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Estimation of N status in Spartina alterniflora (smooth cordgrass) using handheld spectrometry

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K1 project – Master level

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1.0 Abstract

Salt marshes are among one of the most productive ecosystems in the world and are important components of estuarine systems, since it provide food and nutrients to both estuarine and coastal ocean consumers, serves as a habitat for young and adult estuarine organisms, and a refuge for larval and juvenile organisms and regulating important compounds of the estuarine chemical cycle. The location of salt marshes as a buffer zone between land and sea and the continuously increasing N-load from land make it a raising concern regarding monitoring and estimation of its vulnerability to eutrophication and interest in its ability to remove N before its enters the estuaries and coastal ocean waters, along with monitoring of the current N status. Remote sensing is a particular helpful tool when trying to extract information from large areas and to estimate N status of vegetation.

The spectral reflectance signature of *Spartina alterniflora* (a dominant salt marsh species) was investigated in 13 sites with varying N input, within two New England salt marshes (Plum Island Sound and Great Sippewisset Marsh, USA), to survey if remote sensing can be use to sense vegetation response to different nutrient input.

Two different remote sensing tools was used; a Duel Channel Unispec, which measure canopy reflectance and a SPAD-502 chlorophyll meter, which measure leaf reflectance. Three different vegetation indexes (NDVI, GreenNDVI and EVI) were used to model vegetation biophysical variables.

It was not possible to estimate if one index or the other would be better for an overall use to estimate N status but the results indicate the feasibility in predicting N status.

SPAD values give an indirect indication of chlorophyll and nitrogen content in leaf biomass but only a low correlation was observed than correlated with red and green reflectance. More emphasis has to be giving on calibration of SPAD measurements to obtain more reliable results.

Spectral reflectance data obtained from Unispec measurements, clearly illustrated that the vegetation state in the two sites with highest N input (20 and 300-fold larger than reference sites) represented the healthiest green vegetation with a high plant biomass, which correlated with the N input received. In the remaining sites, there was not observed a clear distinguish between the spectral data and observed N input.

Remote sensing can provide information about variations in vegetation and give an insight into important vegetation biophysical features. Therefore using remote sensing to determined N status of vegetation is a low cost useful method, but emphasis on future studies in this area should be a priority.

2.0 INTRODUCTION

Nitrogen is in many environments the limiting nutrient for primarily production, which means that processes related to the availability of fixed nitrogen, is an important regulation for primary production and the function of the ecosystem and the global biogeochemistry (Hulth et al., 2005).

Salt marshes are among one of the most productive ecosystems in the world. The production is attributable to several factors, including tidal mixing and nutrient enrichment from runoff. These are important components of estuarine systems, since it provide food and nutrients to both estuarine and coastal ocean consumers, serves as a habitat for young and adult estuarine organisms, and a refuge for larval and juvenile organisms and regulating important compounds of the estuarine chemical cycle.

Salt marshes act as a buffer zone between land and sea and the continuously increasing N-load from land make it a raising concern to monitoring and estimate its vulnerability to eutrophication and interest in its ability to remove N before its enters the estuaries and coastal ocean waters (Hopkinson & Giblin, 2008), along with monitoring the current N status.

To investigate N status by traditional soil and plant testing are time consuming and labor-intense methods (Li et al., 2008). Remote sensing, which is a small or large-scale acquisition of information of objects or phenomena, is particular helpful when trying to extract information from large areas (Lillesand et al., 2004).

Knowledge about variations in vegetations species and community patterns along with changes in phenological cycles, and modification in the plant physiology and morphology are information that all give valuable insight in to the climate, edaphic, geologic and physiological characteristics of an area (Jensen, 2000). Scientists have devoted a significant work effort to develop sensors and visual and digital image processing algorithms to extract important vegetation biophysical information from remote sensed data.

Remote sensing data can be used to predict photosynthetic rates and biomass production using methologies relating spectral vegetation indexes to leaf chlorophyll, leaf area index, light absorption and nitrogen contents. The direct method for predicting N status using remote sensing is a linear approach by combining spectral reflectance from two or more characteristic wavebands. However, linear combination of spectral bands may results in over-fitting if inadequate methods were used (Thenkabail et al., 2000).

Since the 1960s, scientists have been extracting and modelled various vegetation biophysical variables using remote sensed data. Much of the effort has been into the development of vegetation indexes, which are dimensionless, radiometric measures that function as indicators of relative abundance and activity of green vegetation, often including leaf area index, percentage green cover, chlorophyll contents, green biomass and absorbed photosynthetically active radiation (Jensen, 2000). Compared with direct spectral reflectance, spectral vegetation indexes may be more reliable in predicting plant N status.

2.1 Problem formulation

The spectral reflectance signature in salt marshes with different nutrient input will be investigated, to survey if remote sensing can be use to sense vegetation response to different nutrient input.

Two different remote sensing tools will be use; a Duel Channel Unispec, which measure canopy reflectance and a SPAD-502 chlorophyll meter, which measure leaf reflectance. Emphasis will be on investigate how remote sensing reflectance data can be used to detect nitrogen content or nutrient status of the salt marsh species *Spartina alterniflora* from 13 sites within two New England (MA, USA) salt marshes, Plum Island Sound Estuary system and Great Sippewisset Marsh respectively.

3.0 Theory

3.1 *Spartina alterniflora* – Test organism

Spartina alterniflora (Smooth cordgrass) is a perennial deciduous salt tolerant grass (Figure 1) that dominates the salt marsh community in intertidal wetlands, especially in estuarine salt marshes.

S. alterniflora is native to the Atlantic and Gulf Coast of North America. It grows up to 1-1,5 meter tall and has a smooth hollow stem bearing leaves up to 20-60 cm long and app. 1,5 cm width at the base (GISD, 2005). *S. alterniflora* is famous for its environmental engineering abilities; it will grow out in the water at the edge of a salt marsh and accumulate sediment and enable other habitat-engineering species to settle. The accumulation of sediment and other substrate building species gradually builds up the level of land at the seaward edge allowing higher marsh species to move onto the new land. Typically the taller form of *S. alterniflora* will grow on the outermost edge of a marsh with shorter forms growing up onto the landwards side of the *Spartina* belt (Fang, 2002).

Figure 1: *Spartina alterniflora* (Adapted from USDA NRCS, 2010).

3.2 REMOTE SENSING

3.2.1 GENERAL ASPECTS OF REMOTE SENSING OF VEGETATION

Radiation that reaches the Earths surface consists of solar radiation (wavelength region from app. 250 - 3000 nm) and radiation emitted from the atmosphere (wavelength region from 3000 to >20.000 nm). The energy balance at the surface can be expressed as the following:

$$R_n = G + H + \lambda E \qquad \text{(Equation 1)}$$

R_n is the net absorbed radiation (W m^{-2}), G is the heat flux into the surface (W m^{-2}), H is the sensible heat flux into the air above the surface (W m^{-2}), λE is the latent heat flux to the air (W m^{-2}) and λ is the heat of vaporization.

The net radiation can also be expressed into the different components as the following:

$$R_n = R_S \downarrow - R_S \uparrow - R_L \downarrow - R_L \uparrow \qquad \text{(Equation 2)}$$

$R_S \downarrow$ *is the incoming solar radiation,* $R_S \uparrow$ *is the outgoing reflected solar radiation,* $R_L \downarrow$ *is the incoming long wave radiation from the atmosphere and* $R_L \uparrow$ *is the outgoing emitted thermal long wave radiation.*

The net radiation dependents on the solar intensity and the atmosphere and only slightly on the surface characteristics. On the other hand, the outgoing components are strongly dependent on the surface, whether it is soil, vegetation, constructions or likewise. The magnitude of the wavelength dependence of reflectance- and emitted radiation is determined by the reflective properties and the temperature of the surface feature in question. Thus, a remote measurement of the amount of reflected and emitted radiation at a specific wavelength can be used to derive properties of the surface. This is the basis for remote sensing of vegetation and soil (Jackson, 1986).

3.2.2 ABSORPTION OF RADIATION

Most remote sensing systems operate in wavelength regions where reflected energy predominated. Therefore it is important to consider the reflectance properties of earth features. The reflected energy is equal to the energy incident on a given feature reduced by the energy that is either absorbed or transmitted by that feature. The geometric manner in which an object reflects energy is also important and can be roughly divided into two types: Smooth surfaces act as *specular* reflectors with the direction of the reflectance scattering is predominately away from the incidents direction. This means that it will appear dark to black in image data. Rough surfaces act as *diffuse* reflectors, where scattering of the incident energy goes in all directions, and will appear light in the image data, see Figure 2.

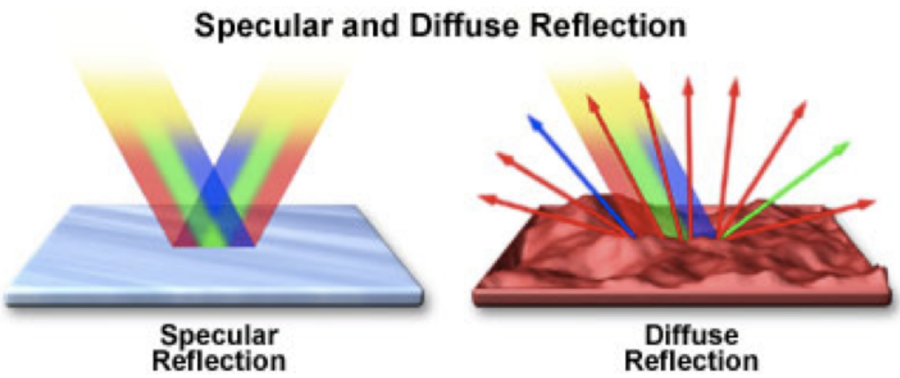

Figure 2: Specular versus diffuse reflectance. Adapted from Abramowitz, 2006.

There are several vegetation factors that can have a significant effecting on the reflectance of incident light toward the used sensor system. The optimal is to hold as many as possible constant while attempting to extract biophysical information using multiple dates of remote sensor data. Even if the variable are kept constant the zenith and azimuth angle of the incident solar radiation and the azimuth and viewing angle of the sensor system can be effecting the data so that it may not be comparable to spectral reflectance measurements obtained at one time with those of another (Lillesand et al., 2004).

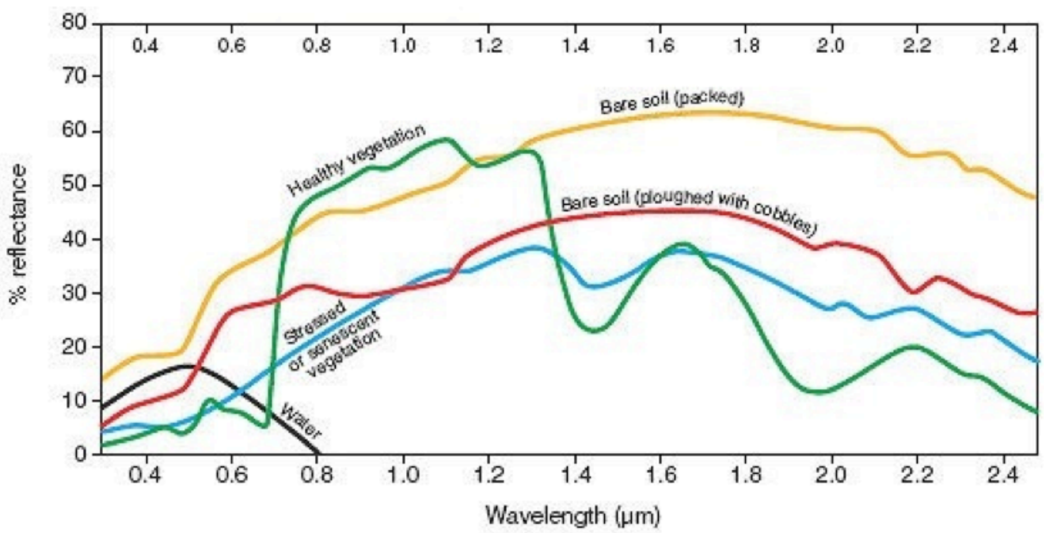

Table 1: Simplified spectral reflectance curve for land cover: Water (black line), natural and agricultural soil (yellow and red line) and healthy or stressed/senescent vegetation (green and blue line). Adapted from Baban, 2005.

3.2.3. Spectral reflectance and emittance

Spectral reflectance curves as a function of wavelength can be divided into three basic types of earth features; A healthy green vegetation, dry bare soil and clear lake water. Table 1 represents average reflectance values made by measuring a large sample of features.

The configuration of these curves is an indication of the type and condition of the features to which they apply, where the curves demonstrate some fundamental points concerning spectral reflectance (Lillesand et al., 2004).

3.2.4 Leaf reflectance characteristics

A healthy green leaf intercept incident radiant flux directly from the sun or from diffused skylight, which is scattered onto the leaf. The electromagnetic incident energy interacts with the pigments, the water and the intercellular air spaces within the plant leaf. Leaf pigments, internal scattering and leaf water content all affects the reflectance and transmittance properties of leaves. The dominant factors controlling leaf reflectance is in the regions of 350-2600 nm (Jensen, 2000).

