Report for 2004MD57B: Leaf-scale hyperspectral reflectance models for determining the nitrogen status of freshwater wetlands

There are no reported publications resulting from this project.

Report Follows

Final Report to
Maryland Water Resources Research Center

Leaf-scale hyperspectral reflectance models for determining the nitrogen status of freshwater wetlands

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Executive Summary

The effects of nitrogen (N) fertilization and species on the hyperspectral leaf reflectance (350 – 1050 nm) of four common emergent freshwater macrophytes (*Acorus calamus*, *Peltandra virginica*, *Phragmites australis,* and *Typha* spp.) were determined in a complete randomized experiment. Plants were cultured in a climate-controlled greenhouse and subjected to five levels of N fertilization (0, 1, 2, 5, and 20 mg-N per L), selected to span levels observed in natural and treatment wetlands. An ASD Handheld SpectroRadiometer (Analytical Spectral Devices, Boulder, CO) with a spectral range of 325—1075 nm and a one degree field of view was used to measure plant leaf reflectance in full sun. Prior to fertilization species exhibited a significant ($p<0.05$) effect on leaf reflectance in the visible and near-infrared with all spectral bands between 450 and 1050 nm affected. The majority of the species effect was due to the differences between *Acorus* and both *Peltandra* and *Phragmites*. After 49 d of N fertilization, N, species and their interaction were all significant factors for visible and near-infrared (NIR) response. Grouping *Acorus* with *Peltandra* and *Phragmites* with *Typha* greatly reduced the species effect and heightened the N effect. The reflectance of the invasive species group (*Phragmites* + *Typha*) in the chlorophyll *a* and *b* absorption bands displayed much higher sensitivity to low N additions the non-invasive group (*Acorus* + *Peltandra)*, suggesting that the invasive group had a greater ability to take up N, build chlorophyll and absorb photosynthetically active and NIR radiation. A partial least squares regression model that used the change in reflectance over N fertilization experiment to predict N treatment level had an R^2 of 0.72. The work demonstrated that the capability to detect the response of wetland plants to elevated N availability via leaf reflectance could be used to assess the N levels of natural emergent marshes. This provides a foundation for scaling the technique to aerial or satellite-based hyperspectral radiometers to perform large scale monitoring and assessment of wetland water quality.

1 Introduction

1.1 Problem Statement

The Clean Water Act stipulates that States report the health and quality of all water bodies, including wetlands, in a National Water Quality Inventory Report, but only 4% of wetlands were included in the most recent edition (USEPA 2002a). By 2012 the USEPA's leniency will end and States will be required to report wetland water quality and ecological health (USEPA 2002b). The lack of reporting stems from technical difficulties associated with sampling wetlands and unresolved issues in defining wetland health. Direct sampling of wetland soils and plant tissues provides the most accurate estimate of the N concentration and prevalence for specific locations, but gives limited information on geographical extent of the problem. Remote sensing with hyperspectral radiometry may offer an ability to assess the likelihood of wetland N availability over broad geographical areas, which would vastly improve wetland assessment capabilities.

Nutrients from agricultural runoff and other sources are widely known to cause eutrophication of open-water aquatic systems such as the Chesapeake Bay, stimulating algal growth and causing dieback of submerged aquatic vegetation and declines in fish and shellfish populations (Jaworski et al. 1992; Chesapeake Bay Program 2002). Anthropogenic input of nutrients to the biosphere is increasingly viewed as a global threat to ecosystems (Vitousek et al. 1997; Fenn et al. 1998). This cultural eutrophication of habitats can alter plant species composition and reduce plant diversity (DiTommaso and Aarssen 1989; Morris 1991). Although Maryland's load of total nitrogen to Chesapeake Bay decreased by 28% from 1986 to 2001 nitrogen loading remains a priority concern for achieving the 2000 Chesapeake Bay Agreement (MDNCWG 2001). Statewide, point source nitrogen loads have decreased from 14,300 MT to 7710 MT (46%) and agricultural loads have dropped from 14,600 MT to 9590 MT (34%). Urban loads, on the other hand, have grown 19%, from 5370 MT to 6390 MT.

Tidal freshwater marshes (TFM) are an extensive type of wetland located along the U.S. coast, often in the upper tidal reaches of rivers flowing into estuaries (e.g., the Patuxent River of Chesapeake Bay) (Odum 1988; Tiner and Burke 1995; Mitsch and Gosselink 2000). TFM's support productive commercial and recreational fisheries, provide recreational opportunities, filter pollutants from land runoff, and add to the biological diversity of the landscape (Odum et al. 1984).

From a water quality management perspective, restoration of wetlands is recommended as a Best Management Practice (BMP) by the State of Maryland for improving Bay water quality (MDNCWG 2001). By 2012 the USEPA will begin enforcing the regulation that all States and Tribes report the water quality and ecological health of wetlands. Thus, not only is there a need to understand the affect of restored and natural wetlands on reducing nutrient and sediment loads to the Bay, we also need to be able to rapidly and easily quantify their nitrogen and phosphorus status. Currently, measuring the nutrient status of wetlands relies on labor-intensive collection and time-consuming water analysis techniques.

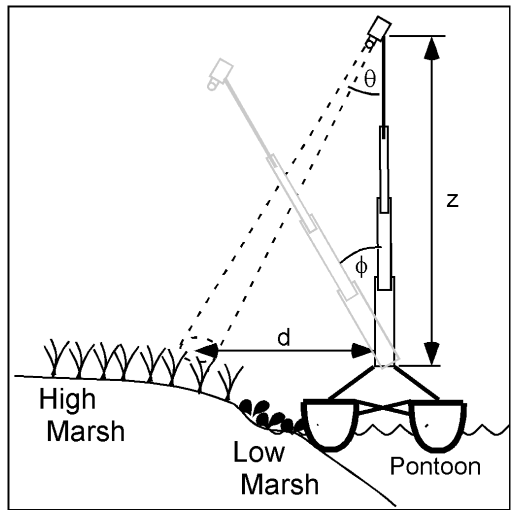


Figure 1.1. Schematic diagram demonstrating measurement positions for the boat-mounted, telescopic spectroradiometer boom.

The emerging field of precision agriculture, whereby satellite, airborne, and handheld spectroradiometers are employed to measure nitrogen status of crops, demonstrates the potential of employing remote sensing technologies to understand the nutrient status of wetland ecosystems. Advancing the capability of wetland remote sensing to quantify the nitrogen status of wetlands can (1) provide a tool for the large scale assessment of water quality in difficult-to-access wetlands, (2) offer a rapid screening method for identifying nitrogen "hot-spots" in a watershed, (3) enable near real-time monitoring in areas suspected of producing significant quantities of non-point source (NPS) pollution, and (4) be used to monitor wetlands used as for nutrient management.

