

# Spatial distribution of *Gracillaria vermiculophylla* in French and Spanish estuaries using Unmanned Aerial Vehicle.

Simon Oiry<sup>1</sup>      Bede Ffinian Rowe Davies<sup>1</sup>      Pierre Gernez<sup>1</sup>  
Laurent Barillé<sup>1</sup>

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To be Written

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## 1 Introduction

The introduction of Non-Indigenous Species (NIS) in terrestrial, freshwater, and marine ecosystems is one of the major threats to biodiversity worldwide. In particular, the proliferation and rapid spread of Invasive Alien Species (IAS) can radically change the structure and functioning of marine ecosystems, , requiring effective inventorying and monitoring programs (Massé et al., 2023). In Europe, 874 NIS have been introduced to the marine environment so far (i.e. until 2020) and it is expected that the rate of biological invasions will continue to increase in the coming years (Zenetos et al., 2022). Macroalgae represent more than 40 % of the NIS introduced to Europe waters, with many species native to the Temperate Northern Pacific (Williams and Smith, 2007). Amongst all invasive macroalgae, *Gracillaria vermiculophylla* (Papenfuss, 1967) (original name *Gracilariopsis vermiculophylla* (OHMI, 1956); also known as *Agarophyton vermiculophyllum* (Gurgel et al., 2018)), has spread extensively from its native distribution range in Japan and Korea (Terada and Yamamoto, 2002) across temperate estuaries in North America, Europe, and other regions, facilitated by aquaculture and maritime activities (Krueger-Hadfield et al., 2017; Rueness, 2005; Weinberger et al., 2008). While *G. vermiculophylla* can provide some ecosystem services, such as habitat for invertebrates and juvenile fish (Davoult et al., 2017), it often outcompetes native vegetation, alters sediment composition (Nyberg et al., 2009), and disrupts trophic interactions (Ginneken et al., 2018).

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<sup>1</sup>Institut des Substances et Organismes de la Mer, ISOMer, Nantes Université, UR 2160, F-44000 Nantes, France

In regions like the Baltic Sea and the eastern United States, it has been documented to negatively affect native fucoids and seagrasses (Firth et al., 2024; Thomsen et al., 2013; Van Katwijk, 2003). These impacts underscore the importance of monitoring and managing the spread of *G. vermiculophylla*, particularly as climate change and anthropogenic pressures continue to facilitate biological invasions. *G. vermiculophylla* success as an invader stems from its tolerance to a wide range of environmental conditions, including temperature (Sotka et al., 2018), nutrient variability (Abreu et al., 2011) and salinity (Weinberger et al., 2008). Its growth capacity at low salinities (Nyberg, 2007; Rueness, 2005) explains its presence in the brackish waters of the Baltic Sea (Weinberger et al., 2008) but also in the mesohaline sheltered part of estuaries of the Atlantic coast of Europe (Surget et al., 2017). It is also present in confined areas of lagoons characterized by low hydrodynamism (Abreu et al., 2011; Sfriso et al., 2012). In Europe, it was first observed in 1996 in the Belon estuary (France) and later in many other estuaries on the Brittany coast of France (Rueness, 2005). It can be found on hard substrates such as invertebrate’s tubes and shells providing a substratum (Thomsen et al., 2007) or attached to pebbles and rocks (Terada and Yamamoto, 2002) but the largest populations are colonizing soft-bottom sediment and particularly estuarine intertidal mudflats (Surget et al., 2017). In this habitat, extensive dark red mats are observed at low tide, covering vast areas that have largely been unquantified in most studies. Therefore, *G. vermiculophylla* is capable of establishing populations in soft-bottom sediment habitats that were previously devoid of macroalgae (Ramus et al., 2017). These mats are usually monospecific with the alga thalli partially buried into the mud (Rueness, 2005; Surget, 2017). Intertidal mats can however be temporarily overgrown by ephemeral green macroalgae (Weinberger et al., 2008). In the estuaries where *G. vermiculophylla* was first documented, large monospecific mats were reported to be confined to the upper intertidal zones (Rueness, 2005); however, their spatial distribution relative to the mudflat topography had not been quantitatively assessed. In fact, *G. vermiculophylla* has never been mapped using remote sensing techniques, and existing descriptions of its distribution lack spatially explicit mapping (Abreu et al., 2011; Sfriso et al., 2012; Thomsen et al., 2007; Weinberger et al., 2008)