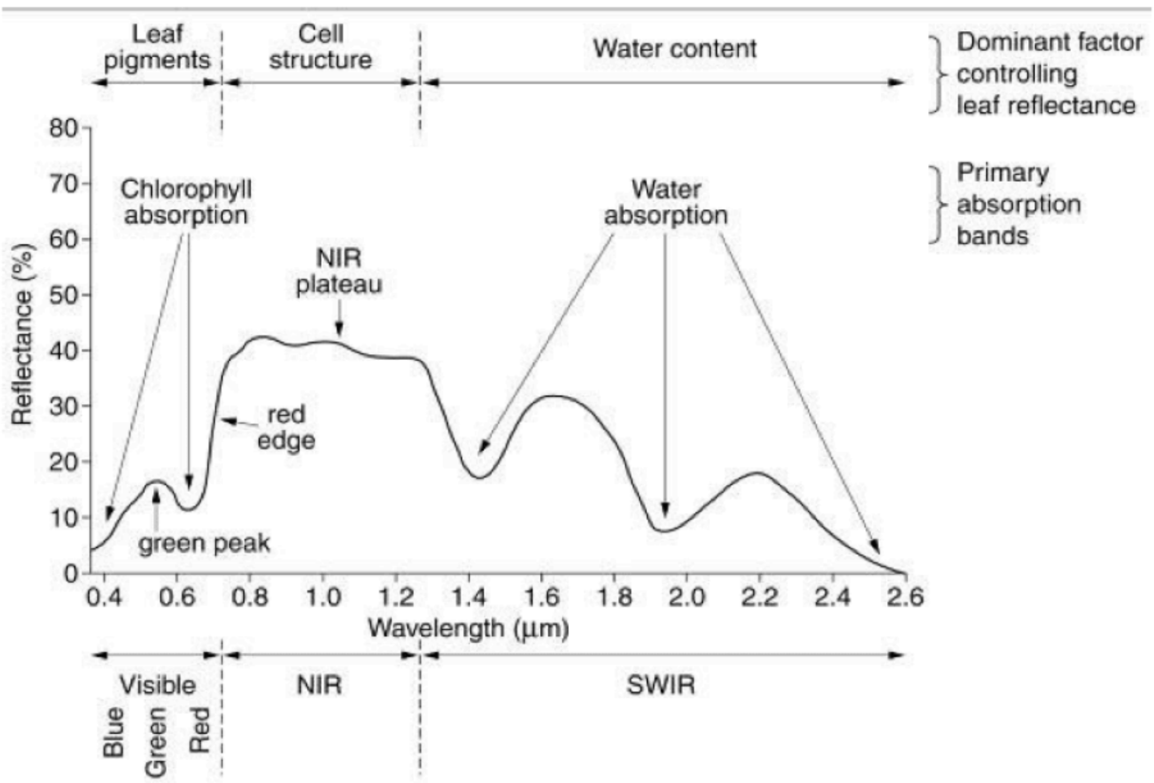

Figure 4: Typical spectral reflectance of healthy green vegetation for the wavelength interval 0.4-2.6 µm. (Adapted from Keyworth et al. 2009). The dominant factors controlling leaf reflectance are the various leaf pigments in the palisade mesophyll, the scattering of NIR energy in the spongy mesophyll, and the amount of water in the plant. The primary chlorophyll absorption bands occur at 0.43-0.45 µm and 0.65-0.67 µm in the visible regions.

A healthy leaf needs four things: carbon dioxide, water, nutrients and irradiance. The first three represents the fundamental raw material where the irradiance powers the photosynthesis. The top layer of the leaf, the upper epidermis cells has a cuticular surface that diffuses but it only reflect a little light. It helps to filter out some of the light and guard against excessive water loses. Conversely leaves growing in shaded areas have a thin cuticular to collect as much sun light as possible. When a molecule is hit by a wave or photon of light it reflects some of the energy or it absorbs the energy and enters a higher energy or exited state. Each molecule reflects or absorbs its own characteristic wavelength of light. An absorption spectrum defines the wavelengths at which it can absorb light and enter the exited state.

Table 2 and 4 illustrated the spectral reflectance of typical healthy green vegetation. The most important pigments for absorbing red and blue light in the visible range are *chlorophyll a* and *b*. There are other pigments present that are masked by the abundance of *chl. a* and *b*. Carotenes, xanthophylls, phycoerythrin and phycocyanin are all example of other pigments.
The two optimum spectral regions for sensing chlorophyll absorption characteristics of a leaf are believed to be 450-520 nm (blue) and 630-690 nm (red). The first region is characterized by carotenes and chlorophyll and the second one is characterized by strong chlorophyll absorption. When a plant undergoes senescence or encounters stress or other variables influencing its normal growth and productivity, it might lead to a decrease in chlorophyll production. The chlorophyll absorption decreases dominantly in the red and blue bands, which allows the carotenes and other pigments to become dominant.
In a healthy green leaf the reflectance increases in the near-infrared region (NIR) at 700-1200 nm. The condition occurs throughout the NIR wavelength range where the direct sunlight reaching the plants has the bulk of its energy. The plants cannot absorb the vast amount of energy without overheating and irreversibly damaging its proteins, so it simply reflects it or transmit it though to underlying leaves. Overall the leaves usually reflect 40-50% of the energy incident upon it. Very little of the remaining energy is transmitted since the reflectance in this spectral region is minimal, and the reflectance results primarily from internal structure in the leaves. The spectral patterns differs highly amount species, and NIR reflectance can be used to distinguish between species. Likewise the reflectance signature in this region is often altered during plan stress (Lillesand et al., 2004 & Jensen, 2000).
When a yellow leaf is undergoing senescence the chlorophyll pigments will be diminishing and a relatively higher amount of green and red light is reflected from the leaf, making the leaf yellow, see Figure 5. The leaf is emitting less in the infrared area compared with the green leaf. However the NIR reflectance is almost the same.

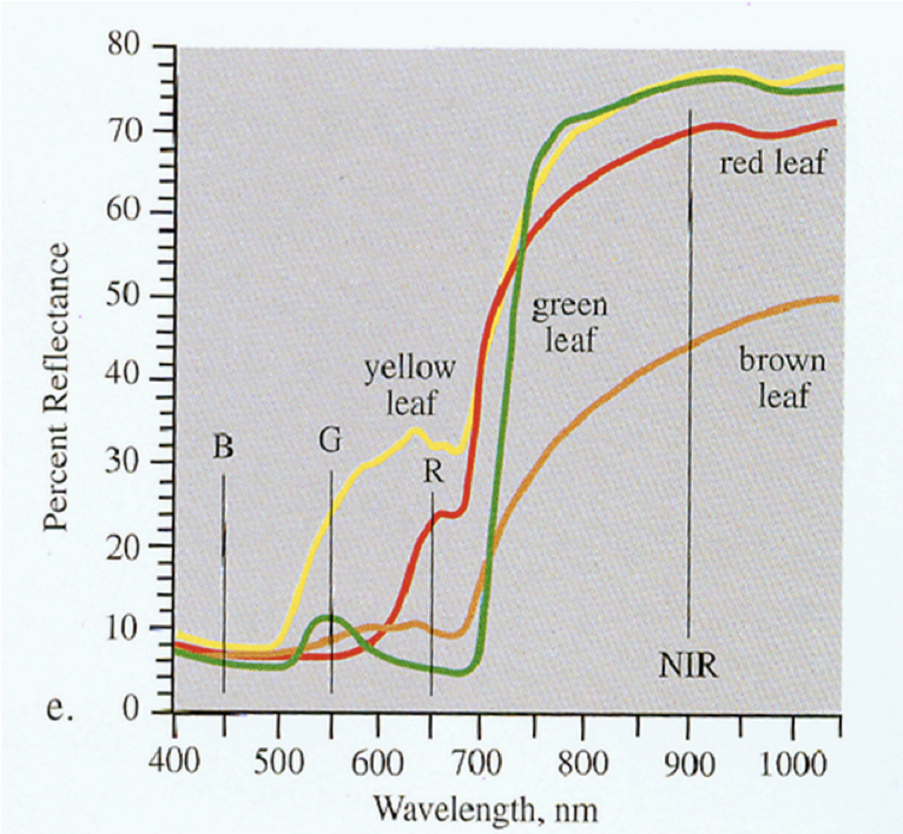

Table 3: 1500 spectroradiometer percent reflectance measurements over the wavelength interval 400-1050 nm. Adapted from Jensen, 2000).

A lack of chlorophyll pigmentation typically causes the plant to absorb less in the chlorophyll absorbance bands. These plants will have a much higher reflectance, especially in the green and red portion of the spectrum and appears yellowish (Jensen, 2000).

Carter 1993 suggested that it is the increasing reflectance in the visible special that is the most consistence leaf reflectance response to plant stress.

Plant stress will first appear in the sensitive 530-640 and 680-700 nm visible light wavelength ranges. A shift towards shorter wavelengths in the region 650-700 nm is evident for the yellow and red leaf. Remote sensing may provide evidence of plant stress not only to individual leaves but whole plants and canopies.

It is also possible to access plants water stress level by looking at the spectral reflectance in the NIR region. The external and internal symptoms of water stress vary among species, but they all have in common that the spectral reflectance changes when water content is decreasing (Jackson, 1986). Peñuelas et al. (1993) has likewise demonstrated a decrease in reflectance when plants are exposed to draught (see Table 4).

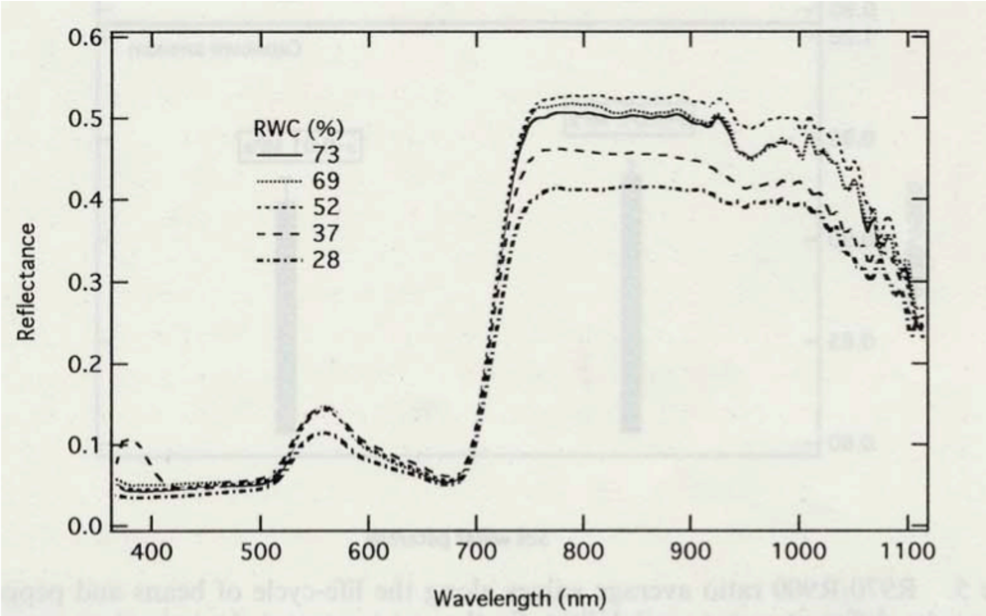

Table 4: Spectral reflectance of detached leaves of beans submitted to progressive desiccation. Adapted from Peñuelas et al., 1993).

3.2.5 Canopy reflectance

Remote sensing of plant canopies involves the detection of electromagnetic radiation coming from a complex matrix of plant leaves and stems above a background of soil and plant litter (Jackson, 1986). This means that when changing scale from leaf to canopy there are complications involved when looking at reflectance abilities, e.g. the effect on reflectance spectra of leaf angle and density, measured as the leaf area index (LAI, m^2 leaf area m^{-2} ground area). As the LAI increases, the contribution from soil or background to the overall reflectance decreases, and the multiply scattering of light caused by the plant cells increases (Yoder & Pettigrew-Crosby, 1995).

Yoder and Pettigrew-Crosby (1995) has investigated whether spectral features would transpose from leaves to canopies scale by selecting bands through stepwise regression on the leaf scale and compared it with those selected on the canopy scale. None of their tests clearly demonstrated signal propagation from leaf to the canopy scale. But when looking at the green and far-red region those regions contained the clearest corresponding in predictive wavelengths on both scales.

Yoder and Waring (1994) investigated the NDVI index of small Douglas-fir canopies under varying chlorophyll concentrations; they found that chlorophyll concentration and LAI had nearly independent effect on canopy reflectance. When the chlorophyll concentration increased, the reflectance in the visible range decreased, with the greatest chance in the green region. Furthermore varying chlorophyll content shows a very low change in the NIR range (Table 5a,b). Changes in LAI had a very little impact on reflectance near the chlorophyll absorbance maxima, in the red and blue range. Conversely, when LAI increased the dominant change in the reflectance spectra, an increase in the NIR reflectance is observed (Table 5,d). As the chlorophyll concentration increased, reflectance at the chlorophyll absorbance maxima in the blue and red changed very little, while reflectance between 500 and 650 nm decreased significantly (Table 5a,b) indicating that when looking for changes in chlorophyll reflectance on canopy scale, the green spectrum is more dominant than the red and blue spectra.

