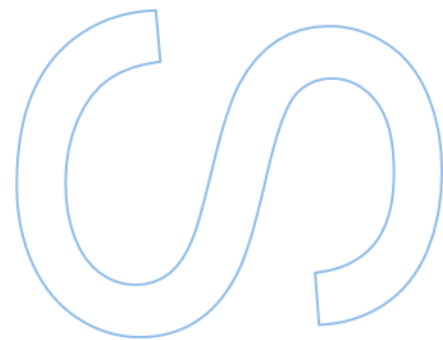
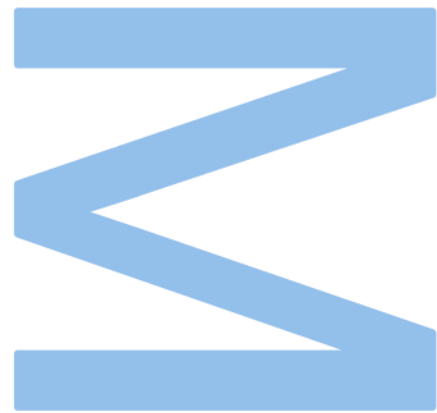




Carpobrotus edulis

The effect of spatial resolution on biomass estimation through multispectral imagery: a case study on *C. edulis*



Manuel de Figueiredo Meyer

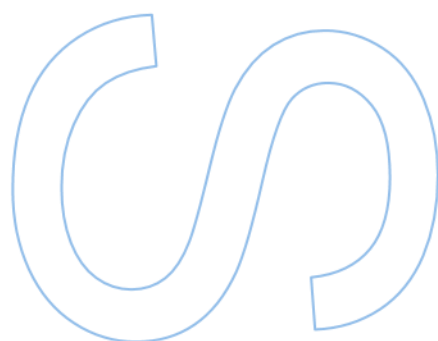
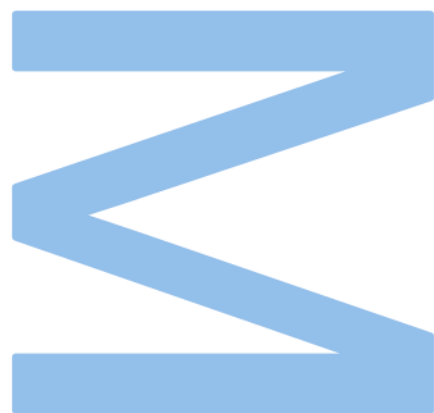
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Supervisor

Dr. Ana Maria Ferreira Bio, Investigador Auxiliar, CIIMAR

Co-supervisor

José Alberto Álvares Pereira Gonçalves, Professor Auxiliar, FCUP



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Resumo

O sensoriamento remoto é uma ferramenta eficiente e de baixo impacto, particularmente útil para obter dados em habitats de difícil acesso e sensíveis. Portanto, ele tem um grande potencial para o monitoramento e conservação de ecossistemas de dunas. Imagens aéreas tiradas com câmeras multiespectrais podem fornecer informações para a identificação de diferentes espécies de vegetação. Este trabalho investiga e compara o desempenho de imagens multiespectrais coletadas em diferentes resoluções espaciais (2.5, 5 e 10 cm de Distância de Amostragem Terrestre) para a identificação e estimativa de biomassa de *Carpobrotus edulis*, uma das espécies invasoras mais proeminentes nos ecossistemas de dunas portuguesas. Uma Aeronave Não Tripulada (Drone) e um avião com câmeras multiespectrais de cinco e quatro bandas, respectivamente, foram usados para capturar imagens das dunas de areia da restinga do Rio Cávado. Simultaneamente, amostras de campo de *C. edulis* foram coletadas para quantificação da biomassa. Uma classificação supervisionada, usando o algoritmo Random Forest, foi aplicada e otimizada (através de filtros) para estimar a área total de *C. edulis*. A validação com base em amostras aleatórias mostrou altas precisões para a espécie-alvo, com F1 Scores de 84.5, 80.5 e 81.1, e precisões globais de 89.5%, 85.3% e 88.6%, para as análises de imagem com GSD de 2.5, 5 e 10 cm, respectivamente. Para estimar a Biomassa Acima do Solo (AGB) de *C. edulis*, nove índices de vegetação (VIs) foram relacionados com a biomassa seca de *C. edulis*, usando quatro modelos de regressão. Todos os modelos de regressão obtiveram significância estatística (valor $p < 0.0001$), com um modelo de regressão exponencial apresentando o melhor ajuste para os diferentes VIs, para todas as resoluções. O Índice de Vegetação com Diferença Renormalizada (RDVI) apresentou o melhor ajuste para os dados com GSD de 2.5 cm ($R^2 = 0.87$; Erro Médio Quadrático Normalizado (NRMSE) = 0.09), o Índice de Vegetação com Diferença Normalizada Melhorada (ENDVI) apresentou o melhor ajuste para os dados com GSD de 5 cm ($R^2 = 0.77$; NRMSE = 0.12), e o Índice de Clorofila Verde (GCI) apresentou o melhor ajuste para os dados com GSD de 10 cm ($R^2 = 0.64$; NRMSE = 0.15). Com base nesses resultados, foram obtidas estimativas da AGB total na área, discutidas e comparadas. A estimativa final de AGB pode ser usada em planos de monitoramento e gerenciamento, especialmente em campanhas de remoção.

Palavras-chave: Imagens multiespectrais, imagens aéreas, veículos aéreos não tripulados, espécies invasoras, índices de vegetação, biomassa acima do solo; QGIS

Abstract

Remote sensing is an efficient and low-impact monitoring tool, particularly useful to obtain data in less accessible and sensitive habitats. It has therefore great potential for dune ecosystem monitoring and conservation. Aerial images taken with multispectral cameras can provide information for the identification of different vegetation species. This work investigates and compares the performance of multispectral imagery collected at different spatial resolutions (2.5, 5 and 10 cm Ground Sample Distance - GSD) for the identification and biomass estimation of *Carpobrotus edulis*, one of the most prominent invasive alien species in Portuguese dune ecosystems. An Unoccupied Aircraft System (UAS) and an Airplane with five-band and four-band multispectral cameras, respectively, were used to capture images from the sand dunes of the Cávado River spit. Simultaneously, field samples of *C. edulis* were collected for biomass quantification. A supervised image classification, using the Random Forest algorithm, was applied and optimized (through sieve filtering) to estimate the total area of *C. edulis*. Validation based on random samples, showed high accuracies for the target species, with F1 Scores of 84.5, 80.5 and 81.1, and Overall Accuracies of 89.5%, 85.3% and 88.6%, for the 2.5, 5 and 10cm GSD image analyses, respectively. To estimate the Above-Ground Biomass (AGB) of *C. edulis*, nine vegetation indices (VIs) were related to *C. edulis* DW, using four regression models. All regression models obtained were significant (p -value < 0.0001), with an exponential regression model presenting the best fit for the different VI, for all resolutions. The Renormalized Difference Vegetation Index (RDVI) presented the best fit for the 2.5 cm GSD data ($R^2 = 0.87$; Normalised Root Mean Square Error (NRMSE) = 0.09), the Enhanced Normalized Difference Vegetation Index (ENDVI) presented the best fit for the 5 cm GSD data ($R^2 = 0.77$; NRMSE = 0.12), and the Green Chlorophyll Index (GCI) presented the best fit for the 10 cm GSD data ($R^2 = 0.64$; NRMSE = 0.15). Based on these results, estimates of the total AGB in the area were obtained, discussed and compared. The final AGB estimate can be used for monitoring and management plans—namely, removal campaigns.

Keywords: Multispectral images, aerial images, unmanned aerial vehicles, invasive species, vegetation indices, above-ground biomass; QGIS.

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List of Abbreviations

CIIMAR	INTERDISCIPLINARY CENTRE OF MARINE AND ENVIRONMENTAL RESEARCH
DJI	DA-JIANG INNOVATIONS (THE COMPANY THAT MANUFACTURES THE PHANTOM 4 UAV)
DVI	DIFFERENCE VEGETATION INDEX
DW	DRY WEIGHT
ENDVI	ENHANCED NORMALIZED DIFFERENCE VEGETATION INDEX
F1 SCORE	A MEASURE OF A TEST'S ACCURACY THAT CONSIDERS BOTH PRECISION AND RECALL
GCI	GREEN CHLOROPHYLL INDEX
GDVI	GREEN DIFFERENCE VEGETATION INDEX
GEBCO	GENERAL BATHYMETRIC CHART OF THE OCEANS
GCP	GROUND CONTROL POINTS
GCI	GREEN CHLOROPHYLL INDEX
GNDVI	GREEN NORMALIZED DIFFERENCE VEGETATION INDEX
GNSS	GLOBAL NAVIGATION SATELLITE SYSTEMS
GPS	GLOBAL POSITIONING SYSTEM
ICNF	INSTITUTO DA CONSERVAÇÃO DA NATUREZA E DAS FLORESTAS
KAPPA	KAPPA STATISTIC
NDVI	NORMALIZED DIFFERENCE VEGETATION INDEX
NIR	NEAR INFRARED
NGA	NATIONAL GEOSPATIAL-INTELLIGENCE AGENCY
NRMSE	NORMALIZED ROOT MEAN SQUARE ERROR

OA	OVERALL ACCURACY
PA	PRODUCER ACCURACY
PNLN	PARQUE NATURAL DO LITORAL NORTE
PRI	PHOTOCHEMICAL REFLECTANCE INDEX
RDVI	RENORMALIZED DIFFERENCE VEGETATION INDEX
RENEP	REDE NACIONAL DE ESTAÇÕES PERMANENTES
RGB	RED GREEN BLUE
R^2	COEFFICIENT OF DETERMINATION
RTK	REAL-TIME KINEMATIC
RVI	RATIO VEGETATION INDEX
SE	STANDARD ERROR
SIO	SCIENTIFIC INTERNATIONAL OCEANOGRAPHY
UAS	UNOCCUPIED AERIAL SYSTEMS
UAV	UNOCCUPIED AIRCRAFT VEHICLE
UA	USER ACCURACY
VI	VEGETATION INDEX
WW	WET WEIGHT

INTRODUCTION

Ocean sandy beaches are a well-known environment, it represents over two-thirds of nonfrozen coastlines (McLachlan & Brown, 2006) and can be found worldwide. As an interface between land and sea, it is a dynamic ecotone with various species and ecosystem services (Vousdoukas et al., 2020). Among the many essential ecosystem services provided by sandy beaches, we can cite food supply, sediment storage, wave energy dissipation, breakdown of organic materials and pollution, water filtration, water storage in dune aquifers and groundwater, nutrient mineralisation and recycling, scenic views and the creation of a link between terrestrial and marine environment (Defeo et al., 2009). This rich and important environment is however under constant pressure driven by meteorological and anthropogenic factors (Luijendijk et al., 2018). It is on this interface, between land and sea, that the coastal dune ecosystem is located.

Coastal dunes are defined as aeolian landforms that develop in coastal areas with sufficient wind and sediments and are stabilised with the support of vegetation near the shoreline (Calvão et al., 2013). Dunes constitute a natural barrier between land and sea, protecting the inland from winds and flood associated risks (Van der Biest et al., 2017), controlling coastal erosion and even, to some degree, mitigating impacts of sea-level rise (Say & Py, 2007) even though sediment shortage and sea-level rise accelerate coastal erosion (Finkl, 2013). More than only a Physical barrier, coastal dune environments present a significant biodiversity and can host habitats for a variety of species (Martínez & Psuty, 2004).

Just as sandy beaches, coastal dunes occur worldwide, they are part of many different biomes and constitute a wide variety of ecosystems. Nonetheless, they only represent a tiny percentage of land cover (Martínez & Psuty, 2004). Due to increasing pressures from anthropogenic activities and unsustainable use, many dune systems are heavily degraded from overexploitation of their natural resources and ecological services and from landscape transformations (Finkl, 2013).

Coastal dunes are commonly explored in many ways for many different reasons. Generically, the direct human impact can be characterised according to four distinct forms: conversion, removal, use and external (French, 2002). Conversion is the direct conversion of the ecosystem into other land uses. It can be due to urbanisation, agricultural expansion or, for instance, the construction of a new golf court. Removal is the actual activity of removing the dune, usually associated with sand mining. Use is

associated with the economic exploitation of the environment without removing the dune, e.g. for tourism and recreation, water extraction or military training. External impacts are related to natural or anthropogenic changes in the surrounding territories that can affect the dune at some point, such as changes in sediment supply, coastal defence interventions and invasive species.

Because of the unique position between land and sea, the vegetation of the dune environment occupies a very distinct and sensitive habitat, which results in a high number of endemic species specially adapted to live in this high-gradient environment (Honrado et al., 2010; Martins et al., 2014). The vegetation community distribution is highly related to the gradient of diverse physic-chemical variables (sea distance, salinity, wind velocity, humidity, pH, grains size, sand stability, etc.), ultimately strongly affecting the species distribution on the dune profile (Frederiksen et al., 2006; Honrado et al., 2010; Bakker et al., 2016). Even though the vegetation community is related to the physical-chemical factors, there has yet to be a consensus on the actual factors causing this sectorial distribution, mainly due to the lack of a conclusive covariance and a large amount of interaction between environmental factors (Dech & Maun, 2005; Forey et al., 2008; Honrado et al., 2010).

The dune's native vegetation plays a central role in dune stabilisation, sand fixation and erosion protection (Lomba et al., 2008). As dune vegetation was removed for the construction of infrastructure and agriculture, at the same time, invasive species were introduced and found fertile new ground to develop (Soares et al., 2002). A prominent invasive alien species in European dunes is the *Carpobrotus edulis* (Campoy et al., 2018).

Carpobrotus edulis is a perennial creeping plant capable of forming mats with individual steams spreading up to 2 meters long (Figure 1). It is a member of the Aizoaceae family with succulent leaves characterised by having a cross-section shaped like an equilateral triangle. Its flowers can vary from yellow to pink. It produces a fleshy fruit of approximately 35mm, shaped like a fig. The species name comes from ancient Greek and Latin, with Karpos, from ancient Greek, meaning "fruit", and brotus, also from Greek, meaning "edible". Edulis, in its turn, means "edible" in Latin.

The *Carpobrotus* genus has approximately 25 identified species, mainly from South Africa, Australia, and New Zealand. *Carpobrotus edulis* is native to South Africa and is reported to grow on coastal and inland slopes with low altitudes. It has been grown in Europe since the seventh century and was initially introduced as an ornamental plant; it

was later used in some sites for soil and dune stabilisation (Preston, 1988; Campoy et al., 2018). It was used and naturalised in many different locations in Europe and probably introduced multiple independent times, with the first record on Portuguese land registered in 1936 in Figueira da Foz (Faulkner et al., 2017). Still, it was only since the 1980s that the records on it increased, indicating that it was either spreading or the interest in the invasive species was rising (Campoy et al., 2018).

Carpobrotus is now found in all coastal areas of the Iberic Peninsula and on Europe's Atlantic coast it can be found up to northern France, Great Britain and most of the Mediterranean (Campoy et al., 2018). It is reported on coastal habitats with rocky and sandy cover and on the central part of coastal dunes zones (Carranza et al., 2010). In Portugal, it can be found either in the shaded pre-forest environment or in coastal areas fully exposed to the sun (Traveset et al., 2008).

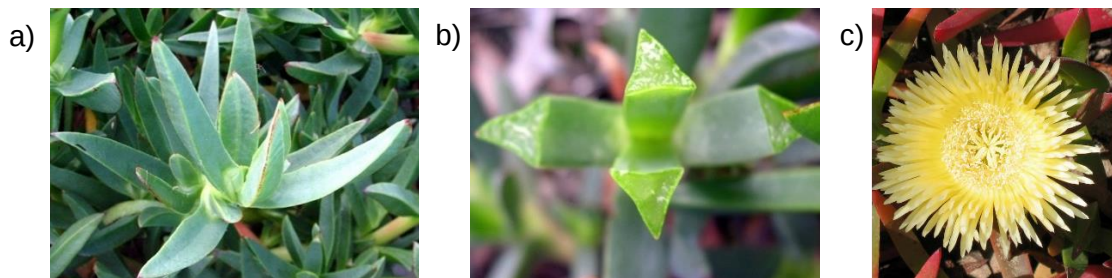


Figure 1 - a) *C. edulis* leaves; b) *C. edulis* leaf section; c) *C. edulis* flower - Source: flora-on (<https://flora-on.pt/#1Carpobrotus>) accessed on 04/07/2023

Many examples in the literature describe various important changes in the environments invaded by *C. edulis* and its negative impact on native flora (Carranza et al., 2010; Santoro et al., 2012; Fried et al., 2014; Rumlerová et al., 2016). It competes with native plants for water, space, and sunlight, reducing and suppressing their growth and reproduction (Molinari et al., 2007; Conser & Connor, 2009). The *C. edulis* dead matter can also affect the soil's physic-chemical and biological parameters, adding more carbon to the substrate and possibly altering the germination and survival dynamics of other native species (Conser & Connor, 2009; Vieites-Blanco & González-Prieto, 2018).

Given the many negative impacts of *C. edulis* invasive species, it is vital to find a viable way to monitor its spread and development along the coastline to prevent the loss of habitat of native flora. There are many alternatives to do such a task, with some of them being more work, economically or environmentally intensive than others. As a non-invasive and (almost) non-destructive method, remote sensing monitoring can provide a viable alternative for this task.