Remote sensing has revolutionized our ability to monitor and manage coastal ecosystems, offering efficient and scalable methods for detecting environmental changes in intertidal vegetation across a wide range of spatio-temporal scales (Calleja et al., 2017; Davies et al., 2024a, 2024b; Valle et al., 2015; Zoffoli et al., 2021). Among remote-sensing technologies, drone-based imagery has recently emerged as a particularly promising tool for studying the spatial distribution of intertidal primary producers such as benthic microalgae (Román et al., 2024, 2021), seagrass (Chand and Bollard, 2021; Duffy et al., 2018; Román et al., 2021) and macroalgae (Diruit et al., 2022; Peidro-Devesa et al., 2024). While it lacks the temporal consistency of satellite missions, drone remote sensing makes it possible to acquire at extremely high spatial resolution (i.e. cm-scale), rapidly target specific areas of interest, and to provide observations in overcast conditions. In particular, the potential of drone remote sensing for monitoring the surface area occupied by IAS has been demonstrated (Roca et al., 2022). Drone-based photogrammetry also makes it possible to characterize the distribution of intertidal vegetation together with mudflat geomorphology, thus improving our understanding of primary producers

patterning (Brunier et al., 2022; Douglas et al., 2024).

In this study, a drone-based multispectral remote sensing approach was applied to map *G. vermiculophylla* spatial distribution at a very-high spatial resolution in three intertidal estuaries of European Atlantic coast. We adapted the neural network classification model DISCOV (Drone Intertidal Substrate Classification Of Vegetation, Oiry et al. (2024b), Oiry et al. (2024a)) by specifically training the model with a new class corresponding to *G. vermiculophylla*. A validation dataset was obtained from *in situ* data to estimate the classification accuracy. LiDAR data were concurrently acquired to accurately map the intertidal elevation. We used a Generalized Additive Model (GAM) to examine the relationship between the seaweed spatial distribution and spatial metrics quantifying the mudflat topography. We expected the presence of *G. vermiculophylla* in mudflats to be associated to a specific height range as well as being more closely related to flat areas of the intertidal zone.

## 2 Materiel & Methods

### 2.1 Study sites

Field campaigns were conducted at three study sites in France and Spain. At each site, two locations were investigated Figure 1. The Aven & Belon Estuary in South Brittany, France (Figure 1 A & C), is a dynamic ria-type system hosting diverse habitats, including sandy tidal flats and subtidal zones with coarse, marine-origin sediments (Castaing and Guilcher, 1995; Michel et al., 2021). These habitats support key benthic species such as *Scrobicularia plana*, *Cerastoderma edule*, and *Tellina tenuis*, which play essential roles in sediment bioturbation and nutrient cycling (Blanchet et al., 2014; Tankoua et al., 2011). The estuary serves as a nursery for juvenile fish and a feeding ground for migratory birds, with its ecological productivity driven by a mix of euryhaline and marine species adapted to salinity gradients (Blanchet et al., 2014). Oyster farming, particularly *Crassostrea gigas*, is a dominant activity, altering sediment dynamics and local biodiversity (Michel et al., 2021). Despite its ecological richness, the estuary faces pressures from nutrient loading and physical alterations, with bioindicators like *S. plana* used to monitor the impacts of salinity, sediment quality, and pollution (Tankoua et al., 2011).

The Saja-Besaya Estuary, situated along the Cantabrian Sea in northern Spain, is characterized by the confluence of the Saja and Besaya rivers near Torrelavega (Figure 1 C). The estuary, also known as San Martín de la Arena or Suances Estuary, has been subject to significant anthropogenic pressures, including industrial developments throughout the 20th century. These activities have led to contamination from mining, paper manufacturing, and carbonate discharges, classifying the estuary as highly polluted near its upper reaches (Ortega et al., 2005). This contamination impacts the estuarine ecosystem, including water quality and biodiversity, with minimal aquatic life and sparse riverbank vegetation in its lower sections (Romero et al., 2008).

