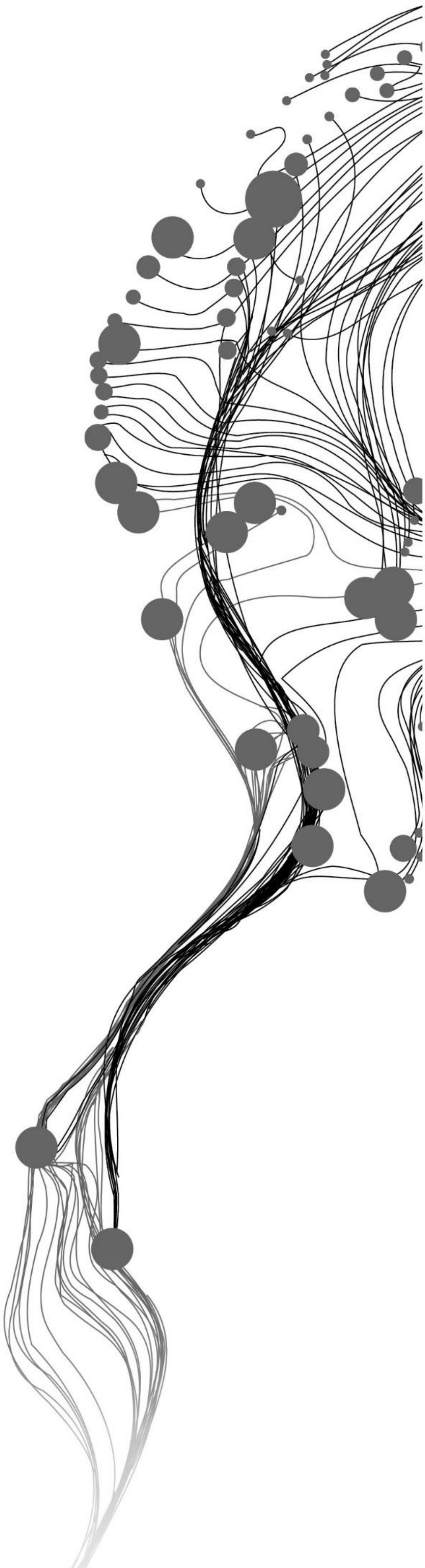
MODELLING AND MAPPING FOLIAR NITROGEN IN A WETLAND USING HIGH RESOLUTION IMAGES

MBAIORGA, SIMON GRACE
February, 2017

SUPERVISORS:
Dr. R. (Roshanak) Darvishzadeh
Prof. Dr. A.K (Andrew) Skidmore



MODELLING AND MAPPING FOLIAR NITROGEN IN A WETLAND USING HIGH RESOLUTION IMAGES

MBAIORGA, SIMON GRACE
Enschede, The Netherlands, February, 2017

Thesis submitted to the Faculty of Geo-Information Science and Earth Observation of the University of Twente in partial fulfilment of the requirements for the degree of Master of Science in Geo-Information Science and Earth Observation.
Specialization: Natural Resource Management

SUPERVISORS:
Dr. R. (Roshanak) Darvishzadeh
Prof. Dr. A.K. (Andrew) Skidmore

THESIS ASSESSMENT BOARD:
Prof. Dr. A.D, Nelson (Chair)
Dr. Moses, Azong Cho (External Examiner, CSIR, South Africa)

ABSTRACT

Regional maps of foliar nitrogen are important for precision agriculture, livestock management, biodiversity conservation and for understanding ecosystem structure and functions. Leaf nitrogen is related to many other leaf constituents and key canopy traits. However, this relationship varies within and across ecosystems. The retrieval of foliar nitrogen is challenging i.e. from a tedious and time consuming contemporary approach to a more expensive and area constrained conventional approach. The optical remote sensing has served as a paramount technique for estimating foliar nitrogen. With the inception of new multispectral sensors with high spectral, spatial, and temporal resolutions covering the red edge region, which provide additional information on vegetation characteristics, the prospect of estimating foliar nitrogen with higher accuracy is promising. Although, vegetation indices are popular and simple means of retrieving leaf nitrogen, they have been rarely examined in wetlands. Therefore, vegetation indices derived from high resolution multispectral images of RapidEye and Sentinel-2 were utilized in this study for nitrogen retrieval. Field measurements of leaf samples were collected from 30 plots in the island of Schiermonnikoog, Netherlands in July 2015 concurrent with the time of the RapidEye image. Moreover, the Sentinel- 2 image from the same phenological date was obtained for the year 2016. Leaf constituents such as nitrogen and carbon were determined from oven dried leaf samples using wet chemical analysis.

First, we assessed the relationships between the measured leaf nitrogen with other leaf constituents (chlorophyll and carbon) as well as with the reflectance of individual spectral bands. Next, a total of 12 standard vegetation indices that were mostly correlated to chlorophyll and nitrogen in the previous literatures were examined. The optimization of using different band combinations in different vegetation index formulation was conducted to assess the relationship between leaf nitrogen and different spectral band combinations. Regression models were then used to study the relationships between the leaf nitrogen and the indices and the results were validated using leave one out cross validation.

The results showed that the measured leaf nitrogen had a relatively low correlation with the measured chlorophyll (R=0.24) and moderate correlation with the measured carbon (R=-0.61). Leaf nitrogen had a high correlation with NIR band in RapidEye and SWIR in Senstinel-2. The standard ratio index (SR index and Clrededge) demonstrated the highest correlation with the leaf Nitrogen (R= 0.70) using the RapidEye data. However, when vegetation indices were optimized using different band combinations, the NIR and red edge band combinations demonstrated to be the most promising index (R=0.70). The results from the cross validation indicated that the best indices selected for RapidEye and Sentinel-2 were not the same.

Foliar nitrogen was estimated from RapidEye data using Clrededge index (red edge and NIR) with R^2_{CV}= 0.41 and $RMSE_{CV}$= 0.40 whereas NDVI like index (red edge and SWIR) yield R^2_{CV}= 0.37 and $RMSE_{CV}$= 0.42 for the Sentinel-2 data. The result of our analysis confirms the importance of the red edge bands for estimation of foliar nitrogen and demonstrate that optimization of vegetation indices using different band combinations improve the accuracy of retrieving foliar nitrogen in a wetland ecosystem from multispectral remote sensing data. The saltmarsh/wetlands are rather heterogeneous ecosystems, so classifying the vegetation based on the species characteristics may further enhance the obtained results.

Keywords: Chlorophyll, Foliar Nitrogen, RapidEye, Red edge band, Sentinel-2, Schiermonnikoog, Saltmarsh grassland/ecosystem, Vegetation Indices.

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LIST OF ABBREVIATION

AVHRR	Advanced Very High Resolution Radiometers
CCCI	Canopy Chlorophyll Content Index
CV	Cross Validation
ETM+	Enhanced Thematic Mapper Plus
EnMap	Environmental Mapping and Analysis Program
MERIS	Medium Resolution Imaging Spectrometer
MODIS	Moderate Resolution Imaging Spectroradiometer
NIR	Near Infrared Region
NRS	Natural Resource Management
NOAA	National Oceanic Atmospheric Administration
RMSE	Root Mean Square Error
RS	Remote Sensing
VI	Vegetation Indices

1. INTRDUCTION

1.1. Background and Motivation

1.1.1. Importance of Foliar Nitrogen

Nitrogen is an essential plant nutrient being an important component in proteins, nucleic acids, and chlorophyll (Reich et al., 1995). Among the available biochemical constituents of protein in leaves, nitrogen plays a primary role of regulating numerous physiological processes such as photosynthesis, leaf respiration and transpiration, as well as been strongly linked to light use efficiency and net primary production (Mutanga & Skidmore, 2007; Skidmore et al., 2010). Nitrogen acts as a limiting factor in plants tissue and serves as an indicator for net photosynthetic capacity in leaves (Reich et al., 1998). Nevertheless, nitrogen and carbon cycles are intimately linked in ecosystem, owing to the role nitrogen exert in controlling rates of several carbon cycling processes including net primary production, regulates carbon assimilation in terrestrial ecosystems, and influences ecosystem processes through decomposition of leaves, uptake of nitrogen in plants and net mineralization (Wright et al., 2004; Goedhart et al., 2010).

Additionally, nitrogen availability influence plant growth and development and the quality of nursery plants hence, an alteration in supply results into non-optimal photosynthesis and subsequently effect the enzyme concentration (Clevers & Gitelson, 2013). Understanding plant functioning and vegetation status, requires information about nitrogen content in addition to other properties (such as leaf area index, biomass, and fraction of absorbed radiation). Moreover, it has been shown that, foliar nitrogen determines the distribution pattern of wildlife most especially in areas with less human interference (Pellissier et al, 2015), influences feeding pattern of livestock (Skidmore et al., 2010), and improves the health status of grazing animals (Ramoelo et al., 2012). Also, foliar nitrogen serves as an input parameter in modelling ecosystem processes (Pereira et al., 2013), and inadequate supply of nitrogen affects the prediction of yield and production of quality crops (Tian et al., 2011). A significantly precise estimate of nitrogen is important for biodiversity conservation, precision agriculture and for understanding ecosystem structure and functions (Asner & Martin, 2008). Besides, nitrogen has also been proposed as an essential biodiversity variable for satellite monitoring towards the progress of Aichi Biodiversity Targets (Pereira et al., 2013; Skidmore et al., 2015).

1.1.2. Remote Sensing: Estimation of Foliar Nitrogen in Vegetation

The three basic physical mechanisms of electromagnetic radiation: absorption, reflection and transmission are responsible for interactions between incident radiation and biochemical pigments in plants (Homolová et al., 2013). In vegetation studies, reflectance from the vegetation canopy is subject to the spatial configuration of the radiative properties of leaves and other non-photosynthetic canopy element. Kumar et al.,(2002) and Homolová et al., (2013) stated that the reflectance spectra of leaves are characterised by the leaf structure in the near infrared region and a strong absorption in the visible region dominated by photosynthetic pigments, particularly chlorophyll. Considering the importance of nitrogen in ecosystem processes, and its spatial variability, there has been efforts made towards retrieving nitrogen using remotely sense data, traced back as to the advent of airborne imaging spectrometers (Martin et al. , 2008a).

Conventional methods involving laboratory spectrophotometric approaches are laborious, time consuming and applicable on small scale (Hansen & Schjoerring, 2003). Remote sensing techniques have been used in the last decade to obtain information on biophysical and biochemical parameters of vegetation including foliar nitrogen (Darvishzadeh et al., 2008). However, remote sensing techniques hinge on the large variety of remotely sensed data provided from different passive and active remote sensing systems, of benefit in vegetation studies. In the process of acquiring data using remote sensing techniques, foliage pigments which are mostly the predominant signal seen from space are reflected captured and stored in spectral bands, either narrow band forming the basic knowledge for terrestrial ecosystems functions. The application of remote sensing techniques in estimating biochemical variables is increasing due its capacity to acquire large scale data and being non-destructive. The estimation of leaf nitrogen has been carried out using hyperspectral and multispectral data in different ecosystems, including forest(Cho et al., 2007; Martin et al.,2008; Wang et al., 2016), crop (Lu et al.,2013) and grassland (Pellissie et al., 2015).

Previous studies have showed that the use of hyperspectral data in estimating leaf nitrogen yields a good accuracy (Feng et al., 2008;Clevers & Kooistra, 2012c;Schlemmer et al., 2013;Wang et al., 2017), however, a number of drawbacks such as the high cost of acquiring data, complexity in processing, and it's avalaibility make it difficult to estimate nitrogen on a regional scale based on such data. Thus, this thesis seeks to evaluate the performance of new high resolution multispectral images in mapping foliar nitrogen.

1.1.3. Remote Sensing Techniques

In the past decades, numerous techniques have been developed to retrieve vegetation biophysical and biochemical variables using remote sensing data; these techniques have been divided into groups in different studies. As such Frampton et al., (2013) recognised them into three broad categories: the use of vegetation indices, machine learning methods like neutral networks (Carpenter et al., 1999) and inversion of Radiative Transfer Models (Shultis & Myneni, 1988). Liang (2005) noted that empirical and physical approaches (or the combination of both) are quantitative methods used for interpreting remotely sensed data to assess plant traits. In more recent studies, these approaches have been categorized as parametric regression methods, non-parametric regression methods, physically based method and hybrid method (Verrelst et al., 2015).

Empirical approaches are computationally fast and more prominent in retrieving vegetation parameters from remotely sensed data, ranging from use of vegetation indices (Miphokasap et al., 2012), and the conventional regression procedure like stepwise multiple linear regression (Kokaly et al.,1999) and partial least square regression (Martin et al., 2008a) to machine learning methods like neutral network, vector regression and Bayesian models (Skidmore et al., 2010; Axelsson et al., 2013; Zhao et al., 2013). Homolová et al., (2013) emphasised that the most appropriate approach of estimating foliar nitrogen is by means of empirical methods and concluded that nitrogen can be retrieved with high accuracy by means of this approach. Additionally, vegetation indices happen to be one of the most widely accepted approach used to estimate leaf biochemical contents especially nitrogen (Wang et al., 2016).

Field measurement of plant trait data are still limited to small areas, to a certain moment in time and to certain number of species only. Therefore, remote sensing (RS) offers potential to complement or even replace field measurements for some plant trait. It offers instantaneous spatially contiguous information, covers larger areas and in case of satellite observation profits from their revisit capacity.