Remote Sensing can be defined as the collection and interpretation of information about an object, area, or event without being in physical contact with the object (Kairu, 1982). Typically, it uses satellites or aircrafts, but in recent years Unoccupied Aerial Systems (UAS) have also been widely used (Toth & Jóźków, 2016). The method allows us to study and monitor various aspects of the Earth's surface and atmosphere without direct physical contact. Remote sensing can also use other static equipment and be applied to underwater and underground environments, but this work will not address these topics.

Remote sensing platforms can carry many different types of sensors that can be categorized in various forms. This study will focus on passive imaging sensors, specifically multispectral cameras. Four main types of resolution must be addressed when considering multispectral cameras sensors: spectral, radiometric, spatial and temporal resolutions.

Spectral resolution refers to the ability of a passive sensor to distinguish different wavelengths of electromagnetic radiation. A higher spectral resolution enables the identification and differentiation of various objects or materials based on their pattern of reflected electromagnetic radiation (spectral signature) (Di Gennaro et al., 2022). It can be defined by the number of bands of a sensor.

Radiometric resolution relates to the sensor's sensitivity in capturing and quantifying the intensity of electromagnetic radiation (Verde et al., 2018). It determines the sensor's ability to detect and differentiate subtle differences in the reflectance energy levels. It can be defined by the number of bits in which the reflectance is recorded.

Spatial resolution can be divided into two aspects. (i) The level of detail in which a sensor can distinguish between individual objects or features within an image. It is usually measured by the pixel size at ground level (Ground Sample Distance - GSD). (ii) The Instantaneous Field of Vision (IFOV), defined by the area viewed by a sensor in a given instant time (Star & Estes, 1990). A higher spatial resolution provides finer details and allows for better identification, characterization, and monitoring of objects on the ground. The field of vision can also influence the time need for survey a specific area.

Temporal resolution addresses the frequency or interval at which a sensor revisits a specific area over time. It determines the sensor's ability to monitor and capture changes on the Earth's surface. Temporal resolution enables tracking temporal phenomena, such as vegetation growth, land cover changes, or weather patterns.

The resolutions may vary a lot from sensor to sensor, and selecting the correct sensor to fit in the monitoring objective is vital for the quality of the final result of a observation. Even though all types of resolutions can influence the outcome of a study, some are more relevant for specific analysis, depending on the study object.

One of the most common analysis made with multispectral cameras is the classification of land cover (Foody, 2002). It involves categorising and mapping different land cover types, such as forests, croplands, water bodies, urban areas etc. Accurate land cover information is used in various applications, including urban planning, natural resource assessment, and environmental management. There are different approaches to generate classification cover maps, such as pixel-based classification, sub-pixel image classification and object-based classification (Phiri & Morgenroth, 2017). Each of these approaches can use multiple mathematical algorithms for classifying multispectral images. This study will focus on a pixel-based approach for image classification, defined as the process of assigning each pixel to a class by treating each pixel as an individual unit (Newman et al., 2011). Pixels within a class are more spectrally similar to one another than those in other classes (Phiri & Morgenroth, 2017).

Another important piece of information that can be extracted from multispectral images are Vegetation Indices (VIs), which are algebraically calculated using the radiation value of each band (Jang et al., 2020). Its value can be used in designing a model for estimating biophysical and biochemical traits, such as health status, chlorophyll contents, water stress, vegetation vigour, above-ground biomass and carbon content (Candiago et al., 2015).

As fundamental as the sensor resolutions, ensuring accurate positional data of the studied object is equally crucial in remote monitoring. The accuracy of the determined position has drastically increased, since the ancient celestial navigation methods, and the first electronic navigation based on radio beacons from the 1930's, both with accuracies of several miles. The first operational positioning satellite systems were launched in the 1960' and had an accuracy within 200 m. Nowadays there is a network of more than 130 satellites (Jin et al., 2022) to provide accurate positioning, being the main Global Navigation Satellite Systems (GNSS) used: Europe's Galileo, the USA's NAVSTAR Global Positioning System (GPS), Russia's Global'naya Navigatsionnaya Sputnikovaya Sistema (GLONASS) and China's BeiDou Navigation Satellite System. Depending on the receiver (the GNSS antenna) these provide accuracies of 2 to 10 m, which can be increased to 1 or 2 cm when a base station is used in combination.

Modern GPS/GNSS methods, particularly those using RTK (Real-Time Kinematics), allow surveys with precise positioning, with data collected both rapidly and at high spatial resolution (Barnard & Warrick, 2010; Rocha et al., 2009). RTK is a high-precision satellite navigation technique used in geodetic and surveying applications. It provides centimeter-level accuracy by measuring the carrier phase of GPS signals in real-time.

This work has two main objectives. Firstly, it seeks to assess the effectiveness of using multispectral cameras mounted on Unoccupied Aerial Vehicles (UAV) and planes for accurately identifying a particular invasive plant - *C.edulis*. Secondly, the thesis aims to explore the integration of Vegetation Indices (VIs) to estimate the Above-Ground Biomass (AGB) of the *C.edulis* plants within the designated study area. This research combines advanced remote sensing techniques with ecological insights to enhance our understanding of invasive plant monitoring biomass estimation and compare the capability of different remote sensing multispectral equipment, in estimating the AGB of *C. edulis* in the study area.

1. Methodology

The present work comprises a preliminary study, carried out to test and optimize the procedures, and a final study in which the optimised methodology is applied to the final datasets collected at different resolutions. Preliminary tests before the final field campaign were essential to ensure good data quality. Since this investigation required three flights over the study area, one UAS survey and two plane surveys at different heights, with the smallest temporal difference possible, these tests were vital to reduce errors, costs and processing hours. Insights from the preliminary study are presented to aid future researchers of the topic facing similar challenges and to justify the final chosen methods. Results of the preliminary study are only summarized in the Appendix A.

Notice that the development and testing of the methodology resulted in a scientific publication (Meyer et al., 2023) and in several participations in national and international conferences.

1.1 Study area

Method improvement

To test the methodology, two preliminary surveys took place in the Reserva Ornitológica de Mindelo (ROM). Located between Mindelo (41°18'54.648"N : 8°44'21.412"W) and the Ave River (41°20'27.463"N : 8°44'41.309" W), in the municipality of Vila do Conde. This natural reserve which was the first protected area in Portugal, created in 1957, was selected for two main reasons: its proximity to the Interdisciplinary Centre of Marine and Environmental Research (CIIMAR) and the existence of a relevant dune ecosystem with a prominent area invade by *C. edulis*.

The study area selected for the final work was the Parque Natural do Litoral Norte (PNLN) (Figure 2). It was officially created in 2005 by the Regulatory decree nº 6/2005 on July 21th, which reclassified the previous Protected Landscape of Esposende Littoral created by Law-decree nº 357/87 on November 17th. It spreads along 16km of the coast, located between the Neiva estuary (41°36'46.56"N, 8°48'32.55"W) and the south border of Apúlia (41°28'10.68"N, 8°46'31.30"O), extending its area 5km into the sea. Covering a total area of 8887 ha, of which 7653 ha are marine areas. The PNLN is administered by the national nature and forest conservation institute (Instituto da Conservação da Natureza e das Florestas – ICNF).

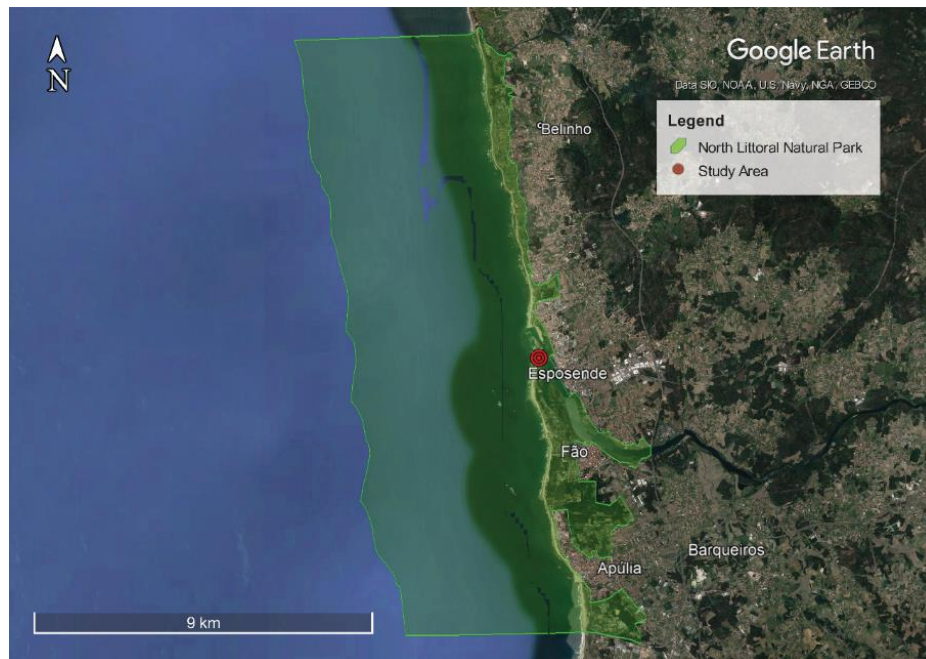


Figure 2 - North Littoral Natural Park and Study Area. - Source: Google Earth – SIO, NOAA, U.S. Navy, NGA, GEBCO

The protection area was created to protect the littoral of Esposende, preserve its natural resources and elements, and promote rational use of the site. With its mainland consisting essentially of a strip of sandy shores, it houses 15 different habitats described in the Habitat Directive, of which four of them are marked as priority habitats:

1150 - Coastal lagoons

2130 - Fixed coastal dunes with herbaceous vegetation ('grey dunes')

2270 - Wooded dunes with *Pinus pinea* and/or *Pinus pinaster*

91E0 - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)

Almost all in-land park area is located under the 10-metre quota, with only some dunes between the 10 and 20 metres. Many water courses are disemboguing into the Atlantic Ocean through the sand stripe, but the two most relevant are the Neiva and Cávado river, with the second being the biggest river in the park.

There are 240 different vegetation species identified on the NLNP, with most of them native to the north Iberic littoral, including some endangered species. This native vegetation is vital for preserving morphological and biotic characteristics (Gomes et al., 2002). Twelve invasive vegetation species were identified within the flora, with the most prominent ones considered the *Acacia longifolia* and *Carpobrotus edulis*, which pose significant pressure on the dunar habitats (Conser & Connor, 2009).

Like many other coastal environments, the dunes on the estuary of Cávado suffer not only from erosion risks but also from the constant pressure of climate change, urbanisation, recreation trampling and invasive species (Soares et al., 2002). Studying and monitoring the Invasive species in the park can play a crucial role in preserving the PNLN habitats. The present study aims to identify the area and estimate the *Carpobrotus edulis* Above-Ground Biomass (AGB) on the sandbanks of the estuary of the Cávado River (marked in red in Figure 3).



Figure 3 - Study Area - Source - Google Earth

1.2 Methodology workflow

The applied methodology follows the workflow show in Figure 4 and is further detailed in the following sections. The study is divided into three phases: *In situ* work, including fieldwork and remote sensing (section 2.3), Laboratory Work (section 2.4), and Imagery processing (section 2.5).

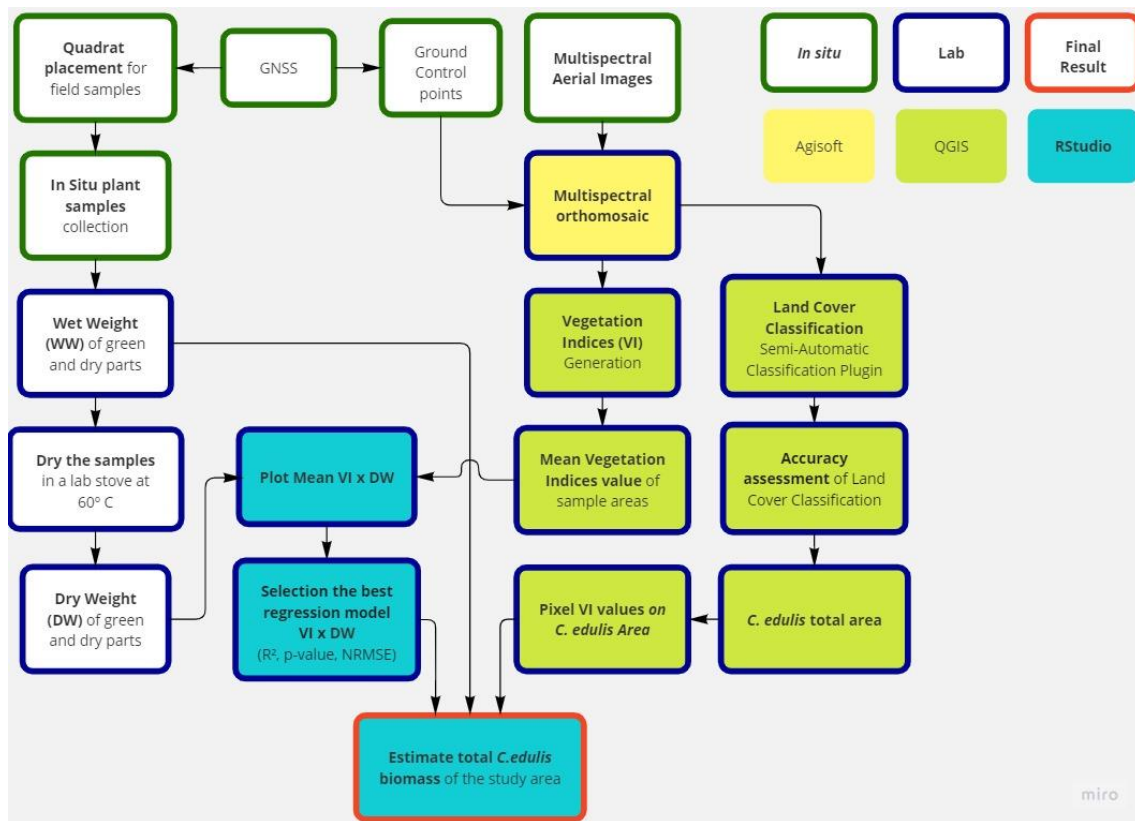


Figure 4 – Methodology Workflow - Source - Author1.3 In Situ work

1.3.1 Quadrat placement

The first activity in the field is the placement of the quadrat to delimit the sample areas. In the first preliminary survey, ten 50x50 cm quadrats were distributed in the study area. Figure 5 a and b show how these quadrats appear in the aerial images. These quadrats have an essential role in identifying the sample areas; they are georeferenced with a GNSS antenna (NovAtel GPS-702-GG) in Real Time Kinematic (RTK) mode with fixed-station corrections using the RENEP base station network, and since they can be identified on the aerial images, they are used as visual marks for the samples.

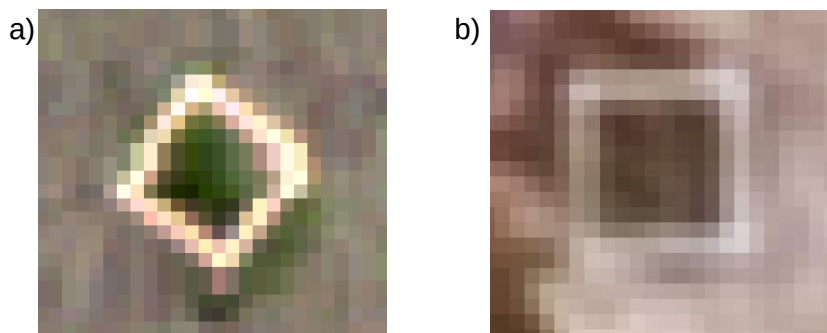


Figure 5 – Aerial image of aquadrat unaligned with the image pixels (a) and quadrat aligned with the image pixels (b)

However, this methodology presented three main problems: (i) The reflectance of the quadrat contaminated the surrounding pixels, which affects the calculation of Vegetation Indices (VI); (ii) the reflectance interference varies with quadrat colour, with the bright with quadrats causing the most interference; (iii) the quadrats unaligned with the image pixels presented a very irregular reflectance interference.

To try to solve these problems, a new fieldwork was carried out without using quadrats. As the GNSS antenna with ground correction had an error of only 2.5 cm, it could be possible to georeference the sample areas in the images, without the quadrat visual aid (Figure 6).

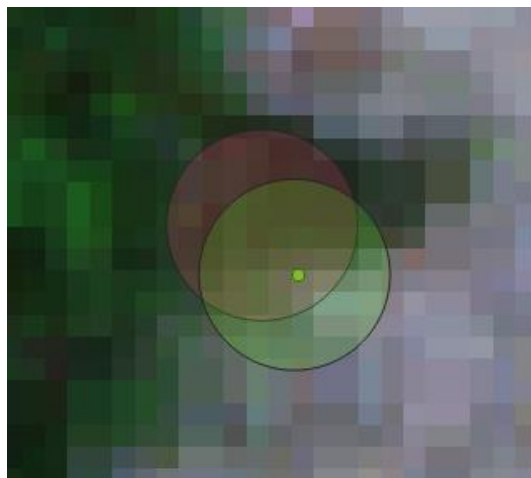


Figure 6 – Sample area using only GNSS antenna with ground correction. In purple is the probable sample area, and in green is the area located with the antenna.