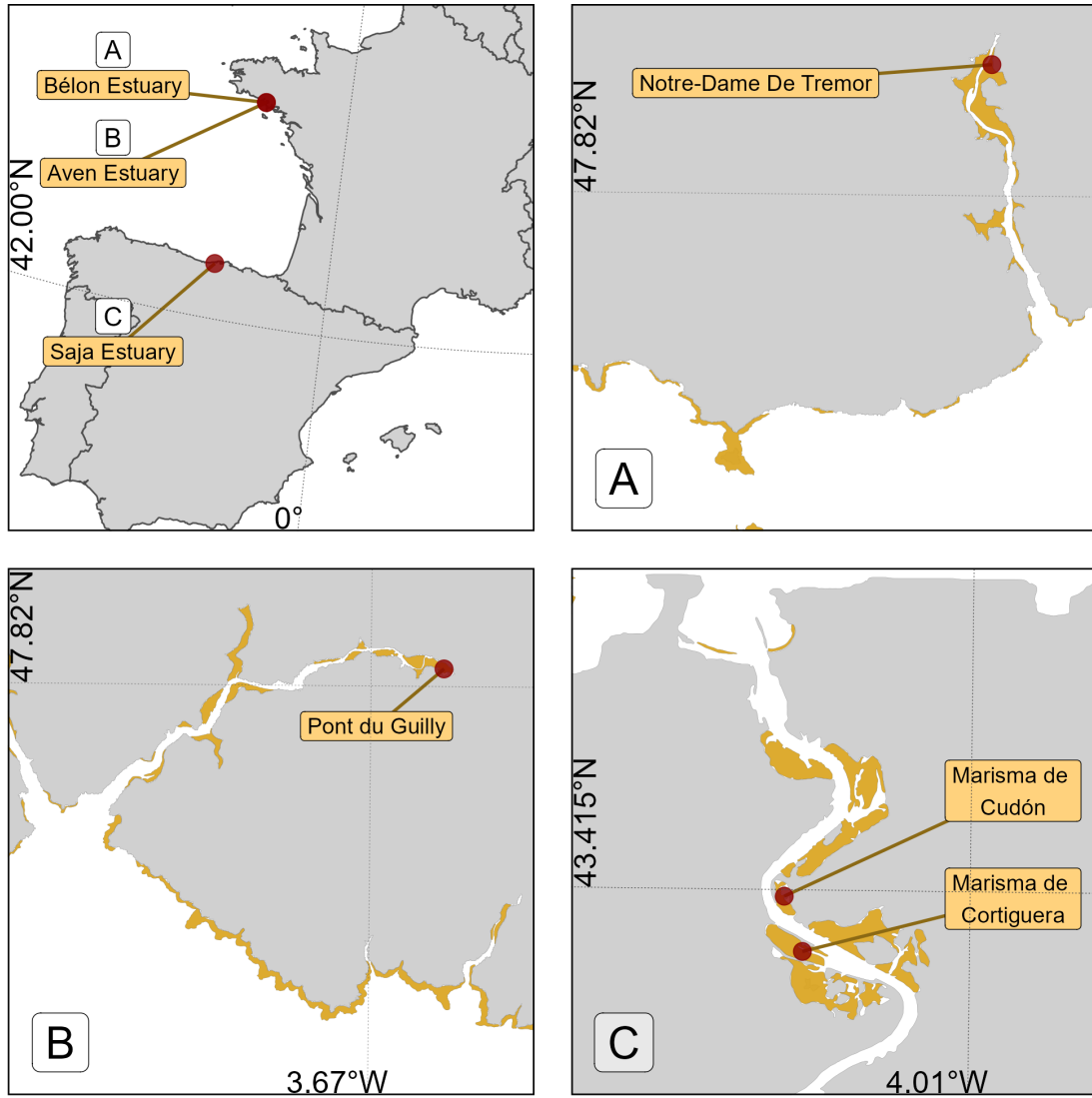


Figure 1: Location of the drone flights. A: Flights made in Aven Estuary, France; B: Flights made in Bélon Estuary, France; C: Flights made in Saja Estuaries, Spain. Golden polygons represent intertidal areas.