1.2. Research Problem

A lot of emphasis has been laid on the importance of foliar nitrogen in forage quality (Skidmore et al., 2010), livestock management (Ramoelo et al., 2012) and biodiversity conservation (Pereira et al., 2013). This propelled researchers to develop an efficient method appropriate for estimating foliar nitrogen, taking into consideration that, the strength of any reliable method depends heavily on the quality of the data used. Despite these advances in remote sensing data and techniques, it's still challenging to continually monitor subtle changes of nitrogen or even use it as a driver in regional to global scale analyses (Lepine et al., 2016).

Although the conventional spectrophotometric laboratory approach can estimate nitrogen with high accuracy, its drawbacks such as the tedious nature, time demanding and the small area coverage, impede the application of this approach on a regional scale. On the other hand, the contemporary approach, utilising hyperspectral remote sensing, provides better estimate of nitrogen compared to the conventional method (Mutanga & Skidmore, 2007); yet application on a regional scale still pose as a challenge, since these data are usually expensive and not readily available on a regional scale (Knox et al., 2012; Ramoelo et al., 2013). Additionally, the use of vegetation indices derived from Red edge band has proved to improve the estimate of nitrogen (Cho & Skidmore, 2006).

However, mapping foliar nitrogen on a regional scale is rare and face with the challenge of unreliable method of extending field measurement to broad scale spatial patterns. But possibilities abound with the inception of broad band multispectral data. The emergence of new generation multispectral sensors with high spatial and temporal resolution which benefits from spectral bands in the Red edge region such as RapidEye and Sentinel-2, create an avenue to map nitrogen with an improved accuracy and on a regional scale. Although, some studies has been carried out using multispectral imagery in ecosystems like grasslands, croplands and forest (Ramoelo et al., 2015) for nitrogen estimation, only few have been conducted in salt marsh ecosystem (Cartaxana & Catarino, 1997; Bertness et al., 2002). Therefore, this study focus on evaluating the performance of high resolution images in mapping foliar nitrogen in salt marsh/grassland ecosystem.

1.3. Research Objective

1.3.1. General Objective

The general objective of the study is to evaluate the performance of high resolution images in mapping foliar nitrogen in salt marsh/grassland ecosystem of Schiermonnikoog.

1.3.2. Specific objectives

- To examine the relationship between foliar nitrogen and other leaf constituents (chlorophyll and carbon) in saltmarsh/grasslands ecosystem of Schiermonnikoog.
- To evaluate the potential of the vegetation indices derived from Rapid Eye images for foliar nitrogen estimation.
- To determine whether the vegetation indices derived from RapidEye are comparable to those of Sentinel-2 for foliar nitrogen estimation.

1.3.3. Research Questions

- What is the relation between foliar nitrogen and chlorophyll/carbon in saltmarsh/grassland?

- Within the studied vegetation indices, which vegetation index derived from RapidEye data can provide an accurate estimate (in terms of highest R^2 and lowest RSME) in estimating foliar nitrogen?
- Does spectral bands and vegetation indices used for retrieval of foliar nitrogen from RapidEye similar to those of Sentinel-2?

1.3.4. Hypotheses

- H_0: There is no significant correlation between foliar nitrogen and chlorophyll/carbon in salt marsh/grasslands of Schiermonnikoog.
- H_1: There is a positive correlation between foliar nitrogen and chlorophyll/carbon in salt marsh/grassland of Schiermonnikoog.

- H_0: Using the RapidEye Red edge band will not provide a high accuracy (in terms of highest R^2 and lowest RSME) for foliar nitrogen estimation.
- H_1: Using the RapidEye Red edge band a high accuracy (in terms of highest R^2 and lowest RSME) for foliar nitrogen estimation will be obtained.

- H_0: The best band combination within the studied vegetation indices derived from RapidEye images are not comparable to those of Sentinel-2.
- H_1: The best band combination within the studied vegetation indices derived from RapidEye images are comparable to those of Sentinel-2.

2. LITERATURE REVIEW

2.1. Remote Sensing of Biochemical Variables: Physical Principles

The interactions between incident radiation and biochemical pigments in plants depends largely on the physical mechanism of absorption, reflection, and transmission of electromagnetic radiation (Homolová et al., 2013). The wavelength of electromagnetic spectrum, incidence angle, surface roughness and biochemical constituents together with leaf and canopy structure are some of the factors that determines the amount and nature of the physical mechanism in plant (Wright et al., 2004b). Since absorption features of vegetation are mostly located within the optical domain (380nm to 2500nm), it has reemphasized the importance of this spectrum in vegetation studies (Kokaly et al., 2009;Gitelson et al., 2009). Admittedly, chlorophyll is a major plant constituent that determines reflectance in the visible (between 400nm and 700nm) region of the spectrum, thus enhancing optical remote sensing techniques in providing reliable information for chlorophyll and nitrogen content (Clevers & Gitelson, 2013). An overall spectral behaviour for foliar pigments and similar biochemical constituent of a vegetation is illustrated in Figure 1

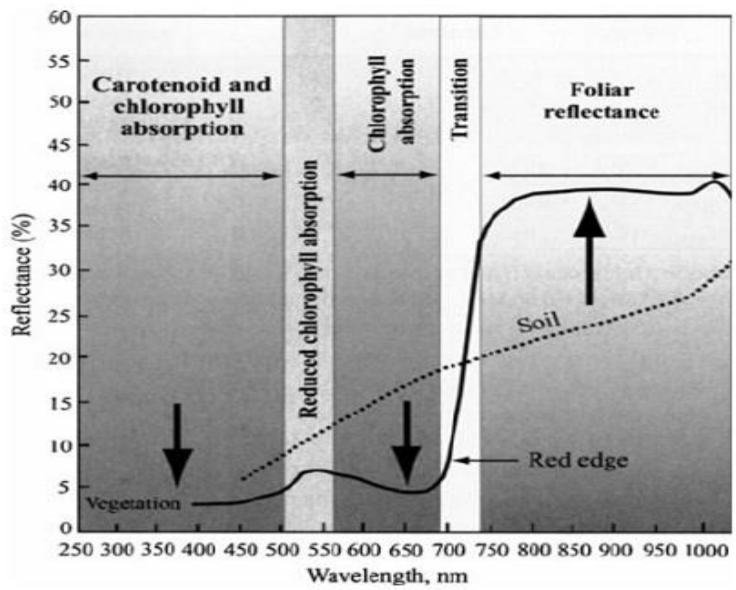

Figure 1. Spectral reflectance characteristics of a healthy vegetation (adapted from Verhoeven, 2012)

Homolová et al., (2013) took a step further to review most of the important and frequently reported wavelengths cited in the literature for estimating nitrogen and summarized them in a graph as shown in Figure 2 below. Based on the above review, it was further stated that these wavelengths can be categorically integrated into three broad spectral regions namely: red-edge region (680–780 nm), near infrared region (1200 nm) and short wave infrared (1680 nm, 2050 nm, and 2170 nm).

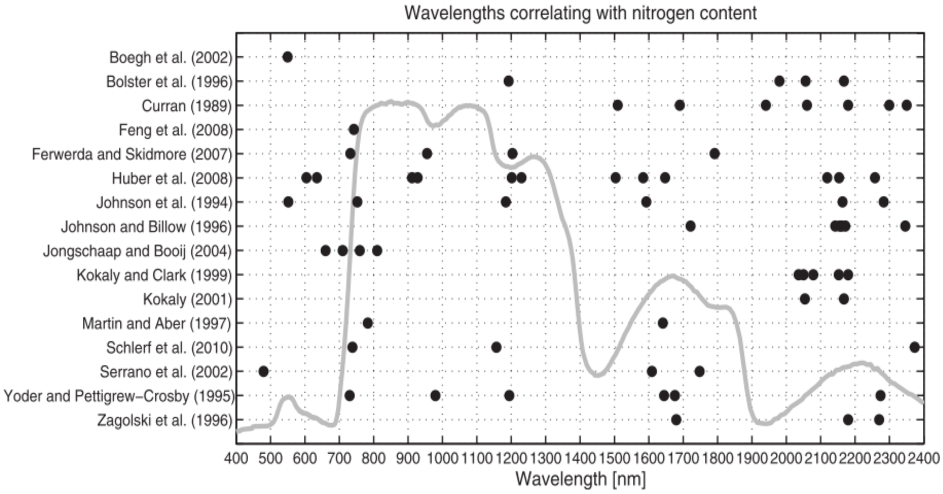

Figure 2. A summary of frequently used spectral wavelength for estimating nitrogen where each dots represents reported wavelength and the grey line representing the reflectance a typical green vegetation(adopted from Homolová et al., 2013)

More recent, studies have demonstrated that the transition between red absorbance and near infrared reflection (NIR) contains additional information related to vegetation characteristics, most of the studies also highlighted that quantification of biochemical traits from NIR are strongly influenced by radiation scattering processes, which must be taken into consideration to obtain reliable results (Homolová et al., 2013). Additionally, reflectance within red and near- infrared (NIR) spectral regions are used for the estimation of biochemical components like chlorophyll imploring remote sensing techniques (Gitelson et al., 2005).

2.1.1. Remote Sensing Instruments

The present prevailing optical remote sensed system used in vegetation studies today are built on, from a range of several remote sensed data obtained from different passive and active satellite sensors. The sensors employed ranges from low-cost spatial multispectral satellite to high-cost imaging spectrometers (AVIRIS, HyMap and Hyperion) (Raymond et al., 2013). Consequently, there is a trade-off between spatial, spectral, and temporal resolution of optical spectroradiometers on the different satellite platforms (ground-based & airborne). However, increased resolution has been recorded right from the inception of coarse spatial broadband multispectral spectroradiometers (AVHRR) around the 1970s and 80s, to an operational satellite based spectroradiometers with a moderate spectral and spatial capabilities (MODIS, MERIS and ETM+) and to a more advance recent satellite sensor with enhanced potentials (Sentinel) (Malenovský et al., 2012; Frampton et al.,2013; Ramoelo et al., 2015).

2.1.2. Remote Sensing Systems in Retrieving Biochemical variables on Regional Scale

The use of remote sensing has obviated the need for field measurement though field measurement remains one of the most reliable means of retrieving accurate measures for biochemical variables. The evolution of optical remote sensing proficiency in estimating plants trait advanced concurrently with the development of remote sensing spectroradiometers (Milton et al., 2009). At first earlier developed spectroradiometers provided coarse spatial and spectral resolution data suitable for broad functional vegetation classes and the used of developed vegetation indices sensitives to broad variations in canopy (Turner et al., 1999). But a more quantitative estimation of biochemical variables (Dash & Curran, 2004) was enhanced further with the generation of medium resolution spectroradiometers (MODIS) simultaneously with the development of radiative transfer model. With the inception of these high spectral resolution imaging spectroradiometers, more quantitative estimate of plant pigments have been retrieved and studied (Mutanga et al.,2004), these studies include chlorophyll and nitrogen which have received much attention (Haboudane et al., 2002; le Maire et al., 2004; Schlerf et al., 2010; Malenovský et al., 2013).

In vegetation studies, remotely sensed data aims at increasing the sensitivity of reflectance towards a biochemical variables (chlorophyll/or nitrogen) However, previous efforts in examining these variables using broad-band sensors have been limited to coarse spectral bandwidths (Haboudane et al., 2002). In addressing this, a ground based sensing system made up of a multispectral optical sensor was developed to measure nitrogen (in cotton) using plant reflectance (Sui & Thomasson, 2006). Most recent, a high spectral resolution aircraft often used for estimating canopy nitrogen on landscape-scale was developed based on the strong correlation between field-measured nitrogen reflectance and the reflectance in some portions of the spectrum (Lepine et al., 2016). In addition, this strong correlation was observed by Ollinger et al., (2008) over broad portions of the NIR region which also correlate with measured nitrogen in temperate and boreal forests.

However, multispectral sensor provides few broad spectral bands, with relatively wide range that can contribute in enhancing the retrieval of biochemical variables such as nitrogen (Bagheri et al., 2013) on a regional scale. Ollinger et al., (2008) and Ollinger (2011) suggested the possibility of estimating nitrogen from spectral features available on sensors which provide broader spectral coverage. Although there are other indications that broad-band spectral features contain information related to variability in canopy nitrogen (Gamon et al., 1995; Zhao et al., 2005; Hollinger et al., 2010). Notwithstanding, Lepine et al., (2016) findings shows that the variability are associated to broad reflectance in the NIR region, so offering many possibilities for estimation of canopy nitrogen on a broad scale from a range of sensors.

2.1.3. Potential of the Red edge Band in Multispectral Sensors

Assessing leaf nitrogen on a regional scale has been challenging owing to insufficient satellite data with spectral configuration appropriate to identify variation in leaf nitrogen content. But with the introduction of satellite sensors containing the red edge band (RapidEye and WorldView-2, Sentinel-2), some improvement has been recorded. The red edge band is a spectral region mostly associated with vegetation, situated within the red absorption maximum and high reflectance in the near infrared. The importance has been acknowledged in vegetation studies for quite a long time. However, in quantifying the red edge, the position which is the maximum slope along the red edge is computed, thus enhancing estimates of concentration of leaf content as well as chlorophyll.