Figure 6 shows the difference between the probable sample area (purple) and the registered sample (green). It was considered a “probable” sample area because there was no way to locate the sample area precisely, but it is known that all the samples were taken from a *C. edulis* covered area. The image clearly shows that the georeferenced area (green) covers a considerable area of bare soil.

Another identified problem was the number of samples. In the first studies, only ten samples were collected, which led to poor results on the correlation between AGB and Vegetation Indices (VI) (See Appendix A)

Therefore, in the final adopted method, thirty quadrats were placed with a north-south orientation over *C. edulis* vegetation with different, visually identified biomass and healthiness characteristics. The north-south orientation reduces the reflectance interference and, consequently, the number of pixels occupied by the quadrat on the image. All the quadrats were placed in areas containing only *C. edulis*. This designed

distribution was made to cover a more extensive biomass sample spectrum to optimise the Dry Weight (DW) x Vegetation Indices (VI) regression models.

1.3.2 Ground control points

Additional Ground Control Points (GCP) were marked on the study area with spray paint. These points were georeferenced with the GNSS antenna and later used, in combination with the georeferenced samples quadrats, to enhance the orthomosaic geometry and geolocation precision during imagery processing.

1.3.3 Multispectral aerial images

Once the quadrats and ground control points were in place, marked, and georeferenced, the aerial photography process was conducted.

During the first preliminary survey, in the ROM, only one image set was collected.

- 1- Multispectral images taken with a MicaSense RedEdge-MX with five bands, transported by a DJI M200 Unoccupied Aerial Vehicle (UAV). The flight was made at 30m height, providing a Ground Sample Distance (GSD) of 2.5 cm.

The second preliminary field survey used two sets of images,

- 1- MicaSense RedEdge-MX camera and GSD of 2.5 cm (same as the first survey).
- 2- RGB images taken with a built-in camera transported by a PHANTOM 4DJI UAV. The flight was made at 30m height, providing a GSD of 1.0 cm.

The use of a second image set proved to be very important. Even though a GSD of 2.5 cm is a very high resolution for a remote-sensing study, it was not always sufficient to identify the landcover class in the images. A finer scale image provided a resource to safely identify the cover class during classification and accuracy assessment.

The final survey, in the PNLN used four different image sets (Table 1):

1. MicaSense RedEdge-MX camera and GSD of 2.5 cm (same as the first and second survey).
2. Built-in PHANTOM 4DJI UAV camera with GSD of 1.0 cm (same as second survey).
3. Multispectral images taken with an UltraCam Falcon f100 mark 1 with four bands, carried by Airplane, providing a GSD of 5 cm.
4. Multispectral images taken with an UltraCam Falcon f100 mark 1 with four bands, carried by Airplane, providing a GSD of 10 cm.

Platform	Sensor	GSD	Bands
UAV DJI Phantom 4	Built IN	1 cm	R, G, B
UAV DJI M200	Micasense RedEdge - MX	2.5 cm	R, G, B, REdg, NIR
Airplane	UltraCam Falcon f100 m1	5 cm	R, G, B, NIR
Airplane	UltraCam Falcon f100 m1	10 cm	R, G, B, NIR

Table 1 – Flights and sensor information. R-Red ; G-Green ; B-Blue ; REdg-Red Edge ; NIR-Near Infrared

As in the second study, the RGB camera from PHANTOM 4DJI UAV was only used to provide a more reliable cover identification. The other three sets of images were assessed on their capability of identifying and predicting the AGB of *C. edulis*.

1.3.4 In situ plant samples collection

The preliminary study showed that the reflectance of the quadrats contaminated part of the image pixels inside the quadrat (Figure 5). Therefore, in the final study, only the central part of the quadrats was sampled and analysed. After the aerial survey, all the ABG within the central 30x30 cm² of each of the 30 quadrats was collected, bagged, tagged and taken to the lab for biomass determination. To do so, the smaller quadrat was visually positioned in the centre of the 50x50 cm² quadrat, as shown in Figure 7, and the AGB was cut out with a saw and scissors.



Figure 7 – placement of a quadrat on a *C. edulis* patch, with other vegetation covers visible to the left (various herbaceous species) and in the back (acacia) (a); delimitation of the central 30 × 30 cm² area within a quadrat for AGB removal (b)

1.4 Laboratory work

1.4.1 Wet weight (WW)

After AGB collection, the samples are taken to the lab and processed. It is essential to address that *Carpobrotus edulis* has the characteristic of growing in two distinct layers, an upper layer – which absorbs and reflects the sunlight and is visible in the aerial images – and a lower layer composed of older and dryer stems and leaves that are generally not

visible from above (Figure 8). To obtain an estimate of the hidden dry layer, the two layers of the collected plant material were separated and weighted on a scale to the nearest 0.01g to obtain the wet weight of each layer for each sample.



Figure 8 – brown layer of *C. edulis* after removal of the top green layer.

1.4.2 Sample drying

The preliminary study provided an estimate of the oven space and time needed to dry the samples of AGB. After weighing, green and brown parts were placed in the lab stove at 60°C to be dried. To verify the drying process, the samples containing green parts were weighed daily until they presented no weight difference between two consecutive weightings. In both preliminary studies, it took six days in the lab stove for all samples to achieve constant weight. The samples of the PNLN we therefore dried in the lab oven during seven days.

1.4.3 Dry weight

After drying, all samples were weighted on the same scale to the nearest 0.01g. The dry weight was later used to (i) relate biomass with VIs (section 2.5.3), (i) compare DW and WW, and (iii) assess the biomass ratio between the green and brown parts of the plants..

1.5 Imagery processing

1.5.1 Multispectral orthomosaic

The multispectral and RGB images were processed with Agisoft Metashape Professional version 1.8.3 built 14331 (64bit) to process a dense cloud point and construct a multispectral orthomosaic. The orthomosaic was later used to help determine training areas for the Land Cover Classification.

1.5.2 Vegetation indices generation

In the preliminary study, sixteen VI were evaluated for their ability to estimate the AGB of *C. edulis*. These include previously evaluated VI (Jang et al., 2020) and two additional indices, computed using the QGIS 3.28.3 raster calculator tool to create VI maps for the study area.

Of the sixteen evaluated VI (Appendix A), nine showed a high potential for the estimation the AGB of *C. edulis* ($R^2 \geq 0.75$). These VI (Table 2) were selected for the comparison between different spatial resolution images performances in estimating *C. edulis* AGB.

Index	Formula	Reference
Green Chlorophyll Index (GCI)	$\frac{NIR}{Red} - 1$	(Gitelson, 2005)
Difference Vegetation Index (DVI)	$NIR - Red$	(Richardson et al., 2002)
Green Difference Vegetation Index (GDVI)	$NIR - Gre$	*
Enhanced Normalized Difference Vegetation Index (ENDVI)	$\frac{(NIR - Gre) - 2Red}{(NIR - Gre) + 2Red}$	(Rasmussen et al., 2016)
Green Normalized Difference Vegetation Index (GNDVI)	$\frac{NIR - Gre}{NIR + Gre}$	(Gitelson et al., 1996)
Normalized Difference Vegetation Index (NDVI)	$\frac{NIR - Red}{NIR + Red}$	(Rouse et al., 1973)
Photochemical Reflectance Index (PRI)	$\frac{Gre - Blu}{Gre + Blu}$	(Gamon et al., 1992)
Renormalized Difference Vegetation Index (RDVI)	$\frac{NIR - Red}{\sqrt{NIR + Red}}$	(Roujean & Breon, 1995)
Ratio Vegetation Index (RVI)	$\frac{Red}{NIR}$	(Pearson et al., 1972)

Table 2 - Vegetation indices formulas used in this work. Bands: Blu—Blue; Gre—Green; RDG—Red Edge; NIR—Near Infrared

1.5.3 Mean vegetation indices

To avoid errors due to the interference of the quadrats in the images, in the final study the mean Vegetation Indices values were extracted from each AGB sample collection area. Notice that the pixels used coincided with the 30x30 cm collection area square in

the centre of the 50x50 quadrats. The obtained VI values were later used to assess their correlation with the DW of green parts. Looking at the RDVI representation of a sample area in Figure 9, notice that the 50x50 cm quadrat and its interference with the reflectance of *C.edulis* is clearly visible in the image. The mean VI value of each AGB sample area was obtained using the following equation.

$$\overline{VI}_m = \frac{(\sum_i^n VI_i)}{n} \quad (i)$$

Where:

$\overline{VI}_m \rightarrow$ Mean vegetation of sample area m

$VI_i \rightarrow$ Vegetation Index Value on pixel i

$n \rightarrow$ Number of pixels inside the sample collection area

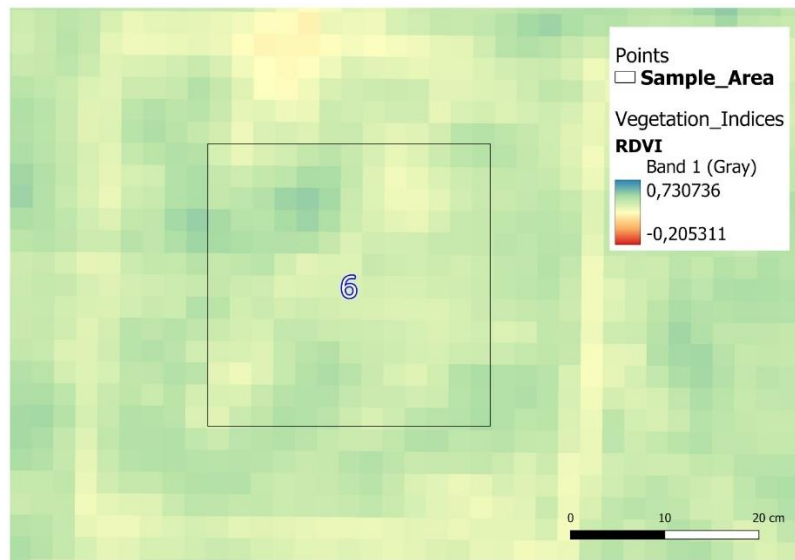


Figure 9 – RDVI Vegetation Index Representation. Source – Author

1.5.4 DW-VI models

Each sample's DW of the green parts (y-axis) was plotted against the mean VI (x-axis) to evaluate the possible correlation between the two parameters.

In the preliminary study, one linear and two exponential regression models were used to evaluate which VI presented the best correlation with the DW of the green parts of the collected plant material. Only the green parts DW was used because of the limitations of the aerial images, which only capture the reflectance of the top layer of *C. edulis*. The best fitting regression model was selected based on the R^2 and p -value. For the final

study comparing spatial resolutions, next to the R^2 and p -value, the selection of the best-fitting regression model also considered the Normalised Root Mean Square Error (NRMSE). Furthermore, an additional exponential regression model was considered.

$$\text{Linear model (lin)} \quad y = a + bx \quad (\text{ii})$$

$$\text{Exponential model 1 (xpo1)} \quad y = ab^x \quad (\text{iii})$$

$$\text{Exponential model 2 (xpo2)} \quad y = ae^{xb} \quad (\text{iv})$$

$$\text{Exponential model 3 (xpo3)} \quad y = ax^b \quad (\text{v})$$

Where:

y = Dry Weight

x = Vegetation Index

a = coefficient 1

b = coefficient 2

The RMSE and NRMSE are calculated as follows:

$$RMSE = \sqrt{\sum_i^n \frac{(\hat{y}_i - y_i)^2}{N}} \rightarrow NRMSE = \frac{RMSE}{y_{max} - y_{min}} \quad (\text{vi})$$

Where:

$\hat{y}_i$ = Predicted Values

y_i = Measured Values

N = Number of Samples

1.5.5 Land cover classification

To evaluate the total area of *C.edulis* cover, different Supervised Land Cover classifications were generated using the multispectral images. In the preliminary study five supervised classification algorithms were tested: Minimum Distance, Maximum Likelihood, Spectral Angle, Support Vector and Random Forest. Their capability of identifying *C. edulis* was measured by the User Accuracy (UA) and Producer Accuracy (PA) of the *C. edulis* class.

The QGIS dzetsaka: Classification Tool Plugin version (3.70) (Karasiak, 2016) was used to perform a supervised classification using the random forest algorithm; all other filters

and accuracy procedures were realized using the QGIS Semi-Automatic Classification Plugin (version 7.10.11) (Congedo, 2021).

The number of cover classes was determined by visual identification of the most relevant covers on the image, and 30 Regions Of Interest (ROI) of 20x20 cm were selected for each class, resulting in approximately 13.000 pixels. In the first approach, the *C. edulis* sample areas were also used as training areas, leading to some problems. Sample areas needed to be selected with visually different coverage and health aspect since they were designed to include the lower and upper end of the DW x VI regression. This approach led to poor classification results for two main reasons: (i) the ROI's spectral signature presented a high standard deviation, making it hard to differentiate *C.edulis* from other vegetation; (ii) The very sparse vegetation in some sample areas resulted in bare soil being included in the *C. edulis* spectral signature. This approach was then discarded, and 30 homogeneous ROIs were created near to the sample areas (Figure 10).

The random forest algorithm, which presented the best performance and accuracy results (Appendix A), was chosen for the classification of the data collected with different resolutions.

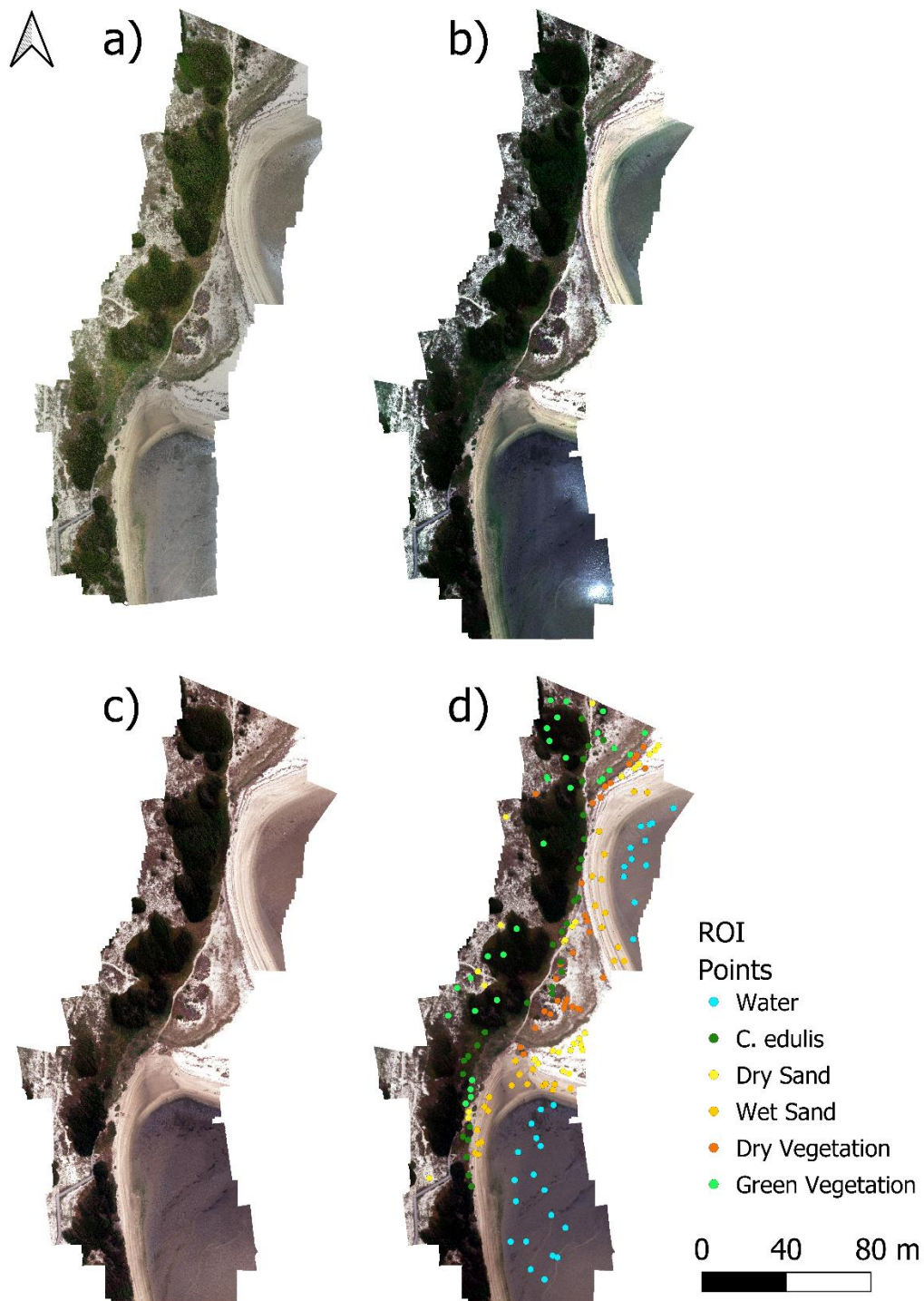


Figure 10 – Othomosaics images - RGB 1cm GSD (a); UAS multispectral 2.5cm GSD(b); Airplane 5cm GSD (c); Airplane 10cm GSD with ROI points marked (d)

Given that a satisfactory classification was achieved with a training area of 13,000 pixels, the number of ROI for the different spatial resolutions was chosen to approximate this number of pixels, maintaining the same amount of information for the classification of the

different resolutions. The number of ROI and training pixels for each resolution is described in Table 3.