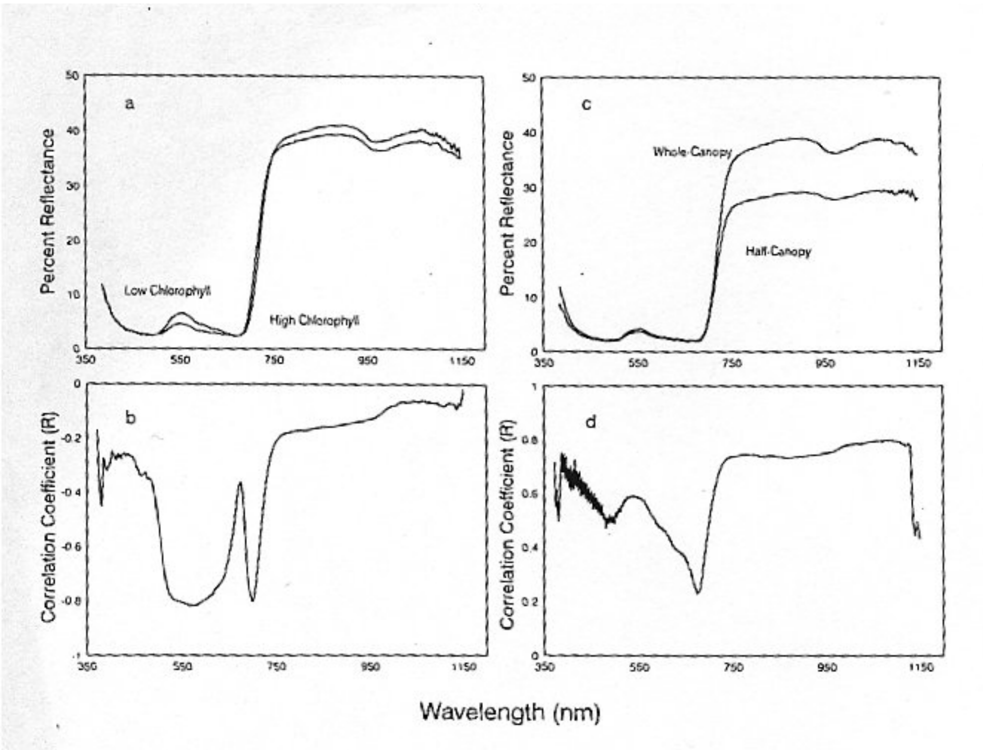

Table 5: Effect of varying chlorophyll concentrations and leaf area index on canopy reflectance. A: typical reflectance spectra of two canopies of similar leaf area having a low and high chlorophyll concentration; B: correlogram relating chlorophyll concentration percent reflectance across the measured spectrum; C: Typical reflectance spectra of a single canopy (chlorophyll concentration is nearly constant) of half and whole density; D: correlogram relating LAI to percent reflectance across the measured spectrum (Adapted from Yoder & Waring, 1994).

3.2.6 VEGETATION INDEXES

Since the 1960s, scientists have been extracting and modelled various vegetation biophysical variables using remote sensed data. Much of the effort has been into the development of vegetation indexes, which can be defined as dimensionless, radiometric measures that function as indicators of relative abundance and activity of green vegetation, often including leaf area index, percentage green cover, chlorophyll contents, green biomass and absorbed photosynthetically active radiation (Jensen, 2000).

NDVI (Normalized Difference Vegetation Index) developed by Rouse et al (1974), is a widely adopted and applied index to estimate changes in vegetation state. It was originally developed to measure green biomass and it has later gotten a solid theoretically background as the measure

of solar photosynthetically active radiation absorbed by the canopy layer. The NDVI relates the reflectance in the red range and NIR range to vegetation variables such as leaf area index, canopy cover and concentration of the total chlorophyll.
The original NDVI index is defined as:

$$NDVI = \frac{(\rho_{NIR} - \rho_{Red})}{(\rho_{NIR} + \rho_{Red})} \qquad \textit{(Equation 3)}$$

ρ_{NIR}=NIR reflectance and ρ_{Red}=Red reflectance. ρ_{NIR} and ρ_{Red} represents surface reflectance averaged over ranges of wavelength in the visible and NIR region.

Vegetation NDVI typically range from 0.1-0.6 where higher values are associated with greater densities and greenness of the canopy. Surrounding soil and rocks have values close to zero. Many studies have shown a positive correlation between NDVI and LAI, although NDVI tend to saturate as LAI increases (e.g. Moges et al., 2004 & Li et al., 2007).
Since vegetation has a low reflectance in the visible range, the index is sensitive to low chlorophyll concentrations, to the fraction of vegetation cover and thereby the photosynthetic active solar radiation (Yoder and Waring, 1994). But it is not sensitive at higher chlorophyll concentration or to rate of photosynthesis for lager vegetation cover.
When chlorophyll concentrations changes it does not affect all part of the visible reflectance spectrum equally. For the individual leaves, the maximum absorbance in the blue and the red regions saturates at a relatively low chlorophyll concentrations. It can then be expected that the sensitivity of NDVI to chlorophyll concentration should depend on the visible band chosen to calculate NDVI (Yoder & Waring, 1994).
Yoder & Wander (1995) found that NDVI increased when either LAI or chlorophyll concentration increased. They found that $NDVI_{625\text{-}675}$ was not a good predictor of LAI across all samples, but by looking at NIR reflectance alone was a comparatively good predictor.
Giteson et al. (1996) has indicated that green NDVI is much more sensitive to the chlorophyll concentrations in a wide range of chlorophyll variations than the original red NDVI.
Furthermore Yoder & Waring (1994) found better correlations between NDVI and photosynthetic activity of miniature Douglas-fir trees with using the green channel (500-600 nm or 565-575 nm) than when using the traditional red NDVI.
Green NDVI index (GNDVI) is defined as:

$$GreenNDVI = \frac{\rho_{NIR} - \rho_{GREEN}}{\rho_{NIR} + \rho_{GREEN}} \qquad \textit{(Equation 4)}$$

ρ_{NIR}=NIR reflectance and ρ_{Green}=Green reflectance. ρ_{NIR} and ρ_{Green} represents surface reflectance averaged over ranges of wavelength in the visible and NIR region.

NDVI and other related indexes for satellite and airborne assessment of vegetation cover has been demonstrated for almost three decades. Global vegetation analysis as been made by basing linearly regressed NDVI values with *in situ* measurements e.g. LAI, absorbed photosynthetically active radiation, percent cover and biomass. But since studies have found empirically derived NDVI products unstable, there is a need for globally accurate NDVI related indexes that do not need to be calibrated by in situ measurements within each geographical area and still remain constant under changing atmospheric and soil background conditions.
EVI (Enhanced Vegetation Index) is an example of a modified NDVI index, which includes a soil adjustment factor, and a correction for atmospheric aerosol scattering in the red band. The index is defined as:

$$EVI = G * \frac{(\rho_{NIR} - \rho_{Red})}{(\rho_{NIR} + C_1\rho_{Red} - C_2\rho_{Blue} + L)} \qquad \textit{(Equation 5)}$$

ρ_{NIR}=NIR reflectance, ρ_{Red}=Red reflectance, ρ_{Blue}= Blue reflectance, G= gain factor, C_1=Atmospheric resistance red correction factor, C_2=Atmospheric resistance blue correction factor, L=Canopy background brightness correction factor. The coefficients are empirically determined as: G=2.5, C_1=6 , C_2=7.5, L=1

This index has shown improved sensitivity to high biomass regions and an improved background signal and a reduction in atmospheric influences (Huete & Justice, 1999). Where NDVI is more chlorophyll sensitive, the EVI is more responsive to variations in the canopy structure, including the LAI, canopy type, plant physiology and canopy architecture (Didan & Yin, 2002).

4.0 Method

4.1 Site description

The study was conducted in two New England salt marshes (Massachusetts, USA) – Plum Island Sound Estuary and Great Sippewisset Marsh, see Figure 3.

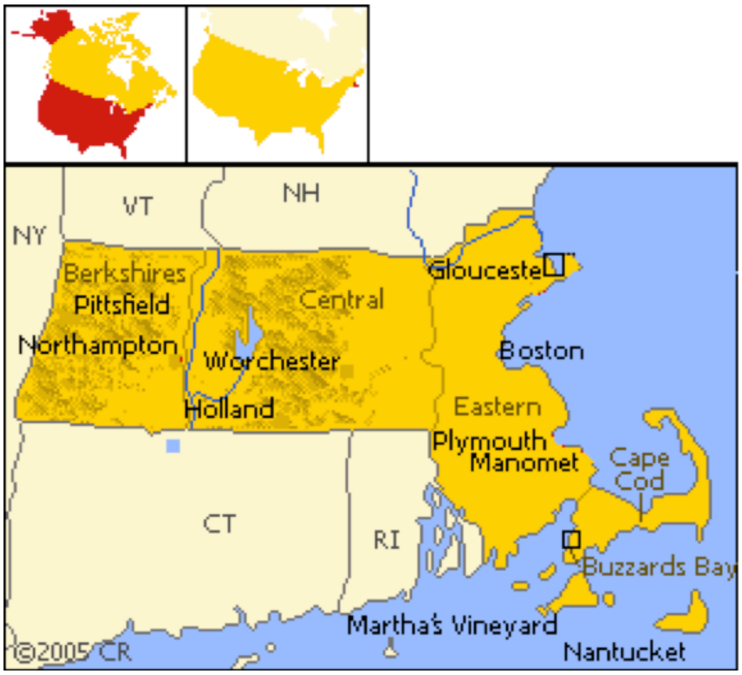

Figure 3: From left to right:
America, The United States highlighted in red;
The States, Massachusetts is highlighted in red;
The State of Massachusetts. Plum Island Sound is illustrated in the upper black square and Sippewisset Marsh by the lower black square.

4.1.1 Plum Island Sound

The Plum Island Sound Estuary in is a classic salt marsh estuary that is unaffected by nutrient loading. The tall *Spartina alterniflora* (~200 cm) is found in as pure stands in the low marsh and along the creek banks that receives a daily tidal inundation. A short form of *S. alterniflora* (~20-60 cm) is also found in the high marsh, often in pure stands and in less well-drained areas. About 80% of the total marsh is high marsh that is flooded during spring tide (Deegan et al., 2007).
5 locations in the Plum Island system are chosen: “Rowley”, “Control”, Lowes Point”, “Greenwood” and “Tides” (See Figure 4 and 5).
Tides, Control and Rowley are located along River Rowley (in the central portion of Plum Island), where Lowes Point is at the mouth of River Rowley towards the Sound. Greenwood is located along Greenwood Creek.

Figure 4: Plum Island Sound and the 5 selected sites: Tides, Control, Lowes Point, Rowley and Greenwood. The black squares illustrate the area shown individually on Figure 6. Adapted from TerraMetrics, 2010)

Tides is a part of the Trophic cascades and Interacting control processes in a Detritus-based aquatic Ecosystem (TIDE)-project. Since 2003, the tidal marsh was and is fertilized continuously on every incoming tide throughout the growing season (May-Oct). The fertilizer-additions aimed for a nitrate concentration of 70 μm, which is equivalent to a 15-fold increase in the nitrogen loading to the marsh (Deegan et al., 2006). The nitrate level is representative for estuarine waters designated as moderate to highly entrophic, according to the Environmental Protection Agency (EPO, 2002).

Greenwood is a tidal salt marsh in the Plum Island Sound system along Greenwood Creek, which has been a site of sewage effluent input from secondary wastewater treatment facility to the town of Ipswich, Massachusetts for the last 40 years. The sewage effluent input has elevated nitrate concentrations. At low tide, the nitrate concentration is over 300 times larger then nearby reference creek near the effluent source of the sewage creek and declined downwards to 50 times when it empties out into Plum Island Sound (Twichell et al. 2002).

Control and Rowley are located upstream of River Rowley with more freshwater sources than Lowes Point.

Figure 5: Plum Island Sound sites; Rowley, Control, Greenwood, Lowes Point and Tides. The black area and red lines illustrates surveyed area for Unispec measurement and SPAD chlorophyll meter measurements respectively. Adapted from TerraMetrics, 2010.

Water samples from all sites where collected in August and October 2009 and analysed for nitrite. Tides was found to be app. 20-fold larger than Control, Lowes Point and Rowley, where Greenwood has more than 300-fold larger nitrite concentration.

4.1.2 GREAT SIPPEWISSET MARSH

Great Sippewisset Marsh (see Figure 6 & 7) consists of vegetation mostly dominated by *Spartina alterniflora* and a few other species. The distribution of *S. alterniflora* varies along the intertidal gradient in the salt marshes, where the shorter forms are founds above the creek banks on the high parts of the marsh and the taller form is found lower in the intertidal regions and along the creek banks. The intermediate form is found between the short and tall form. 10 experimental salt marsh plots has been subjected to experimental enrichment since 1970. Each plot is 10 m in radius and is bisected by small creeks connected to Buzzards Bay via a main creek (Figure 7). Each year a fertilizer (a commercially available sewage-sludge based material) has been spread by hand every 2nd week throughout the April-October growing season, at dosages of 0,85 g N m-

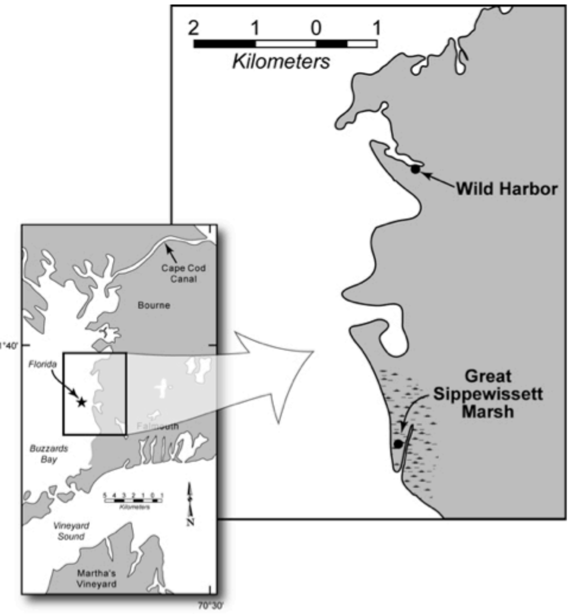

Figure 6: Great Sippewisset Marsh. Map by Jack Cook, adapted from WHOI, 2007.