Table 1.1 indicates how Maryland Water Resources Research Center (MWRRC) funding fit into our long-term research and development effort to develop wetland hyperspectral radiometry as a remote sensing tool for assessing the water quality of emergent marshes. Our previous research demonstrated the proof-of-concept of leaf-scale reflectance models for assessing marsh water column N status in brackish treatment marshes (Tilley et al. 2003). The present project focused on advancing reflectance modeling by testing previously developed reflectance indices and exploring the efficacy of the multivariate data analysis technique of Partial Least Squares to assess the availability of N to emergent macrophytes. Simultaneous to the work of this project, we were developing a boat-borne imaging radiometer system (BIRS, Fig. 1.1) with funds awarded from Maryland Sea Grant College, which evaluated the feasibility of collecting marsh canopy reflectance using BIRS. Now we are seeking further funding to conduct pilot-scale testing of BIRS in a variety of wetlands and to improve the data collection and reflectance modeling methods. Our goal is to have the technology ready in 2 years for full-scale testing in other wetlands by environmental managers and to have a wetland radiometric system operational by 2012 when the U.S. EPA will begin to enforce sections of the Clean Water Act that stipulate States must include wetland water quality in their biennial reports on water quality (303b Reports).

Table 1.1. Tasks required to develop hyperspectral boat radiometry as a commercial tool for assessing wetland nutrient status.

Tasks	Status	Comments
Proof-of-Concept	Completed 2002	Tilley et al. (2003)
Leaf Reflectance Models	2003-2004	This MWRRC project
Boat-radiometer Proof-of-Concept	2004-2005	MD Sea Grant
Pilot-scale testing of boat-radiometer	Near-term	funding TBD
Full-scale testing w/ end users	Mid-term	funding TBD
Commercial Operation	2012	

1.2 Hyperspectral Reflectance and Ecosystem Assessment

Hyperspectral reflectance refers to the ability to measure plant reflectance of solar radiation in hundreds of narrow (1—2 nm) spectral bands. Hyperspectral reflectance data continue to be evaluated for their ability to represent biological and ecological functions and structural properties (see review by Treitz and Howarth, 1999). At the leaf cellular level, reflectance indices have been developed to infer physiological and biochemical attributes (e.g., chlorophyll and carotenoid content, Jago et al. 1999). At the canopy level of terrestrial ecosystems, spectral indices have been developed to infer leaf area index and canopy nitrogen (Boegh et al. 2002), and water use and evapo-transpiration (Szilagyi 2000, Wiegand and Richardson 1990a, 1990b). The reflectance of upland plants are known to respond to various environmental factors—soil nitrogen (Read et al. 2002), soil moisture (Strachan et al. 2002), salinity (Wang et al. 2002), and ozone concentration (Penuelas et al. 1995), demonstrating the versatility of narrow spectral band reflectance indices to detect plant response to environmental stimuli.

Although hyperspectral reflectance has been widely used to assess water quality conditions of open-water aquatic ecosystems--classifying the trophic status of lakes (Koponen et al. 2002, Thiemann and Kaufmann 2000) and estuaries (Froidefond et al. 2002), characterizing algal and red tide blooms (Stumpf 2001, Kahru and Mitchell 1998), and identifying and classifying

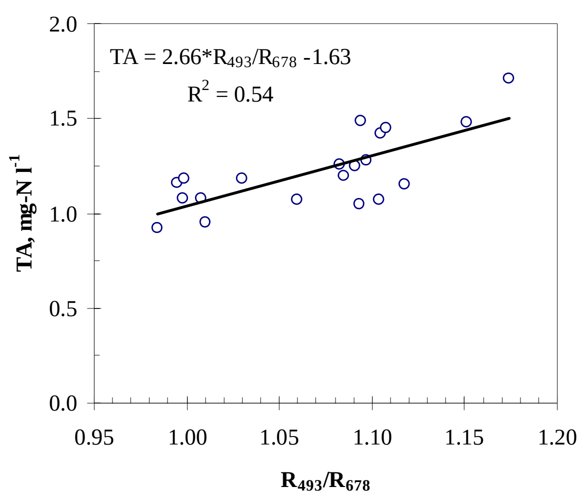


Figure 1.2. A simple reflectance index (R493/R678) was indicative of the wetland's ammonia (TA) concentration over a narrow range.

submerged aquatic vegetation (Williams et al. 2003)--assessment of the ecological health of emergent wetlands based on narrow band spectral reflectance appears limited to plant photosynthetic radiation use efficiency (Penuelas et al. 1997) and redox potential (Anderson and Perry 1996). Thus, quantification of wetland water quality from plant reflectance appears to be a new area of investigation and application (Tilley et al. 2003).

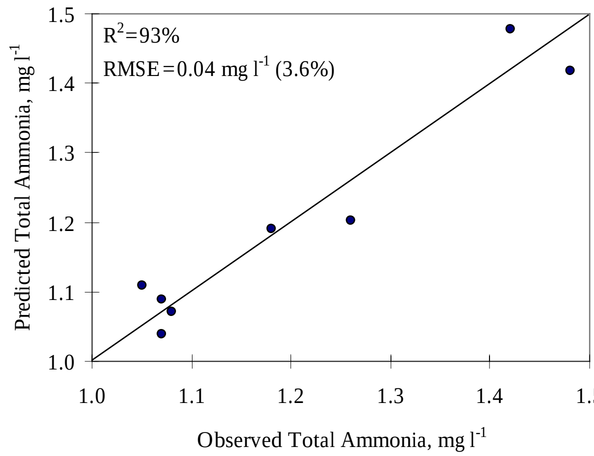


Figure 1.3. A multiple regression equation with PRI and RE of *T. latifolia* as independent variables explained 93% of ammonia variation in the wetland and had a root mean square error (RMSE) equal to 3.6% of the mean ammonia concentration.

During investigations of a brackish (2.5—4.5 ppt) marsh in south Texas, we found the Photochemical Reflectance Index [PRI, defined as $(R_{531}-R_{570})/(R_{531}+R_{570})$], the red edge (RE, defined as the wavelength of maximum slope at the red—NIR transition) and simple reflectance ratios (e.g., R_{493}/R_{678})—determined from handheld spectroradiometric readings of marsh macrophyte leaves (*Typha latifolia* and *Borrichea frutescens*)—to be indicative of marsh water column ammonia concentrations (Tilley et al. 2003; Ahmed 2001). We also found that the normalized difference vegetation index (NDVI) and floating water-band index (fWBI) were responsive to small (1 ppt) changes in salinity (Tilley et al., in review). Others have found the PRI to be positively related to nitrogen, phosphorus, and potassium fertilization rates for annual, deciduous and evergreen upland species (Gamon et al. 1997). Penuelas et al. (1997) determined that the PRI of emergent wetland macrophytes was negatively related to photosynthetic radiation-use efficiency (PRUE), which was similar to non-wetland plants (Gamon et al. 1997).