GSD (cm)	Individual ROI area (cm)	Pixels per ROI	Number of ROIs per class	Total training pixel count
2.5	20 x 20	64 – 81	30	13269
5.0	20 x 20	16	120	11520
10.0	20 x 20	4	460	11524

Table 3 – ROIs and training pixels count per resolution

The ROI added to the lower resolution orthomosaics were positioned as close as possible to the ROI of the 2.5 cm GSD orthomosaic.

It is important to notice that the ROI of 2.5 cm is also part of the ROI of 5 cm, which, in turn, is also part of the ROI for the 10 cm GSD orthomosaic. Therefore, in Figure 11 a) and b), there is 1 ROI for 2.5 cm GSD, 4 ROIs for 5 cm GSD, and 16 ROIs for 10 cm GSD.

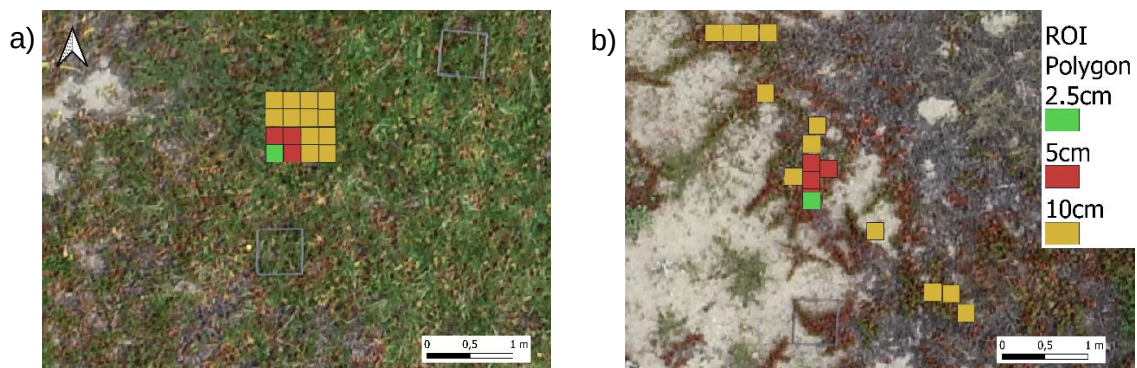


Figure 11 – ROI for *C. edulis* when located over a homogeneous area (a), and located over a non homogeneous area (b).

1.5.6 Accuracy assessment of land cover classification

Accuracy assessment was thoroughly tested in the preliminary study. In a first approach, of the 30 ROI selected for each class, only 25 were used for the classification algorithm. The remaining five were later used on the accuracy test to validate the classification. The accuracy test on these five selected regions resulted in a 100% Overall Accuracy, which is unrealistic for any landcover classification and for any model (Foody, 2002). This result showed that it was necessary to implement another accuracy assessment approach.

In a second approach, the accuracy of each classification was assessed based on a large set of randomly selected pixels, from which the ground truth cover class was

visually identified in the RGB image and compared to the classified class. This comparison resulted in an error matrix for each classification (Appendix A).

The number of pixels for the accuracy test was defined based on the proportion of each class and the expected standard deviation of each class (Olofsson et al., 2014) according to the following equation.

$$N = (\sum_{i=1}^6 (W_i * S_i) / S_0)^2 \quad (\text{vii})$$

N = total number of pixels

W_i = mapped area proportion of class i ;

S_i = standard deviation of class i ;

S_0 = expected standard deviation of overall accuracy;

The coincident overlapping orthomosaic generated from the Phantom 4 DJI flight, with RGB images with 1 cm GSD, was used to allow for a better visual identification. The accuracy results were compared and are presented in Appendix A

The different classifications were evaluated in terms of overall accuracy (OA), calculated by dividing the total number of correctly classified pixels by the total number of selected pixels; the Kappa statistic, which measures the agreement between classification and ground truth pixels - where Kappa = 1 means a perfect agreement while Kappa = 0 means a random agreement; the User Accuracy (UA) for the *C. edulis* class, calculated by dividing the number of correctly classified *C. edulis* pixels by the total number of pixels classified as *C. edulis*; and the Producer Accuracy (PA) for the *C. edulis* class, calculated by dividing the number of correctly classified *C. edulis* pixels by the number of pixels known to be of *C. edulis*.

After the first accuracy test, two filters with progressive strength were applied to reduce the number of small patches of incorrectly classified pixels (considered noise), aiming to improve accuracy. An example of the noise is shown in Figure 13. The sieve filter removes all the raster pixel polygons smaller than a specified threshold size (in pixels), replacing them with their largest neighbouring class. Two types of sieve filters were used (Figure 12): a) only the pixels on the edge of the polygon are considered a neighbour pixel b) all connected pixels are considered neighbours.

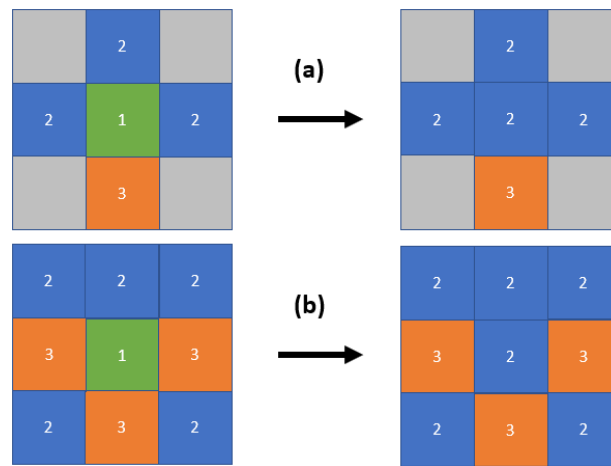


Figure 12 – The two sieve filters types considered. In image, class 1 is substituted by class 2 as the most common neighbour.

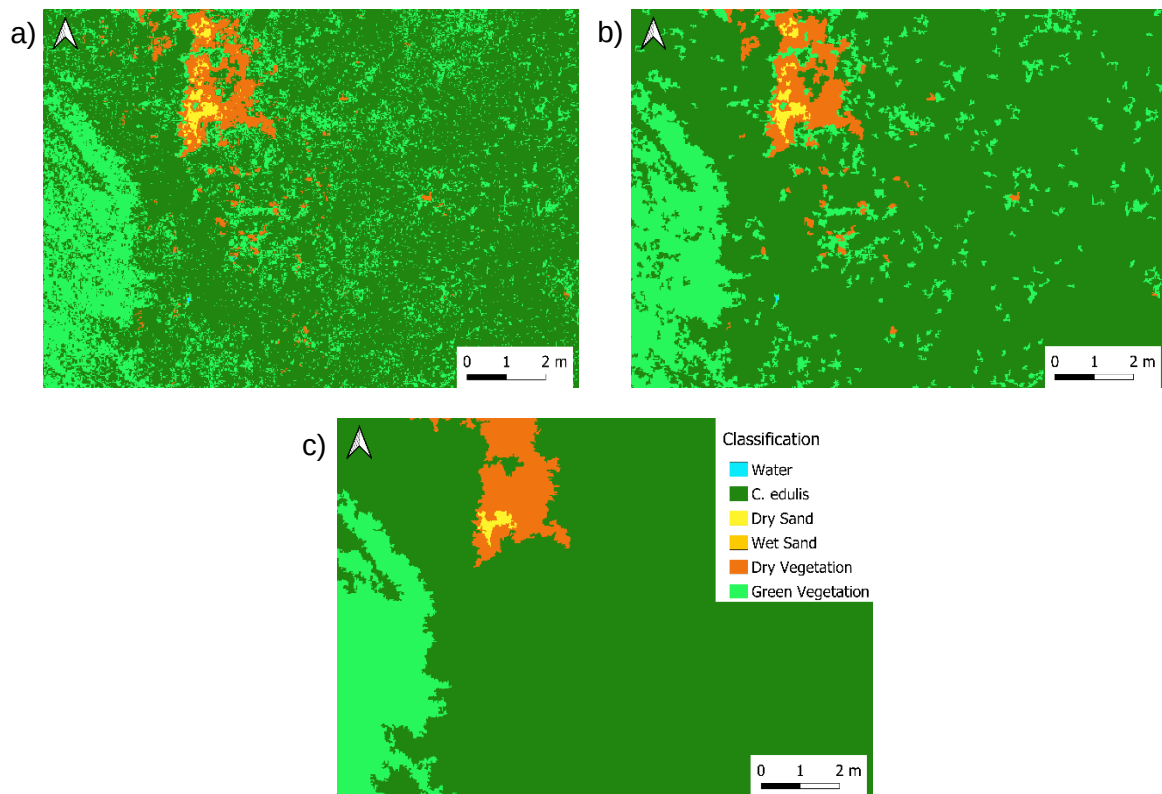


Figure 13 – Example of classification obtained without any filtering, showing the typical noisy class distribution (a); classification after application of a sieve filter with a threshold of 16 (b); classification after application of a sieve filter with threshold of 512 (c)

The two filters were used with progressively larger thresholds (i.e. larger neighbouring areas used to calculate pixel values) to assess the accuracy improvement. The threshold was doubled at every interaction (1, 2, 4, 8, 16, (...), 2048) until the accuracy of the classification stopped increasing and started to fall. The increase in the accuracy with

increasing threshold can be linked to classification noise reduction, but as the filter becomes larger, the classification begins to lose information, reducing the accuracy.

For the final classification, as only the Random Forest classification algorithm was used, the accuracy assessment was based on one set of random pixels for each of the three orthomosaics. After the selection of the random pixels for accuracy assessment, progressively larger sieve filters were applied, again doubling the threshold at every interaction (1, 2, 4, 8, 16, (...), 32.768) until the accuracy of the classification stopped increasing and started to decrease.

1.5.7 *C. edulis* total area

The total area of *C. edulis* was obtained by selecting the land cover classification with the highest F1 score, i.e. considered a harmonic mean between UA and PA.

$$F_1 \text{ score} = 2 \frac{UA \times PA}{UA + PA} \quad (\text{viii})$$

1.5.8 Pixel VI values on *C.edulis* area

The area classified as *C. edulis* was used to create a new VI map containing only pixels classified as *C. edulis* and their respective VI value.

1.5.9 Estimate the total *C. edulis* biomass of the study area

To estimate the total *C. edulis* biomass through the classified area, the following information is necessary, (i) *C. edulis* classified area; (ii) pixel VI values in the *C. edulis* area; (iii) the regression model correlating the VI value with the DW of the green parts of *C. edulis*; (iv) the relation between the DW and WW; (v) the ratio between the WW of green and brown parts.

For the biomass estimation, the pixel VI values were converted to AGB DW, using the best-fitting regression model. This allowed calculation of the green-part DW of each pixel classified as *C. edulis*. After that, the DW was converted to WW, using the previously established WW-DW relationship. Finally, to assess the total weight of *C. edulis* in the study area, an estimate of the brown-part AGB had to be added, since only the green parts have been accounted for in the regression model. This was achieved using ratio between the WW of green and brown parts.

1.5.10 Error consideration

There are different errors to be considered using the proposed methodology: (i) geometric distortions, which depend on sensor perspective and motion, platform stability, terrain relief and the curvature and rotation of the earth (less relevant for small surveyed areas); (ii) sensor errors, causing image deformation; (iii) Classification errors; (iv) Regression model errors; (v) the natural variability of the relationship between DW and WW, largely dependent on environmental and, consequently, plant moisture, which can produce AGB estimation errors; and (vi) the variability in the ratio between green and brown parts, depending on the age and development of the vegetation, which also contributes to AGB estimation errors. For this study, only the (iii) classification and (iv) regression errors were considered in the final AGB estimation. Further discussion can be found in section 2.2.

The classification error (expressed per m^2) is directly related to the accuracy evaluation procedure, from which an area Standard Error (SE) can be extracted. This error was considered in the total AGB estimation, applying the mean VI of the classified area to the estimated area SE (Olofsson et al., 2014).

The RMSE (in kg/m^2) was extracted from the regression model and also considered in the total AGB estimation. To obtain an error for the total AGB, the RMSE per m^2 was multiplied by the classified area.

The two errors were added for a conservative evaluation of the methodology.

2. Results

2.1 Classification results

2.1.1 Cover classification

The cover classes were visually identified in the orthomosaic. Six most relevant covers could be identified: Water, *C. edulis*, Sand, Wet Sand, Dry Vegetation and Green Vegetation. The Dry Vegetation and Green Vegetation classes included all vegetation species in the study area that were not classified as *C. edulis*. The mean spectral signatures of the ROI training areas for each class and for the different image resolutions are displayed in Figures 14, 15 and 16.

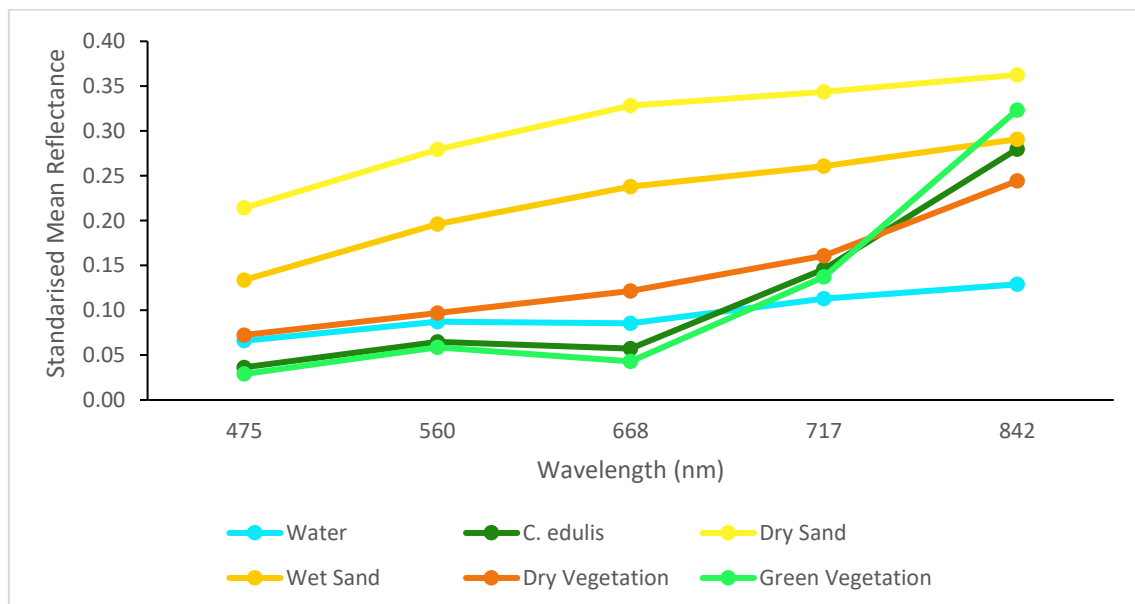


Figure 14 – Mean spectral signatures of the cover class ROIs for the high-resolution UAS survey (GSD 2.5cm)

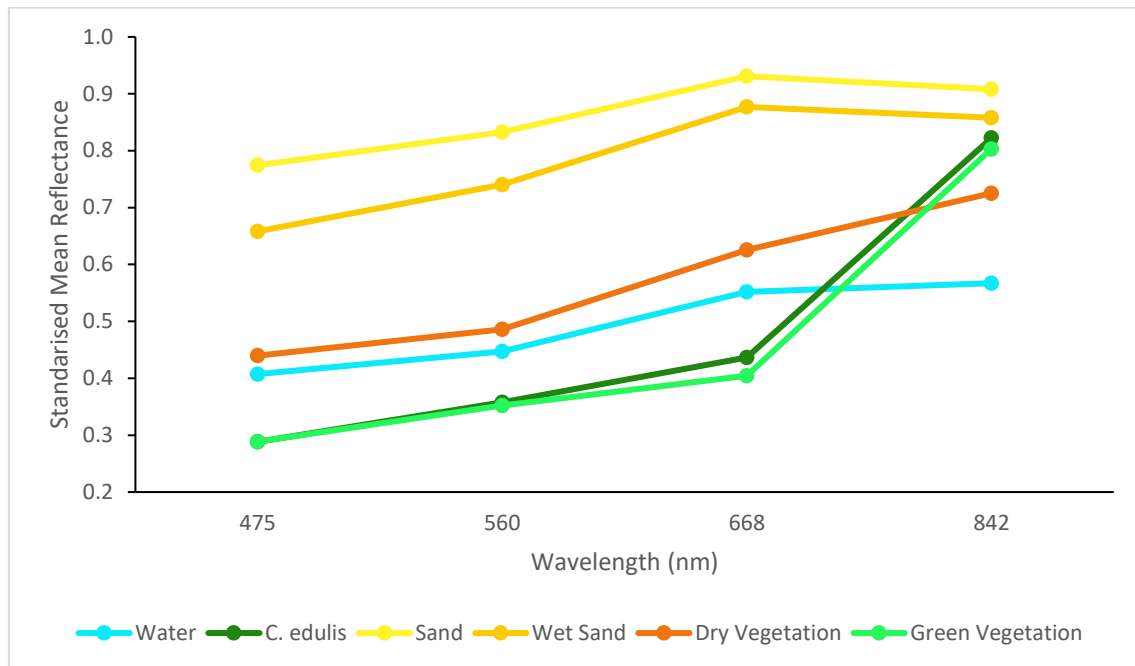


Figure 15 – Mean spectral signature for the cover class ROIs for the medium-resolution airplane survey (GSD 5cm)