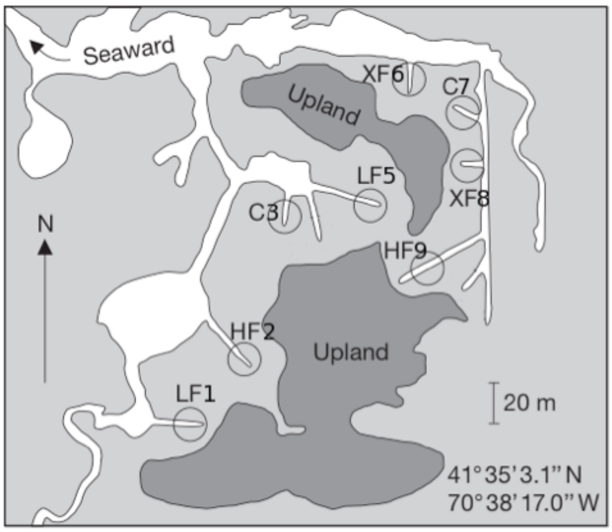

Figure 7: Experimental plots in Great Sippewisset Marsh. Control [C], Low fertilization [LF], High fertilization [HF], Extra high fertilization [XF]. Adapted from Brin et al., 2010

2 wk-1 (LF=Low fertilization), 2,5 g N m-2 wk-1 (HF=high fertilization) and 7,6 g N m-2 wk-1 (XF=extra high fertilization), which app. equalling 0.7, 2.2, and 6.5 times the annual short *S. alterniflora* N demand, respectively (White and Howes, 1994). Untreated plots are referred to as C (Rogers et al., 1998 & Brin et al., 2010). 2 replicate plots of 4 treatment levels were chosen for this survey.

4.1.3 LOW AND HIGH N INPUT

Based on the information presented from each site within Great Sippewisset Marsh and Plum Island Sound, the sites will be divided into low and high N input.

Low N input: Control, Rowley, Lowes Point, C3, C7, HF1 and HF5

High N input: Tides, Greenwood, HF2, HF9, XF6 and XF8.

4.2 SAMPLING METHODS

4.2.1 UNISPEC MEASUREMENTS

Dual Channel Unispec, is a field portable instrument which is capable of unattended, simultaneously measurements of incident and reflected light (PP Systems, 2009).

Canopy reflectance of *Spartina alterniflora* was measured using a Duel Channel Unispec, in cloudless conditions. The device measures the visible and NIR spectrum in the 310-1100 nm wavelength domain. The reflectance of the vegetation is calculated with the calibration measurements of a dark current and a white reflectance canal with known reflectance properties (Caves, 2009). The integration time (that controls the amount of light entering the probe) is set to 20 ms. Two sensors (an upward foreoptic for incident light and a downward foreoptic for canopy reflectance) was mounted on a stand and held in a nadir orientation app. 143 cm above the ground level. A 12 ° FOV lens was used on the downward foreoptic, giving a field of view of 30 cm diameter on ground level. To reduce the possible effect of sky and field conditions, spectral measurements were taken 20-24 times within each plot and averaged to represent the canopy reflectance of each plot.

Unispec measurements were collected in Plum Island Sound the 27th of October 2009 (see Figure 2 for surveyed areas) and in Great Sippewisset Marsh the 18th of November 2009 (randomly collected within experimental plots).

The study was conducted with the consideration of using broad band satellite remote sensing image for sensing N status in plants over a larger scale so reflectance measurements at the canopy scale was averages to reflectance of Thematic Landsat Mapper bandwidths: band 1 (blue: 450-520 nm), band 2 (green: 520-600 nm), band 3 (red: 630-690 nm) and band 4 (NIR: 760-900 nm). The three normalized vegetation indexes: NDVI, GreenNDVI and EVI has been calculated based on the reflectance data, see 5.0 Results & Discussion.

Multispec 5.0 (Gamon, 2010,) a software for creating standardized reflectance files from Unispec measurements was used to transform the reflectance data.

4.2.2. SPAD-502 CHLOROPHYLL METER

SPAD-502 (Minolta, Spain) is a handheld chlorophyll meter that is used for extracting leaf chlorophyll, leaf nitrogen or nutrient status by measuring leaf reflectance. The meter gives non-destructive instantly measurement of the relative chlorophyll content or greenness of plants. Meter reading are given in Minolta Company-defined SPAD (Soil Plant Analysis Development) values that indicate relative chlorophyll contents (Li et al. 2008). Measurements are obtained by inserting a leaf into the head of the SPAD-502 meter. The principle behind the measurements of

the SPAD meter is based on the difference in light attenuation at 650 and 940 nm. The transmittance at 940 nm (in the NIR region) functions as a reference to compensate leaf variables while the 650 nm source is sensitive to chlorophyll concentration. From the difference in light attenuation a dimensionless SPAD unit, ranging form 0 to 80 is calculated (Azia & Steward, 2001).
There has been shown a linear relationship between SPAD values and leaf nitrogen and chlorophyll concentrations (Spectrum Technologies, 2010).

Leaf chlorophyll concentration has been measured using a SPAD-502 chlorophyll meter. For each site 20-40 leaves from individual *S. alterniflora* species are measured twice. For each speciment the top mature leaf is measured app. 10 cm from the stem. In Plum Island Sound measurements where made along the creek side (see Figure 5 for details) and randomly within the experimental plots of Sippewisset Marsh (see Figure 7 for details).

In order to use the SPAD values for validating leaf nitrogen by remote sensing, a relationship with leaf nitrogen and chlorophyll must be established. For this purpose app. 20 leaves of *Spartina alterniflora* was collected in different colour bands (greenish-yellowish) from 2 sites in Plum Island Sound (Control & Tides) and 4 sites in Great Sippewisset Marsh (C7, LF5, HF9 and XF8) in August 2009. All leaves were thoroughly measured with the SPAD meter and subsequently cut into 5 cm pieces, divided into SPAD unit intervals and kept frozen until further process for % N and chlorophyll extraction. Only leaves form Sippewisset Marsh is used for chlorophyll extraction.
% N: All leaves are dried for 24 hours at 100 °C and grinded. 3-5 mg of samples was transferred to a tin cup, sealed and analysed on a CHN analyser, providing the total % N concentration per mg dry weight. The relationship between % N and SPAD unit is established. The best-fit relationship was found with leaves collected from LF5 (R_2: 0.6, which was applied to all SPAD values from Great Sippewisset Marsh) and from Tides (R_2: 0.8, which was applied to all SPAD values from Plum Island Sound).
Chlorophyll: App. 0.1-0.4 g of sample was collected from each interval. The samples were immediately placed into 8 ml of 100% methanol and sonicated for 30 seconds at medium strength (overall method described by Ritchie, 2006). The pigments were allowed to extract in the dark at 5°C for 24 hours. Absorbance of the clear extract at 652.0, 665.2 and 750 nm were recorded and concentration of chlorophylls *a, b* and *a+b* were calculated as describes by Porra et al., 1989).
The best fit relationship of *chl a* and SPAD values was found at LF5 (R_2: 0.86), which is applied to all SPAD data.

5.0 Results & Discussion

In the first section of the results the reflectance data from Unispec measurements will be presented. The reflectance signature from each site will be presented separately first, then sites per marsh and at the end the two marshes combined. The calculated indexes will be presented and debated. In the second section SPAD measurements will be presented and correlations between the Unispec reflectance data and SPAD measurements and calibrations will be discussed.

5.1 Spectral signatures of Plum Island Sound:

CONTROL:

See Table 6. There is not observed any peak in the green region (4% at 550 nm) of the visible spectrum, which is the major characteristic of a healthy green leaf. Instead there is observed a steady increase in leaf reflectance from app. 400-700 nm (1 % at 450 nm and 7% at 650 nm), followed by an increase in reflectance in the NIR region (17% at 900 nm). The missing peak of reflectance in the green region along with an increase of reflectance from the blue to the red region indicated a yellow senescence vegetation, since the diminishing chlorophyll pigments leads to a relatively higher amount of green and red light reflected from the leaf. This observation corresponds with the reflectance signature of senescence vegetation illustrated in Table 2 & 3. The spectral signature in the visible region indicated stressed vegetation and the relatively low reflectance in the NIR region indicate a low biomass or low LAI.

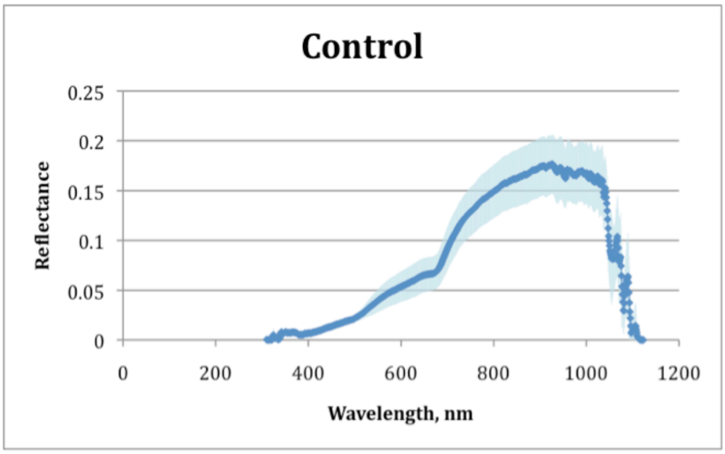

Table 6: Vegetation reflectance signature of *Spartina alterniflora* at "Control" in Plum Island Sound. Average values ± SD are illustrated.

GREENWOOD:

See Table 7. There is a low reflectance in the blue (4 % at 550 nm) and red (9% at 650nm) region, with a high peak in the green (19% at 550 nm) region of the visible spectrum and app. 46 % of the incident NIR flux was reflected from the leaf at 900 nm. It is the spongy mesophyll layer in the green leaf that controls the amount of NIR energy reflected. On a canopy level the LAI will have a greater influence on the reflectance than the individual leaf structures. The high diffuse reflectance in the NIR region is due to internal scattering at the cell wall-air interfaces within the leaves. The spectral signature of Greenwood indicates a high chlorophyll absorption in the blue and red regions of normal healthy green vegetation, with a high reflectance in the NIR region indicating a large biomass, corresponding with a high chlorophyll concentration. The variations within the site are largest in the NIR regions, which likely could reflect differences in vegetation height and site variation.

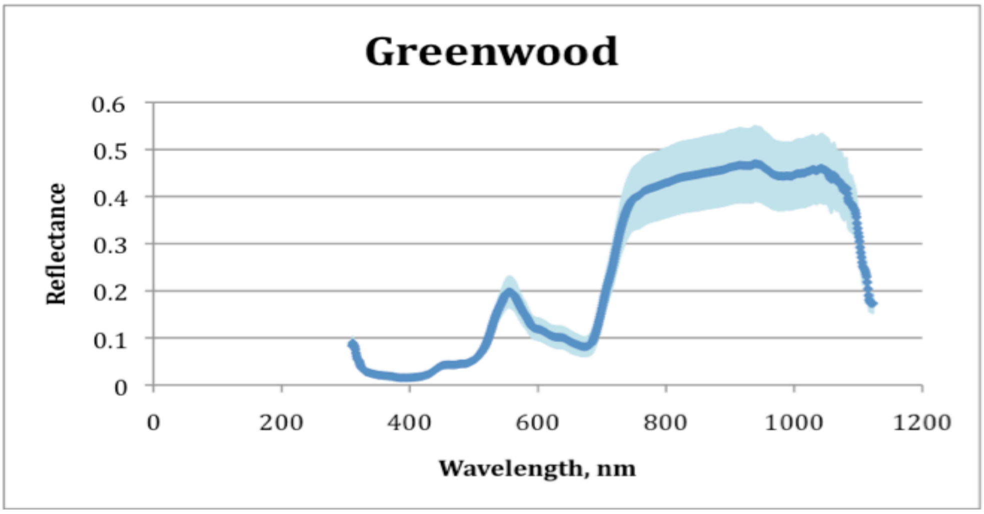

Table 7: Vegetation reflectance signature of *Spartina alterniflora* at "Greenwood" in Plum Island Sound. Average values ± SD are illustrated.

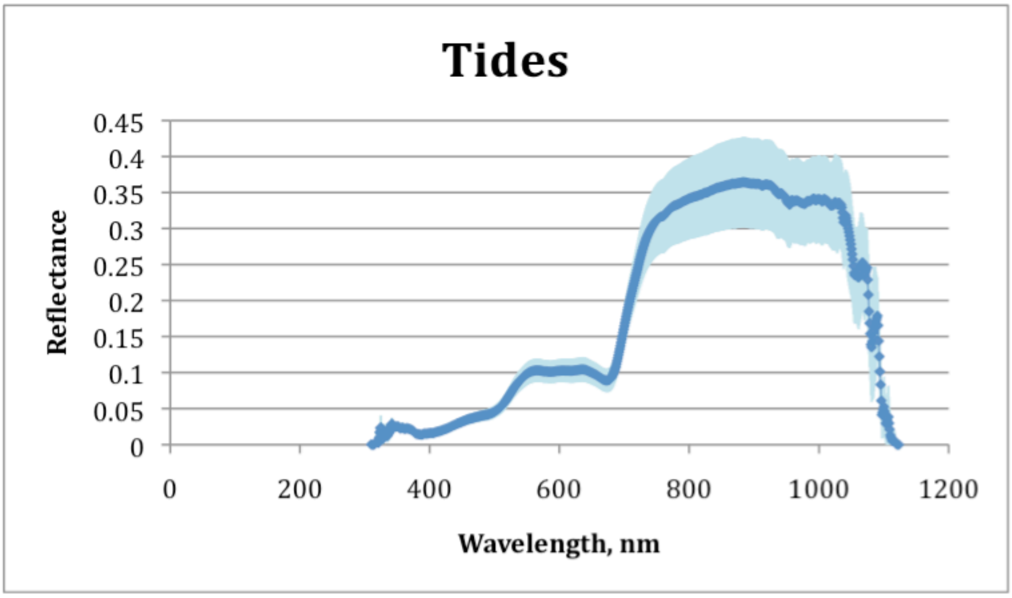

Table 8: Vegetation reflectance signature of *Spartina alterniflora* at Tides" in Plum Island Sound. Average values ± SD are illustrated.

