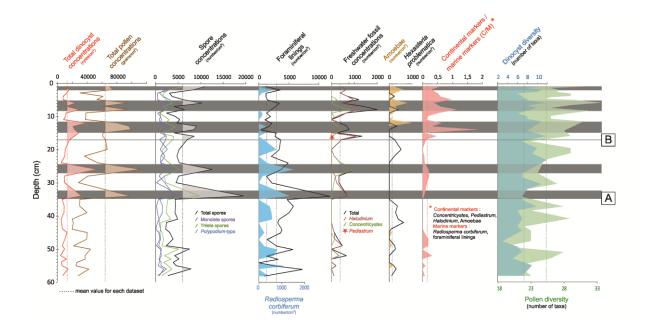
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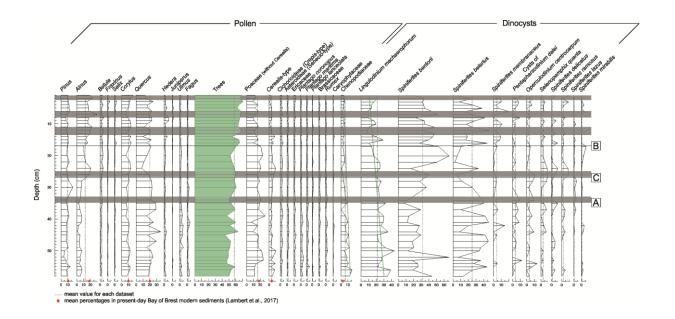
Figure 1













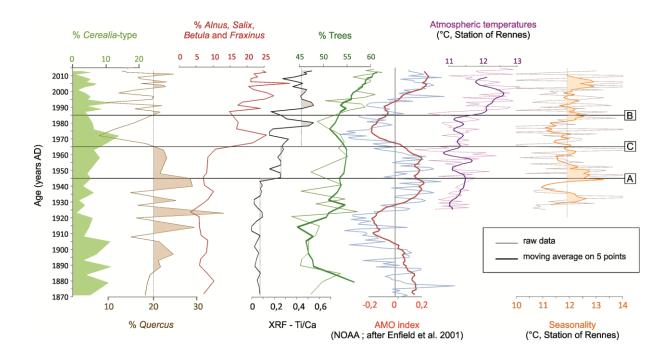


Figure 5

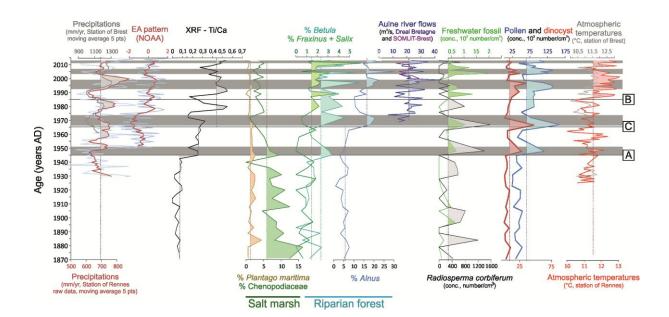


Figure 6

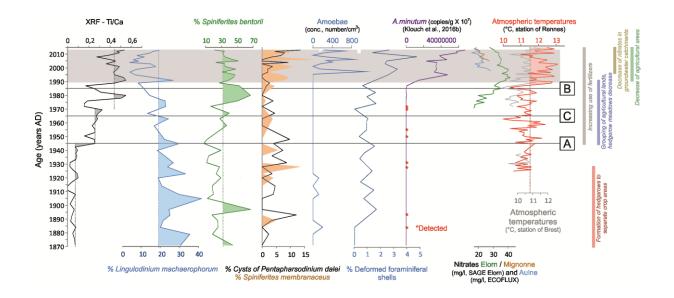


Figure 7