Spatial distribution of *Gracillaria vermiculophylla* in French and Spanish estuaries using Unmaned Aerial Vehicule.

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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, *Gracilaria 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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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).

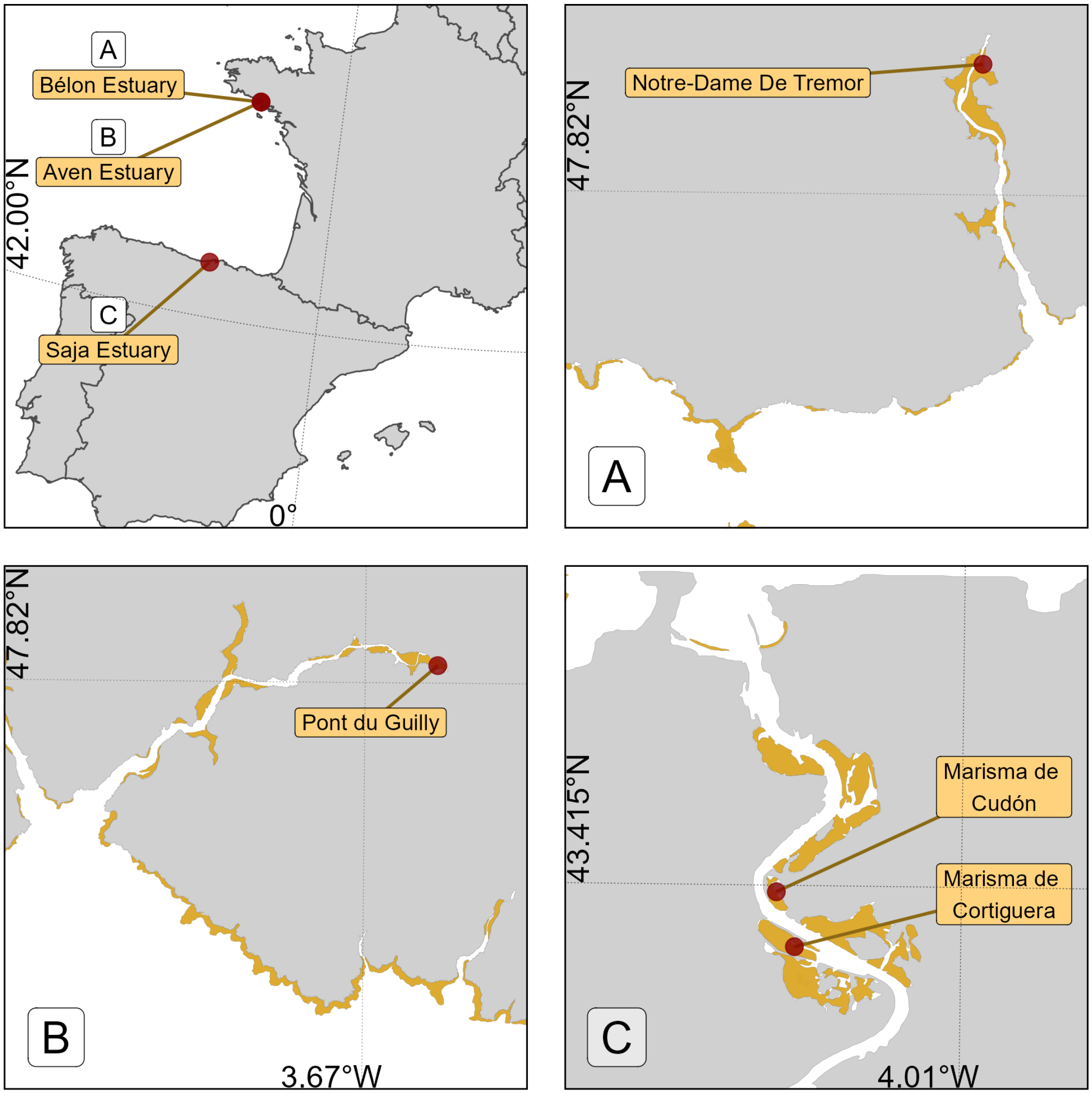

Figure 1: Location of the drone flights. A: Flights made in Aven Estuary, France; B: Flights made in Bélon Estuary, France; C: Flights made in Saja Estuaries, Spain. Golden polygons represent intertidal areas.