The red edge (RE), defined as the wavelength of maximum slope at the red—NIR transition, increases with higher chlorophyll content, which is strongly influenced by nitrogen levels. In general, red spectral reflectance is more responsive to nitrogen changes than blue or green spectra due to changes in chlorophyll *a* and *b* (Carter and Miller 1994). Strachan et al. (2002) included PRI along with RE, as necessary members of a multi-index reflectance model developed for classifying nitrogen application rates in corn (*Zea mays*).

Combining individual spectral reflectance bands as simple ratios (e.g., R_{493}/R_{678}) to reduce data noise is a common approach for relating hyperspectral reflectance to biological and ecological properties (Carter and Miller, 1994). Normalization of sensitive wavebands to non-sensitive wavebands minimizes noise and is readily scaled to airborne or satellite platforms. Recently, Read et al. (2002) found a simple blue to red reflectance ratio (R_{415}/R_{710}) as a strong indicator (R^2 = 0.70) of leaf nitrogen in cotton (*Gossypium hirsutum*).

Partial least squares (PLS) regression is a type of eigenvector analysis that can reduce full-spectrum data to a small set of independent latent factors (i.e., PLS-components) that explain response in dependent variables, such as the leaf chlorophyll or nitrogen content (Esbensen 2002). PLS regression is related to principal components regression (PCR), which proceeds by first determining the principal components of the independent variable matrix (**X**) without considering any information contained in the dependent variable matrix (**Y)** and then uses ordinary least squares to relate the principal components to the dependent variables (**Y**). The advantage of PLS regression is that it selects latent components of **X** that explain the most about **Y**. PLS reduces the potential for selecting spectral bands not associated with absorption features, which is a concern with a method such as stepwise multiple linear regression (MLR)—which deals poorly with multicollinearity among spectral bands (Grossman et al. 1996). PLS has become a preferred method for interpreting the hyperspectral reflectance of ecosystems to ascertain biochemical information. Townsend et al. (2003) and Smith et al. (2003) used PLS to develop highly predictive reflectance models of temperate forest canopy nitrogen concentration from airborne and satellite hyperspectral images. We found PLS could estimate the marsh cover occupied by the invasive species *P. australis* (R^2=88%) and the non-invasive dominant species *Polygonum arifolium* (R^2=62%) (Tilley et al. 2004).

1.3 Previous Research

Fig. 1.2 gives an example from our previous work (Tilley et al. 2003) showing that the ratio of leaf (*T. latifolia*) reflectance in a narrow blue band centered at 493 nm and a red band centered at 678 nm explained 54% of the variation in the total ammonia concentration of the wetland's water column. Less leaf reflectance at 678 nm results from higher leaf chlorophyll *a* concentration, which is affected by nitrogen availability. Thus, the technique is a remote sensing method for directly measuring plant reflectance, which infers wetland water quality. We also found that the explanatory power of a multiple linear regression equation containing the Photochemical Reflectance Index (PRI) and red-edge (RE) reflectance indices was highly precise (root mean square error, RMSE = 0.04 mg-N l^{-1}) over a narrow range (1.0—1.5 mg-N l^{-1}) of ammonia (Fig. 1.3).

Our earliest field research on relationships between canopy reflectance and N fertilization conducted on tidal freshwater marshes of the Nanticoke river on Maryland's eastern shore suggested that N supply changes radiation balance. Our initial analysis of canopy reflectance indicates that nitrogen fertilization significantly ($P<0.05$) increased the reflectance in a majority of the near-infrared (NIR) bands, but in none of the visible bands (Fig.1.4). Presumably, the more developed canopy (i.e., higher LAI) of the fertilized sites allows less NIR radiation to reach the soil surface of the wetland where it is effectively absorbed (i.e., not reflected); consequently the canopy of the fertilized plots reflects more NIR. This has interesting ramifications for assessing the global affects of anthropogenic nitrogen fertilization on the energy budgets of wetlands.

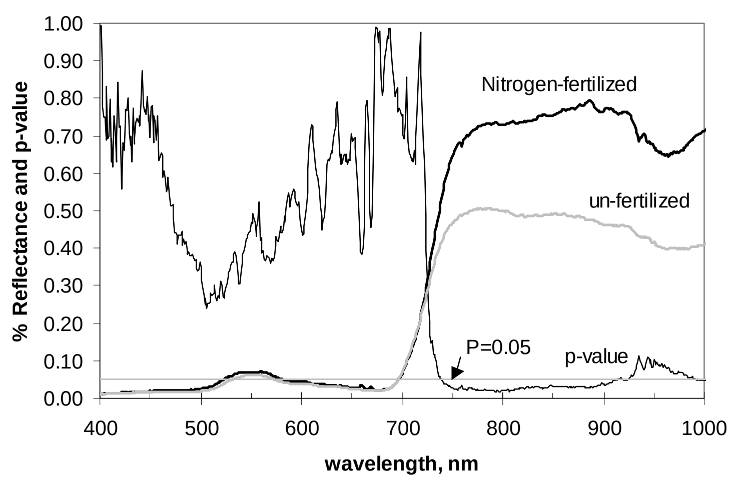


Figure 1.4. Mean canopy reflectance of fertilized and un-fertilized Nanticoke sites and p-value of t-test.

1.4 Objectives

In our original proof-of-concept work (Tilley et al. 2003) the range of ammonia concentrations was small (0.9—1.8 mg-N l^{-1}) and we only investigated two emergent macrophyte species. To develop advance wetland hyperspectral radiometry's ability to assess wetland N availability, we needed to determine the effect of species on reflectance and we needed to understand the relationship between narrow spectral band reflectance indices and wetland N over a broader range. Presumably, at some high nitrogen concentration N will no longer limit plant growth, which would lead to a saturation of chlorophyll and low reflectance sensitivity, which indicates the limits of the tool as an indicator of wetland N availability.

Therefore, our objective for this project was to test the applicability of previously developed leaf-scale reflectance models over a broader nitrogen range than previously used and to assess the differences among wetland species. A specific objective of our proposed research was to experimentally examine how narrow spectral band reflectance indices (e.g., PRI, RE, R_{678}/R_{493}) and individual spectral bands vary between four common species of wetland plants in their response to a wide range of nitrogen availability. This objective was an integral component of our larger effort to develop BIRS for assessing wetland water quality via remote sensing techniques.