## 2.2 Remote sensing data acquisition and pre-processing

A total of 6 drone flights were done spread in the 3 study sites. Each time, flights were done at an altitude of 120 m and at a speed of 10 m.s<sup>-1</sup> (Table 1).

Table 1: List of drone flights, summarising the location, the date, and the total extent of each flight (in hectares).

Country	Site	Flight	Date
France	Aven & Belon	Notre-Dame De Tremor	2024-04-11
		Pont du Guilly	2024-04-11
Spain	Saja Estuary	Marisma de Cortiguera	2024-06-25
		Marisma de Cudón	2024-06-25

### 2.2.1 Multispectral data

At each location, reflectance images with a resolution of 1.2 million pixels were captured using a DJI Matrice 300 quadcopter drone equipped with a Micasense RedEdge Dual MX multi-spectral camera. The camera recorded data across ten spectral bands, spanning from blue to near-infrared (NIR) wavelengths (444, 475, 531, 560, 650, 668, 705, 717, 740, and 840 nm) (). To ensure consistent lighting conditions, the drone’s flight trajectory was aligned to maintain a solar azimuth angle of 90 degrees. Image acquisition was carried out with an overlap of 70% between side-by-side images and 80% between successive images along the flight path. A downwelling light sensor (DLS2) was used to measure real-time irradiance, enabling the correction of reflectance values for variations in light intensity caused by cloud cover during the flight. The raw image data were subsequently calibrated to reflectance using a calibration panel with ~50% reflectivity, provided by the camera’s manufacturer. Images were processed using structure-from-motion photogrammetry software (Agisoft, 2019) to generate multispectral ortho-mosaics for each flight. The ortho-mosaicking workflow was consistent across all flights. Initially, key tie points were identified within each image and across overlapping images to create a sparse point cloud. This point cloud was refined by removing noisy points using a reprojection accuracy metric. Subsequently, a dense point cloud was generated using a structure-from-motion algorithm. A digital surface model (DSM) was then created through surface interpolation of the dense point cloud, which served as the basis for reconstructing the multispectral ortho-image (Nebel et al., 2020). The resolution of the multispectral ortho-mosaic obtained were 8 cm per pixel.

## 2.2.2 LiDAR data

LiDAR standing for Light Detection and Ranging uses lasers to measure distances by timing reflected pulses, creating detailed 3D maps of surfaces.

Using the Matrice 300 Series Dual Gimbal Connector, a DJI Zenmuse L1 LiDAR and RGB sensor was mounted on the drone alongside a multispectral camera. This setup enabled the simultaneous capture of LiDAR point clouds, high-resolution RGB images, and multispectral images collected by the MicaSense RedEdge Dual MX during the same flight. The same processing workflow as Section 2.2.1 was applied to process LiDAR RGB images, resulting in ortho-mosaic with a resolution of 2.5 cm per pixel. Since the mapping focused solely on flat surfaces without dense vegetation, the LiDAR measured only a single return. Operating in repetitive scanning mode with a sampling rate of 240 kHz, the system achieved a point density of 350 points per square meter. The LiDAR point cloud was extracted and converted into LAS format using DJI Terra software. The LAS point cloud was then imported into Agisoft Metashape (Agisoft, 2019) to generate a DEM with a resolution of 2.5 cm.

## 2.3 Scene classification

A neural network classification model (DISCOV; Oiry et al. (2024b); Oiry et al. (2024a)), previously applied with success to Micasense reflectance data for mapping intertidal vegetation along the Portuguese and French Atlantic coasts, has been used in this study. The training dataset of DISCOV v1.0 has been updated. As shown by Oiry et al. (2024b) the DISCOV v1.0 model (Oiry et al., 2024a) was trained using only 5771 Rhodophyceae pixel (3% of the training dataset). To fill this gap the original training dataset of DISCOV v1.0 was updated using new training pixel coming from the 5 drone flights (Section 2.2).