The red edge band also has a positive correlation between chlorophyll and nitrogen, however the region between 680-780nm and 550nm and 700nm spectral wavelength have been used continuously in assessing chlorophyll (Li et al., 2014) and also used mostly for quantification of vegetation indices for estimating leaf nitrogen especially where biomass and leaf nitrogen interaction are minimal. (Ramoelo et al., 2012; Skidmore et al., 2010). Equally there have been researches that highlighted that reflectance in the green and red edge regions is sensitive to a wide range of chlorophyll (Gitelson et al., 1996; Gitelson & Merzlyak, 1996).

2.1.4. Using RapidEye & Sentinel-2 in Retrieving Biochemical Variables

Among most of the multispectral sensors containing the Red edge band are RapidEye and Sentinel-2 sensor. Sentinel-2 is a satellite recently launched by the European Space Agency, which possess a high possibility tailored towards using the sensor to retrieve biochemical variables while utilizing its high spatial, spectral, and temporal resolution (Ramoelo et al., 2015). Due to the fact that it contains spectral bands covering red edge region which is useful in retrieving chlorophyll content (Dash & Curran, 2004; Gitelson et al., 2005; Delegido et al., 2011). Estimates of chlorophyll content using the red edge band of Sentinel-2 has been shown to be highly significant (Clevers et al., 2001; Dash & Curran, 2004). Delegido et al., (2011) shows the significance of red edge bands of Sentinel -2 in estimating LAI and chlorophyll.

Additionally, Wu et al.,(2008) laid more emphasis on the importance of red-edge bands where the red and NIR spectral bands in the MCARI/OSAVI and TCARI/OSAVI indices was replaced by red edge and improved linearity with the canopy chlorophyll and nitrogen content was obtained. Various studies have shown that the use of red edge bands in ratio indices or normalized vegetation indices produce good estimates for nitrogen and chlorophyll content.

2.2. Remote sensing Methods in Retrieving Biochemical Variables

2.2.1. Use of Vegetation Indices

Spectral indices are an important method of retrieving information from remote sensed data. Vegetation indices are widely used in providing quantitative ground measurements of biophysical varaibles of vegetation by contrasting and comparing spectral reflectance characteristics of varying plant species (Frampton et al., 2013). The benefit of using vegetation indices include; simplicity in computation, not site specific and universally applicable. Spectral indices has been shown to be of great importance especially in analyzing imaging spectrometer data (Gitelson, 2011). Although most of these developed spectral indices are calculated using ratios or normalised differences between two or three bands but this depends on the spectral properties of individual plant species.

Considerably, each of this developed vegetaion indices has it’s own strength and weakness in application, so some are more optimal at retrieving certain varables than others. However, vegetation indices might be affected by different factors like soil, topography and angular view (Hatfield et al.,2008), but notwithstanding they try to reduce the effects of the above mentioned factors but not completely eliminating them. One of the best method of reterieving biophysical parameters of a vegetation regionally with a high temporal covearage still remains with the use of vegetation indices derived from remote sensed data (Frampton et al., 2013).

2.2.2. Estimating Nitrogen using Vegetation Indices

Vegetation indices is an empirical approach used mostly for estimating foliar nitrogen. It is an old but simple technique for estimating leaf biochemical content like nitrogen (Verrelst et al., 2015). These vegetation indices consist of a combination of Near Infrared (NIR) spectral band and a visible band where the former represents the scattering of radiation at the canopy level and the latter represents the absorption by chlorophyll. Studies have shown that the presence of nitrogen in leaves influences spectral reflectance due to its presence in protein and chlorophyll contents of leaf cells (Kokaly et al., 2009).

There are two main categories of vegetation indices: Vegetation indices developed for broadband multispectral sensors; and hyperspectral vegetation indices built on discrete narrows bands (Dorigo et al ., 2007). A number of vegetation indices (VIs) have been propound for estimating chlorophyll and nitrogen content with a range of strengths (Daughtry et al., 2000; Boegh et al., 2002; Dash & Curran, 2004; Gitelson et al., 2005) and with more emphasis on the red edge region (Clevers & Kooistra, 2012a).

Since the visible and NIR reflectance indices are sensitive to chlorophyll content, indices developed with these spectral bands increases sensitivity to chlorophyll. The positive correlation existing between chlorophyll and nitrogen have resulted into different mathematical transformation of vegetation indices (Main et al., 2011).

However, Sentinel-2 has the potential of retrieving nitrogen content using suitable band position for use in vegetation indices, these indices have been used in studies for estimating nitrogen and chlorophyll (Clevers & Kooistra, 2012: Wang et al., 2012: Wang et al., 2016).

2.3. Statistical Approach

In most vegetation studies, statistical approaches are used to model empirical relationship of spectral features and the biophysical parameter of interest which are driven primarily by reflectance over broad portions of the near infrared (NIR) region, with little contribution from the visible or mid infrared regions. Most of the attempts carried out in estimating nitrogen and other biochemical constituents involves the development of an empirical prediction model (Wessman et al., 1988; Coops et al., 2003; Townsend et al., 2003; Martin et al., 2008a; McNeil et al., 2008).

In modelling the relationship existing between spectral features and the biophysical parameter of interest, we mathematically correlate reflectance for different wavelength ranges or broad spectral bands with the biophysical vegetation parameters of interest (e.g., leaf area index (LAI), leaf chlorophyll content (LCC), fractional vegetation cover, nitrogen content) (River et al., 2014). This is achieved by fitting a function (exponential, power, logarithmic and polynomial) using a simple linear regression.

The regression function is dependent on the following factors: selecting the most sensitive spectral band relative to the retrieval of the biophysical parameter in question, formulation of spectral index that establish accurate empirical relationship and the selection of an accurate fitting function (River et al., 2014).

Lately, a conceptual model which relates remotely sensed reflectance with pigment content in different components of plant (leaves, crop canopy and phytoplankton) was developed and used for non-destructive estimation of chlorophyll (Gitelson et al., 2003). Although the development of vegetation indices, which are mostly based on spectral regions have been used successfully, but their calibration coefficients are species-specific because they were tested under single-species canopies (Daughtry et al., 2000; Boegh et al., 2002; Dash & Curran, 2004).

However, the relationships between field measured nitrogen and canopy spectral properties as shown by (Martin et al., 2008a) appears to be highly boosted by NIR reflectance patterns, and were consistent enough across boreal, temperate, and tropical forests. In addition, Feng et al. (2008) deduced a model for reliable estimation in wheat by employing hyperspectral bands and estimation indices.

2.4. Using Remote sensed Chlorophyll as a Proxy for estimating Nitrogen

Vegetation indices, spectral models and transformations have been developed to estimate chlorophyll from reflectance data (Homolová et al., 2013). Remotely sensed chlorophyll can be used as a proxy to estimate nitrogen using operational approach (Le Marie et al., 2008) as usually high correlation exist between nitrogen and chlorophyll in plant (Evans, 1989). Baret et al., (2007) also concurred that nitrogen could be determined from chlorophyll estimate. However, this relationship has proven to be specie and ecosystem specific and therefore makes it more interesting for communities with lower species diversity.

2.5. Conclusion

Based on the above review, empirical approach involving the use of vegetation indices are proven in many application areas and we would like to test how they perform in Schiermonnikoog. This approach has been adopted for use in this present study.

3. MATERIAL AND METHODS

3.1. Study Site and Data

3.1.1. Site Description

The study area is the saltmarsh in the islands of Schiermonnikoog (53°30' N, 6°10' E), (figure 3) the Netherlands and is part of the Dutch Waddenzee ecosystem (Schmidt & Skidmore, 2003). The wetland is made up of a salt-marsh with a total area of 39.9km^2 with 16 km long and a width of 4 km, a yearly temperature of $10.2^0 \pm 0.72^0$C (mean ± SD), and rainfall of 824 ± 149.1 mm (Schrama et al., 2012). The west and northwest part of the island is fortified against erosion by the sea. The artificial sand dikes protect the salt marsh towards the western part whereas the natural dunes protect the eastern part of the salt marsh (Schmidt & Skidmore, 2003). It's a contiguous nature reserve formed because of the deposition of sediments from the river Rhine. The eastward sea current extends to the islands towards the east, where the salt marsh chronosequence has been present for over 100 years (Olff et al., 1997).

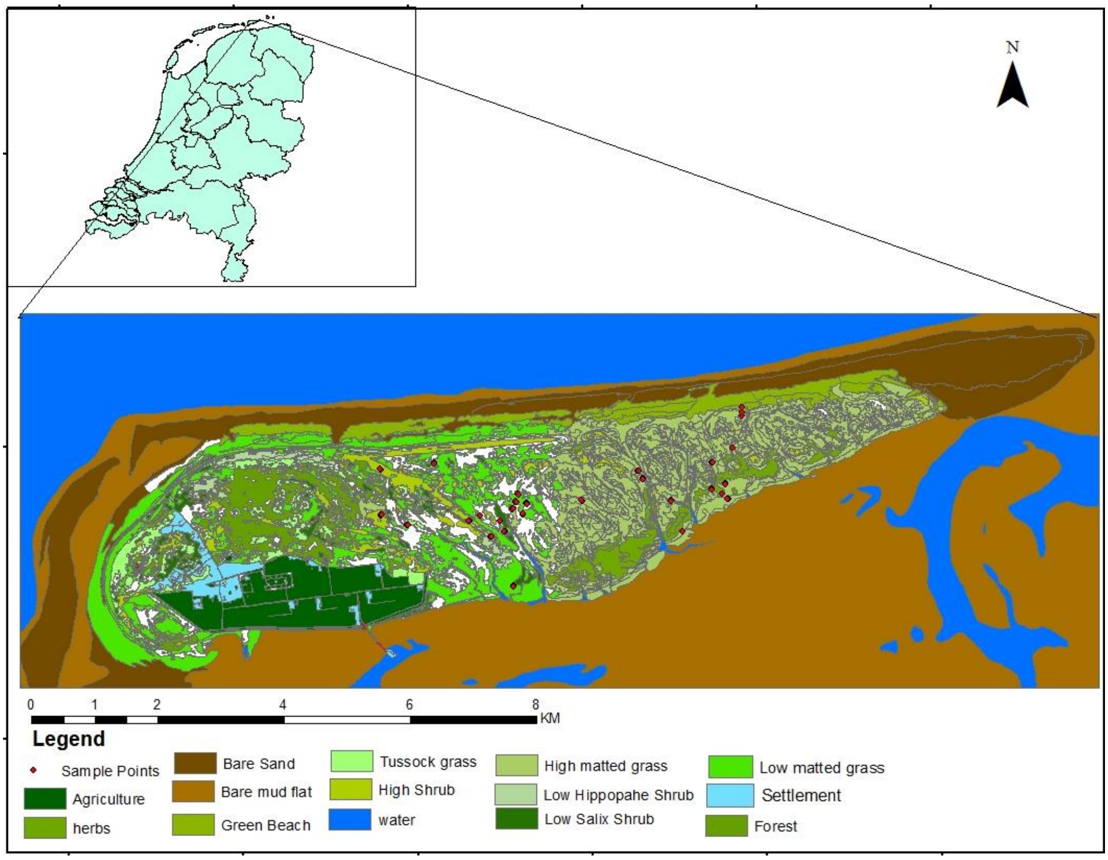

Figure 3: Location of the Schiermonnikoog islands, the vegetation structure, and the distribution of the sample plots.

3.1.1.1. Vegetation Type

The National Park Management known as Natuurmonument is responsible for managing the National Park of Schiermonnikoog since the island was left to nature since 1965 (Olff et al., 1997). The island characteristics features include beach, dunes, forest, a polder, salt marshes and the mud flats. The saltmarsh vegetation is made up of grasses, sedges, rushes, and herbs. Canopy within the salt marsh are on average of 25 cm which are either electrophiles or circular (Schmidt & Skidmore, 2003). There are patches of different degree of bare soil between vegetation and a relatively uniform soil characteristics within the vegetation (Schmidt & Skidmore, 2003). Examples of grass species dominate in the island are *Festuca rubra* (Poaceae), *Elytrigia atherica* (Poaceae) and *Juncus maritimus* (Juncaceae) (Ruifrok et al.,2014). Figure 3 shows the vegetation structure and the distribution of the sample plots in Schiermonnikoog.

3.1.2. Data

3.1.2.1. Field Data

The field data used for this study was collected during a field campaign carried out by ITC, NRS staff in July 2015. Field measurements of different vegetation traits were collected in a total of 30 plots (30m x 30m), which were generated within the salt marsh grassland strata subject to the land cover map used by the National Reserve Park's management Authority. In each plot, a range of one to four subplots (1m x 1m) was stratified based on the homogeneity and heterogeneity of the grass species found on the main plot.

Leaf chlorophyll content (LCC) was non-destructively measured in the field with a SPAD-502 leaf chlorophyll meter, measuring the transmittance in the red (650nm) and near infrared (920 nm) wavelength region. A total of 30 leaves representing the dominant species was randomly selected in each plot and the SPAD reading averaged and converted into LCC in unit of $\mu g\ cm^{-2}$. Further, for leaves with very small surface area, the leaf chlorophyll content (Cab $\mu g\ cm^{-2}$) was measured non-destructively in the field using a CCM-300 chlorophyll content meter (Opti-Sciences, 2011).

3.2. Laboratory Chemical Analysis

3.2.1. Sample Preparation

Leaf samples were collected in each subplot representing the species diversity and were placed in labelled plastic zip-locked bags and subsequently transported to the laboratory. In the laboratory, the fresh weight and area of the leaf samples were recorded and they were dried for 48 hours using an oven at 65^{o}C. It is paramount to note that 27 sample plots were used further in the analysis, three sample plots were lost in the process of storage. The dried samples were pulverized into fine particles with a mortar and pestle to pass through a 180μm mesh screen. The properly dried prepared leaf samples were label and stored properly in paper bags in an oven dried at 50 c for chemical analysis.