2 Materials and Methods

2.1 Experimental Design

Plant materials were collected in mid-June of 2004 from a tidal freshwater marsh on the Patuxent River near its intersection with U.S. Hwy 301, which is approximately 5 km east of Washington, D.C. Plants with soil residue intact were transferred to 4 L plastic pots containing a peat-perlite mixture. Above-ground tissue was cut back to within 15 cm of the soil surface so plant tissue age would be more uniform. One individual of each potted plant was transferred to one of fifteen 170 L black tubs (i.e., four pots/species per tub) located in the University of Maryland Research Greenhouse Complex. For a period of 21 d following potting and prior to fertilization, plants were irrigated weekly with municipal tap water to flood levels that were 5 cm below the surface of the soil. Each irrigation included emptying the tubs of residual water and refilling to the specified level. Climate conditions within the greenhouse were maintained within ranges indicative of the local summer.

Four species of common wetland emergent macrophytes were used: cattail (*Typha* spp.), common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), arrow arum (*Peltandra virginica* L.) and sweet flag (*Acorus calamus* L.). These species were chosen because they are common in mid-Atlantic freshwater marshes and are large clonal perennials that are easy to collect and grow. Also, cattail and reed are invasive plants of interest to natural resource managers and researchers due to their potential for colonizing disturbed and natural (cattail is native, while exotic genotypes of reed are common in the U.S.). The treatments were 0, 1, 2, 5, and 20 mg-N/L, which were made by mixing municipal tap water with 5-0-1 (N-P-K) fertilizer (4.7% NO^-_3, 0.3% NH_3). In addition to N, all tubs received 2 mg-P L^{-1} and 1.6 mg-K L^{-1} from 0-5-4 fertilizer (5% P_2O_5) to ensure that phosphorus did not limit growth. Treatment began on July 27, 2004, 21 d after plants were potted. Fertilized irrigation water was changed approximately weekly on 8/3, 8/10, 8/18, and 8/27. Each tub was randomly subjected to one of the five nitrogen additions (Table 2.1).

Table 2.1. Summary of experimental treatments for greenhouse study of effects of species and nitrogen concentration on spectral reflectance indices.

		Nitrogen concentration (mg/L)				
		0	1	2	5	20
Species	*Typha*	3 reps	3 reps	3 reps	3 reps	3 reps
	Phragmites	3 reps	3 reps	3 reps	3 reps	3 reps
	Peltandra	3 reps	3 reps	3 reps	3 reps	3 reps
	Acorus	3 reps	3 reps	3 reps	3 reps	3 reps

These levels were selected to span a range from natural marsh concentrations (1-2 mg/L Tilley et al. 2004) up to levels typically seen in treatment wetlands that receive municipal or animal farm wastewater. The design and setup of this study was similar to studies investigating ammonia toxicity in wetland plants (Clarke and Baldwin 2002).

2.2 Radiometric measurements

An ASD Handheld SpectroRadiometer (Analytical Spectral Devices, Boulder, CO) was used to measure plant leaf reflectance. Each pot was taken outside of the greenhouse, and spectroradiometric measurements then taken on clear sky days between 10:00 am and 3:00 pm. Percent reflectance was calculated by dividing canopy reflectance by the reflectance of a calibrated Spectralon white panel (Labsphere, Inc., North Sutton, NH), measurements of which were taken immediately before canopy measurements. We used a 1º field-of-view (FOV) fore-optic positioned at 30º from nadir aimed at the leaf surface from the north side (to reduce shadow) at a distance of approximately 10 cm. The spectroradiometer collected ten sub-samples of each leaf which were later averaged to define the mean reflectance.

2.3 Data analyses

The experimental design resulting from the experimental setup described previously is a 4 × 5 factorial arrangement of treatments (4 species, 5 N levels per species), with 3 replicates of each treatment combination in a completely randomized design. Analysis of variance (ANOVA) was conducted to examine treatment effects on reflectance indices and spectral band reflectance using SPSS for Windows (SPSS Inc., Chicago, IL). Differences among means were distinguished with Bonferroni's test. Mixed effects two-factor ANOVA, which assumed nitrogen as fixed and species as random, was used to determine whether nitrogen or species affected leaf reflectance response across the full spectrum. Significant differences were defined at the 0.05 probability level. Plots of the p-values for spectral differences were plotted as functions of wavelength to identify patterns in effects. ANOVA and multiple comparison tests were conducted using SPSS for Windows (SPSS Inc., Chicago, IL). We also analyzed effect of N on reflectance by considering N treatment level as a continuous variable in simple linear regression models, which were conducted using SPSS. Spectral reflectance indices included Photochemical Reflectance Index (PRI), a simple reflectance ratio of blue to red (R_{493}/R_{678}), and a standard Normalized Difference Vegetation Index (NDVI).

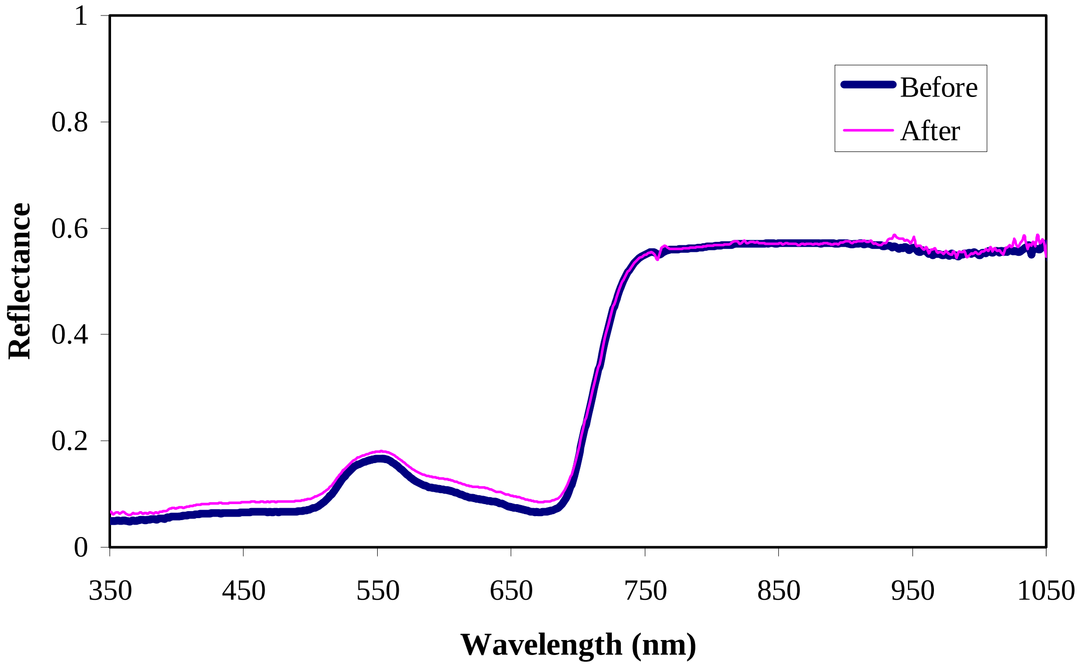
PLS was used to develop a regression model of hyperspectral reflectance predictive of N availability. We used Unscrambler 9.0 (Camo Process, Oslo, Norway) to conduct PLS. To validate the PLS model we used full-cross validation (i.e., leave one sample out). The number of PLS components to include in the final model was chosen based on the minimum root mean squared error of prediction (RMSEP). The efficacy of the final PLS model to predict N exposure was expressed by the RMSEP and the coefficient of determination.