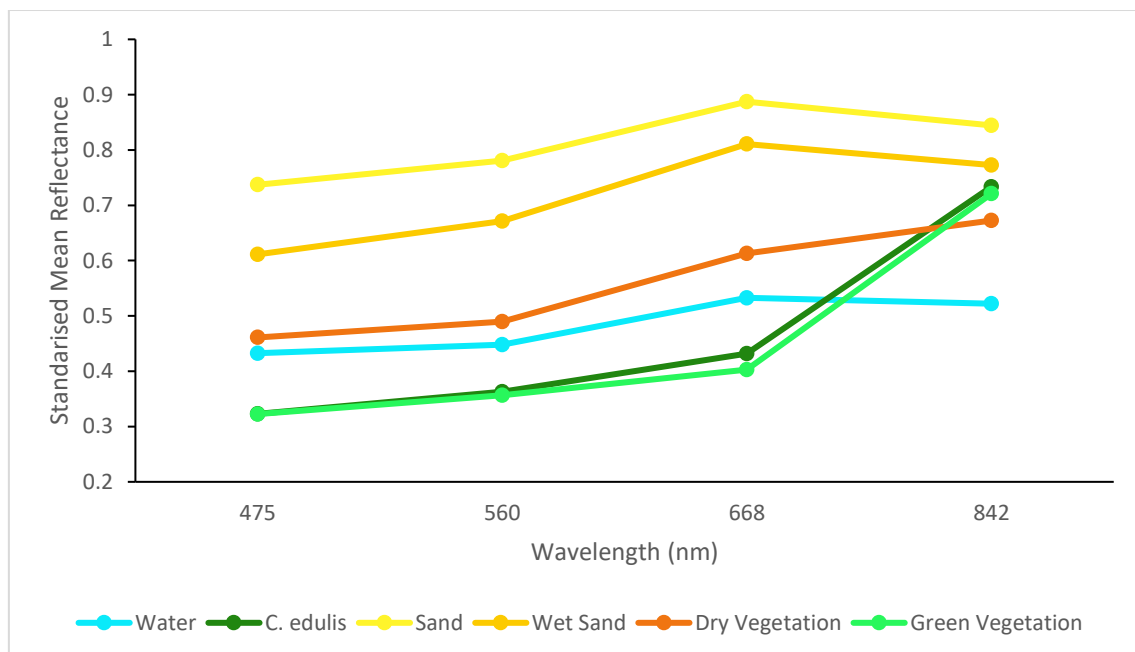


Figure 16 – Mean spectral signature for the cover class ROIs for the lower-resolution airplane survey (GSD 10cm)

The different resolution images resulted in classification with different cover class distributions (Figure 17), with varying proportions, particularly for the of target class *C. edulis* and for green vegetation.

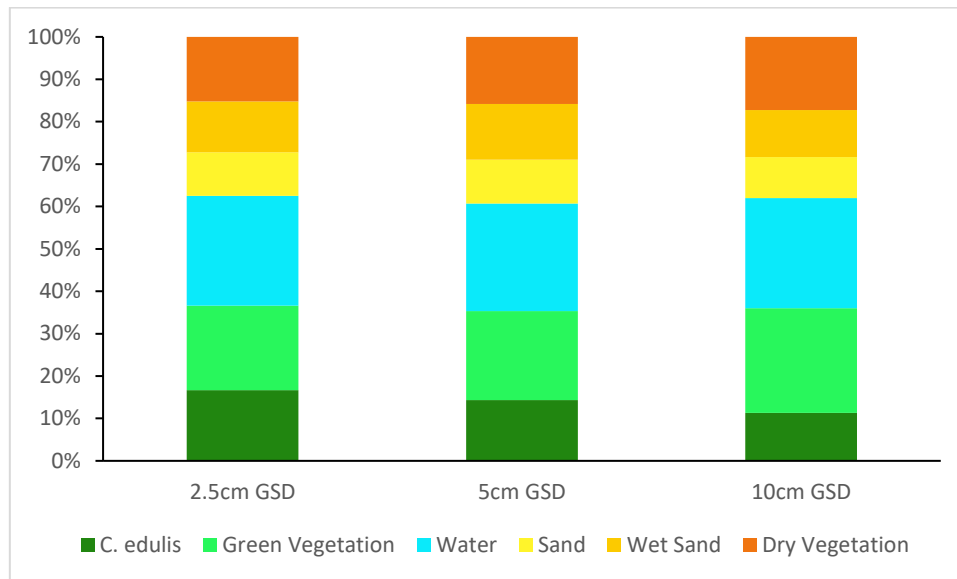


Figure 17 – Cover class distribution for the classification results obtained with each spatial resolution.

2.1.2 Classification accuracy assessment

Applying Equation (vii) for every classification resolution, while accounting for a class-specific standard deviation of 0.3 and an overall accuracy standard deviation of 0.01, resulted in an accuracy assessment using 780 pixels. The following methods of pixel distribution per class can be considered: (i) equal distribution, (ii) area proportional distribution, and (iii) combination of equal and area proportional. For the target class *C. edulis*, the equal pixel distribution method will achieve a more reliable accuracy than the proportional distribution or mixed methods. Since *C. edulis* relative area is one of the smallest (~10%), a proportional distribution would lead to more random pixels being allocated to the larger classes, which would generate a less reliable accuracy for *C. edulis*. Therefore, an equal distribution was applied with 130 randomly generated pixels for each class, enhancing the reliability of accuracy for the study cover.

Accuracy results are presented in Table 4

	2.5cm	5cm	10cm
F1 Score <i>C. edulis</i>	72.6	70.6	75.5
kappa hat	0.827	0.841	0.842
Overall Accuracy	85.9	87.1	87.2

Table 4 – Accuracy of the classification for different resolution without filter

2.1.3 Sieve filter effects

In the post-processing procedure, a sieve filter was applied to investigate if the accuracy could be improved. The different resolution classifications presented overall similar behaviour (Figure 18). In general, as the filter threshold increased, the F1 Score for *C. edulis* increased, while the classification noise decreased, until it dropped significantly, when too much information was lost. This happened at different thresholds for the different imagery resolutions.

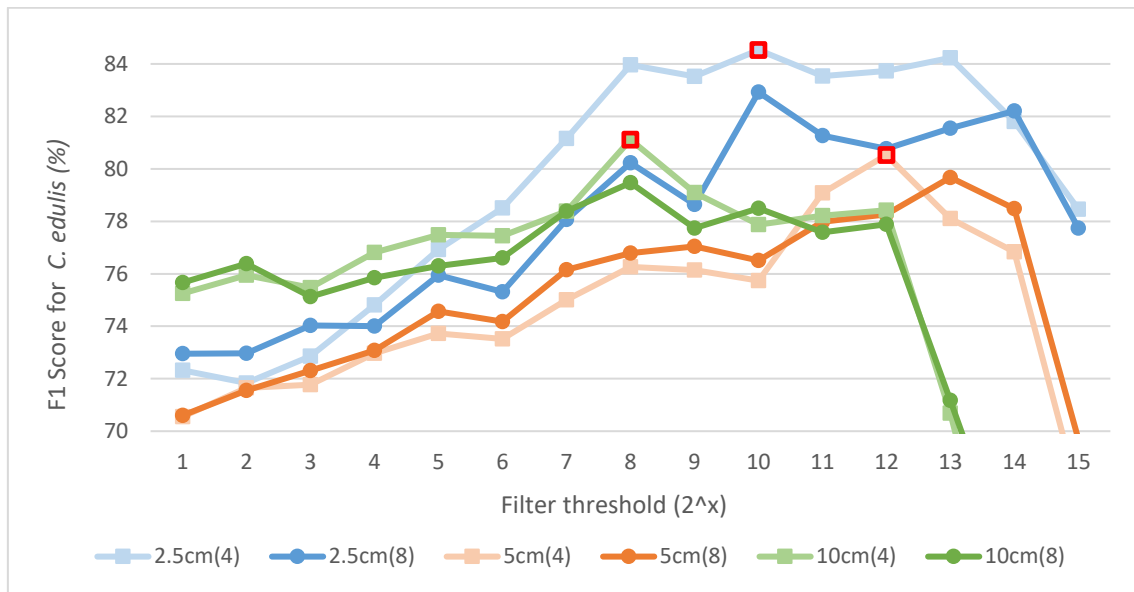


Figure 18 – Comparison of F1 Score for the *C. edulis* class for different filters thresholds and resolutions. Highest F1 score marked in red.

The sieve filter that achieved the highest F1 Score for *C. edulis* for each survey resolution (identified in Figure 18), was used for the *C. edulis* area estimation. Given that *C. edulis* is the target species and its class accuracy is the central object in the present study, the F1 Score was the only criterion considered for the sieve filters selection. However, the overall accuracy (OA) was also plotted to evaluate the information loss for the other classes (Figure 19).

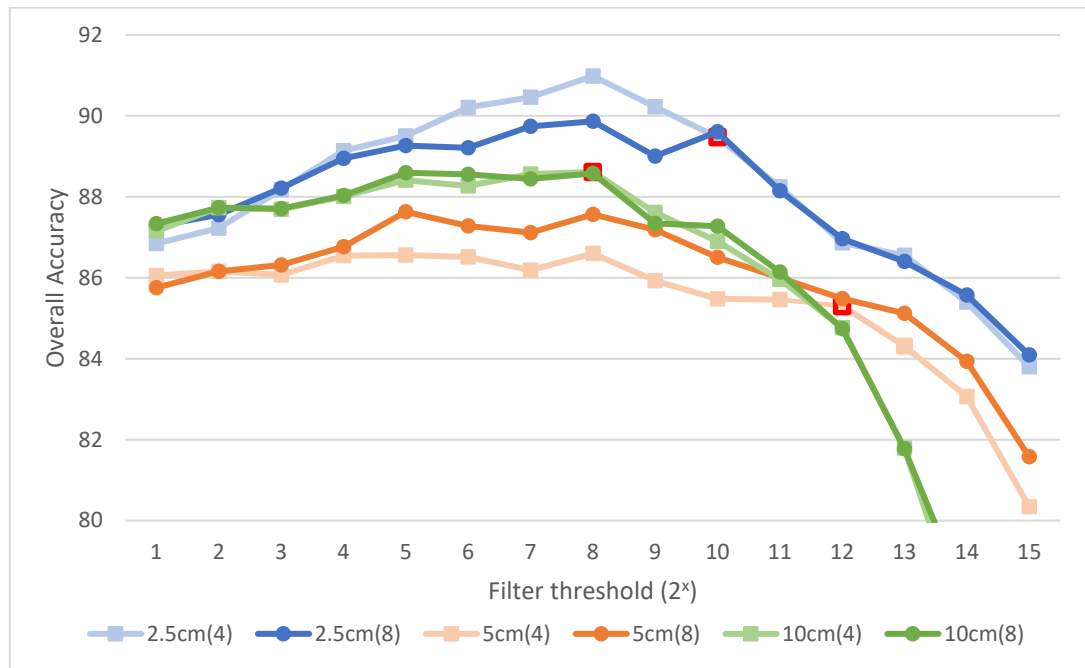


Figure 19 – Comparison of Overall Accuracy for different filters thresholds and resolutions. Highest F1 score marked in red.

For 2.5 and 10 cm GSD, the OA increased if compared with the classification with no filter applied, but for the 5 cm GSD, the OA dropped 1% if compared with the initial OA, and 2% if compared to the highest OA. Even though the 5cm GSD OA dropped, the F1 Score for *C. edulis* increase almost 10%, which justified the use of the filter.

The best results for F1 scores for all resolutions were achieved using the sieve filter with a connectedness of 4 pixels. The threshold was different for every resolution, and each classification resulted in a different final classified *C. edulis* area (Table 5)

2.1.4 Final classification results

	2.5cm	5cm	10cm
Sieve Filter Threshold	1024	4096	256
F1 Score <i>C. edulis</i>	84.5	80.5	81.1
<i>C. edulis</i> classified area	3441	3217	2305
<i>C. edulis</i> reference raster estimated area	2982	2686	2431
Standard Error	176	171	160
Overall Accuracy	89.5%	85.3%	88.6%
kappa hat	0.871	0.808	0.859

Table 5 – Statistics for the best *C. edulis* classification sieve/filter threshold and F1 Score per image resolution

The classification accuracy results for each resolution are detailed in Tables 6, 7 and 8.

2.5 cm GSD		Reference							
		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	% of Area	Area
Classified	Water	0.262	0.000	0.000	0.002	0.000	0.000	26.5%	5980
	<i>C. edulis</i>	0.000	0.120	0.000	0.000	0.002	0.030	15.2%	3441
	Dry Sand	0.000	0.000	0.091	0.000	0.010	0.002	10.3%	2329
	Wet Sand	0.002	0.000	0.002	0.108	0.000	0.000	11.2%	2533
	Dry Vegetation	0.001	0.005	0.016	0.005	0.134	0.019	18.1%	4100
	Green Vegetation	0.000	0.006	0.000	0.000	0.002	0.179	18.7%	4223
	% of Area	26.6%	13.2%	11.0%	11.5%	14.7%	23.0%	100.000	
	Area (m ²)	6011	2982	2478	2606	3331	5198		22605
	SE area	66	176	128	90	177	201		
	95% CI area	129	345	252	177	347	393		
	PA	98.7%	91.0%	83.3%	93.5%	91.2%	77.7%		
	UA	99.2%	78.9%	88.7%	96.2%	74.1%	95.7%		
	Overall accuracy	89.5%							
	Kappa hat	0.871							

Table 6 - Area-based classification error matrix for the **2.5 cm** GSD classification

5cm GSD		Reference							
		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	% of Area	Area (m ²)
Classified	Water	0.247	0.000	0.000	0.006	0.000	0.000	25.2%	5705
	<i>C. edulis</i>	0.000	0.101	0.002	0.000	0.002	0.030	13.6%	3070
	Dry Sand	0.000	0.000	0.083	0.001	0.010	0.001	9.5%	2140
	Wet Sand	0.001	0.000	0.005	0.107	0.003	0.001	11.6%	2628
	Dry Vegetation	0.007	0.004	0.030	0.012	0.139	0.014	20.6%	4651
	Green Vegetation	0.000	0.011	0.002	0.000	0.006	0.177	19.5%	4416
	% of Area	25.5%	11.6%	12.1%	12.6%	16.1%	22.2%	100.0%	
	Area (m ²)	5761	2616	2727	2845	3638	5023		22610
	SE area	104	160	160	133	200	190		
	95% CI area	203	314	313	261	392	372		
	PA	96.7%	87.5%	68.4%	84.9%	86.5%	79.6%		
	UA	97.7%	74.6%	87.2%	91.9%	67.7%	90.6%		
	Overall accuracy	85.3%							
	Kappa hat	0.820							

Table 7 - Area-based classification error matrix for the **5 cm** GSD classification

10 cm GSD		Reference						
Classified		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	% of Area
								Area
	Water	0.247	0.004	0.000	0.000	0.000	0.006	25.7%
	<i>C. edulis</i>	0.000	0.085	0.000	0.000	0.003	0.014	10.2%
	Dry Sand	0.000	0.000	0.090	0.000	0.003	0.000	9.3%
	Wet Sand	0.000	0.000	0.003	0.099	0.000	0.000	10.1%
	Dry Vegetation	0.000	0.006	0.023	0.028	0.136	0.006	20.0%
	Green Vegetation	0.000	0.013	0.000	0.000	0.005	0.230	24.8%
	% of Area	24.7%	10.8%	11.5%	12.7%	14.8%	25.6%	100.0%
	Area	5578	2431	2603	2865	3339	5795	22610
	SE area	100	160	123	127	186	176	
	95% CI area	196	313	241	248	364	345	
	PA	100.0%	79.0%	77.8%	77.9%	92.3%	89.8%	
	UA	96.1%	83.3%	96.5%	97.6%	68.3%	92.8%	
	Overall accuracy	88.6%						
	Kappa hat	0.859						

Table 8 - Area-based classification error matrix for the **10 cm** GSD classification

2.2 Biomass estimation

The samples' wet and dry weights for the green and brown parts of the collected *C. edulis* AGB are presented in Table 9 . Notice that some samples did not present any brown plant parts.

	GREEN		BROWN	
	WW (g/m ²)	DW(g/m ²)	WW (g/m ²)	DW(g/m ²)
Maximum	27120	2860	3650	2637
Minimum	2088	466	0	0
Mean	9684	1268	1005	615
Median	8163	1068	511	290
SD	6430	707	1127	732

Table 9 - Summary statistics of *C. edulis* sample wet weights and dry weights for the plants' green and brown parts (SD; standard deviation).

To estimate the proportions of the WW of the Green (WWgreen) and Brown (WWbrown) plant parts, a simple mean ratio was calculated using all the samples, WWgreen/WWbrown = 15.9. The relationship between the WW and DW of the Green parts was clearly linear, with the regression equation being WWv = 9.1DWv – 168.5. The regression presented a *p*-value < 0.001, R² = 0.93 and a NRMSE = 0.06. To simplify the Total AGB estimation and reduce computation resources, a mean ratio

WWgreen/DWgreen = 7.0 was used as an approximation. The mean ratio considered the mean of the ratio between WWgreen/DWgreen of each sample area.