3.2.2. Chemical Analysis

Next, the leaf nitrogen and carbon (% dry weight) were determined by the dry combustion method using the Perkin Elmer 2400 CHNS/O Elemental Analyzer (PerkinElmer, 2005). The analyzer combusts sample elements in a pure oxygen environment, with a temperature range of 1200-2400F to convert the sample element into simple gases like CO_2, H_2O and N_2, separated under a steady state. However, CO_2, H_2O and

N_2 represents carbon, hydrogen, and nitrogen contents respectively. In retrieving nitrogen percentage, the following step was adhered to.

- a steady state condition was established in the instrument by using a high purity oxygen and helium gases, running the leak diagnostic because the column was changed, conditioning the system by running several unweighed capsules of acetanilide, and adjusting the sample size (within the range of 1.6 – 1.8mg);
- establishing a blank value at the beginning and throughout the run sequence;
- Running accurately weighed leaves samples;
- Establishing a K-factor by running pure standards.

The ideal operating procedure includes running conditioners, blanks, calibrants and standards throughout the run sequence. The percentage of nitrogen, carbon and hydrogen are enclosed exported as a csv file.

The above method is similar to the method adapted by Ramoelo et al., (2015), where the potential of sentinel-2 was explored in measuring leaf nitrogen in grasslands. Additionally, the possibility of retrieving biochemical concentration from dried leaves has been emphasized by Kokaly & Clark, (1999)

Table 1: Summary Statistics of the measured variables

Summary Statistics							
Variables	Mean	Median	Min	Max	Stdev	Range	CV
%Nitrogen	2.47	2.46	1.65	3.54	0.54	1.88	0.22
Chlorophyll	17.19	16.77	5.23	34.69	7.75	29.46	0.45
%Carbon	42.48	44.15	36.47	45.57	2.82	9.10	0.07

3.3. Image Data Acquisition and Processing

3.3.1. RapidEye Data

The RapidEye is a multispectral sensor constellation, consisting of five satellites with identical sensors and capable of collecting large volume of data covering over 6 million square kilometres per day (Tyc et al., 2005). These satellites are all in the same orbital plane (sun-synchronous orbit) and provides imagery with high repetitive rate and a spatial resolution of 6.5m x 6.5m equipped with a multi spectral push broom focal plane. The constellation provides five multi spectral bands covering the blue (440–510 nm), green (520–590 nm), red (630–685 nm), and near infrared spectral ranges (760–850 nm). Schuster & Förster (2008) demonstrated that the red edge spectral band was sensitive to sudden rise in reflectance induced by vegetation's chlorophyll status. The specification of the multispectral instrument (MSI) on the RapidEye satellite system is shown in Table 2.

The RapidEye imagery used for this study was obtained on the 18th July 2015 concurrent to the time of the field campaign. The data obtained was pre-processed at level 3A, which means that the radiometric, geometric corrections and geo-referencing were applied. The image covers 25 km × 25 km with orthorectified pixel size of 5 m × 5 m. The reflectance spectra of the sample plots were extracted from RapidEye imagery within a kernel size of 5 by 5 for the field plots locations, resulting in a spectral calibration datasets of 27 reflectance spectra.

Table 2: RapidEye Constellation and Sensor Specifications

Mission Characteristics	Information
Number of satellites	5
Orbit Altitude	630 km in Sun-synchronous Orbit
Equator Crossing Time	11:00 am local time (approximately)
Sensor Type	Multispectral push broom
Spectral Bands	Blue 440-510nm
	Green 520-590nm
	Red 630-685nm
	Red edge 690-730nm
	NIR 760-850nm
Ground Sampling Distance(nadir)	6.5m
Swath Width	77km
Maximum Image Strip per Orbit	Up to 1500km of image data per orbit
Revisit Time	Daily (off-nadir)/5.5 days (at nadir)
Image Capture Capacity	>6 million km^2/day
Camera Dynamic Range	12-bit

3.3.2. Sentinel-2 Data

In 2015, the European Space Agency launched a polar orbiting satellite called Sentinel-2, carrying a multispectral instrument(MSI) with four bands at 10m, six bands at 20m and three bands at 60m spatial resolutions. The swath width covers 290km with a 20^o field of view window (Drusch et al., 2012). The Sentinel-2 image also contains two spectral bands within the red edge region centred at 705nm and 740nm with a spatial resolution of 20m. The specification of the multispectral instrument on Sentinel-2 is presented in Table 3.

Table 3: Specifications of the Multi Spectral Instruments(MSI) on the Sentinel-2 satellite system

Spectral band	Wavelength (nm)	Band width (nm)	Spatial resolution (m)
B1	443	20	60
B2	490	65	10
B3	560	35	10
B4	665	30	10
B5	705	15	20
B6	740	15	20
B7	783	20	20
B8	842	115	10
B8a	865	20	20
B9	945	20	60
B10	1380	30	60
B11	1610	90	20
B12	2190	180	20

In this study, Sentinel-2 image was acquired for 20 July 2016 in which vegetation were in the same phenological stage of the field campaign. Both satellite sensors (RapidEye and Sentinel-2) contain the Red edge spectral bands making them distinguished amongst other satellites and important for vegetation studies especially in retrieving biochemical variables like nitrogen (Ustuner et al., 2014). The reflectance spectra of the sample plots were extracted from Sentinel-2 imagery using the coordinates of the centre of field plots, resulting in a spectral calibration datasets of 27 reflectance spectra.

3.4. Selection of Vegetation Indices for Nitrogen Estimation

Generally, a strong positive correlation exist between nitrogen and chlorophyll across plant species has been noted (Hansen & Schjoerring, 2003; Haboudane et al., 2004) Most of the vegetation indices linking chlorophyll and nitrogen to vegetation indices have used spectral wavelength within 550 nm -780 nm range.

The selection of vegetation indices in this study was based on their performance and sensitivity to leaf chlorophyll and nitrogen in earlier studies. The selected vegetation indices were modified using spectral bands of the images used. The selected indices include the combination of visible, near infrared and red edge band (see table 4 below). The vegetation indices shown in table 4 were selected based on RapidEye band settings using the closest bands available, suited within nitrogen absorption features from the reflectance spectra many of which has been reported in literatures for nitrogen estimation.

Table 4: Selected Vegetation Indices in this study based on their sensitivity to leaf nitrogen and chlorophyll

S/N	Spectral Index	Algorithm	Reference
1	SR705	R_{NIR}/R_{RED}	Gitelson & Merzlyak (1994) Jordan, (1969)
2	Clrededge	(NIR/Red Edge) −1	Gitelson et al. (2003,2006)
3	Cl green	(NIR/Green) − 1	Gitelson et al. (2003,2006)
4	Green NDVI	$(R_{NIR} – R_{green})/ (R_{NIR} + R_{green})$	Gitelson et al., (1996)
5	NDVI	$(R_{NIR} − R_{RED})/ (R_{NIR} + R_{RED})$	Rouse et al., (1974)
6	NDVIred edge	$(R_{NIR} − R_{RED\text{-}edge})/ (R_{NIR} + R_{RED\text{-}edge})$	Gitelson & Merzlyak (1994)
7	SR Index	$R_{NIR}/R_{RED\text{-}edge}$	Gitelson & Merzlyak (1994)
8	RDVI	$(R_{800} – R_{670})/ (SQRT (R_{800} + R_{670}))$	Roujean & Breon, (1995)
9	MSAVI	$0.5(2R_{800} + 1 – SQRT ((2R_{800} + 1)^2 – 8 (R_{800} – R_{670})))$	Qi et al., (1994)
10	GI (Green Index)	R_{554}/R_{677}	Smith et al., (1995)
11	OSAVI	$(1 + 0.16) (R_{800} – R_{670})/ (R_{800} + R_{670} + 0.16)$	Rondeaux et al., (1996)
12	OSAVI (705,750)	$(1 + 0.16) (R_{750} – R_{705})/ (R_{750} + R_{705} +0.16)$	Wu et al., (2008)

The use of vegetation indices comes with enormous benefits of retrieving nitrogen (Mutanga et al., 2005; Kruse et al., 2006), including serving as a means of representing variability in leaf area index and biomass which are relative to percentage nitrogen. Most recently, vegetation indices explored in studying characteristics features of vegetation, concentrate on the red edge region. The red edge region is define as a region with a sharp rise in the reflectance of green vegetation between 670nm and 780nm containing information useful for chlorophyll and nitrogen estimation and can serve as a measure of plant condition (Horler, Dockray, & Barber, 1983). Horler et ., (1983) demonstrated that the region is a transition of the red NIR, containing information for vegetation spectra. However, the position and slope of red edge is subject to change under a stress condition. Although indices like NDVI are affected by factors like soil and atmosphere whereas chlorophyll red-edge and green chlorophyll index do not affect the saturation effects.

3.5. Regression Analysis

Regression analysis has been a popular empirical method of linking biochemical variables (such as nitrogen) to remote sensing data to provide continuous estimates for these variables (Cohen et al., 2003). In most studies, vegetation indices are related to vegetation variable through linear or exponential regression models. In this study the percentage nitrogen obtained from the laboratory analysis was averaged to mean nitrogen concentration and then were related to vegetation indices using simple linear regression models which were developed to interpret the relationships between the vegetation variable (nitrogen) and the vegetation indices. Measured percentage nitrogen per plots was related to the RapidEye and Sentinel-2 reflectance spectra through linear regression model to derive a predictive model. Likewise, the extracted reflectance of each individual band and the selected vegetation indices were iteratively linearly regressed against measured percentage nitrogen.

3.5.1. Band Combination

A systematic assessment of possible band combinations, vegetation indices formulations and curve fitting procedures are required before choosing a vegetation index model to retrieve biophysical or biochemical variables from a remotely sensed data (Rivera et al., 2014). An interesting approach therefore is by calculating all possible band combinations for vegetation indices formulations. For instance, the mostly used vegetation index is the generic Normalized Difference Index (NDVI).

The index calculates all possible two-band narrowband combinations per the formulation:

$$\mathrm{NDVI} = (R_{NIR} - R_{RED}) / (R_{NIR} + R_{RED})$$

where NIR and Red represent the reflectance bands for the entire optical spectral range. These supposed generic spectral indices allows the selection of a best performing index when correlated with nitrogen. In the review conducted by le Maire et al., (2004), the formulation of vegetation or a spectral index are categorized into four broad classes; indices using a single reflectance or a difference between reflectance at two wavelengths; simple ratio of reflectance's (SR); normalized difference ratios of reflectance (ND); and indices based on reflectance signature derivatives.

In this study, in addition to selected indices, the correlation between nitrogen and individual bands as well as the correlation with other leaf constituents (chlorophyll, carbon) were examined. The fitting model was restricted to ordinary least-squares linear regression.

3.6. Model Calibration and Validation

The regression models were validated using leave one out cross validation LOOC (Stone, 1974; Ramoelo et al., 2015) due to the small sample size(n=27). During this process, individual samples were predicted based on the remaining samples. For instance, out of the 27 samples available, iteratively, 26 samples were used to predict the sample which was left out. Cross validated root mean square error (RMSE) and coefficient of determination (R^2) were determined where RMSE represents the overview a measure of the standard deviation of the error in the model prediction (Lepine et al., 2016) and R^2 expresses the dispersion of the estimated point from the best fitting line and expresses how good the model captures the relationship between nitrogen and the selected vegetation indices.

3.7. General workflow of the Methodology

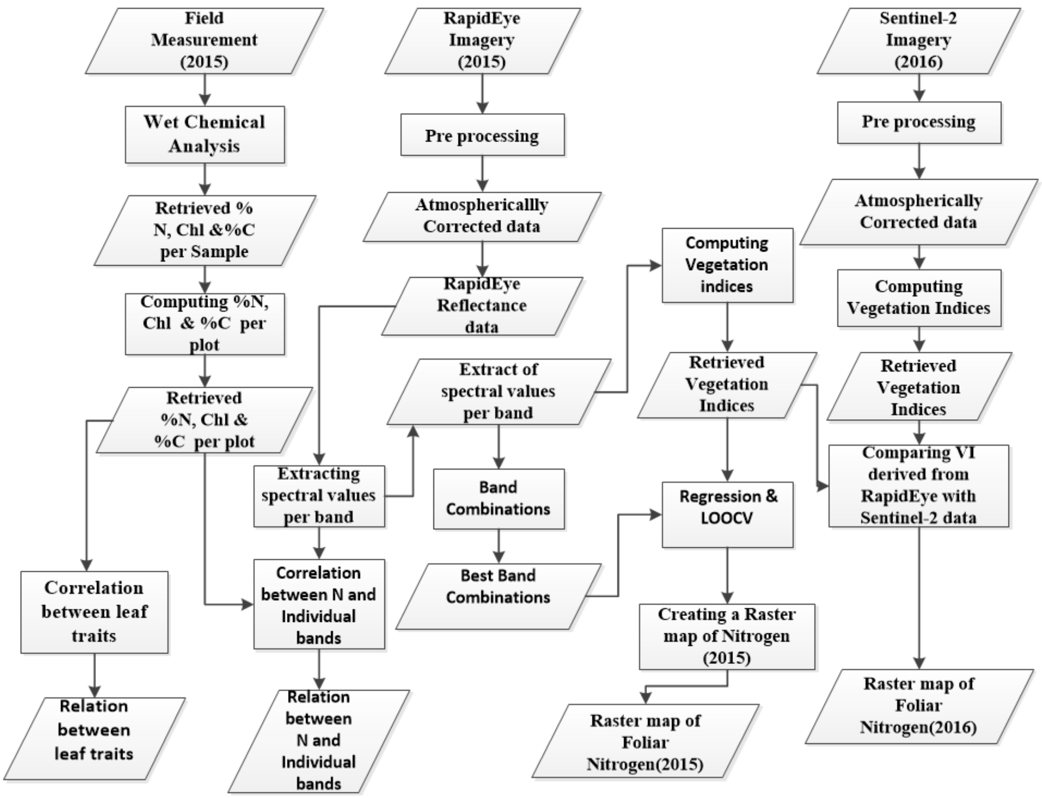

Figure 4: Overall workflow of the present study

4. RESULTS

This chapter presents the results of the analysis performed in the study including exploration of field and remote sensing data, the relationship between foliar nitrogen and other leaf constituents (such as chlorophyll and carbon) in the saltmarsh of Schiermonnikoog, calculation of the selected vegetation indices for foliar nitrogen estimation, evaluation of the best band combination derived from RapidEye imagery to estimate foliar nitrogen and finally, comparing the best band combination of RapidEye to those of Sentinel-2 for the estimation of foliar nitrogen.