TIDES:

See Table 8. There is small peak in the green region (10 % at 550 nm) with a very low reflectance in the blue (3% at 450 nm) region and a low reflectance in the red (10% at 650 nm) region. The reflectance is 36% in the NIR region. The reflectance signature indicates the vegetation is showing initial signs of stress with an increased reflectance in the red region, but also with a lower reflectance in the green region, indicating lower chlorophyll concentration in the vegetation. The NIR reflectance is high and could illustrate a high biomass. Maybe the plants are losing their pigments but a large part of the biomass is still present.

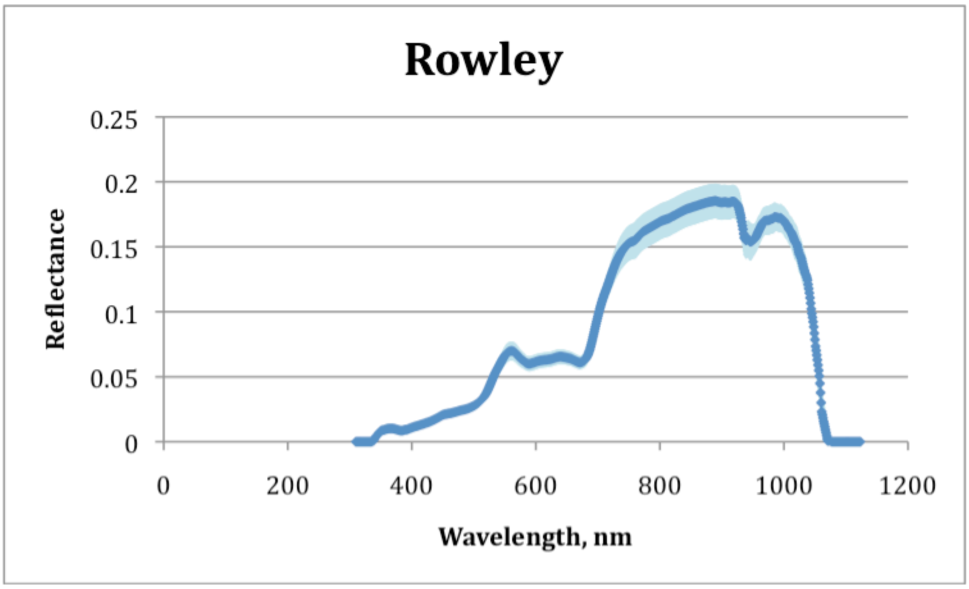

Table 9: Vegetation reflectance pattern of *Spartina alterniflora* at "Rowley" in Plum Island Sound. Average values ± SD are illustrated.

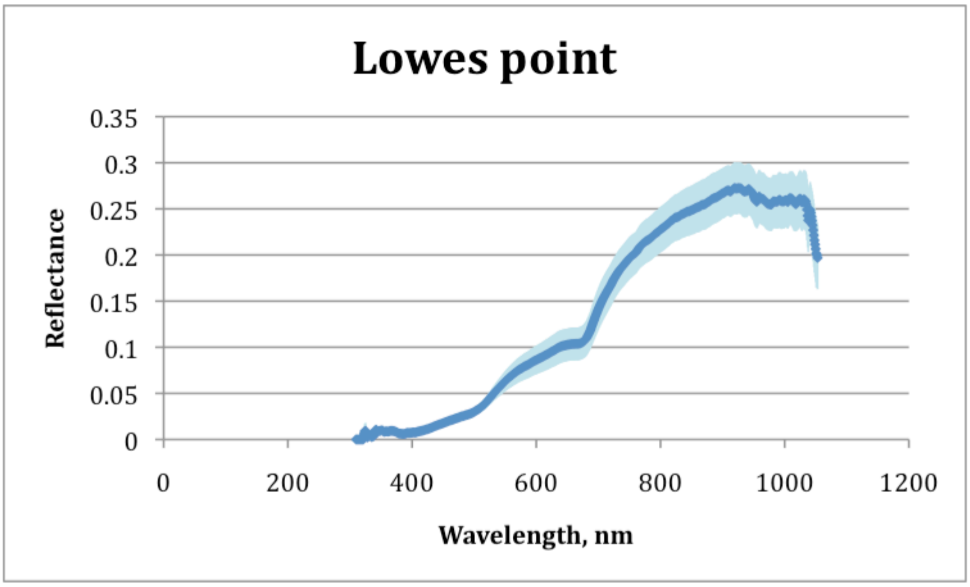

Table 10: Vegetation reflectance pattern of *Spartina alterniflora* at "Lowes Point" in Plum Island Sound. Average values ± SD are illustrated.

ROWLEY:

See Table 9. The reflectance is increasing steadily from the blue region (2 % at 450 nm) to the red region (6 % at 650 nm), with no significant peak in the green region (7 % at 750 nm), with a 19% reflectance in the NIR region (at 900 nm). The reflectance signature is showing same tendency as in Tides, indicating senescening vegetation, but with a lower NIR reflectance.

LOWES POINT:

See Table 10. The reflectance is increasing slowly from the blue region (2 % at 450 nm) to the red (10 % at 750 nm) with no peak in the green reflectance (6 % at 650 nm). The reflectance signature indicates senescening vegetation. As in Tides and Rowley, the chlorophyll concentration is diminishing leading to a higher red reflectance.

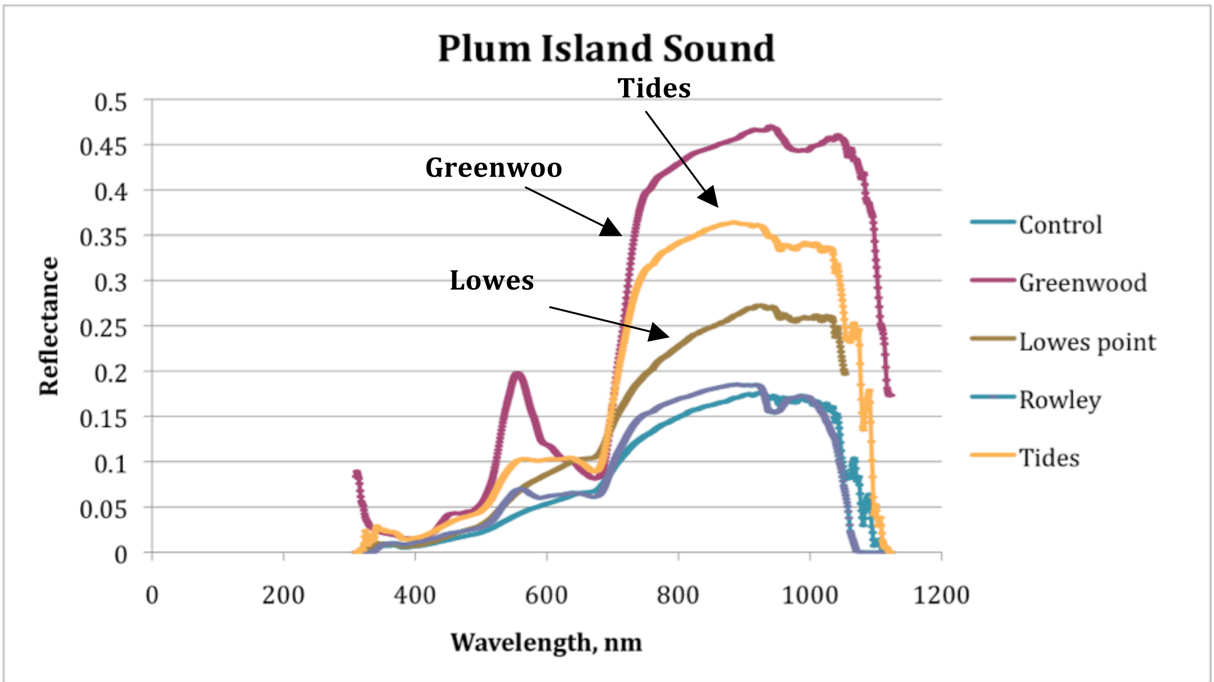

Table 11: Spectral reflectance pattern for Plum Island Sound. Average values are illustrated.

PLUM ISLAND SOUND:

The spectral reflectance signature of *Spartina alterniflora* in all sites, with the exception of Greenwood, indicates that the vegetation is in a senescening state, see Table 11. In the visible spectra the changes in the blue and red region is very low (1-4 % and 6-10 % reflectance respectively). The NIR reflectance signature in the spongy mesophyll layer will tend to decrease in senescing yellowish vegetation, where the highest reflectance is seen in Greenwood and Tides, which are the two sites with the highest nutrient input. This also correlates well with the assumption that a high LAI index (and thereby a higher biomass) will lead to a higher reflectance

in the NIR region, where the lowest NIR reflectance is seen in Control and Rowley, which are the two sites with the highest nutrient input.
When looking at the reflectance in the visible region across the sites, it is difficult to make a estimating of the chlorophyll concentration when comparing with standard curves over leaf reflectance, e.g. Table 3. The increase in the green and red region, as a consequence of senescing vegetation does not seem to be so obvious, and it seems more likely that it is only the decrease in green reflectance that is a clear indication of a decreasing chlorophyll concentration overall.
When looking at the NIR reflectance there is a clear distinction between the sites. The highest reflectance is from Tides and Greenwood, which has the highest nutrient input. The high reflectance is very likely to represent a high LAI and thereby a high plant biomass. But the lower NIR reflectance could also reflect lower water content in the vegetation.
In Tides and Greenwood, it would be expected that the reflectance in the red region is lower, due to the high chlorophyll concentration (since *chl a* and *b* has a high absorption in the blue and red band). But the lowest reflectance of red light it seen in Control and Rowley. This could on the other hand reflect that the chlorophyll concentration is higher, even though that the biomass is lower. When measuring reflectance on canopy level it is likely that the red reflectance will be saturated and the reflectance signature is not sensitive enough, to notice spectral features in that range.

5.2 Spectral signatures of Great Sippewisset Marsh.

The reflectance signature from Great Sippewisset Marsh (data from XF8 is excluded due to abnormal data) within the visible spectrum is without major individual differences (3-5 % reflectance at 450 nm, 8-10 % at 550 nm and 6-7% at 650 nm). See Table 12.

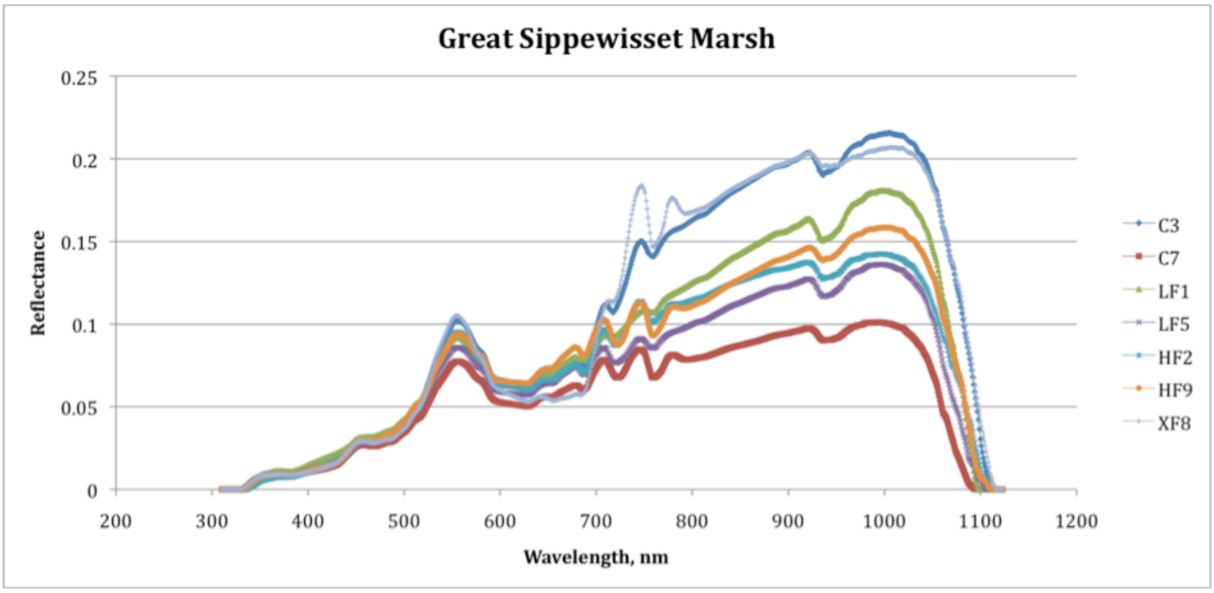

Table 12: Spectral reflectance signature from Great Sippewisset Marsh. Average values are illustrated.

In the infrared region the reflectance is between 9-20% (at 900 nm) with the highest reflectance seen in C3 and XF8 (19 & 20%). When plants are stressed, the reflectance signature in the NIR region will decrease which could indicate a lower biomass and LAI. But the lower reflectance could also reflect lower water content.

When the plant biomass or LAI is low a decreased chlorophyll concentration is expected. If this is the case, less chlorophyll could lead to a lower reflectance in the red and blue region. This seems to be the case, with the exception of XF8, which has a low reflectance in the red region along with a high NIR reflectance. This could be due to variation (e.g. in density and vegetation height) within the marsh.

Overall the data indicated that the vegetation in site C3 and XF8 is the healthiest of all sites, but none of them have an overall healthy green vegetation signature.