..

The relationship between the sample's DWgreen and the sample area mean VI values, for best correlating VIs, are presented in Table 10 for each resolution. For a complete list of the best DWgreen—VI regression models results see Appendix 1. Regression models correlating the DWbrown + DWgreen with VI presented a much lower R^2 and p -value. All regression models for DWbrown parts and were not significant for any of the indices (p -values > 0.05). All DWgreen—VI regressions were significant (p -value < 0.001).

GSD (cm)	Index	R^2	RMSE	NRMSE	Model	Coef a	Coef b
2.5	RDVI	0.87	227.18	0.09	$y = ab^x$	151.94	81.11
5	ENDVI	0.77	300.67	0.12	$y = ab^x$	2596.48	13.48
10	GCI	0.64	364.55	0.15	$y = ab^x$	506.44	3.07

Table 10 - Best DWgreen – VI regression model for each resolution with respective R^2 , p -value, RMSE, NRMSE, model and respective coefficients a and b .

Different indices performed best for the different resolution images: The Renormalized Difference Vegetation Index (RDVI) was the best performing for 2.5cm GSD; the Enhanced Normalized Difference Vegetation Index (ENDVI) was the best performing for 5cm GSD; and the Green Chlorophyll Index (GCI) was the best performing for 10cm GSD. These indices were therefore applied to estimate the total biomass of *C. edulis* in the study area, computing the DWgreen for all pixels classified as *C. edulis*. Notice that, even though these were the best-performing indices, many of the other indices tested also presented a satisfactory performance. Seven of eight other indices from the 2.5 GSD images presented an R^2 that was less than 10% lower than the R^2 of the best performing index. Likewise, seven of the eight other indices applied to the 5 and 10 cm GSD images presented R^2 values that were less than 5 % lower than that of the best VI. The regression models relating *C. edulis* DW to the best performing VI for each GSD resolution, and their respective residuals plotted are show in Figure 20, 21 and 22.

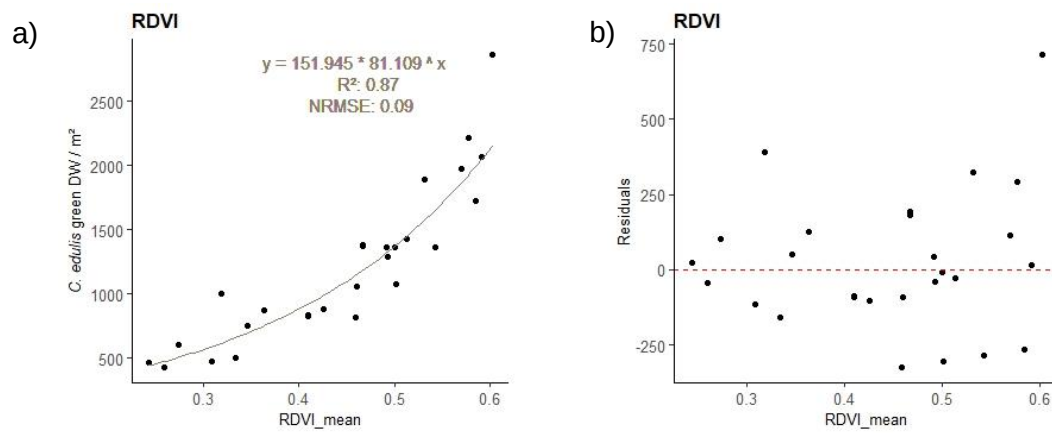


Figure 20 – Regression model relating *C. edulis* DW to the best performing VI for the **2.5 cm GSD** survey - sample points and regression line with equation (a), and residuals plot (b).

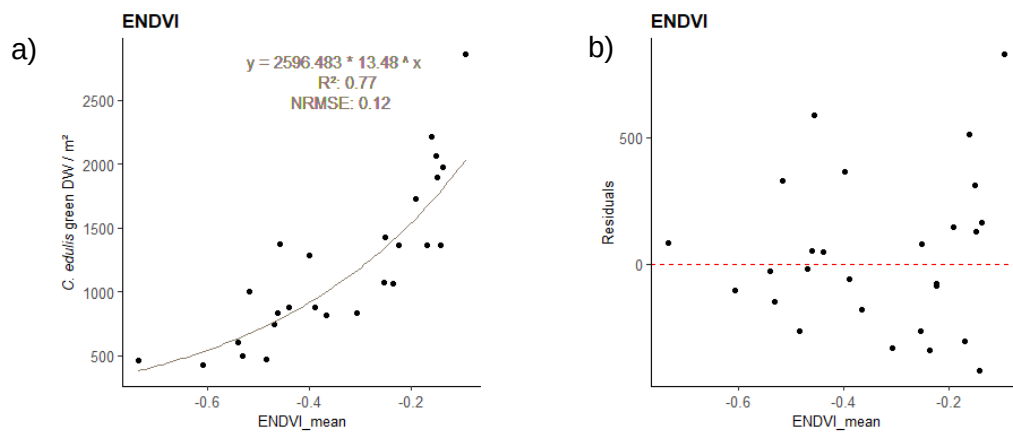


Figure 21 – Regression model relating *C. edulis* DW to the best performing VI for the **5 cm GSD** survey - sample points and regression line with equation (a), and residuals plot (b).

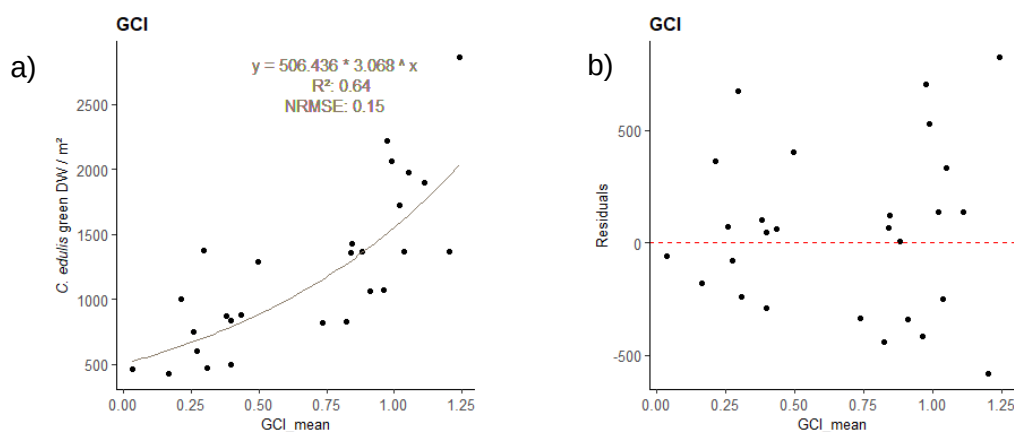


Figure 22 – Regression model relating *C. edulis* DW to the best performing VI for the **10 cm GSD** survey - sample points and regression line with equation (a), and residuals plot (b)..

Notice that, even though the classification accuracy did not decrease much with the increase of the GSD (i.e. decrease in image resolution), the coefficient of determination

R² of the regression model significantly decreased with increasing GSD. This is probably due to the amount of information available in the sample areas. For the 2.5 cm GSD there are up to 144 pixels in each sample area, while for 5 and 10 cm GSD there are only 36 and 9 pixels, respectively. The lower amount of information could therefore reduce the efficiency of the regression models.

The application of the regression models to the classified area of *C. edulis* resulted in estimates for the DW of green parts, for each image resolution. These values were subsequently converted to WW_{green} using the above-mentioned WW/DW ratio of 7.0. Finally, the area's total AGB of *C. edulis* was estimated using the WW_{green}/WW_{brown} ratio of 15.9, resulting in values of total AGB for each classification (Table 11).

	2.5cm	5cm	10cm
Classified Area (m ²)	3441.1	3070.0	2305.1
Total AGB (kg)	28327	29171	22136
Highest Value (kg/m ²)	29.13	29.18	24.50
Lowest Value (kg/m ²)	1.23	1.80	3.58
Mean (kg/m ²)	8.23	9.50	9.60
SD (kg/m ²)	3.66	3.16	2.88

Table 11 – Relative and total AGB estimates considering the regression model results.

There are different errors related to this methodology that must be acknowledged: (i) the regression model error, and (ii) the classification error. The regression error considered the root mean square error of the model, the RMSE (kg/m²), which was multiplied by the total classified area, resulting in an error in kg for the total AGB. The classification error was based on the Standard Error (SE) of the estimated classified area. A more detailed explanation of estimated area and its SE can be found in section 4.4 of Olofsson et al. (2014). Even though the SE is related to the estimated reference area, it is intrinsically linked to the accuracy of the classification. The SE was multiplied by the mean value of AGB to estimate the total classification error (Table 12).

	2.5cm	5cm	10cm
Classified Area (m2)	3441	3070	2305
SE of Area (m ²)	176	160	160
Total classification error (kg)	1449	1520	1536
RMSE (kg/m ²)	0.23	0.30	0.36
Total regression model error (kg)	782	923	840
Total AGB (kg)	28327	29171	22136
Total AGB error (kg)	2231	2443	2377
Relative error	0.08	0.08	0.11

Table 12 – Classified area, mean and total AGB, and respective error estimates.

3. Discussion

3.1 Classification results

Unoccupied Aerial Systems (UAS) equipped with multispectral sensors have been increasingly used to identify and monitor different invasive species in many ecosystems, and their application can be considered a well-established technique (Gonçalves et al., 2022; Mallmann et al., 2020; Abeyasinghe et al., 2019; Huete et al., 2016). Adding to that, multispectral UAS have also gained importance in commercial agriculture, where VI are used for crop health assessment and yield estimation (Bian et al., 2022; Yang et al., 2021; B. Li et al., 2020; Santos, L.M. et al., 2020). Some recent studies of natural habitats have combined a species identification methodology with yield estimations to assess vegetation's Above-Ground Biomass (AGB) using multi/hyperspectral UAS (Wengert et al., 2022; Wijesingha et al., 2020).

In the present study, the cover of the invasive species *C. edulis* was assessed classifying multispectral imagery (orthomosaics) of different resolutions. The supervised random-forest classification presented satisfactory results in identifying of *C. edulis* (Table 13) for all three analysed resolutions, if compared to similar studies (Papp et al., 2021; Sabat-Tomala et al., 2020; Michez et al., 2016). However, it is important to notice that, in spite of the satisfactory accuracies, the areas classified as *C. edulis* varied in size. The difference is more prominent for the 10cm GSD image classification, which *C. edulis* area differed a lot from the area of the 2.5 GSD and 5 cm GSD image classifications

	2.5cm	5cm	10cm
Sieve Filter Threshold	1024	4096	256
F1 Score <i>C. edulis</i>	84.5	80.5	81.1
<i>C. edulis</i> classified area (m ²)	3441	3070	2305
<i>C. edulis</i> reference raster estimated area (m ²)	2982	2616	2431
Standard Error (m ²)	176	160	160
95 % Confidence Interval (m ²)	345	314	313
Overall Accuracy	89.5%	85.3%	88.6%
kappa hat	0.871	0.820	0.859

Table 13 – *C. edulis* accuracy results and estimated area errors.

Examination of Tables 13 and 10 (results of the regression models) indicates that some aspects deserve further investigation, in order to: (i) determine where the differences in the area of *C. edulis* between classifications occur; (ii) identify the different attributed classes for the different resolutions and investigate the possible reasons for these differences; (iii) assess if the reference raster estimated area can be utilised with the mean vegetation values to estimate total AGB, considering that the *C. edulis* reference raster estimated areas for all three resolutions are within the 95% Confidence Interval of each other.

There is a significant difference in *C. edulis* classified area between resolutions. To further detail these differences, the classified area was compared between resolutions. A representative area of the orthomosaics cover changes is presented in Figure 23.

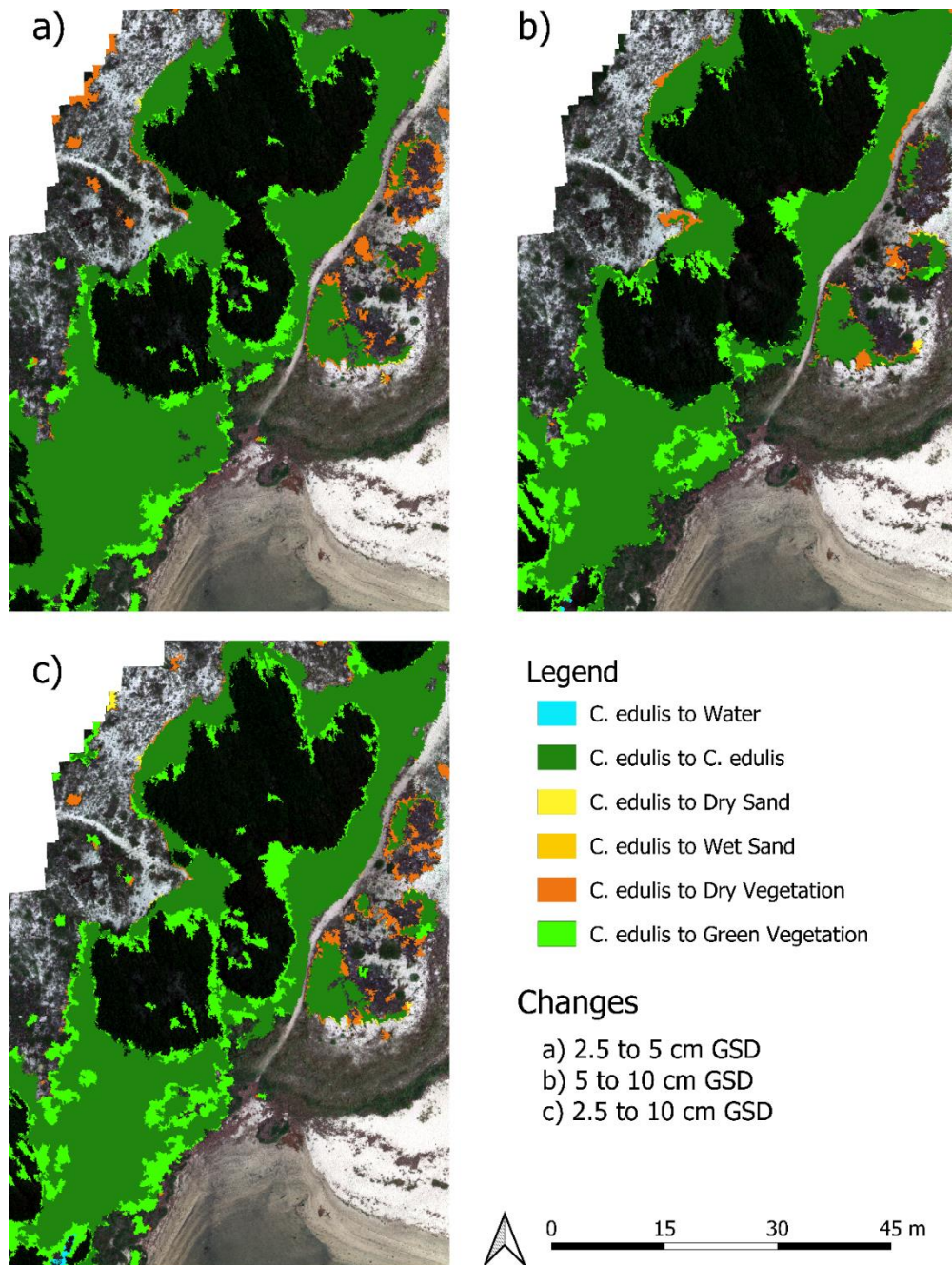


Figure 23 – Orthomosaic cover changes comparison between resolutions, for the areas classified as *C. edulis* using the 2.5cm GSD imagery.

Considering that the highest-resolution results are the most reliable, comparison between the 2.5 GSD classification and the other imagery classifications (Figure 23) showed that the changes from smaller to larger GSD are characterised by the reduction of *C. edulis* classified cover area (Table 12), replaced mainly by Green and Dry Vegetation. These changes occur in small patches, close to the border of *C. edulis* areas as well as inside bigger *C. edulis* areas (Figure 23). The observed differences may be due to a scale effect of the resolution, an imprecision in the superposition of

orthomosaics, a miss classification due to overlapping spectral signatures or a mixture of these effects. The scale effect can be defined as the influence of spatial resolution on classification accuracy (R. Li et al., 2023). Further investigation is required to determine the main cause(s) for this phenomenon.

The different cover classes observed at the border of the *C. edulis* areas might be attributed to imprecision in the superposition of orthomosaics and to the scale effect. These effects are more relevant at the border of classified cover areas, where the reflectance of *C. edulis* areas may mix with the reflectance of neighbouring covers due to the larger GSD. As seen in Figure 23, some of these changes are at the border between *C. edulis* and Green Vegetation cover, are the two classes with the less distinct spectral signatures. It is also interesting to notice that these differences are distributed on all sides of *C. edulis* borders, suggesting that these changes cannot be explained by orthomosaic superposition imprecisions alone (as these would produce a lateral shift). Some changes are also on the border between *C. edulis* and dry sand, but were classified as green vegetation, also likely due to the scale effect and spectral signature inaccuracy in larger pixels.