3 Results and Discussion

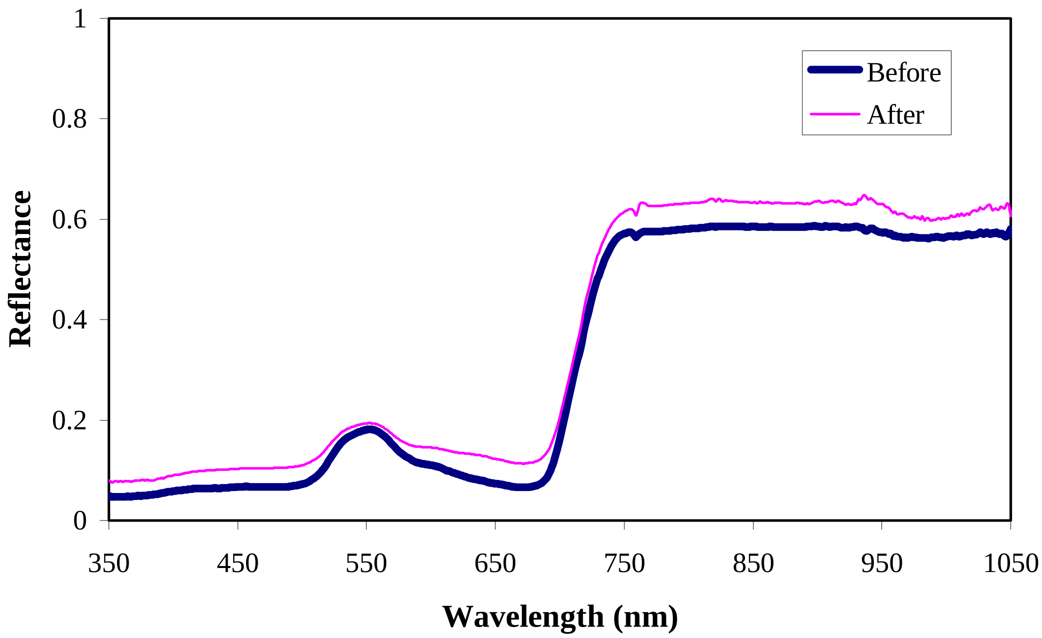
3.1 Effect of Nitrogen Fertilization on Hyperspectral Reflectance

3.1.1 Mean reflectance before and after N fertilization

Figure 3.1 shows the mean hyperspectral reflectance of all four species before (July 15, 2004) and after (September 1, 2004) nitrogen fertilization for each of the 5 fertilization levels.



(a)



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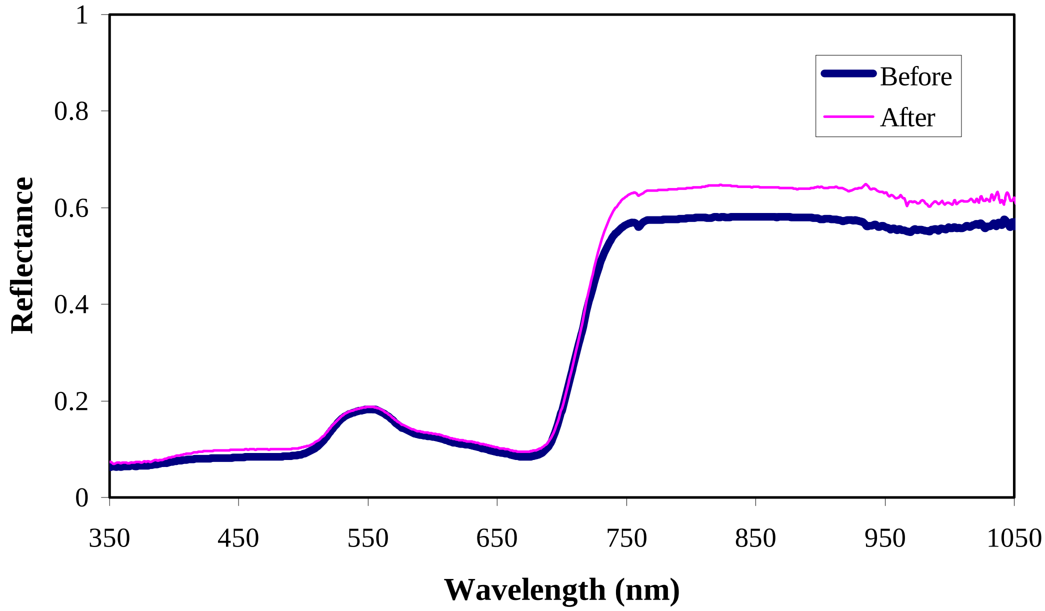
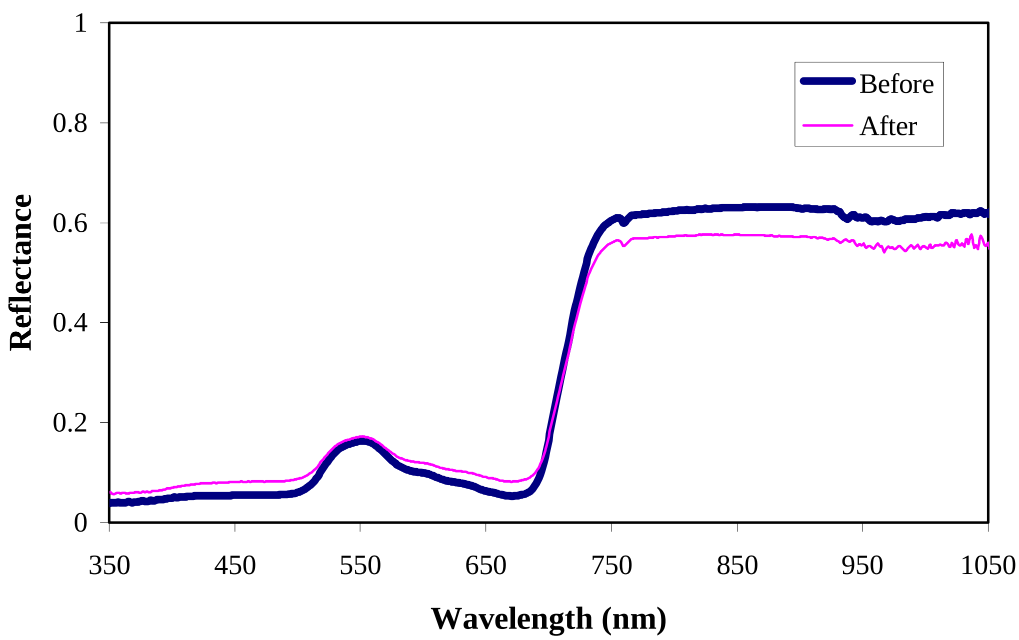
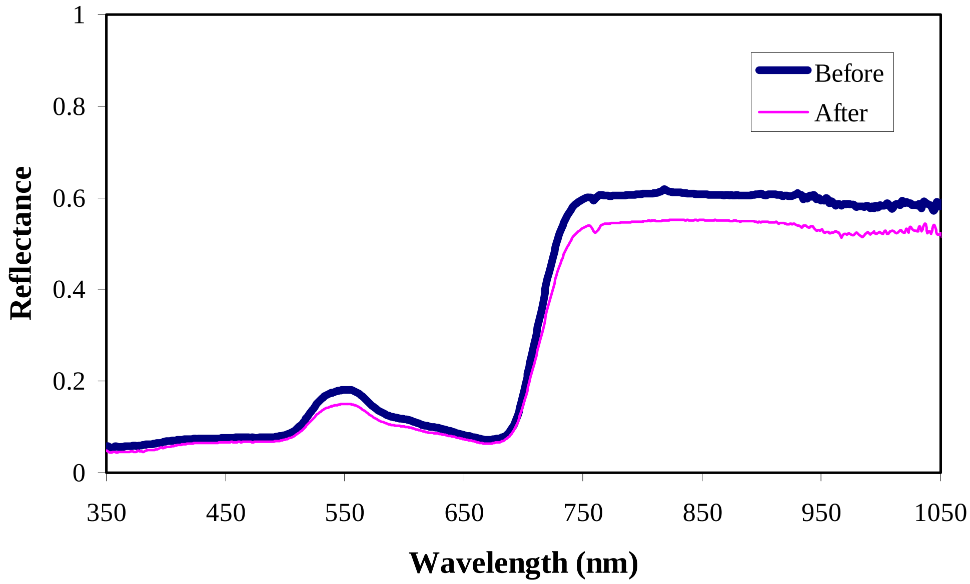