5.3 Spectral signatures of Great Sippewisset Marsh and Plum Island Sound

Table 13 shows the reflectance signature from all sites in the two selected marshes. When looking at the data together, it is clear that there is a big difference in the NIR reflectance between the sites. The highest NIR reflectance is found in Tides, Greenwood and Lowes Point, which have the highest biomass among the sites, where all sites from Sippewisset Marsh have a low NIR reflectance, with the highest found in C3 and XF8. Since water stress is also detectable in the NIR region, it is difficult to rule out if the vegetation in Sippewisset Marsh is in a more stress state and therefore the NIR reflectance is overall lower than Plum Island Sound.

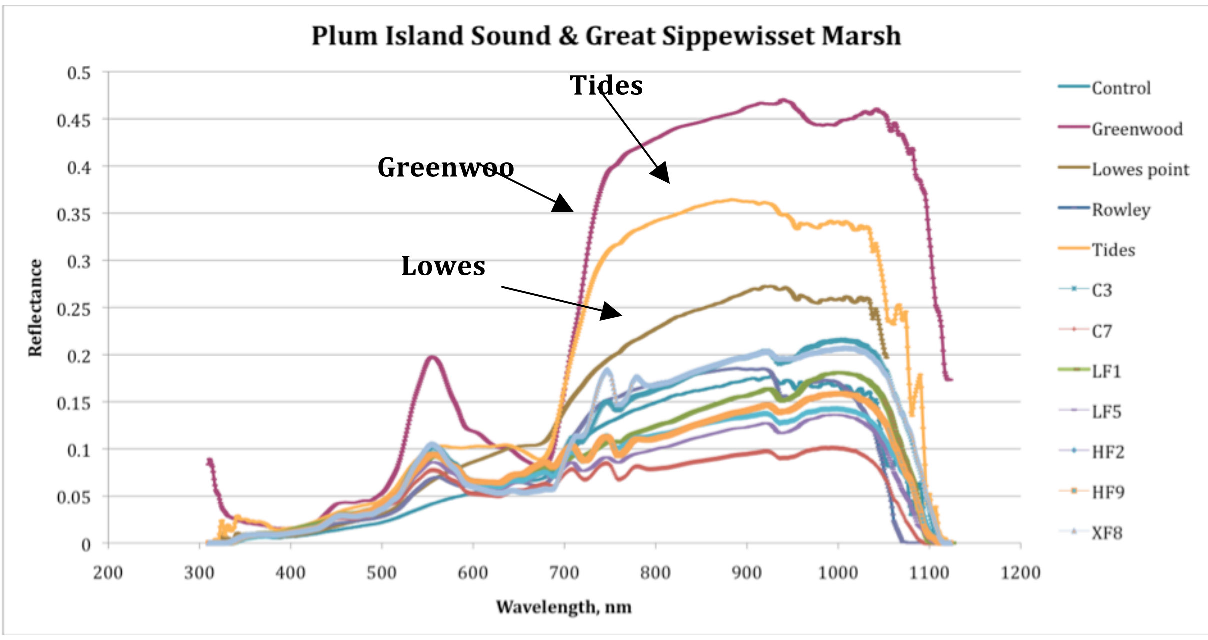

Table 13: Spectral reflectance signature from Great Sippewisset Marsh and Plum Island Sound. Average values are illustrated.

Variances within the marshes could also be a reason.
When looking at the chlorophyll concentration based on the red and green reflectance, it seems that the variance in the blue and red region is minimum and the variance in the green region is more likely to illustrate a chlorophyll difference. This correlates with other people's work, which suggests that on canopy level, the green reflectance as a chlorophyll indicator is more sensitive than the blue and red region (e.g. Giteson, 1996; Yoder & Pettigrew-Crosby, 1995).
Based on the overall spectral data, it is clear that Greenwood and Tides are the two sites with the highest N input, followed by Lowes Point, C3 and XF8. Even though there is a nutrient different between the replicate plots of Sippewisset, it does not seem as the reflectance data can distinguish clearly between them along with the other lower nutrient sites from Plum Island Sound.

5.3 Vegetation indexes

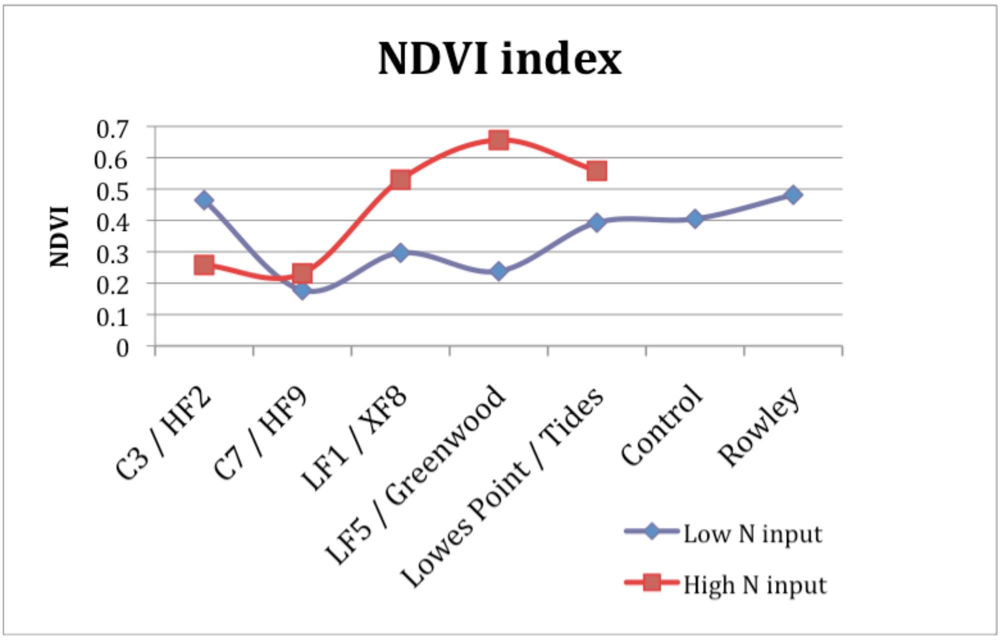

Table 14: Average NDVI values from sites with high N input (HF2, HF9, XF8, Greenwood and Tides) and low N input (C3, C7, LF1, LF5, Lowes Point, Control and Rowley).

When looking at the NDVI values from all sites (see Table 14), there seem to be a tendency with a higher NDVI value (and thereby a more dense, green vegetation) in the sites with a high N input compared with sites with a low N input. Especially, XF8, Tides and Greenwood have high values.

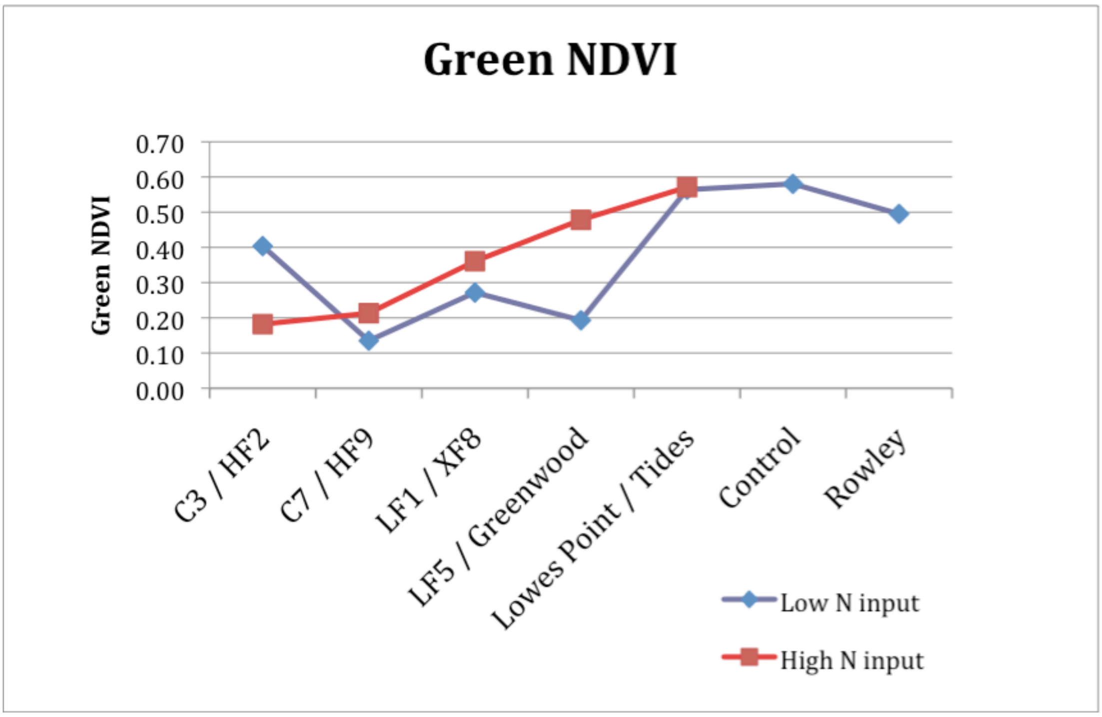

Table 15: Average GreenNDVI values from sites with high N input (HF2, HF9, XF8, Greenwood and Tides) and low N input (C3, C7, LF1, LF5, Lowes Point, Control and Rowley).

When looking at the GreenNDVI values (see Table 15), which are more sensitive to leaf chlorophyll than NDVI, the data looks different from the NDVI results. There is not a clear tendency between the sites with low or high N input and the highest values are found in Tides (high N input) and Lowes Point, Control and Rowley (low N input).

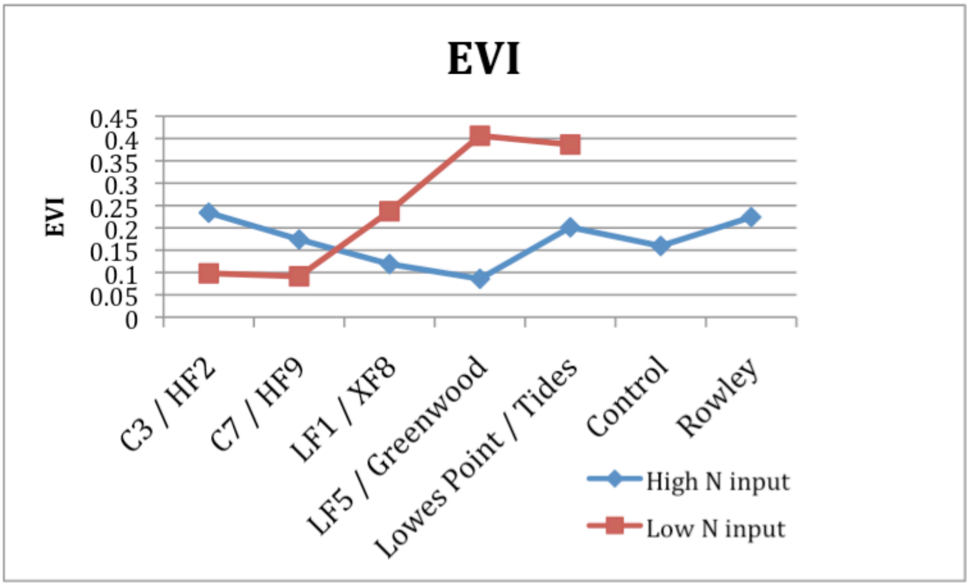

Table 16: Average EVI values from sites with high N input (HF2, HF9, XF8, Greenwood and Tides) and low N input (C3, C7, LF1, LF5, Lowes Point, Control and Rowley)

When looking at the EVI values (see Table 16), which is more sensitive to plant biomass, the data look more alike the NDVI data (Table 9). Here the highest index is found in Greenwood and Tides, where the three other high N input sites (HF2, HF9 and XF8) all is within the same index as the low N input sites.

Comparing the vegetations indexes against each other (see Table 17) shows an interesting difference between site with low and high N input. Table 17a illustrates the calculated indexes from the sites with high N input. When NDVI is calculated the values range between app. 0.25-0.65, where the highest values are defined as very dense, green vegetation. Especially Greenwood, Tides and XF8 have highest values. GreenNDVI could illustrate that the chlorophyll concentration in XF8 and Greenwood is overestimated by NDVI, leading to lower GreenNDVI values and HF9 and Tides are underestimated, leading to higher values. EVI illustrate the leaf biomass and the curve looks similar to NDVI, which could mean that NDVI in this case are more sensitive to the biomass than chlorophyll.

Table 17b looks at the sites with low N input. The NDVI values are ranging between app. 0.2-0.5, where the highest index is seen in C3, Lowes Point, Control and Rowley. The GreenNDVI is following the same trend, but has much higher values in Lowes Point and Control than NDVI values, which could indicate that the chlorophyll concentration is much higher in these sites, then pictures by NDVI. The EVI values are following the same pattern at NDVI, but with higher values in C7, but with lower biomass estimation.