# 3 Results

## 3.1 Historical records in the Belon estuary

A clear shift in sediment coloration over the past 70 years were observed, closely aligned with the subsequent proliferation of the invasive red macroalga *Gracilaria vermiculophylla* (Figure 2). Before 1976, the sediments appeared relatively light, indicating no detectable presence of this species. Following its initial appearance in 1976, subtle darkening of the sediment became discernible, coinciding with the early establishment of *G. vermiculophylla*. During the subsequent decades, the late 1970s through the 1990s, this darkening trend became more pronounced and widespread, reflecting an increasing spatial coverage and biomass of the algae. By the early 2000s, and especially by 2024, the sediment exhibited consistently darker tones, indicative of extensive and persistent colonization by *G. vermiculophylla*.

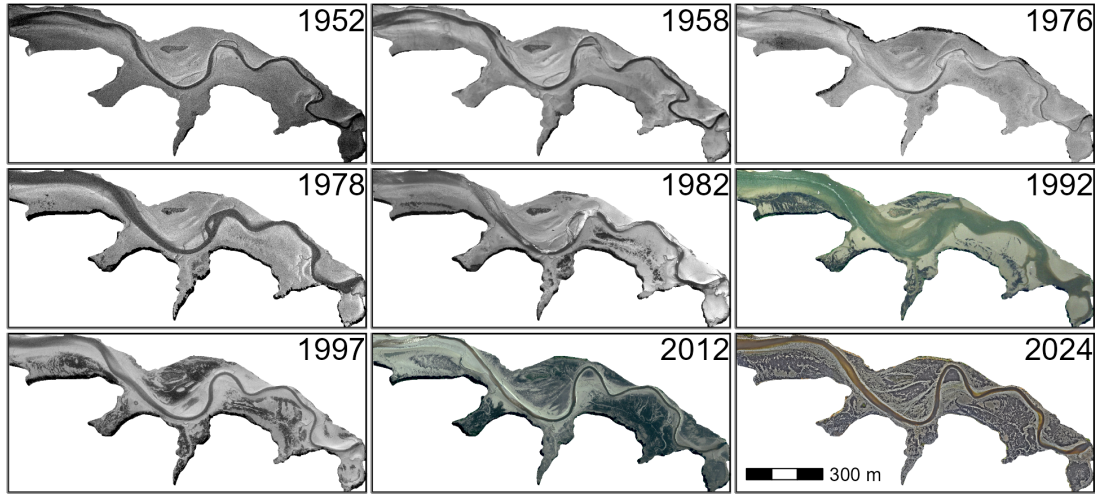


Figure 2: Historical images of Pont du Guilly between 1952 and 2024.

From the early recordings in the 1950s through the late 1970s, *Gracilaria vermiculophylla* coverage remained effectively at 0% (Figure 3). Shortly after the introduction of *Crassostrea gigas* in the estuary (see vertical red dashed line in the figure), the first detectable presence of *G. vermiculophylla* emerged. By 1976, it covered 2.5% (0.7 ha) of the Pont du Guilly area, and by 1978 it had increased slightly to 3.0% (0.9 ha). From 1982 onward, coverage expanded more rapidly, increasing from 6.6% (2.0 ha) in 1982 to 14.7% (4.5 ha) in 1992 and nearly 30% (9.0 ha) by 1997. This upward trend continued into the 21st century, peaking at 43.8% (13.3 ha) in 2012. Although coverage fluctuated somewhat thereafter (40.6% in 2019 and 40.2% in 2024), it remained consistently high, indicating sustained and widespread colonization.