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 $m.s^{-1}$ (Table 1).

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

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 multispectral 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

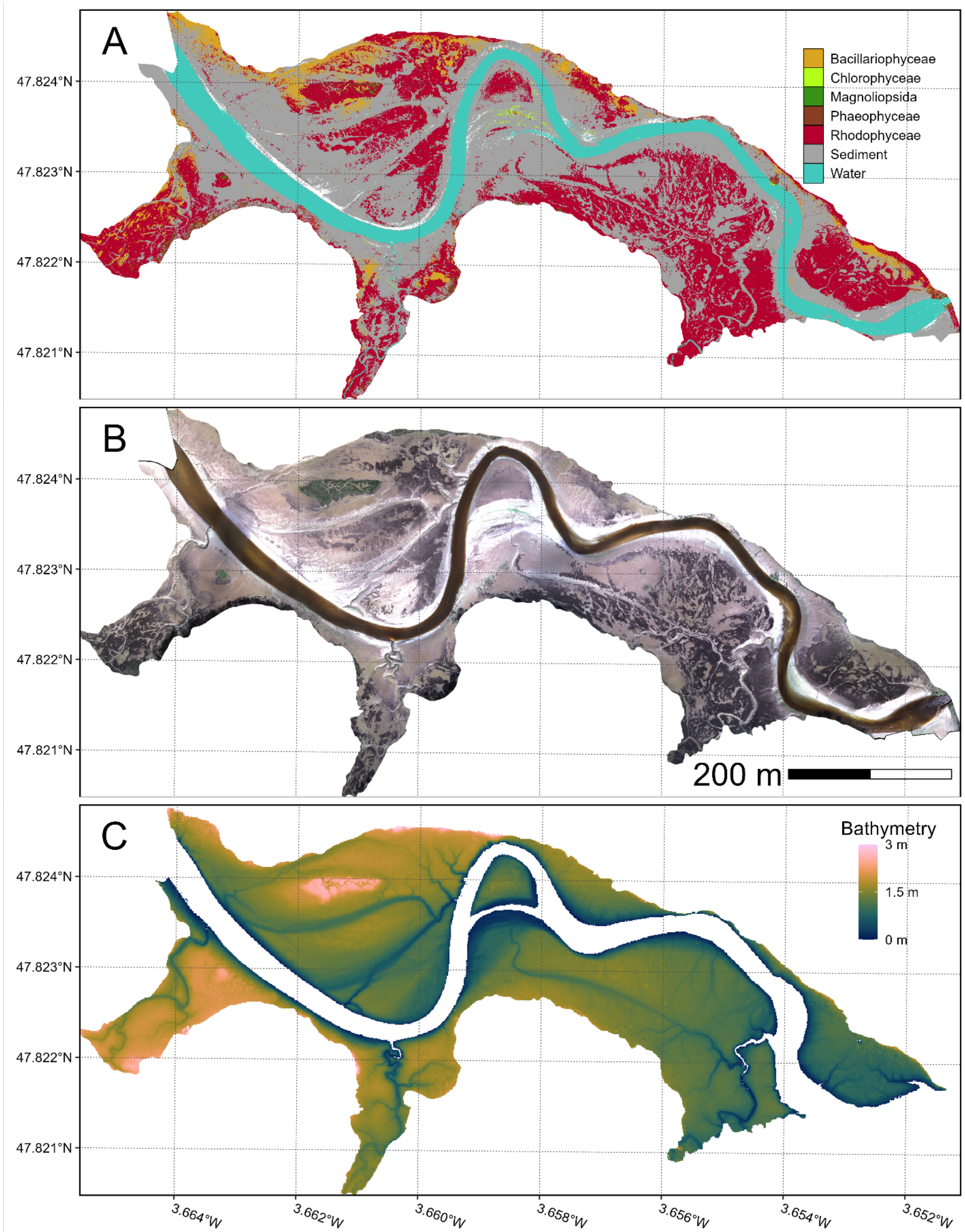

Figure 2: DISCOV Prediction (A), RGB composition (B) and Bathymetry (C) of the Bélon estuary site in Brttany, France. The total extent of this flight was 21 hectars with a resolution of 8 mm per pixel. Bathymetry is represented as the height above mean sea level.

4 Discussion

5 Conclusion

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