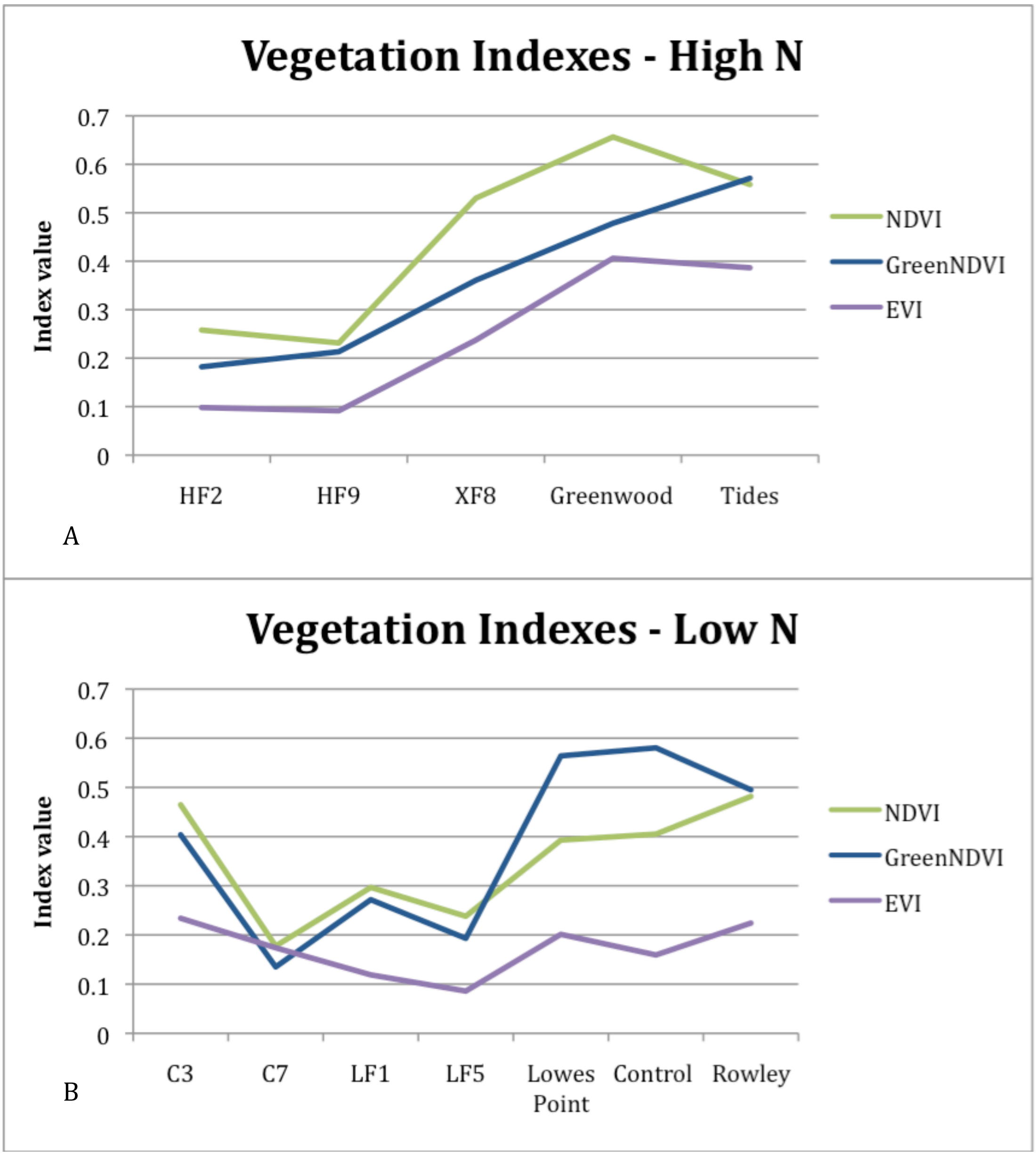

Table 17: A and B illustrates the three indexes (NDVI, GreenNDVI and EVI) from sites with a high N input (HF2, HF9, XF8, Tides and Greenwood) and low N input (C3, C7, LF1, LF5, Lowes Point, Control and Rowley) respectively.

Remote sensing techniques to estimate vegetation characteristics from reflective optical measurements have been based either on empirical-statistical approaches where surface measurements of canopy variables are related to single spectral reflectance or vegetation indexes, or on physically based canopy reflectance models. Both approaches have pros and cons. Vegetation indexes potentials have been demonstrated in numerous studies (e.g. Huete & Justice, 1999; Didan & Yin, 2002; Yoder & Wander, 1994) and the simplicity of the approach makes it desirable for large-scale remote sensing applications. But a fundamental problem with the indexes is the estimation of biophysical variables, which easily becomes generalised. Canopy reflectance depend on a complex interaction of internal and external factors (Jackson, 1986), that may vary in time and space and form between one vegetation type to another and is difficult to establish a universal relationship between a single canopy variable and a spectral signature (Houborg & Boegh, 2008).

The results indicate that the performance of NDVI, GreenNDVI and EVI when predicting plant biomass and chlorophyll content is affected by varieties that differs between the sites and it is difficult to state if one index would be to prefer over the other. On the other hand, they clearly demonstrates the difficulties in creating accurate indexes that without calibration can remain constant under changing atmospheric and soil background conditions.

5.4 SPAD-502 CHLOROPHYLL MEASUREMENTS

In this section only data from Tides & HF9 (high N input) and XF8 and control (low N input) will be presented.

XF8:

See Table 18 (The table structure for Table 18-21 is similar to each other). SPAD measurements give an indirect indication of the leaf chlorophyll concentration. Since red reflectance on a leaf scale and green reflectance on a canopy are the dominant indicators of chlorophyll content in vegetation sensed through remote sensing, it is assumed that there will be a negative correlation between SPAD values and the two colour bands (SPAD values represent absorption and not reflectance and the correlation should thereby be negative instead of positive).

The correlation for XF8 is illustrated in Table 18a and 18b. There is observed a low negative correlation with SPAD values.

To minimize influence from leaf biomass, Table 18c and 18d is calibrated for leaf biomass by dividing the red and green reflectance with NIR reflectance respectively. A low correlation is observed.

SPAD values are calibrated for % leaf N and *chlorophyll a* content. Table 18e,f and 18g,h represents % N and *chlorophyll a* content against red and green reflectance respectively. A low negative correlation is observed, indicating that there is not a connection between red or green reflectance and %N and leaf chlorophyll.

C3:

See Table 19. The correlation between the red and green band and SPAD values is a low positive correlation, which turns negative, when SPAD values are calibrated against NIR. There is a positive correlation between % N and leaf chlorophyll and the visible spectra.

Control:

See Table 20. There is observed a low positive correlation with SPAD values, illustrating a missing connection between SPAD and reflectance data. When calibrating SPAD values again NIR the correlation decreases and turns slightly negative. There is positive correlation for % leaf N and *chlorophyll a* content for both spectra.

Tides:

See Table 21. There is observed a negative correlation between the visible range and SPAD values, which turns positive when calibrated against NIR.

There is a negative correlation between % N and leaf chlorophyll against red and green reflectance.

XF8:

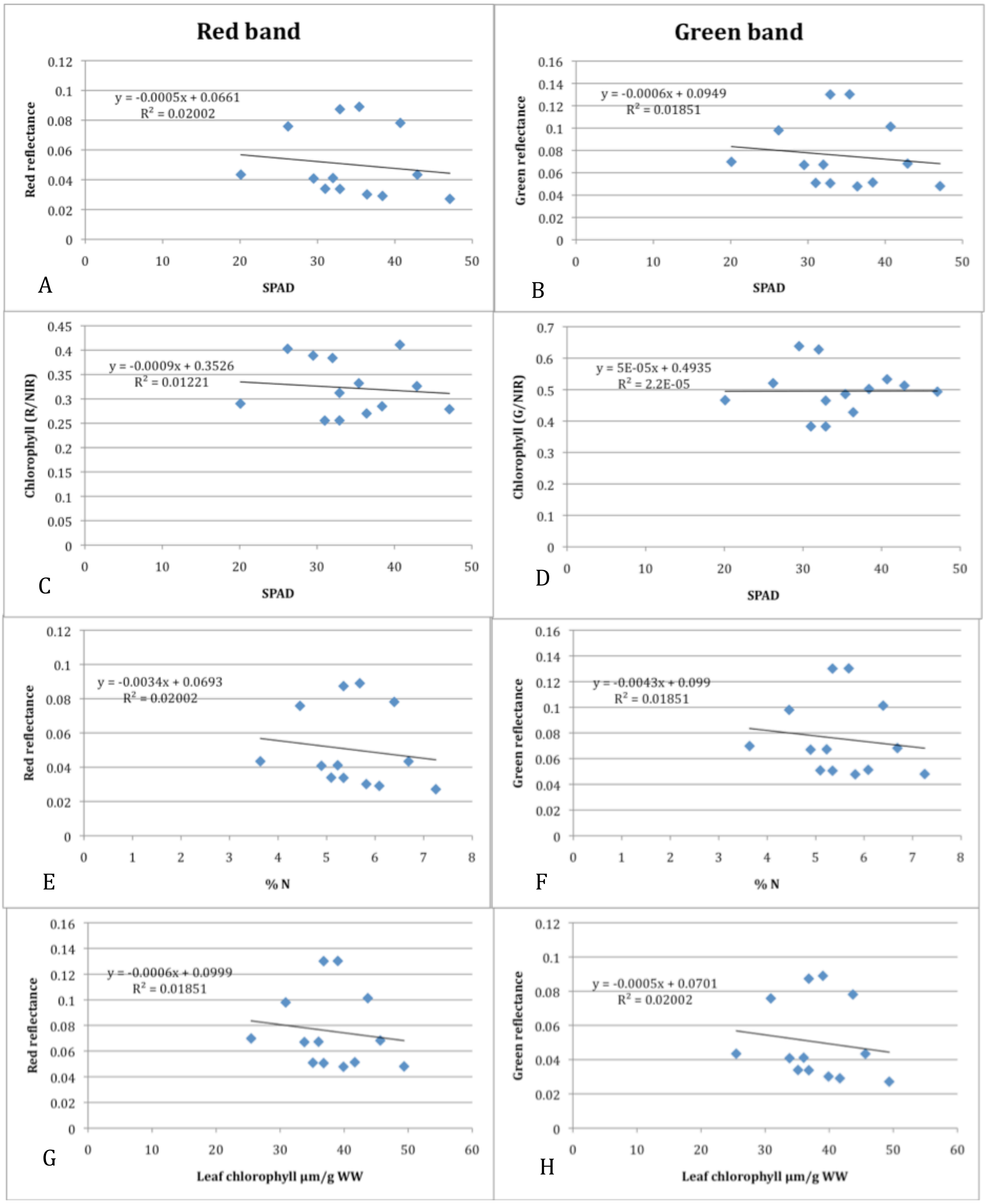

Table 18: "XF8" - A & B: SPAD values plotted again red and green reflectance. C & D: SPAD values plotted against R/NIR and G/NIR (which is to minimize the influence of leaf biomass). E & F: % N plotted again red and green reflectance. G & H: Leaf chlorophyll (µm/g WW) plotted against red and green reflectance. % N and leaf chlorophyll are transformed SPAD values.

C3:

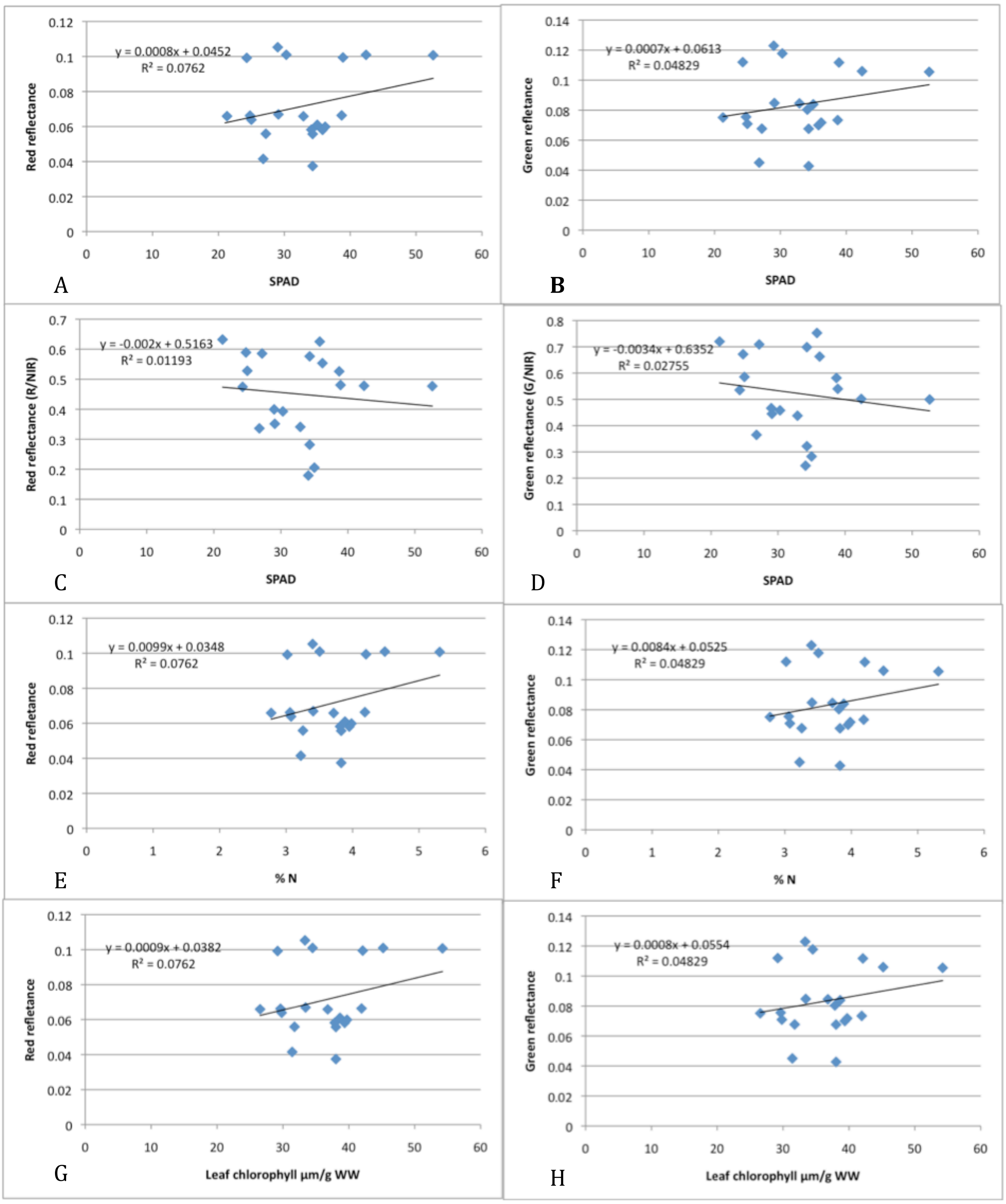

Table 19: "C3" - A & B: SPAD values plotted again red and green reflectance. C & D: SPAD values plotted against R/NIR and G/NIR (which is to minimize the influence of leaf biomass). E & F: % N plotted again red and green reflectance. G & H: Leaf chlorophyll (µm/g WW) plotted against red and green reflectance. % N and leaf chlorophyll are transformed SPAD values.