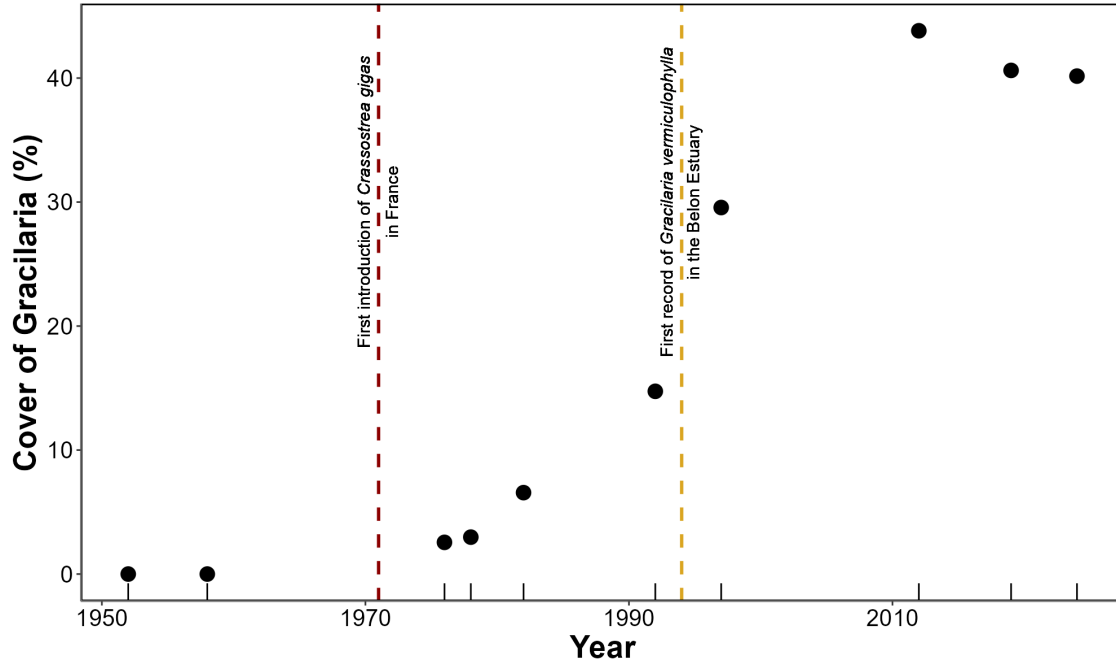


Figure 3: Evolution of the *Gracilaria vermiculophylla* cover at Pont du Guilly in the Belon Estuary. The red vertical line indicates the date of *Crassostrea gigas* introduction in France (Grizel and Heral, 1991), while the golden line represents the date of the first documented mention of *Gracilaria vermiculophylla* invasion in France in the literature (Rueness, 2005).

### 3.2 Classification

The classification map illustrates the diversity of benthic communities and substrates in the study area (Figure 4 A and B). Rhodophyceae (red) emerges as the dominant algal cover, forming extensive, continuous patches aligned with the mid-intertidal zones. In contrast, Bacillariophyceae (orange) and Chlorophyceae (green) exhibit more localized distributions, typically restricted to smaller, fragmented patches where specific microtopographic or hydrodynamic conditions favor their presence. Phaeophyceae (brown) is confined to limited patches, often found near transitional zones between sediment and water or in the upper intertidal area, where it is attached to rocky substrates. The water class (blue) delineates the main tidal channel, which meanders through the center of the area and influences the distribution of adjacent habitats.

The bathymetric map reveals a continuous gradient in elevation relative to mean sea level (Figure 4 C). A comparison of bathymetry and vegetation distribution highlights a clear elevation-driven pattern in algal presence. Higher intertidal zones, located above the deeper channel



areas, are associated with more extensive algal communities. In contrast, lower intertidal zones closer to the channel consistently exhibit reduced macroalgal cover.