Larger areas of cover change, especially in the central part of *C. edulis* classified areas, cannot be explained by superposition imprecision or by the scale effect. These changes are probably related to misclassifications due to overlapping spectral signatures. Figure 14, 15 and 16 shows that even though there was sufficient differentiation between the spectral signature of Green Vegetation and of *C. edulis* to provide a satisfactory classification accuracy, a considerable overlapping of these signatures must be acknowledged. This overlapping might result in uncertainties and miss classification of *C. edulis* and Green Vegetation. Mis-classifications may further be accentuated by the lack of the RedEdge band in the plane-based aerial photographs, i.e. the 5 and 10 cm GSD images. A further and deeper investigation is needed to identify the most significant factors influencing the cover change in larger areas. An analysis of the classification confidence map for these areas may provide some helpful information.

A full comparison of cover classes obtained for the different resolutions can be seen in Tables 14, 15 and 16.

		5 cm GSD						Total 2.5cm GSD Area (m ²)
		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	
2.5 cm GSD	Water	5555	3	2	103	298	16	5979
	<i>C. edulis</i>	0	2548	9	6	300	574	3440
	Dry Sand	80	15	1685	26	504	16	2328
	Wet Sand	37	9	88	2349	45	0	2532
	Dry Vegetation	23	143	327	131	3274	197	4098
	Green Vegetation	4	349	23	8	225	3609	4221
Total 5cm GSD area (m ²)		5701	3069	2138	2627	4649	4416	22602

Table 14 – Comparison of the cover class areas resulting from the 2.5 to 5 cm GSD classifications

		10 cm GSD						Total 5cm GSD Area (m ²)
		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	
5 cm GSD	Water	5604	0	0	14	85	0	5704
	<i>C. edulis</i>	1	2029	5	0	84	946	3069
	Dry Sand	0	9	1774	76	263	13	2138
	Wet Sand	83	10	13	2187	324	8	2627
	Dry Vegetation	41	79	295	8	3654	565	4645
	Green Vegetation	66	175	6	0	96	4069	4413
Total 10 cm GSD area (m ²)		5798	2304	2095	2287	4509	5603	22599

Table 15 – Comparison of the cover class areas resulting from the 5 to 10 cm GSD classifications

		10 cm GSD						Total 2.5cm GSD Area (m ²)
		Water	<i>C. edulis</i>	Dry Sand	Wet Sand	Dry Vegetation	Green Vegetation	
2.5 cm GSD	Water	5564	0	5	30	367	11	5979
	<i>C. edulis</i>	10	2075	14	0	143	1194	3438
	Dry Sand	80	15	1772	32	414	12	2327
	Wet Sand	53	8	36	2172	256	6	2532
	Dry Vegetation	33	87	252	48	3204	468	4093
	Green Vegetation	52	118	14	3	122	3909	4219
Total 10 cm GSD area (m ²)		5794	2304	2094	2287	4508	5602	22592

Table 16 – Comparison of the cover class areas resulting from the 2.5 to 10 cm GSD classifications

Analysing the tables above is possible to see that most cover changes occurred between *C. edulis* and Green Vegetation, which may also point to an inaccuracy related to the lower spectral resolutions from the 5 and 10 cm GSD. The extra RedEdge band of the UAS camera may provide some additional information to enhance classification accuracy.

3.2 Biomass estimation

To investigate alternatives calculations AGB estimation, a comparison was undertaken assessing the, already presented, total AGB obtained using the VI of individual classified *C. edulis* pixels against the total AGB derived using the mean VI in conjunction with the reference raster estimated *C. edulis* area. The results, presented on Table 17, suggest that the mean VI and the reference raster estimated area of *C. edulis* can provide a reasonable estimative of the total AGB in the study area. Even though the error was higher since the 95% CI Area was used, 0.17 relative errors might still provide some relevant insights. The final result can be compared with the result using classified area, with both sharing a relevant overlap considering the errors.

	2.5cm	5cm	10cm
<i>C. edulis</i> reference raster estimated area (m ²)	2982	2616	2431
Mean AGB (kg/m ²)	8.23	9.50	9.60
95% CI Area (m ²)	345	314	313
95% CI Area AGB Error (kg)	2839	2983	3005
RMSE (kg/m ²)	0.227	0.301	0.365
Regression Error (kg)	677	787	886
Total AGB reference raster estimated area (kg)	24542	24852	23338
Total estimated area AGB Error (kg)	3517	3770	3891
Relative error	0.14	0.15	0.17
Total AGB classified area (kg)	28327	29171	22136
Total AGB error (kg)	2231	2443	2377
Relative error	0.08	0.08	0.11

Table 17 – Result comparison between Total AGB calculation using classified estimated raster area and classified area.

It is essential to address that even though all resolutions presented some relevant results, the total biomass of *C. edulis* obtained from the 2.5 cm GSD images captured by the MicaSense RedEdge-MX was considered the most accurate. This consideration accounted for the higher number of bands available for classification - 5 bands vs. 4 bands from lower resolutions – which possibly resulted in better classification results (Figures 18 and 19), and the best regression model results, which increased the confidence in the total AGB estimation.

For the quantification of vegetation through regression models, RDVI, ENDVI and GCI exhibited the best performance for 2.5, 5 and 10 cm GSD respectively. Several previous investigations have achieved promising outcomes when employing NDVI to assess various measurable attributes of vegetation (Guan et al., 2019; Hassan et al., 2019; Pandey et al., 2019; Huang et al., 2021; Tenreiro et al., 2021), consolidation NDVI's status as the predominant index in vegetation research (Xu et al., 2022). Nevertheless, within the context of this study, NDVI occupied a relatively low position as the eighth, seventh and fifth most effective model for DW prediction for 2.5, 5 and 10 cm GSD respectively (Attachment 1). This observation corroborates with prior research indicating that various vegetation indices (VIs) may exhibit stronger correlations with vegetation AGB and quantitative attributes, compared to the conventional NDVI (Huang et al., 2021). Consequently, the development of a specific methodology for evaluating the predictive accuracy of diverse VIs in relation to vegetation attributes still cares of investigation. To do so, is crucial to consider of a wide spectrum of variables, including species diversity, topographical features, as well as weather and lighting conditions

(Sabat-Tomala et al., 2020). Additionally, opposed to commercial crops, the inherent morphological variability among natural vegetation species poses an additional challenge when seeking a universal correlation between image-derived data and quantifiable vegetation attributes. Consequently, a possible correlation between plant AGB and VI must be investigated case by case (Huang et al., 2021).

3.3 Estimation uncertainties

While this investigation has achieved favourable outcomes in forecasting DWgreen through utilising VI, certain uncertainties persist in the computation of total above-ground biomass (AGB). In addition to the widely acknowledged uncertainties inherent in classification and regression models, it is imperative to account for additional uncertainties that remain unquantifiable. For instance, the WW–DW ratio demonstrates a linear correlation, and employing a mean ratio can be a reasonably accurate approximation. Nevertheless, this approach involves many variables that exhibit spatial and temporal variations. For instance, certain plants may thrive in more humid microenvironments compared to others, and their life stages may also differ, potentially affecting the ratio.

An even higher uncertainty is associated with the morphology of *C. edulis*, characterised by the presence of two distinct layers: an upper succulent green layer and a drier brown layer. This peculiarity poses a considerable challenge when estimating AGB for a generic location using a model-based approach. Notably, no identifiable correlation was noticed between DWgreen and DWbrown, and all regression models presented *p*-values greater than 0.05. Consequently, the most viable approach used a mean ratio as the best estimate. This ratio could be influenced by many factors, including plant age, growth rate, decay velocity, seasonality, and availability of water and light.

The ability to distinguish between various vegetation covers may be more or less successful, depending upon the season and the plants' state, with spectral signatures likely varying between seasons and across regions. In the current investigation, data collection occurred during the spring season, specifically prior to flowering. This choice was made based on the belief that flowers could potentially influence on classification outcomes and biomass estimation via vegetation indices. However, a recent study (Innangi et al., 2023), which involved the classification of *C. edulis* during the flowering season, revealed that flowers do not poses a significant impact on image classification results.

The estimation of *C. edulis*' AGB plays an important role in the management of invasive species, where the biomass estimates offer critical insights for planing and executing removal campaingns. Nonetheless, the approach adopted in this research holds the potential for broader applications, extending beyond *C. edulis* and can be reproduced with various low stratum plant species. Furthermore, it can be leveraged for estimating the total carbon content in ecosystems, employing established biomass–carbon correlations.

Moreover, the exploration of the feasibility of constructing a general model for predicting *C. edulis* VI–DW relationships could be an interesting future investigation. This endeavour necessitates conducting an array of new tests, mirroring the methodology employed in the current investigation, to recognize potential patterns associating VIs with AGB. These future investigations can also search into the utility of VIs for refining land cover classifications, thus assessing their viability in enhancing the identification of *C. edulis*. Relevant future research may also include an evaluation of the applicability and precision of this methodology using imagery characterized by even lower resolutions, including satellite-based images. Such assessments can serve to monitor the distribution and biomass of *C. edulis* in regional scale.

Conclusion

In conclusion, results suggest that the multispectral imagery, obtained for the surveillance of *C. edulis* within this investigation, has the potential to provide valuable insights into vegetation species distribution and biomass. The applied methodology can be applied to a wide variety of coastal (and other) environments monitoring. This includes the tracking of coastal dune evolution and erosion dynamics (Laporte-Fauret et al., 2019), as well as the monitoring of diverse vegetation species(Suo et al., 2019), in addition to mapping and identifying litter accumulations(Gonçalves et al., 2022; Gonçalves & Andriolo, 2022). The prospect of collecting aerial data for multipurpose applications stands as a promising approach for efficient data acquisition processes and, at times, it can also create opportunities for diverse remote sensing projects that use aerial images.

Attachment 1

Resolution Rank	GSD (cm)	Index	R^2	p -value	RMSE	NRMSE	Model	Coef a	Coef b
1	2.5	RDVI	0.867564	< 0.0001	227.18	0.0935	$y = ab^x$	151.945	81.109
2	2.5	DVI	0.846444	< 0.0001	234.42	0.0965	$y = ab^x$	204.547	380.533
3	2.5	GDVI	0.840966	< 0.0001	234.85	0.0967	$y = ax^b$	15205.471	1.984
4	2.5	RVI	0.828356	< 0.0001	271.86	0.1119	$y = ax^b$	264.764	-0.733
5	2.5	GCI	0.808096	< 0.0001	292.92	0.1206	$y = ax^b$	355.145	0.613
6	2.5	ENDVI	0.806527	< 0.0001	306.38	0.1261	$y = ab^x$	530.993	5.144
7	2.5	GNDVI	0.79492	< 0.0001	301.81	0.1242	$y = ae^{bx}$	31.944	5.201
8	2.5	NDVI	0.793329	< 0.0001	316.80	0.1304	$y = ab^x$	130.453	19.155
1	5	ENDVI	0.766573	< 0.0001	300.67	0.1238	$y = ab^x$	2596.483	13.480
9	2.5	PRI	0.76636	< 0.0001	334.37	0.1376	$y = ab^x$	199.192	111.099
2	5	GCI	0.763262	< 0.0001	286.28	0.1178	$y = ab^x$	439.550	2.762
3	5	RVI	0.757382	< 0.0001	305.37	0.1257	$y = ax^b$	357.892	-1.826
4	5	GNDVI	0.755466	< 0.0001	312.08	0.1285	$y = ab^x$	128.654	326.561
5	5	NDVI	0.752989	< 0.0001	311.99	0.1284	$y = ab^x$	341.068	53.151
6	5	GDVI	0.752104	< 0.0001	299.78	0.1234	$y = ab^x$	118.118	174.225
7	5	RDVI	0.750094	< 0.0001	310.31	0.1277	$y = ab^x$	327.380	41.328
8	5	DVI	0.740037	< 0.0001	314.43	0.1294	$y = ab^x$	316.124	31.858
1	10	GCI	0.644142	< 0.0001	364.55	0.1501	$y = ab^x$	506.436	3.068
2	10	DVI	0.641136	< 0.0001	363.28	0.1495	$y = ab^x$	420.967	33.223
3	10	RDVI	0.63942	< 0.0001	369.60	0.1521	$y = ab^x$	429.327	38.289
4	10	RVI	0.636954	< 0.0001	375.04	0.1544	$y = ax^b$	448.837	-1.787
5	10	ENDVI	0.634277	< 0.0001	374.65	0.1542	$y = ab^x$	3111.199	11.267
6	10	NDVI	0.634028	< 0.0001	378.19	0.1557	$y = ab^x$	439.312	43.577
7	10	GDVI	0.630055	< 0.0001	358.41	0.1475	$y = ab^x$	213.865	123.690
8	10	GNDVI	0.619652	< 0.0001	383.23	0.1578	$y = ab^x$	223.962	155.699
9	5	PRI	0.601178	< 0.0001	411.63	0.1694	$y = ab^x$	257.451	6134560
9	10	PRI	0.449098	0.0001312	457.04	0.1881	$y = ab^x$	381.250	4.75E+09

Appendix A

Flights

The first Multispectral images, taken with a MicaSense RedEdge-MX with five bands, provided a set of 5135 different images adding up to 11.9 GB of data for processing with a Ground Sample Distance (GSD) of 2.5 cm.

The second flight captured RGB images with a built-in camera transported by a DJI PHANTOM 4 UAV. This flight provided a set of 136 different images adding up to 1.1 GB of data for processing with a Ground Sample Distance (GSD) of 1.0 cm.

The three images' datasets were processed using Agisoft Metashape Professional version 1.8.3 built 14331 (64bit), resulting in two co-incidents orthomosaics

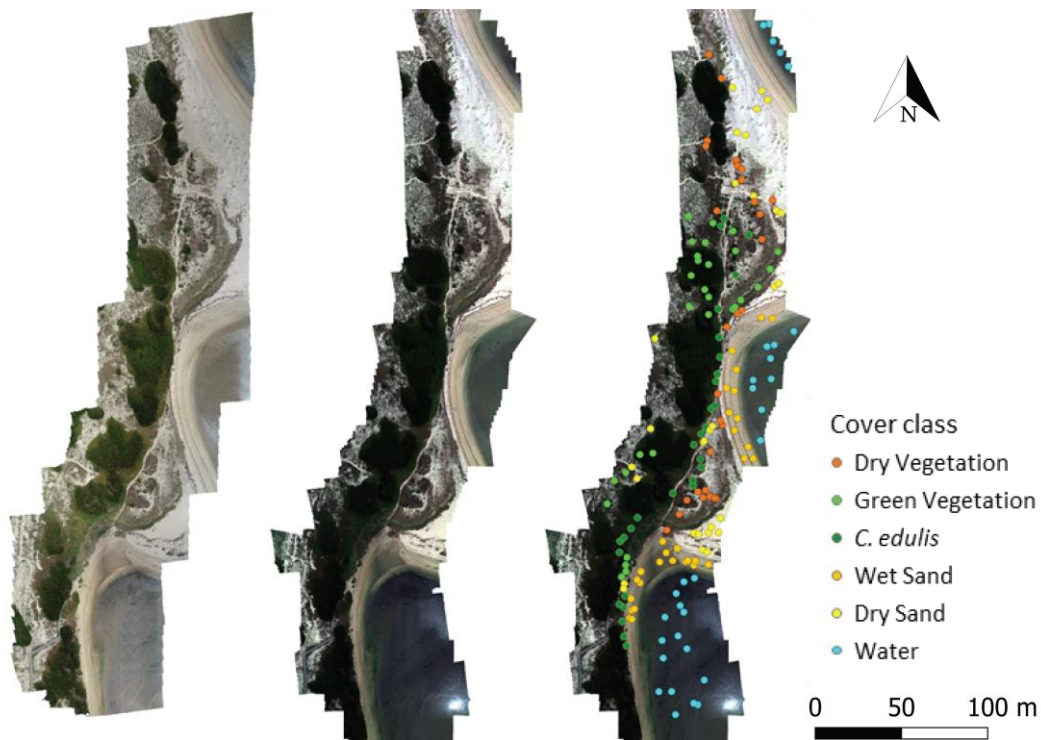


Figure A1 - Orthomosaics of the RGB (a) and multispectral (b) images, and multispectral images with the location of the classification training and validation areas (c)

Wet Weight and Dry Weight measurement

After collection, the 30 30x30 cm samples were taken to the lab to be dried. Because of space limitations in the lab stove, it was only possible to dry ten samples simultaneously. The others 20 samples were laid to dry in the open air, protected from the rain. After seven days, the first samples on the lab stove were completely dried, and their respective

Dry Weight was obtained. The following ten samples were weighed and placed in the stove. The exact process was made with the ten reminiscent samples.

It is essential to acknowledge that the WW of the first ten samples was measured on the day of the sample collection. After the first ten samples were dried, the next ten had their WW measured seven days after collection. The last set of 10 samples dried on the stove did not have its WW registered since they were already 14 days drying in the open, and its WW would have already changed a lot since the collection day.