4.1. Leaf triats and their interactions

AS can be observed from Table 1 (presented in chapter 3) the measured leaf nitrogen ranged from 1.65% to 3.54% with a mean of 2.46% while the measured carbon ranged from 36.47% to 45.57% and with a mean of 42.48%. The variability of nitrogen and carbon were relatively low while chlorophyll showed a larger variation with a coefficient of variation of approximately 22%, 7%, and 45%, respectively.

Further, the relationship between the field measured leaf nitrogen and other leaf constituents were examined to understand how leaf traits are interacting in the saltmarsh of Schiermonnikoog and the results are illustrated in table 5. As can be observed from the table, the relationship between leaf nitrogen and leaf chlorophyll content among the measured samples were not very strong (R=0.24). On the other hand, the relationship between leaf chlorophyll and leaf carbon was somehow stronger (-0.54). Moreover, carbon also showed a high negative correlation with leaf nitrogen (R=-0.61).

Table 5 Interaction between leaf traits among the measured samples (N=27).

Leaf Constituents	Nitrogen	Chlorophyll	Carbon
Nitrogen	1		
Chlorophyll	0.24	1	
Carbon	-0.61	-0.54	1

4.1.1. Reflectance variations among samples

Reflectance spectra of the sample plots were extracted from the RapidEye and Sentinel-2 images based on the coordinates of the plots. This spectral reflectance for the 27 sample plots are demonstrated in figure 6 (for RapidEye) and Figure 7(for Sentinel-2), respectively. From these figures, it can be observed, that the reflectance spectra obtained from both satellites have similar general shapes corresponding to the shape of vegetation. Although there seems to be few crossovers around the visible and near infrared regions which can be attributed to the differences within spectra of different vegetation types or species in relation to the different spectral regions.

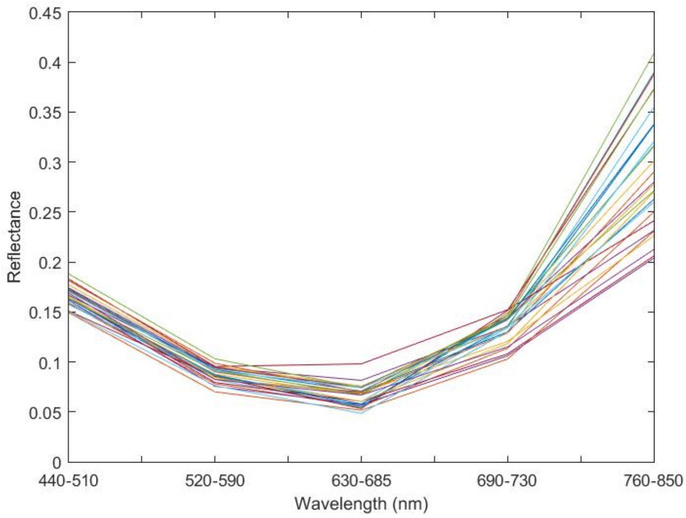

Figure 5: Spectral Reflectance of the 27 sample plots extracted from RapidEye image (2015)

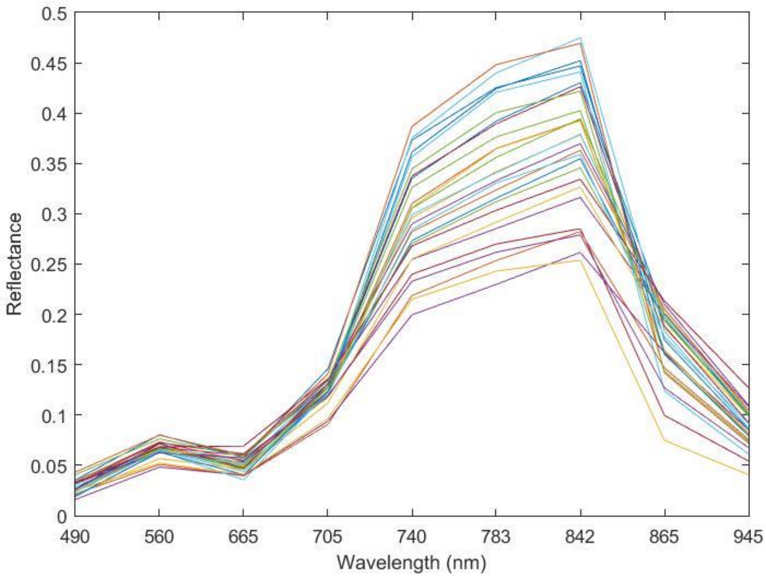

Figure 6: Spectral Reflectance of the 27 sample plots extracted from the Sentinel-2 image (2016)

As can be realized from figure 6 and figure 7, the reflectance of the 27 samples plots are very distinct from each other which indicate a large variability within the sample plots and consequently the study area. This variation may further explain the heterogeneity of the vegetation cover in our study area.

4.2. Relationships between individual spectral bands and leaf nitrogen

It is important to determine the relationship between two measured quantities before fitting a model function. Therefore, we studied the existing relationships between leaf nitrogen and individual spectral bands of the remote sensing data. In this regard, we first examined the relationships between leaf nitrogen and the reflectance of the sample plots extracted from RapidEye image (table 6). Next, to further extend to our analysis to larger scale, we studied the relationship between leaf nitrogen and the reflectance of individual spectral bands of Sentinel-2 from (2016) data which belonged to the same phonological stage. These relationships are presented in Table 7.

Table 6: Correlation between reflectance of individual RapidEye bands and the measured leaf nitrogen (n=27).

Bands	Blue	Green	Red	Red edge	NIR	Nitrogen
Blue	1					
Green	0.83	1				
Red	0.83	0.73	1			
Red edge	0.31	0.72	0.36	1		
NIR	-0.03	0.35	-0.28	0.71	1	
Nitrogen	-0.08	-0.06	-0.44	0.03	0.53	1

As can be observed from table 6, nitrogen shows high correlations to NIR and red bands of RapidEye, however, the relationship between the Red edge band of RapidEye and leaf nitrogen are not very strong.

Table 7: Correlation between reflectance of individual sentinel-2 bands and leaf nitrogen (n=27)

Bands	Blue	Green	Red	Red edge	Red edge	Red edge	NIR	SWIR	SWIR	N
Blue	1									
Green	0.76	1								
Red	0.77	0.65	1							
Red edge	0.61	0.93	0.68	1						
Red edge	0.31	0.70	0.01	0.69	1					
Red edge	0.29	0.68	-0.03	0.65	0.99	1				
NIR	0.24	0.69	-0.03	0.66	0.99	0.99	1			
SWIR	-0.13	0.34	0.28	0.44	0.18	0.16	0.27	1		
SWIR	-0.09	0.28	0.40	0.41	0.03	0.01	0.12	0.97	1	
N	0.17	0.14	-0.19	0.09	0.41	0.40	0.33	-0.47	-0.54	1

As can be observed from table 7 nitrogen shows a high correlation to NIR and two red edge bands of Sentinel-2, however the relationship between the leaf nitrogen and bands in SWIR region appears to be also strong, while the relation between leaf nitrogen and band from Red region is rather low (R=0.19).

4.3. Relation of standard spectral vegetation indices and foliar nitrogen

The correlation of the studied vegetation indices (in standard formulation) with foliar nitrogen are shown in table 8. As can be observed from the table, Clrededge and SR indices show strong positive correlations to nitrogen (R=0.70) for the RapidEye image, while using the Sentinel-2 image, Clrededge, NDVIrededge, SR indices and OSAVI with almost similar value of R (0.46) performed better in comparison to other indices.

Table 8: The relation between standard vegetation indices and leaf nitrogen using reflectance extracted from both images.

Vegetation Indices	RapidEye	Sentinel-2
	R	R
NDVI	0.59	0.35
NDVIrededge	0.69	-0.46
SR705	0.57	0.30
Clrededge	0.70	-0.46
Clgreen	0.57	0.31
Green NDVI	0.57	0.31
SR Index	0.70	-0.46
RDVI	0.59	0.36
MSAVI	0.58	0.35
GI (Green Index)	0.56	0.31
OSAVI	0.59	0.35
OSAVI (705, 750)	0.67	0.47

As can observed from table 8, the performance of the standard vegetation indices for RapidEye are similar to those of Sentinel-2. This indicate that the performance of the satndard vegatation indices for both images can be compared.

In figure 7 the relationships between the measured leaf nitrogen and the standard indices (Clrededge) for RapidEye (2015) and Sentinel-2 (2016) are presented.

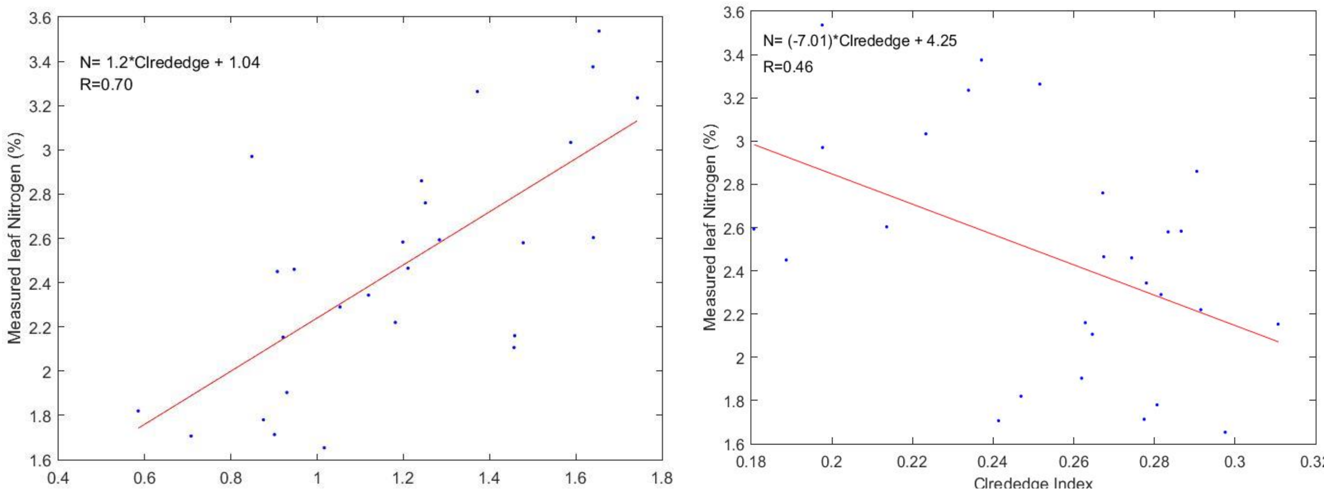

Figure 7: The relationships between foliar nitrogen and the standard vegetation index; (a)Clrededge for RapidEye (2015) (b) Clrededge Sentinel-2 (2016)

4.4. Best Band Combination

To further analyse which band combinations are most suitable for estimation of leaf nitrogen, the relationships between leaf nitrogen and all band combinations in RapidEye (5*5) and Sentinel-2 (9*9) for the selected vegetation indices (12 indices) were examined and the best performing combination (in term of highest $R^{2)}$ was selected and are presented in table 9. It is paramount to note that only two band vegetation indices were used for this formulation. Table 9, demonstrates the best possible two band combinations using the reflectance form RapidEye and Sentinel-2 data.

Table 9: The correlation between the best possible two band combinations for nitrogen estimation using vegetation indices

Vegetation Indices	RapidEye		Sentinel-2	
	Best band combination	R	Best band combination	R
NDVI	band_5 & band_4	0.69	band_5 & band_9	0.67
SR705	band_5 & band_4	0.70	band_5 & band_9	0.65
Clrededge	band_5 & band_4	0.70	band_5 & band_9	0.65
RDVI	band_5 & band_4	0.65	band_5 & band_8	0.67
MSAVI	band_1 & band_3	0.68	band_8 & band_5	-0.66
OSAVI	band_5 & band_4	0.67	band_5 & band_8	0.66

As can be observed from table 9, the best performing vegetation index using the best band combinations for RapidEye are simple ratio (SR705) and Clrededge using the red edge and NIR bands, while on the other hand NDVI and OSAVI indices using NIR and SWIR bands performed best for the sentinel-2 data.