1
0.8
0.6
0.4
0.2
0
Reflectance
350
450
550
650
750
850
950
1050
Wavelength (nm)
Before
After

(c)


1
0.8
0.6
0.4
0.2
0
Reflectance
350
450
550
650
750
850
950
1050
Wavelength (nm)
Before
After

(d)

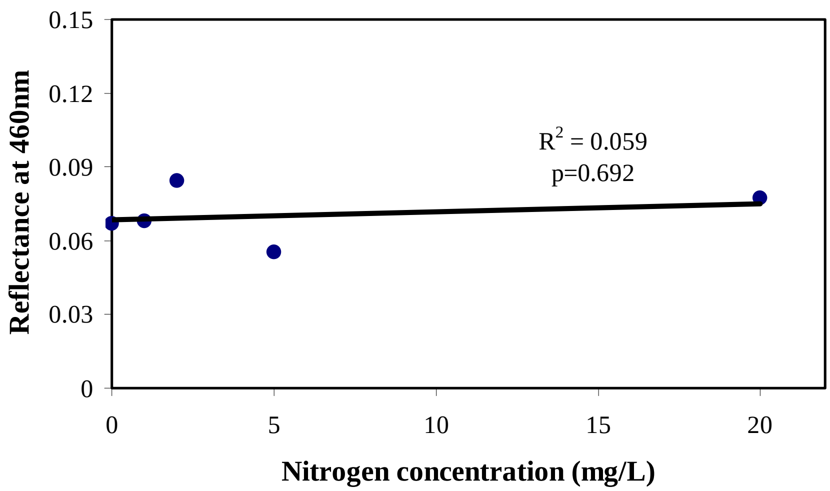


(e)

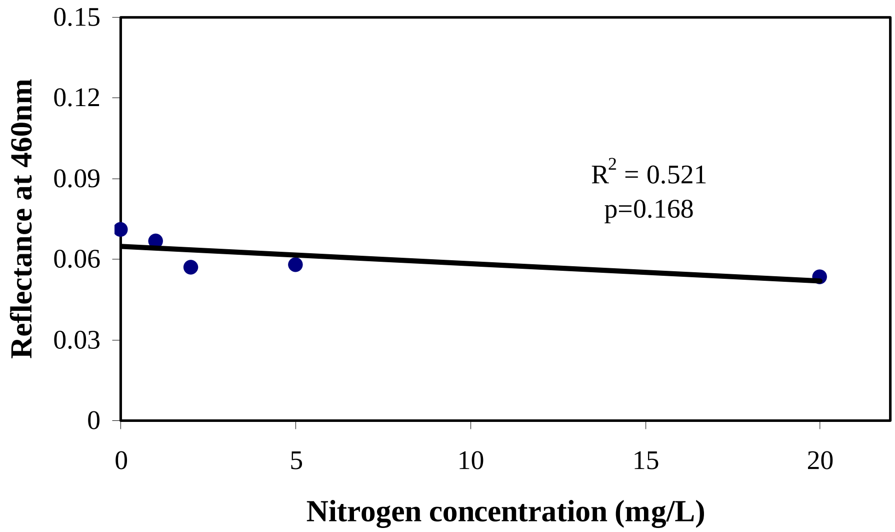
Figure 3.1. Mean hyperspectral reflectance of four species (3 replicates each) before and 49 days after fertilization for (a) 0 mg-N/L, (b) 1 mg-N/L, (c) 2 mg-N/L, (d) 5 mg-N/L, and (e) 20 mg-N/L treatment levels.