	Green		Brown	
	WW (g/m²)	DW (g/m²)	WW (g/m²)	DW (g/m²)
Maximum	27,120	2860	3650	2637
Minimum	1781	421	117	60
Mean	8912	1188	1093	680
Median	8035	1034	642	370
SD	6175	657	1052	687

Table A1 – WW and DW of Green and Brown parts¹

To estimate the whole AGB of *C. edulis* in the area, it is necessary to obtain a relation between DW and WW of green parts and the ratio between Green and Brown parts. To estimate the proportions of the WW of the Green (WWgreen) and Brown plant parts (WWbrown), a simple mean ratio was calculated using all the samples—the mean ratio WWgreen/WWbrown = 15.9. The relationship between the WW and DW of the Green parts was clearly linear, with a mean ratio of WWgreen/DWgreen = 7.0.

¹ Table A1 slightly differs from Table 9. Table 9 only considers 27 samples, as three samples were not computed as they were located outside of larger resolutions imagery areas; Table 9 also considers the samples with zero values, different from Table A1, which only considers samples with samples weights higher than zero.

DW-VI models

Three regression models (Section 1.5.4) were used to assess the relation between the VI (Table 02) and the DW of green parts. Different VI (x-axis) were plotted against the DW (y-axis), and the top ten best-fitted regressions are presented in the table below.

Ranking	VI	R ²	Model
1	RDVI	0.86	$y = ab^x$
2	GDVI	0.84	$y = ae^{xb}$
3	DVI	0.84	$y = ae^{xb}$
9	RVI	0.81	$y = ae^{xb}$
10	GCI	0.80	$y = ae^{xb}$
11	ENDVI	0.80	$y = ab^x$
12	NDVI	0.79	$y = ab^x$
13	PRI	0.79	$y = ab^x$
14	GNDVI	0.78	$y = ab^x$
22	MGRVI	0.72	$y = ab^x$
23	ARVI	0.71	$y = ab^x$
25	ExR	0.70	$y = ab^x$
33	RB	0.62	$y = ae^{xb}$
34	ExG	0.62	$y = ab^x$
36	NDREI	0.59	$y = ab^x$
46	CVI	0.00	$y = a + bx$

Table A3 – Best-fitted VI x DW regression models

The best fitting model was achieved in the plot of the Renormalized Difference Vegetation Index, using the exponential regression model 1.

$$RDVI = \frac{NIR - Red}{\sqrt{NIR + Red}} \quad y = ab^x$$

Where:

NIR = Reflectance value on the Near Infrared Band

Red = Reflectance value on the Red Band

y = Dry Weight

x = Vegetation Index

a = coefficient 1

b = coefficient 2

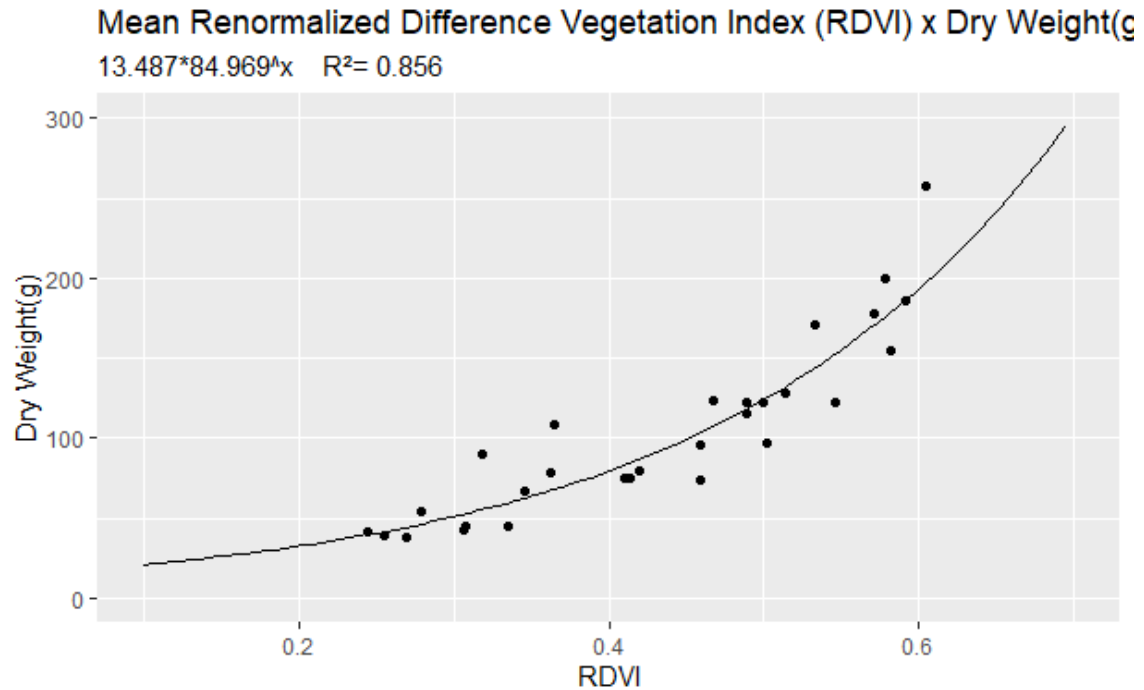


Figure A2 – Mean RDVI x DW

The conversion from VI to DW was calculated using the following equation.

$$DW = 13.4874415913714 * 84.9690438366571^{VI}$$

Classification and accuracy

The classifications were made using five supervised classification algorithms: landcover classifications, Minimum Distance, Maximum Likelihood, Spectral Angle, Support Vector and Random Forest. Six class major important classes could be identified in the image:

- 1- Water
- 2- *C.edulis*
- 3- Sand
- 4- Wet Sand
- 5- Dry Vegetation
- 6- Green Vegetation

The resulting classifications can be compared in Figure A3.

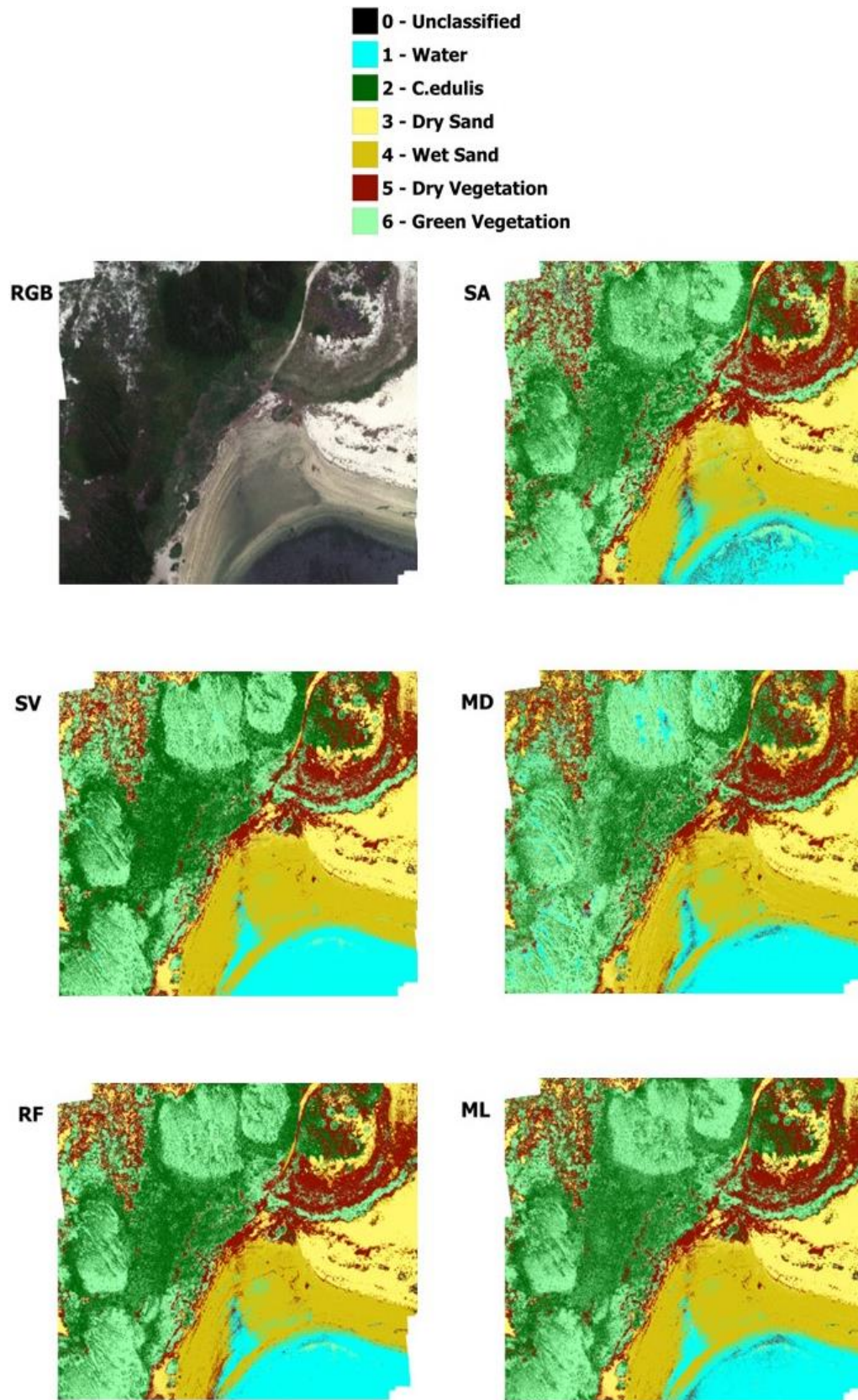


Figure A3 – Supervised Classification results comparison –RGB is the original orthomosaic; SA – Spectral Angle; SV – Support Vector; MD – Minimum Distance; RF- Random Forest; ML – Maximum Likelihood

Three variables were used to assess which classification was the most accurate: Overall Accuracy, Kappa and User Accuracy of *Carpobrotus edulis*. The total number of randomly generated pixels for accuracy evaluation was based on Equation (vii)

	pixel	Wi	Si	Wi*Si
1	11454240	0.201375	0.3	0.060412
2	7856090	0.138116	0.4	0.055246
3	9945184	0.174844	0.35	0.061195
4	7100103	0.124825	0.4	0.04993
5	9831870	0.172852	0.35	0.060498
6	10692794	0.187988	0.35	0.287283

Tabel A4– Class values for calculation of the total number of pixels selected for the accuracy test

Using $S_0 = 0,02$ the total number of randomly selected pixels is 600. There are different methodologies for selecting these pixels and how many will be chosen for each class. (Olofsson et al., 2014) Since there were no extreme differences in the number of pixels for each class, the number of randomly generated pixels was the same, 100 for each class. From the 600 randomly generated pixels, only 518 could be securely identified on the image.

Comparing the randomly selected pixels with ground truth pixels results in an error matrix with different parameters to evaluate the precision of the classification. This work considered the following parameters: Overall Accuracy, kappa hat, User Accuracy and Producer Accuracy.

Algorithm	<i>C. edulis</i> PA *	<i>C. edulis</i> UA *	Kappa	Overall Accuracy
Minimum Distance	0.65	0.72	0.76	0.80
Maximum Likelihood	0.74	0.60	0.80	0.84
Random Forest	0.83	0.73	0.84	0.87
Spectral Angle Mapper	0.79	0.66	0.76	0.77
Support Vector Machine	0.81	0.67	0.82	0.85

Table A4 – Supervised Classification results comparison

The best classification was obtained using the Random Forest Algorithm, which presented a higher value on all four evaluation criteria. It is essential to notice that this evaluation was made with the raw classification, which means that the classification was not submitted to any filter or other techniques to enhance the accuracy.

A sieve filter (section 1.5.3) was applied to assess higher accuracy values. The strength of the filter was doubled each time. The best accuracy was obtained with the sieve size smaller than 512, with 8 neighbours' pixels.

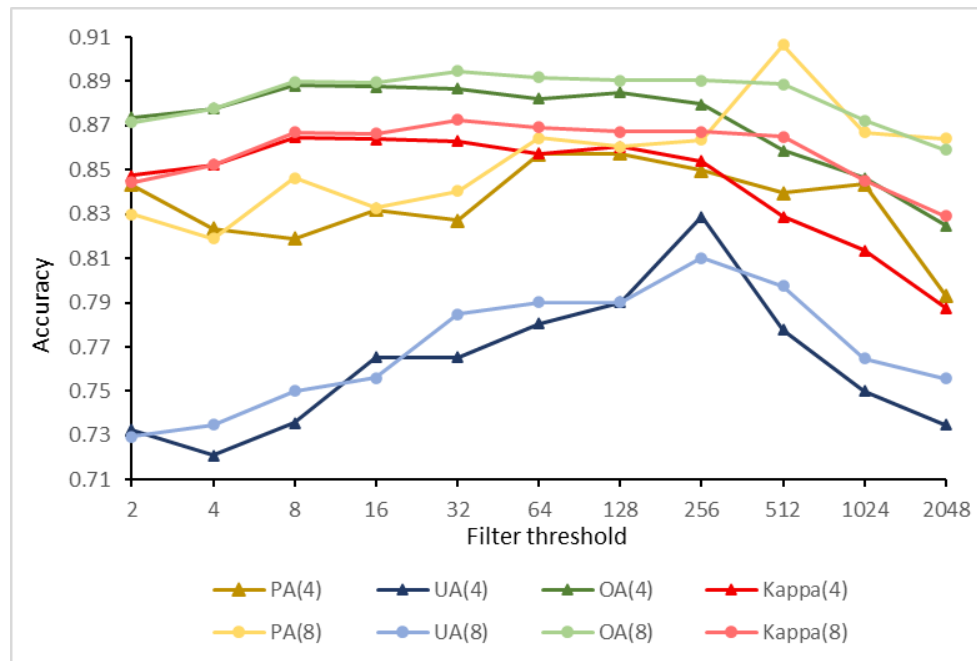


Figure A4. Accuracy measures (PA: Producer Accuracy, UA: User Accuracy, OA: Overall Accuracy) for the Random Forest classification results after the application of sieve filters with increasing thresholds, using a 4 or 8-pixel neighbourhood (between brackets).

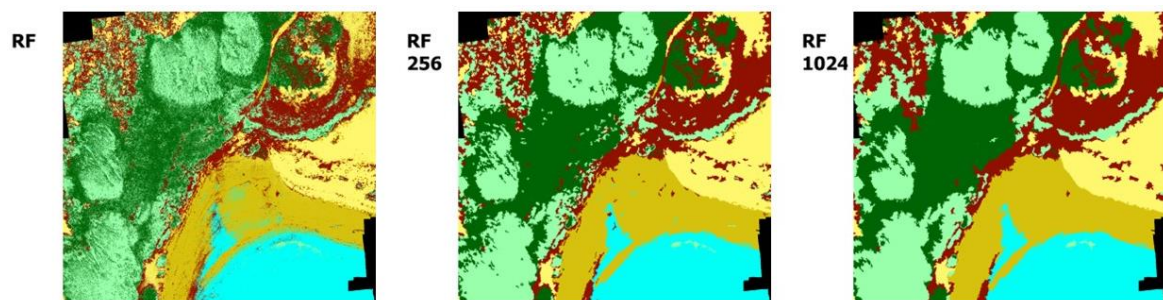


Figure A5 – Filter comparison

Figure A5 presents a comparison of 3 images with different filter threshold values. It is reasonable to conjecture that at some point, the filter will result in a loss of information, meaning that the accuracy will start to go down after it reaches a maximum. The full error-matrix of the Random Forest classification is displayed below.

		Reference							
		Water	<i>C. edulis</i>	Sand	Wet Sand	Dry Vegetation	Green Vegetation	% Area	Total Area m ²
Classified	Water	0.198	0.000	0.000	0.005	0.000	0.002	20.5	6300
	<i>C. edulis</i>	0.000	0.099	0.000	0.000	0.004	0.021	12.4	3821
	Sand	0.000	0.002	0.164	0.006	0.009	0.000	18.1	5565
	Wet Sand	0.005	0.000	0.003	0.097	0.002	0.000	10.7	3279
	Dry Vegetation	0.000	0.002	0.015	0.006	0.163	0.017	20.3	6242
	Green Vegetation	0.000	0.006	0.000	0.000	0.006	0.168	18.0	5540
% Area		20.3	10.9	18.2	11.4	18.5	20.8	100	
Total Area m ²		6232	3362	5596	3497	5673	6387		30,748
Standard Error m ²		148	219	245	214	319	290		
Producer Accuracy		0.98	0.91	0.90	0.85	0.88	0.81		
User Accuracy		0.97	0.80	0.91	0.91	0.80	0.93		

Table A5 - Area-based classification error matrix.

Total *Carpobrotus edulis* Above-Ground Biomass

The calculation of the *C. edulis* total AGB used mainly four central information: (i) each Pixel VI value in the area classified as *C. edulis*; (ii) the regression model correlating the VI value with the DW of *C. edulis*; (iii) the relation between the DW and WW of green parts; (iv) the ratio between the WW of green and brown parts. The VI value on each pixel classified as *Carpobrotus* was extracted and then converted to DW using the regression model. Then, the DW was converted into WW and multiplied by the DW/WW ratio. Finally, there is an approximate value in kg for the AGB of *C.edulis* in the study area.

<i>C.edulis</i> total mass (kg)	Tota area of <i>C.edulis</i> (m2)
32528.57	3775

Table A6 – Final Results of *C.edulis* AGB

Finally, it is important to notice that the study area in this Appendix was slightly larger than the area presented in the main text.

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