4.5. Cross Validation

To assess the performance of the vegetation indices calculated from the best band combinations for estimation of foliar nitrogen, cross validation was used for model validation. A scatterplot of measured nitrogen and estimated nitrogen using the best band combination in the form of simple ratio (NIR and RedEdge bands) index using RapidEye and in the form of Clrededge index (NIR and SWIR bands) using Sentinel-2 data are illustrated in figure 8.

The cross validated results using the best band combinations of the selected vegetation indices are presented in table 10 and the cross validated R^2 and RMSE between measured and estimated leaf nitrogen are presented. The R^2_{CV} and $RMSE_{CV}$ indicate that the best indices can only explain 41% variability in RapidEye and 33% variability in Sentinel-2.

Table 10: The relation between measured and estimated foliar nitrogen

	RapidEye		Sentinel-2	
Index	R^2_{CV}	$RMSE_{CV}$	R^2_{CV}	$RMSE_{CV}$
NDVI	0.40	0.41	0.37	0.42
NDVIrededge	0.40	0.41	0.37	0.42
SR705	0.41	0.41	0.33	0.43
Clrededge	0.41	0.41	0.33	0.43
Clgreen	0.41	0.41	0.33	0.43
Green NDVI	0.40	0.41	0.37	0.43
SR Index	0.41	0.41	0.33	0.43
RDVI	0.33	0.43	0.36	0.43
MSAVI	0.37	0.42	0.34	0.43
GI (Green Index)	0.41	0.41	0.33	0.43
OSAVI	0.35	0.43	0.36	0.43
OSAVI (705, 750)	0.35	0.43	0.36	0.43

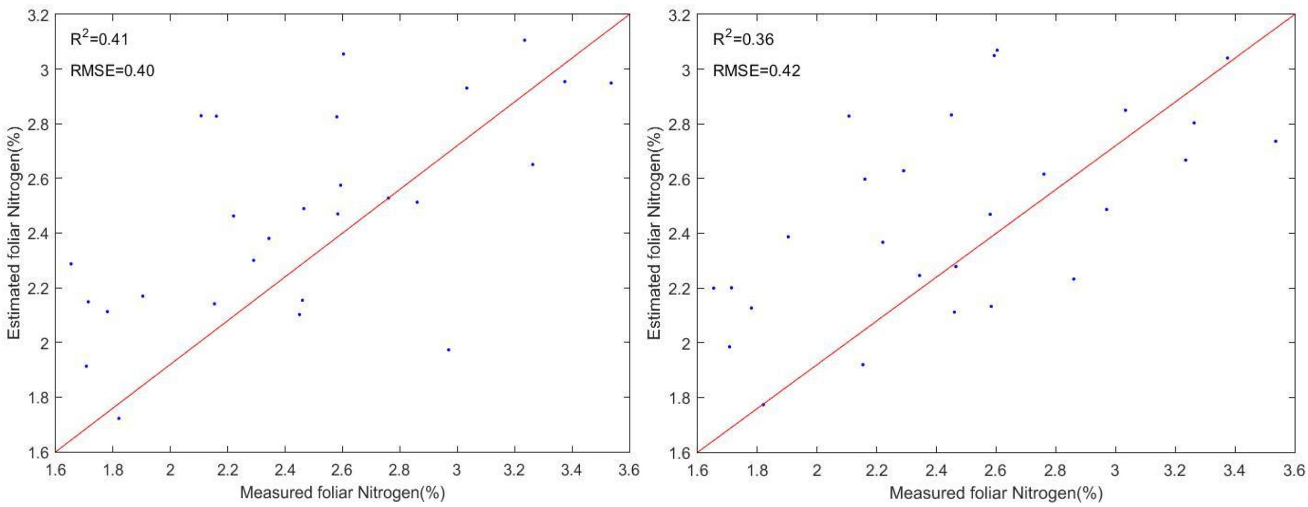

Figure 8: The relationship between the estimated and measured foliar nitrogen based on the best band combinations: (a)Clrededge index (NIR and RedEdge bands) (RapidEye, 2015) and NDVI index (Red and SWIR bands (Sentinel-2, 2016). R^2 and RMSE are cross validation

4.6. Mapping foliar Nitrogen

The best performing vegetation index for Sentinel-2 using red and NIR bands in were utilized to map nitrogen in the study area. For this first the waterbodies and residential areas were identified and masked out from the images. Next the best vegetation index (NDVI using band red and NIR) was calculated in the image and then the regression model that was developed on the best performing index was applied to the Sentinel-2 image resulting into the map of foliar nitrogen for the salt marsh area of Schiermonnikoog.

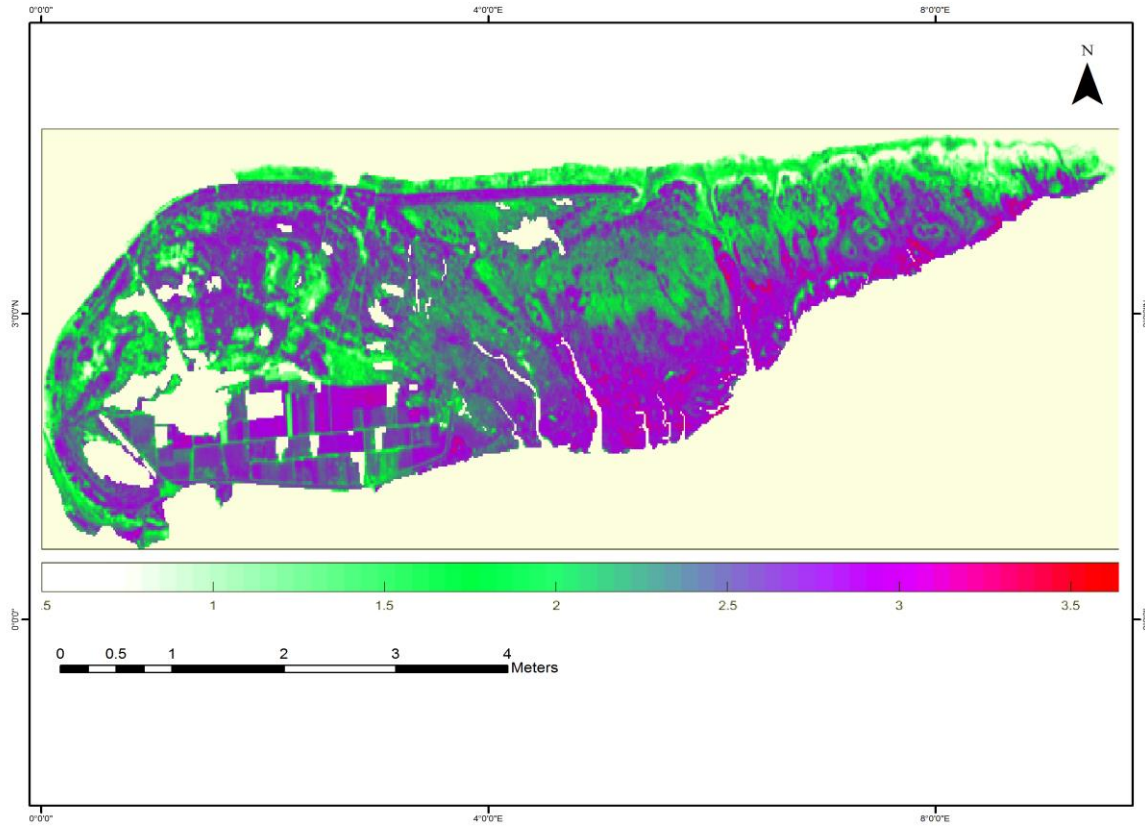

Figure 9: Map of foliar Nitrogen for the saltmarsh area of Schiermonnikoog using Sentinel image (2016)

As can be observed from figure 9, foliar nitrogen varies for a scale of 1.65 to 3.56. The area around 3 to 3.4 have higher concentration of leaf nitrogen compare to other areas of the salt marsh grasslands. The measured nitrogen from of the field samples plots illustrated in figure 3 are well characterized in the map of foliar nitrogen. Different vegetation cover types such as agriculture, forest, high shrubs, and the grasslands are captured in the map.

5. DISCUSSION.

Two satellite images RapidEye and Sentinel-2 images were used in evaluating the performance of high resolution images in mapping foliar nitrogen in the salt marsh/grassland ecosystem of Schiermonnikoog with the aim of making a contribution towards understanding how remote sensing can estimate foliar nitrogen on a regional scale

5.1. Relationship Between Nitrogen, Chlorophyll and Carbon in the grassland/saltmarsh

Most previous studies demonstrated the existence of a strong correlation between nitrogen and chlorophyll (Hansen & Schjoerring, 2003; Haboudane et al., 2004). However, the results obtained in this study indicated that the relationship between nitrogen and chlorophyll was rather weak (R=0.24). A factor explaining the significantly weak correlation might be the type of ecosystem, attributed to the differences between field and reflectance data obtained from the remote sensed images (RapidEye and Sentinel-2), or the existing heterogeneity between and within plots as the nitrogen of multiple species were averaged within a plot and used to determine the relationship between these two variables (Ferwerda et al.,2005). Although our finding is inconsistent with the past studies which established a strong-moderate positive correlation between nitrogen and chlorophyll (Clevers & Kooistra, 2012b; Evans, 1989; Homolová et al., 2013; Le Marie et al., 2008) using chlorophyll alone has been insufficient to explain nitrogen variation, because their correlation becomes lower in nitrogen rich ecosystems (Asner & Martin, 2008). The combination of other leaf traits (carbon, dry matter, and water content) in addition to chlorophyll may explain the variance of nitrogen and improve the nitrogen estimation (Wang et al., 2016). As was expected carbon showed rather strong correlation with nitrogen (R=0.61). Moreover, the relationship between carbon and chlorophyll was moderate (R=0.61). Nitrogen availability constrains carbon assimilation and, it plays a vital role in terrestrial ecosystem carbon dynamics(Heimann & Reichstein, 2008; Ollinger et al., 2008b).

5.2. Vegetation indices, Red edge and NIR

The spectral transformation of two bands vegetation indices used in this present study were designed to provide more reliable spatial and temporal physiological variations in the saltmarsh ecosystem. Vegetation index helps in monitoring seasonal, inter-annual and long-term variation of vegetation structure, phenology and biophysical parameter (Liu et al., 2016). Therefore, the concept behind using broad band indices in estimating foliar nitrogen is to evaluate the performance of spectral bands that falls within the absorption features and are also sensitive to vegetation properties in estimating leaf nitrogen.

Vegetation indices extracted from RapidEye image were correlated to the measured nitrogen using a linear regression model. The obtained result showed that among the studied popular vegetation indices Clrededge index was the best index for explaining the leaf nitrogen (R^2=0.49) variation using the RapidEye imagery. The result showed that Clrededge can be used as a linear estimator of nitrogen, thus confirming the finding by (Clevers & Kooistra, 2012b) that the use of red edge band in a vegetation index band combination, would increases the correlation with nitrogen. This is also similar to the findings of Clevers, (1999) who found that the red edge band contains additional information (although; an imaging spectrometry was used). In addition, Clevers et al., (2000) noted that the red edge band contribute immensely to the quantitative information obtained in terrestrial ecosystem.

The response of the red edge band with nitrogen in this study indicated that RapidEye Red edge reflectance data have weak correlation with nitrogen (RapidEye: R=0.03, as shown in table 6. Although this correlation was differed using the Sentinel-2 data. As can be observed from Table 7, while the correlation of first Red edge band with nitrogen was low (R=0.09), this correlation become stronger using the other two Red edge bands (R=0.41 and R=0.40). This indicate that the position of Red edge band is probably very important in defining its relation with biochemical properties of vegetation. The obtained result differed from the study done by Mutanga & Skidmore (2007) where using hyperspectral data the red edge region had a high correlation with nitrogen concentration with respect to a grassland ecosystem. This probably can be justified by the position of the red edge bands which is sensitive in predicting leaf nitrogen and further confirmed using Sentinel-2 data as it can be seen that the correlation increased to R=0.41 and 0.40 (Table 7). Although, since the saltmarsh is very humid, some factors like water interferes with the estimation of some biochemical variables because they conceal in the absorption features (Kokaly and Clark, 1999), and alters reflectance spectra of vegetation. It is also important to note that in this study we considered the leaf nitrogen which probably plays a small role in canopy reflectance obtained from the satellite sensors. Considering the nitrogen at the canopy level (e.g. by multiplying it to LAI) will surely increase its correlation with reflectance.

The findings in this studies contribute to the growing number of studies that observed a moderate to strong correlation between nitrogen and NIR reflectance (Goel et al., 2003; Kruse et al.,2006; Martin et al., 2008a). In this present study, the correlation of NIR band and nitrogen is relatively moderate using both satellite images (R= 0.53 and R= 0.33). However, this results might be subject to the fact that NIR region is indicative of structural features that influences scattering (Curran, 1989) instead of an absorption feature that is driven by biochemical constituents. Furthermore, research has shown that RapidEye bands 4 and 5 happens to produce reliable results in optimal band combinations (Frampton et al., 2013). Moreover, the study conducted by Wang et al., (2016) showed that NIR reflectance within the range of 800-850 nm produces a good accuracy in estimating percentage nitrogen (R^2cv =0.75,. Martin et al., (2008b) also support the findings about correlation between NIR reflectance and canopy foliar nitrogen and concluded that the spectral region is important for predicting nitrogen but this can be attributed to NIR reflectance and canopy structure (Knyazikhin et al., 2013). Thus, the structural properties of the saltmarsh ecosystem must be considered, so as to account for the effects of estimating plant traits based on reflectance.