Control:

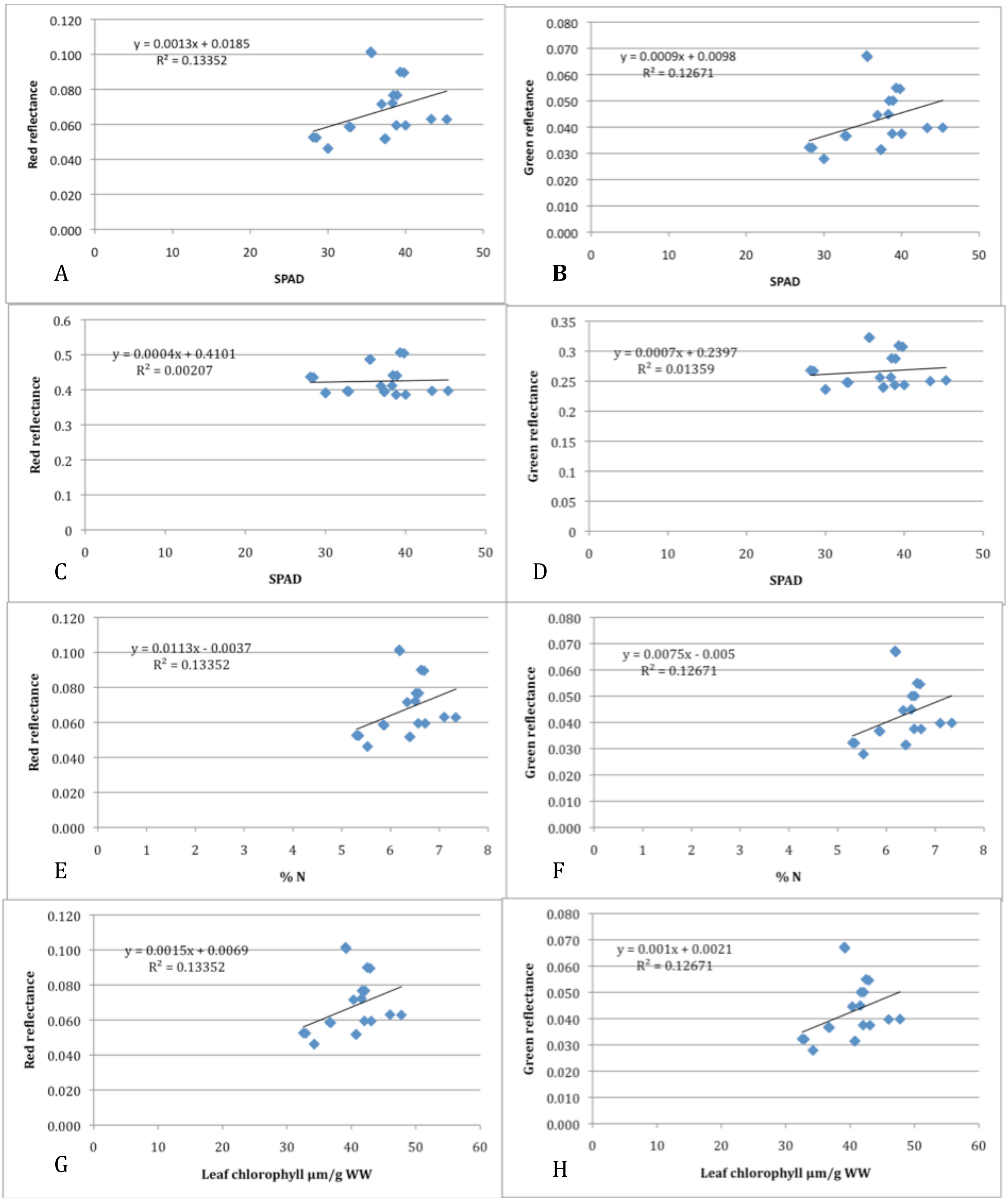

Table 20: "Control" - A & B: SPAD values plotted again red and green reflectance. C & D: SPAD values plotted against R/NIR and G/NIR (which is to minimize the influence of leaf biomass). E & F: % N plotted again red and green reflectance. G & H: Leaf chlorophyll (µm/g WW) plotted against red and green reflectance. % N and leaf chlorophyll are transformed SPAD values.

Tides:

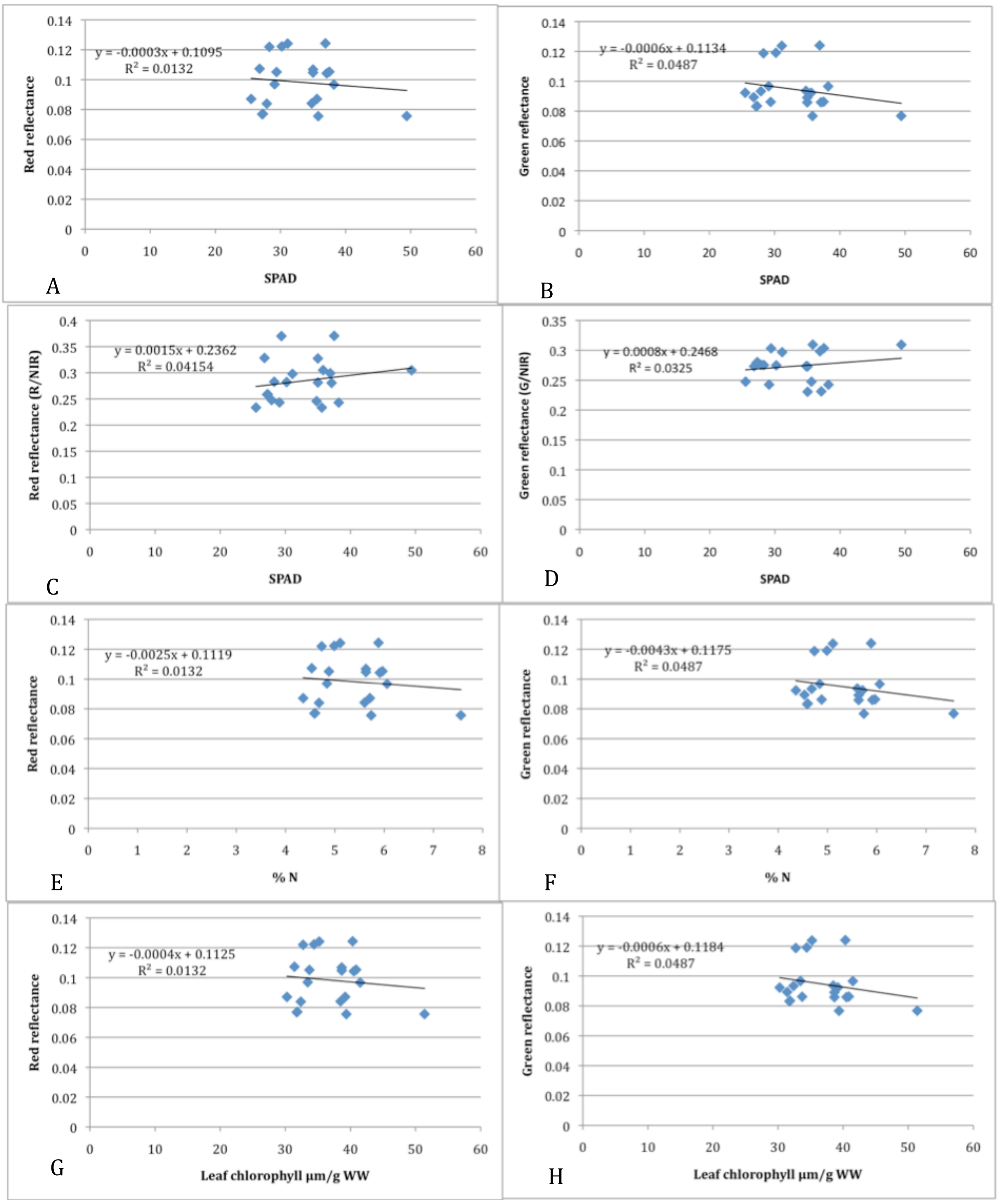

Table 21: "Tides" - A & B: SPAD values plotted again red and green reflectance. C & D: SPAD values plotted against R/NIR and G/NIR (which is to minimize the influence of leaf biomass). E & F: % N plotted again red and green reflectance. G & H: Leaf chlorophyll (µm/g WW) plotted against red and green reflectance. % N and leaf chlorophyll are transformed SPAD values.

The correlation between SPAD values and red and green reflectance is not strong. There are several possible reasons for this:
The SPAD values, which are raw data, should have the strongest correlation with red and green reflectance data, since the data is not transformed. When looking at % N and leaf chlorophyll, it is expected to see a higher correlation between chlorophyll and the visible region than for nitrogen. This is because spectral properties between 400 and 700 nm are determined primarily by chlorophyll and relationships between nitrogen and visible spectral features are thus indirectly due to associations with chlorophyll (Yoder and Pettigrew-Crosby, 1995). There is not observed a major different in correlation with chlorophyll or nitrogen.

There are two primary limitations when using a SPAD meter. First, a reference is needed to acquire accurately quantified N concentrations in the plants. Secondly the SPAD meter collects point measurements from a single leaf on a single plant. To obtain a representative average value, many leafs from numbers of plants must be surveyed, also to adequately assess the spatial variability (Xue et al. 2004). The data presented did not show a very strong correlation between SPAD values along with % N and leaf chlorophyll concentration against red or green reflectance. There are several possible reasons for this: First of all the reflectance data is on a canopy level and even though the data was transform by dividing with NIR reflectance, which mostly represent leaf biomass, it might not have been enough and other features might be influencing the reflectance data, which makes it unsuccessful to compare with SPAD values.

Calibration of SPAD values for % N and leaf chlorophyll concentration was highly correlated for a few sites and three equations was used to calibrate the data. The low overall correlation could be due to errors in the laboratory technique or that other features are influencing the results. If better correlations were obtained the calibration might correlate better with reflectance data.

SPAD measurements are obtained on a larger area than Unispec measurements. The number of measurements along with the density between them could have an effect on the results. More measurements within a denser area could lead to very different results, then the one presented, especially since the measurement represent leaf chlorophyll, which can vary greater on a leaf scale than an more overall canopy scale.

When using remote sensing to sense leaf characteristics it is important to keep focus on the different scales. The spectral reflectance pattern from vegetation measured on a leaf scale differs from measurement of a canopy scale, where other features can become dominant. As demonstrated by other authors (e.g. Yoder & Pettigrew-Crosby 1995; Yoder & Waring, 1994) scaling from leaf to canopy is difficult and the remote sensed data presented in this report could also illustrates that either more complex model has to be applied or more specific wavelength has to be investigated to more clearly see if stress or other features have an influence on the results.

When using remote sensing, reflectance from other sources will inevitable mix with the signal from the plant. The effect of soil background reflectance, leaf biochemical parameters, leaf internal structure, leaf dry matter, canopy biophysical parameters (LAI) and the influence from the atmosphere (Fensholt & Sandholt, 2003) can be an issue and the main questions is whether the effect of chlorophyll and plant biomass on plant reflectance is still distinguishable from these influences.

Temporal characteristics of vegetation will have an influence of the reflectance data. Timing means everything, since the difference in percent canopy closure, soil moisture, biomass or difference in orientation might have a dramatically difference in reflectance pattern even between two very similar vegetation types. This means that choosing the right time for obtaining measurements are of importance (Jensen, 2000). When working with reflectance data from Unispec measurements and SPAD meter, data from several seasons would be interesting. The data presented is obtained late in the vegetative season, where reflectance data from the high peak season, might give more distinguishable spectral data between the sites along an indication of spatial changes.

7.0 Conclusion

Spectral reflectance obtained from Unispec measurements, clearly illustrates that the vegetation state in Greenwood and Tides are the healthiest green vegetation with a high plant biomass, which correlated with the N input received. In the remaining sites, there is not observed a clear distinguish between the spectral data and observed N input.
The calculated NDVI, GreenNDVI and EVI based on Unispec reflectance data shown different estimations of chlorophyll and plant biomass. In sites with low N input it is likely that NDVI have fine biomass estimation but it is overestimating the chlorophyll concentration in XF8 and Greenwood and underestimating it in HF9 and Tides, when comparing with GreenNDVI.
In sites with high N input, chlorophyll concentration is underestimated by NDVI in Lowes Point and Control and the biomass is underestimating in C7. Based on this information, it is not possible to estimate if one index or the other would be better to use to estimate N status but the results indicate the feasibility in predicting N status.
Spectral reflectance obtained from SPAD values is low correlated with red and green reflectance in XF8 and Tides. Transformed SPAD values (with minimized LAI influence) are low correlated with XF8 and C3.
% N and leaf chlorophyll derived from calibrated SPAD values are low correlated with Control and C3. There is not any clear connection between SPAD values and high and low N input.
SPAD values are shown to give an indirect indication of chlorophyll and nitrogen content in leaf biomass but more emphasis has to be giving on calibration of SPAD measurements to obtain more reliable results.
Remote sensing can provide information about variations in vegetation and give an insight into important vegetation biophysical features. Therefore using remote sensing to determined N status of vegetation is a low cost useful method, but emphasis on future studies in this area should be a priority.

8.0 Reference list

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