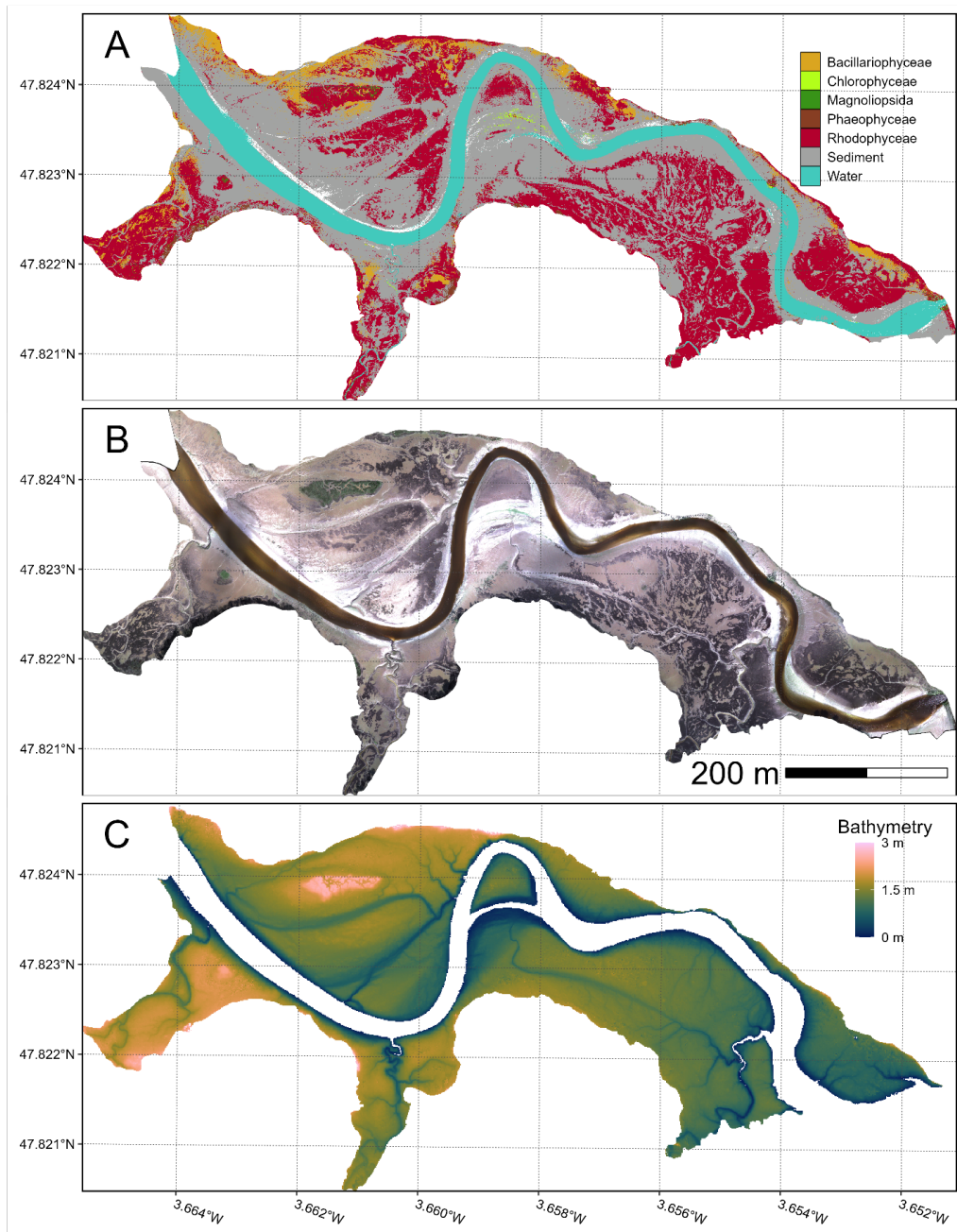


Figure 4: DISCOV Prediction (A), RGB composition (B) and Bathymetry (C) of the Bélon estuary site in Brttany, France. The total extent of this flight was 21 hectares with a resolution of 8 mm per pixel. Bathymetry is represented as the height above mean sea level.

### 3.3 Spectral description

### 3.4 Spatial distribution

## 4 Discussion

### 4.1 Drone mapping *G. vermiculophylla* with machine learning

In this study, we produced the first spatial distribution maps of the invasive red alga *Gracilaria vermiculophylla* using a multispectral drone survey conducted at low tide in Atlantic estuaries representing varied environmental conditions. In southern Brittany, the species formed monospecific mats, while in the Cantabrian region of Spain, it was intermixed with other intertidal vegetation. Distinguishing among these vegetation types was a key prerequisite for the analysis.

To achieve this, we adapted the deep learning-based classification model DISCOV (Oiry et al., 2024b), initially developed to discriminate seagrass from green macroalgae. Although the original model included Rhodophyceae as a class, this group constituted less than 3% of its training dataset. In contrast, the updated model presented here was trained on a dataset in which *G. vermiculophylla* covered 26% of approximately one million pixels. This improved dataset allowed the model to achieve an accuracy of **X%**.