5.3. Best band combination in predicting foliar nitrogen in saltmarsh/grassland variables

Heege et al., (2008) found that the red edge inflection point (REIP) based on four bands (R_{670}, R_{700}, R_{740} and R_{780}) are linearly correlated to nitrogen concentration (R^2=0.97). Clevers, (1999) and Clevers et al., (2000) have shown that the red edge band is less sensitive to soil background and atmospheric effects and can provide information on vegetation properties. In present study, more emphasis was laid on the red edge because the radiation within this region penetrates deeply into leaves of crop canopies compared with visible light (blue and red) (Ramoelo et al., 2012).

Hatfield et al., (2008) concluded that indices which include the red edge and NIR bands in canopy nitrogen concentration perform better than indices which only use the NIR and Red bands in relation to maize plant. In this study, the best performing vegetation indices (SR705 and Clrededge) made use of the spectral information in the red edge region which resulted into the estimation of nitrogen with R^2 of 0.41 and RSME of 0.40, compared to other indices used. This shows that red edge region with a low reflectance in red band and a high reflectance in the NIR band can be sensitive to nitrogen estimation.

5.4. Comparing the Retrieval of Nitrogen from RapidEye and Sentinel-2 Data

In this study, the best band combination of the vegetation indices like SR705, Clrededge, Clgreen and Green Index performed better than the normalised differences indices (NDVI and NDVIrededge) as evaluated by their correlation in the RapidEye data whereas the reverse is the case when Sentinel-2 data was used. Correlation coefficient is used here as an indicator to express the relationship between vegetation indices and percentage nitrogen captured by the best fit function.
Our results are further supported by similar findings obtained by Li et al., (2014) and Perry et al., (2012) on showing promising results using RapidEye satellite data for canopy nitrogen estimation in wheat.

Using the Sentinel-2 bands, almost all the vegetation indices were related linearly to measured leaf nitrogen. The results which are presented in table 10 shows the performance of these indices in relation to leaf nitrogen. The overall performance of the normalized (NDVI, and NDVIrededge) vegetation indices were better compared to the ratio indices (SR705 and Clrededge) using the Sentinel-2 data whereas in using RapidEye data the reverse was observed. Clevers & Gitelson (2013) observed that simulated bands of Sentinel-2 used in ratio index like Clrededge provided a good estimate of chlorophyll and nitrogen in a grassland landscape.

5.5. Effects of spectral Resolution and upscaling the retrieval

Although Inoue et al. (2012) found that nitrogen at canopy level could be mapped on a regional scale with the application of hyperspectral measurement, factors such as cost and limited area coverage plays a significant role in hindering this regional application. Another factor to consider is the spectral noise within the leaf reflectance spectra brought about by upscaling leaf to canopy nitrogen reflectance, often influenced by plant structure and leaf background.

In this study, the strength of leaf nitrogen and reflectance relationship declined when RapidEye pixels were up scaled to 20m. This can be partly attributed to the fact that nitrogen considered in this was only at the leaf level. However, similar results was also reported by Lepine et al., (2016) where weak relationship between degraded AVIRIS pixels from 18m to 30m was observed. There was no significant correlation between leaf chlorophyll and the reflectance from RapidEye as well as Sentinel-2. Though band 4 (Red edge) and band 5 (NIR) from the RapidEye were significantly related to nitrogen. For Sentinel-2 not only Red edge bands but also bands from NIR and SWIR region correlated to nitrogen (see table 7). It was also noted that there was little deviation of accuracy from the results obtained from RapidEye and Sentinel-2 meaning some of the synergy captured by RapidEye at 5m was also captured by Sentinel at 20m.
Additionally, nitrogen correlated with Sentinel-2 reflectance much better than with RapidEye, reasons might be not only the position of the wavelengths in Sentinel-2 which covers further the NIR and SWIR electromagnetic spectrum but also attributed to the pixel size of Sentinel-2 which is 4 times larger than the pixel size of RapidEye. Lepine et al., (2016) suggested that pixels' sizes can accounts for the differences between the measured nitrogen reflectance but other related effects such as sensor fidelity should also be taken into consideration. However, saltmarsh vegetation types are statically significantly different for various spectral regions.

5.6. Likely source of Prediction Errors and ways to improve accuracy

Nitrogen in plants are available in a variety of compounds, most of which lack spectral signature or a designed defined approach of estimation (Homolová et al., 2013), this contributes to the challenges experienced in providing an accurate estimation of nitrogen. However, the application of remote sensing techniques augmented with an empirical approach, it has open useful feasible path. In this study, possible errors could be linked to heterogeneity within the plot and averaging the leaf nitrogen per plot and not based on vegetation types or species. Another possible source of error could be the inter annual variation in foliar nitrogen that occurs due to offsets between field and image data collection, this might cause a potential source of error in the analysis. Or perhaps the samples collected were very few to make any reasonable prediction because it does not serve as a true representation of the entire ecosystem.

As indicated before, leaf nitrogen plays a small role in canopy reflectance obtained from the satellite sensors, considering the nitrogen at the canopy level would increase its estimation accuracy form satellite data.

6. CONCLUSION AND RECOMMENDATION.

6.1. Conclusion

From the present studies, the most important conclusions are as follows:

- Although chlorophyll and nitrogen content have been shown to be correlated in a number of ecosystems, their relationship in the saltmarsh ecosystem was weak and thus requires additional studies and information to explain the variability.

- Refinement in the band combinations derived from RapidEye of the selected vegetation indices were comparable to those of Sentinel-2 but the upscaling was affected with some spectral noise leading to slight deviation of accuracy compared to the former.

- Ratio indices such as simple ratio and Clrededge index were the best performing index using RapidEye whereas the normalised differential indices performed better in Sentinel-2 data

- A combination of the red edge region and NIR in a formula would yield a better estimate of nitrogen for RapidEye image, where using Sentinel-2, SWIR bands were played an equal role with these bands.

- Nitrogen has been demonstrated that it can be estimated through empirical methods achieving a moderate accuracy amongst other biochemical constituents in plants.

- However, there are technical problems associated with using field data as well as remotely sensed images for estimating biochemical variables

6.2. Summary of Research Question Answers

Based on the result presented in chapter four (4), short answers to the research question are presented below:

Question 1: What is the relation between foliar nitrogen and chlorophyll/carbon in saltmarsh/grassland?
Answers: From this study, the relation between nitrogen and chlorophyll in the saltmarsh/grassland was relatively weak (R=0.24) but stronger with carbon (R=0.61). This was obtained by correlating measured percentage nitrogen and the chlorophyll values for the 27 plot. However, this result can be because of insufficient sample points or classifying samples based on the vegetation types.

Question 2: Within the studied vegetation indices, which vegetation index derived from RapidEye data can provide an accurate estimate (in terms of highest R^2 and lowest RSME) in estimating foliar nitrogen?
Answers: Amongst all selected vegetation indices used in this study, simple ratio (SR705) and Clrededge provided a moderate estimate of nitrogen (with R^2 =0.41 and RMSE = 0.40).

Question 3: Does spectral bands and vegetation indices used for retrieval of foliar nitrogen from RapidEye similar to those of Sentinel-2?
Answers: The spectral bands (refined in the band combinations) derived from RapidEye for the selected vegetation indices were comparable to those of Sentinel-2, however, it should be noted that for Sentinel-2 bands from SWIR region play an important role.

6.3. Recommendation for Subsequent Studies

- A more refined research narrowed towards developing method to map nitrogen which accommodate a broad range of ecosystem and landcover types should be considered.

- An understanding of how plants nitrogen varies in relation to NIR and red edge should be considered.

- In understanding the inference of making reliable estimate of nitrogen at landscape, regional and global scale, sensor characteristic should be considered.

- Development of a method with the inversion of radiative transfer models that explore the mechanism of photons interaction with plants thus enhancing better estimate of properties of reflectance.

- Development of red edge vegetation index based algorithms for monitoring interanual seasonal variation of nitrogen in saltmarsh grassland/ecosystem.

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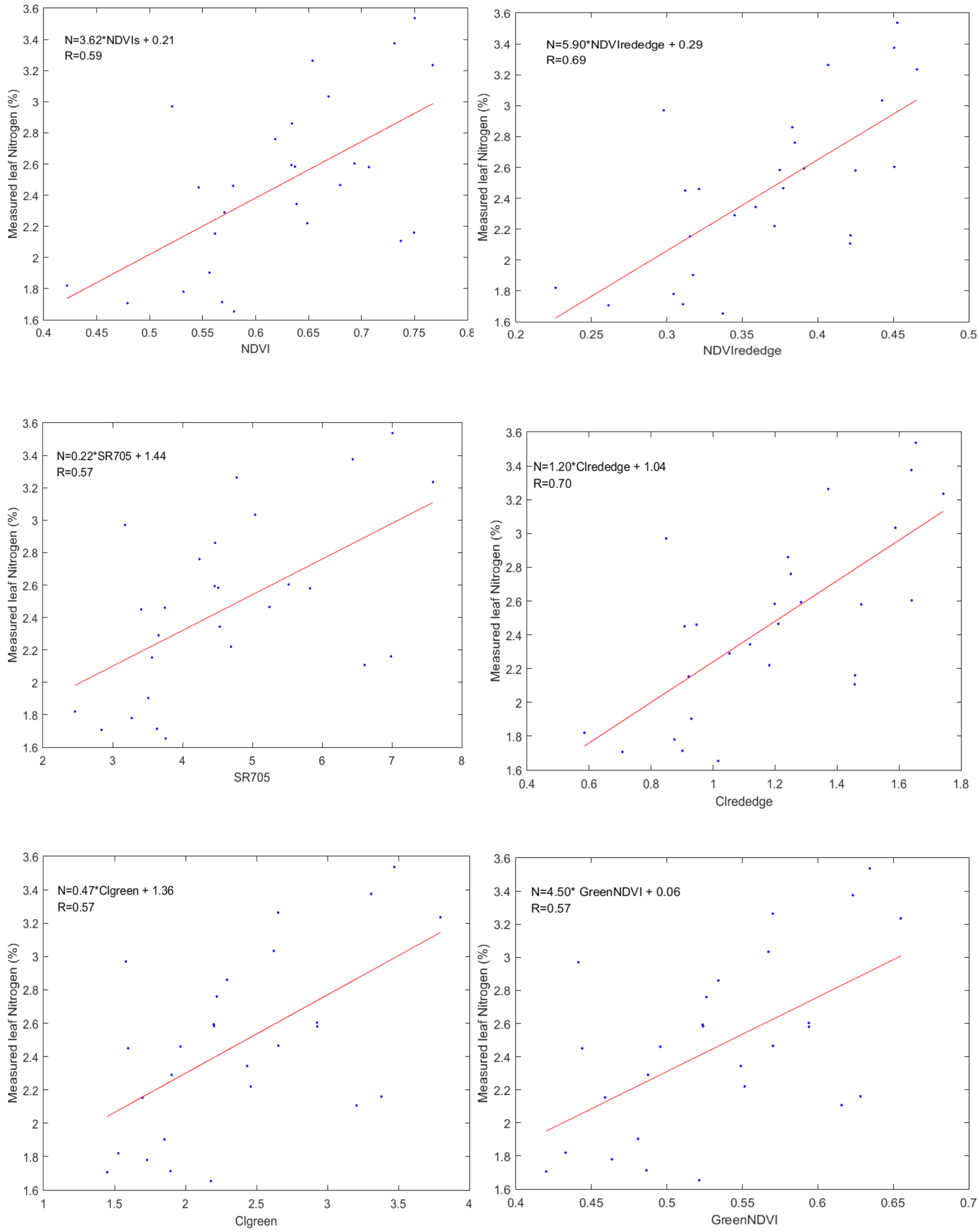
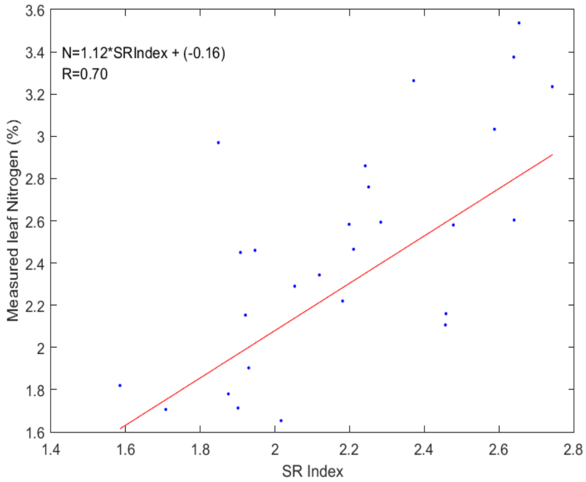
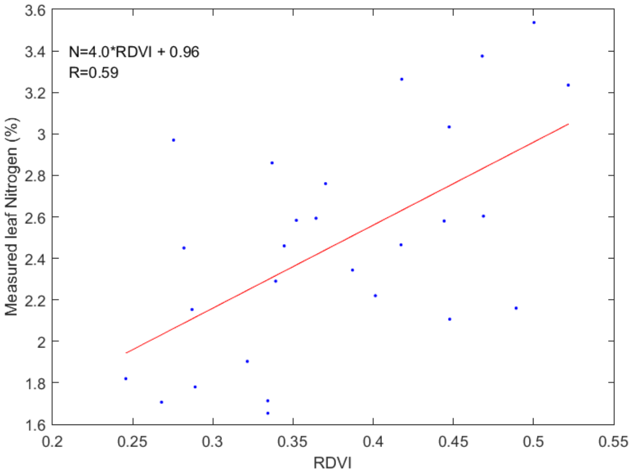
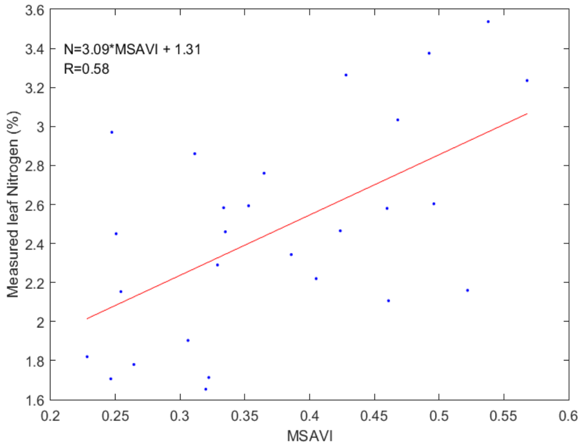
APPENDIX