3.1.2 Response of blue, green, red and NIR spectral bands to N fertilization

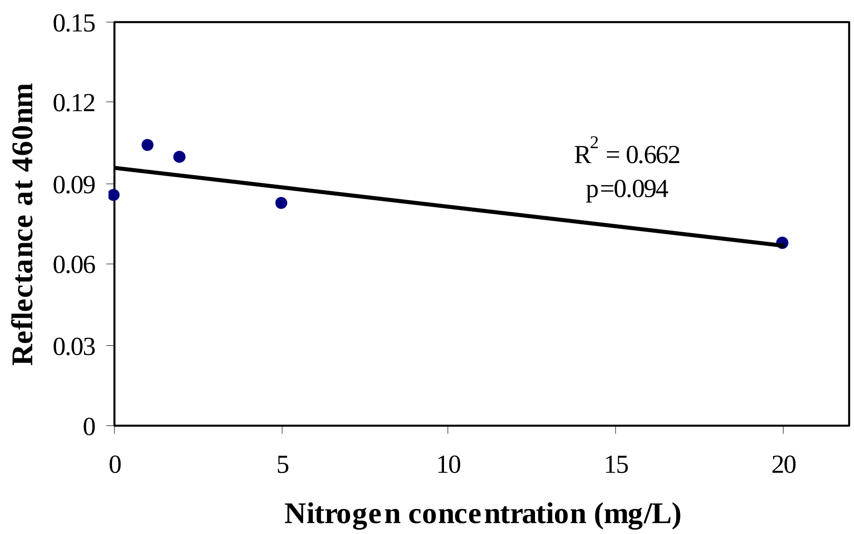
Figure 3.2 shows the effect of N treatment concentration on mean reflectance at 460 nm, which is a representative blue waveband, when all four species were combine. After 49 d of N fertilization R_{460} was suppressed by higher N availability although p-value was only 0.094.



(a)



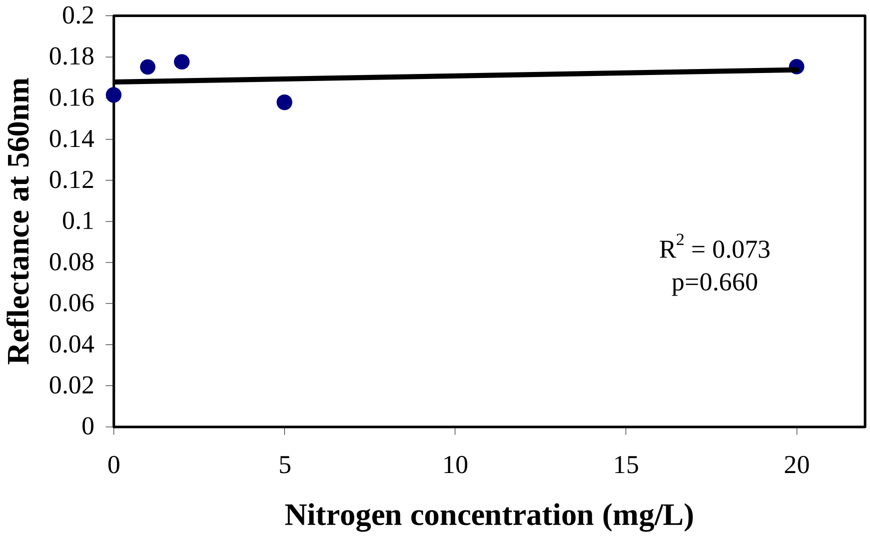
(b)



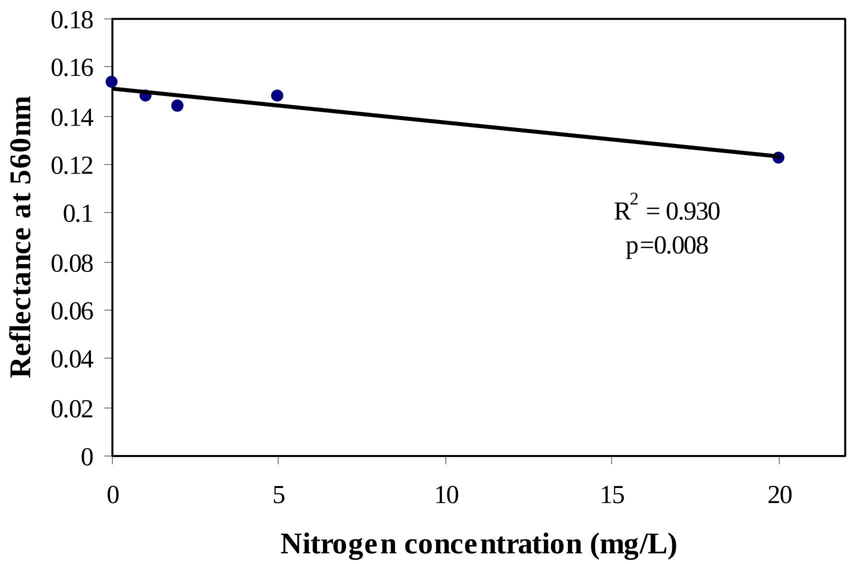
(c)

Figure 3.2. Mean reflectance of four species at the 460nm (blue waveband) across five fertilization levels (0, 1, 2, 5, and 20 mg-N/L) on (a) July 15, 2004 (pre-fertilization). (b) August 9, 2004 (post-fertilization), and (c) September 1, 2004 (post-fertilization). Coefficient of determination and significance of slope (p-value) included.

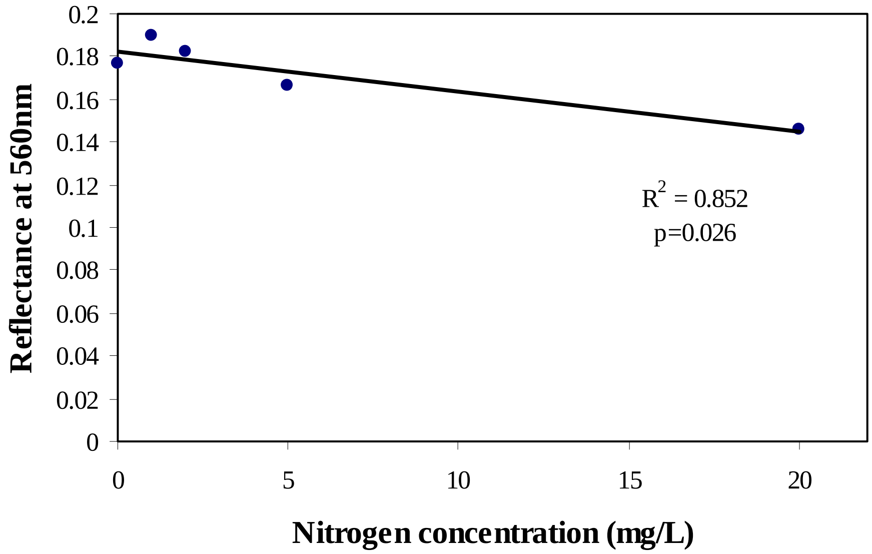
Figure 3.3 shows the effect of N treatment concentration on mean reflectance at 560 nm, which is a representative green waveband, when all four species were combine. After 25 d of N fertilization R_{560} was suppressed by higher N availability (Fig. 3.3b), which continued after 49 d of N fertilization (Fig. 3.3c).