Rhodophytes possess unique phycobilin pigments, enabling their spectral distinction from other macroalgal groups (Douay et al., 2022; McIlwaine et al., 2019; Olmedo-Masat et al., 2020). Even with the ten-band multispectral sensor used in our study, it remained feasible to discriminate the major classes of intertidal macrophytes (Davies et al., 2023; Oiry et al., 2024b; Román et al., 2021). However, the model identifies *G. vermiculophylla* at the class level (Rhodophyceae) rather than at the species level. Although hyperspectral approaches may allow finer taxonomic resolution (Douay et al., 2022; Olmedo-Masat et al., 2020), it is unlikely that *Gracilaria* species can be precisely distinguished using standard multispectral sensors.

Ecological factors also aid in differentiating *G. vermiculophylla*. Unlike many other macroalgae that require hard substrates,\* *G. vermiculophylla*\* establishes itself on soft-bottom sediments. In fact, it is commonly found on mudflats, anchoring its thalli in the top 10 cm of mud (Surget, 2017), and inhabits the upper intertidal zone—an unusual trait for a Rhodophyte (Abreu et al., 2011; Davoult et al., 2017). By reliably detecting *G. vermiculophylla* in these soft-substrate, upper intertidal habitats, our method provides a framework for identifying environmental conditions that favor its spread, potentially offering managers early-warning indicators to control its expansion before it reaches nuisance levels. Thus, combining spectral data with sediment characteristics provides a strong indicator of *G. vermiculophylla* presence in European Atlantic estuaries, complementing the physical variables already used in species distribution modeling (Mendoza-Segura et al., 2023).

In addition, the scalability of drone-based surveying facilitates repeat mapping to detect temporal shifts in the distribution and abundance of *G. vermiculophylla*. Such continuous monitoring could capture seasonal patterns of colonization, allowing researchers and environmental managers to evaluate the effectiveness of mitigation measures, track long-term ecological impacts, and anticipate future shifts in habitat suitability under changing climate conditions.

## 4.2 *G. vermiculophylla* spatial distribution and mudflat topography

## 4.3 Spatio-temporal monitoring of invasive macroalgae

Accurate, high-resolution maps of invasive or alien species are extremely scarce (**REF**), yet they enable in-depth evaluations of these species' ecology, temporal dynamics, and niche behavior in relation to their environment. In this study, using individual flights over monospecific algal mats, we quantified how this invasive alga associates with local mudflat topography, demonstrating that ... Repeated monitoring of this type can further reveal phenological patterns, invasion dynamics, and local conspecific biological interactions—such as co-occurrence, displacement, or avoidance (**REF**).

Remote sensing using multispectral drone mapping can provide high-resolution, spatially explicit data, but it must be combined with repeated, in situ field measurements to maximize its potential (**REF**). As noted, temporal repetition makes it possible to assess dynamic processes, and integrating these mapping approaches with in situ analyses of local infauna, carbon cycling, riverine inputs, and sedimentology would yield valuable insights for local managers. Such an integrated approach could help determine how the invasive alga affects the local ecosystem and, more broadly, forecast its potential impact on other estuarine environments facing similar invasion events. This holistic approach can guide strategic interventions aimed at mitigating the alga's spread, maintaining ecological balance, and protecting native biodiversity, ensuring that management efforts are informed by accurate, timely, and spatially explicit data.

Invasive species like *Gracilaria vermiculophylla* and *Rugulopteryx okamurae* can be identified using drones equipped with multispectral sensors, taking advantage of the characteristic reflectance of rhodophytes (Roca et al., 2022). However, this capability has not yet been tested using standard RGB sensors found in readily available commercial drones. These drones are easy to deploy, can cover large areas when flying at speeds of  $15 \text{ m s}^{-1}$  at an altitude of 120 m, and still maintain sufficient overlap between images to support photogrammetric reconstruction. Expanding these methodologies to RGB-based detection would significantly lower barriers to entry, allowing local stakeholders with limited resources to access valuable monitoring tools for early detection and rapid response. A promising avenue for operational applications lies in testing machine learning techniques on RGB imagery that do not rely on enhanced spectral resolution. Considering the low cost of RGB and multispectral commercial drones, coupled with ongoing advancements in machine learning, drone-based remote sensing has now matured into a practical tool for adoption by environmental authorities in coastal management. Integrating these technologies into routine monitoring protocols can enhance

surveillance capabilities, improve understanding of invasive species dynamics, and ultimately contribute to more effective conservation and restoration strategies.

## 5 Conclusion

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