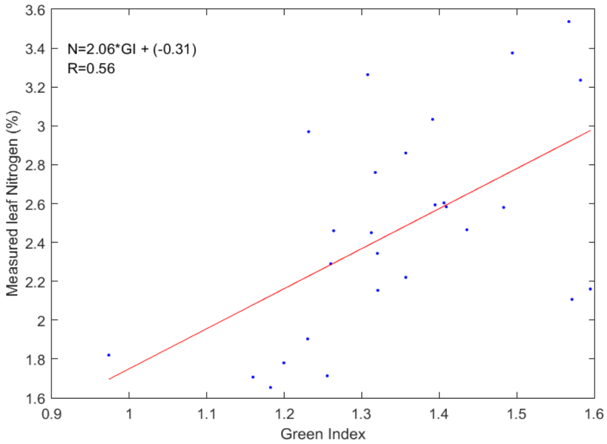
A. The figure shows the Correlation between vegetation indices and leaf nitrogen; RapidEye (2015)

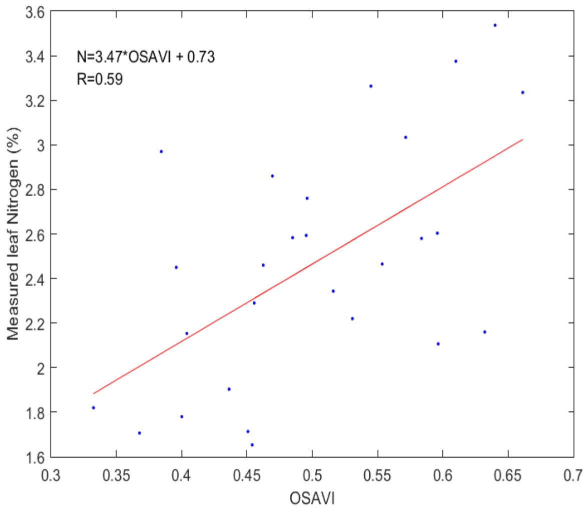


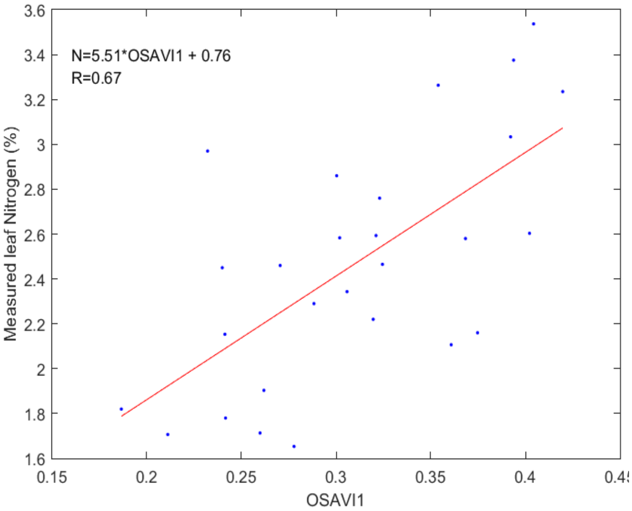

N=1.12*SRIndex + (-0.16)
R=0.70
Measured leaf Nitrogen (%)
SR Index


N=4.0*RDVI + 0.96
R=0.59
Measured leaf Nitrogen (%)
RDVI

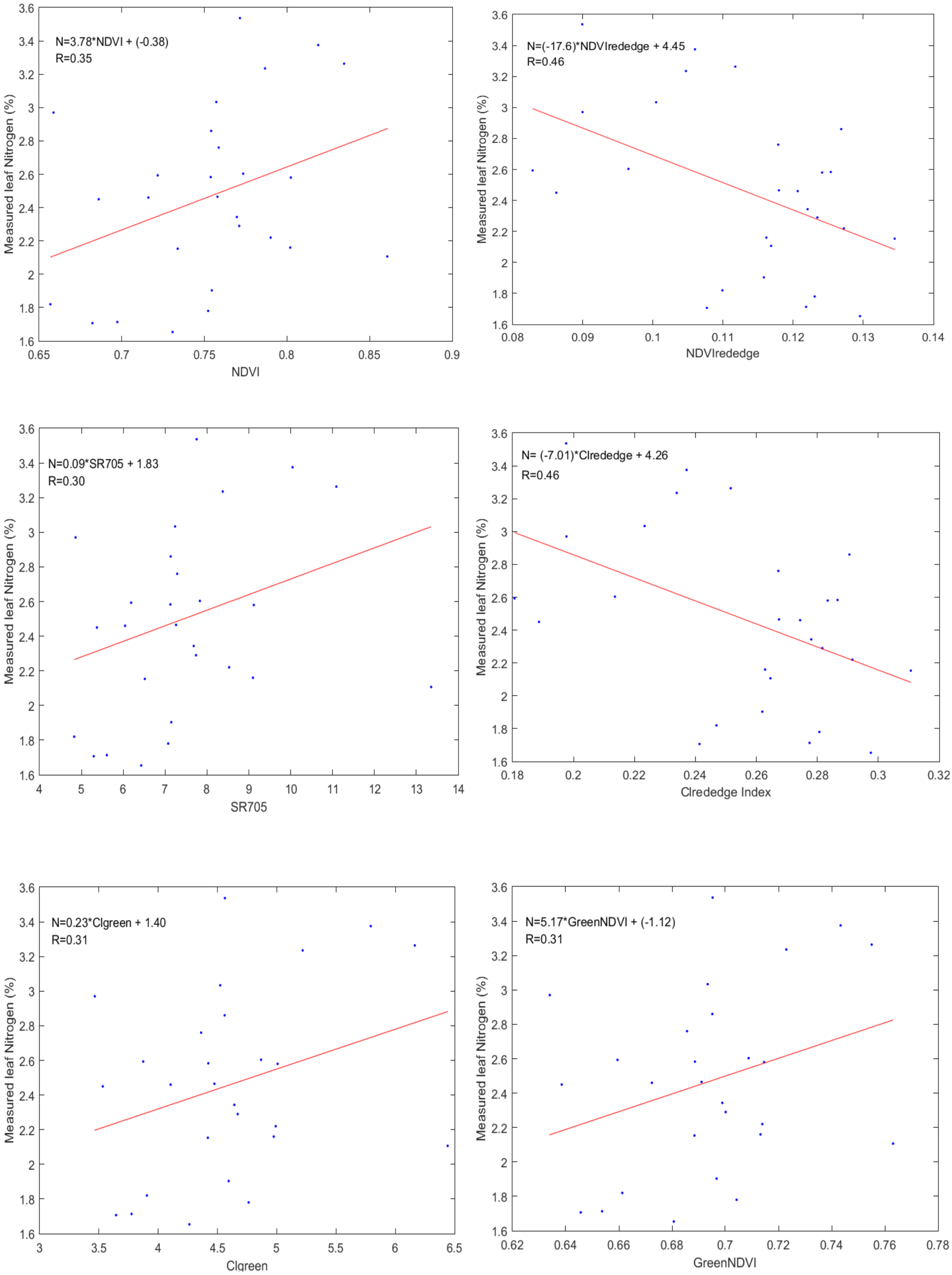

N=3.09*MSAVI + 1.31
R=0.58
Measured leaf Nitrogen (%)
MSAVI

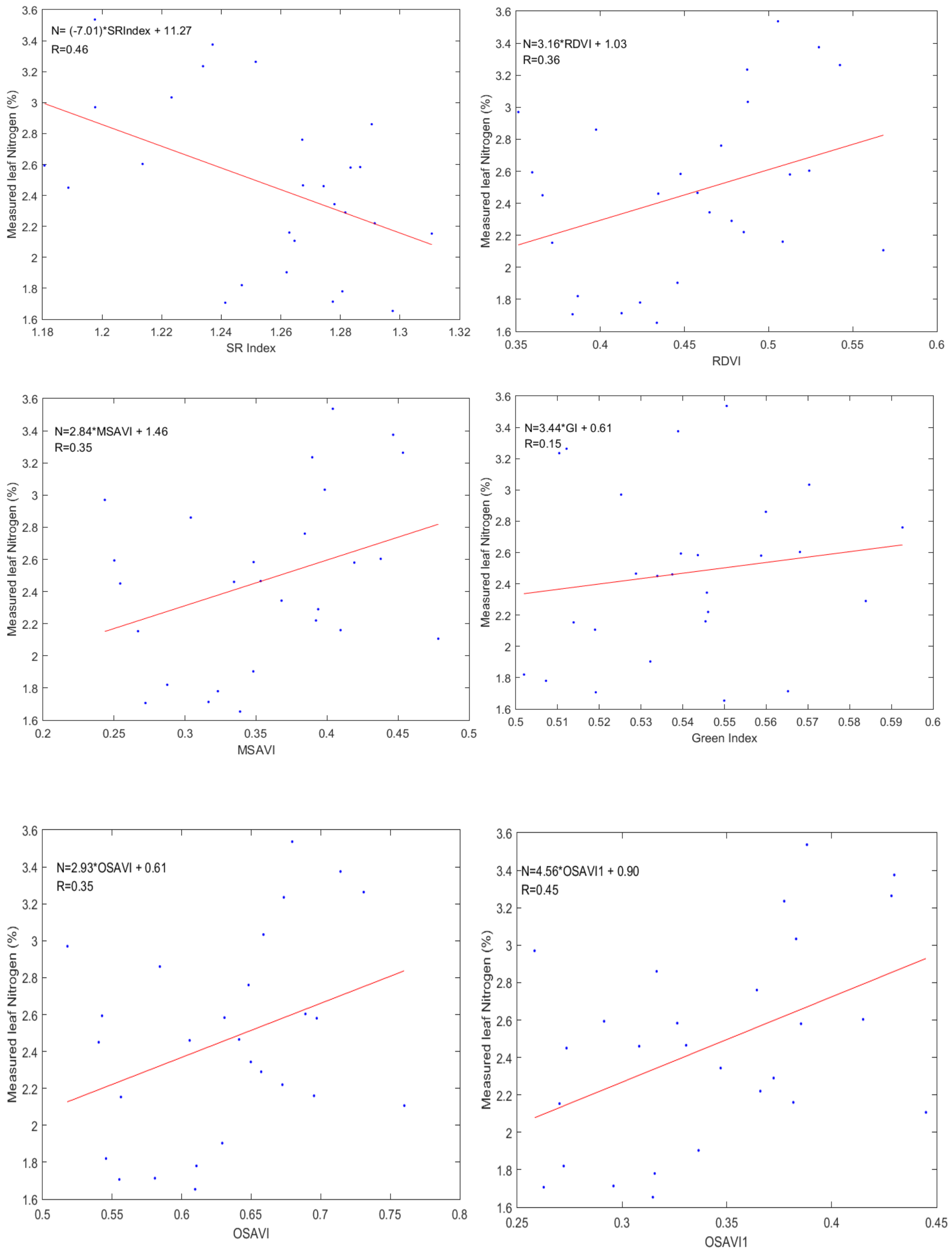

N=2.06*GI + (-0.31)
R=0.56
Measured leaf Nitrogen (%)
Green Index


N=3.47*OSAVI + 0.73
R=0.59
Measured leaf Nitrogen (%)
OSAVI


N=5.51*OSAVI1 + 0.76
R=0.67
Measured leaf Nitrogen (%)
OSAVI1

B. The figure shows the Correlation between vegetation indices and leaf nitrogen; Sentinel (2016)




N= (-7.01)*SRIndex + 11.27
R=0.46
Measured leaf Nitrogen (%)
SR Index
N=3.16*RDVI + 1.03
R=0.36
Measured leaf Nitrogen (%)
RDVI
N=2.84*MSAVI + 1.46
R=0.35
Measured leaf Nitrogen (%)
MSAVI
N=3.44*GI + 0.61
R=0.15
Measured leaf Nitrogen (%)
Green Index
N=2.93*OSAVI + 0.61
R=0.35
Measured leaf Nitrogen (%)
OSAVI
N=4.56*OSAVI1 + 0.90
R=0.45
Measured leaf Nitrogen (%)
OSAVI1