0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Reflectance at 560nm
0
5
10
15
20
Nitrogen concentration (mg/L)
R2 = 0.073
p=0.660

(a)


0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Reflectance at 560nm
0
5
10
15
20
Nitrogen concentration (mg/L)
R2 = 0.930
p=0.008

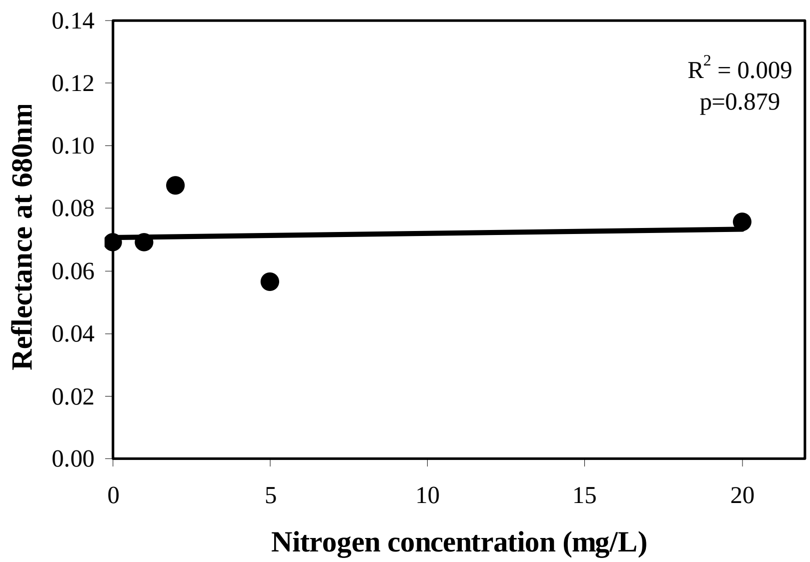
(b)



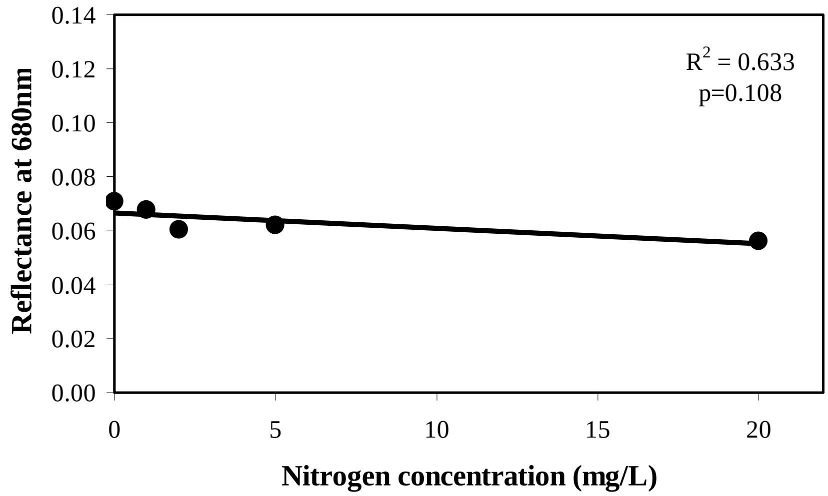
(c)

Figure 3.3. Mean reflectance of four species at the 560nm (green waveband) across five fertilization levels (0, 1, 2, 5, and 20 mg-N/L) on (a) July 15, 2004 (pre-fertilization). (b) August 9, 2004 (post-fertilization), and (c) September 1, 2004 (post-fertilization). Coefficient of determination and significance of slope (p-value) included.

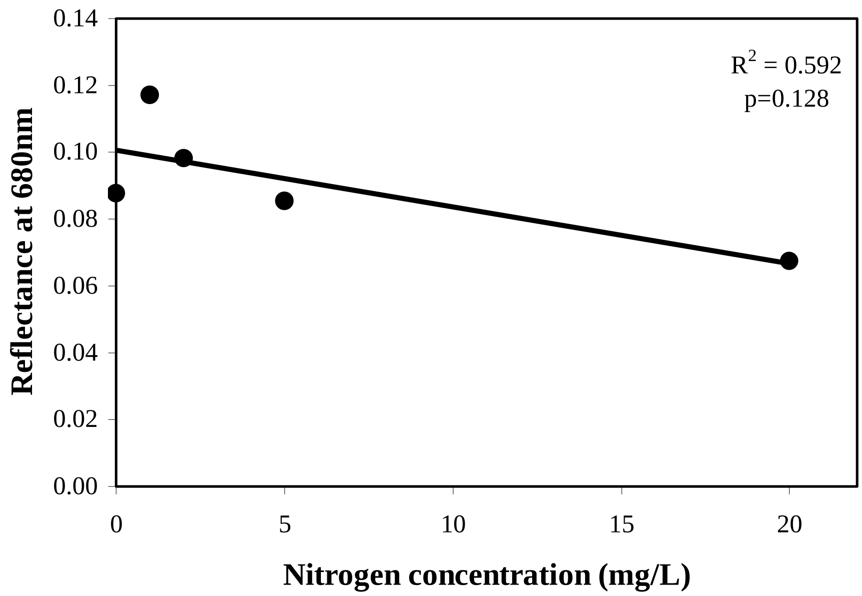
Figure 3.4 shows the effect of N treatment concentration on mean reflectance at 680 nm, which is a representative red waveband, when all four species were combine. After 25 d of N fertilization R_{680} was suppressed by higher N availability, but only moderately significant (Fig. 3.4b). R_{680} was lower at higher N levels after 49 d, but was not significant (Fig. 3.4c)



(a)



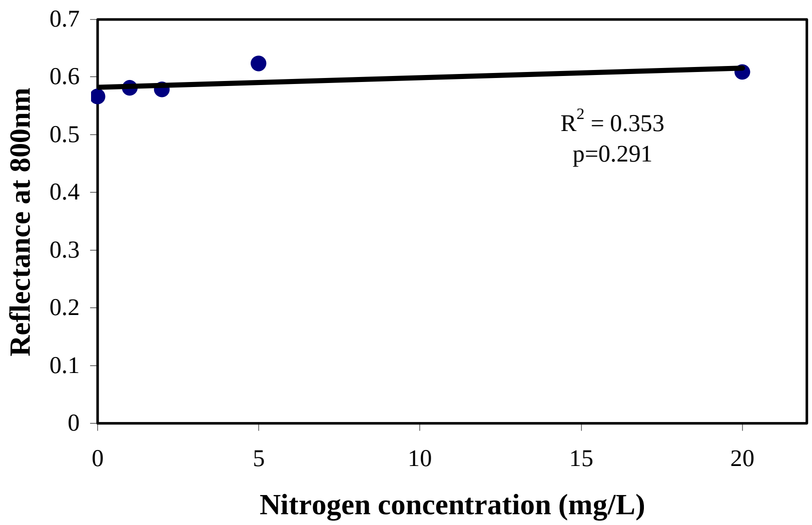
(b)



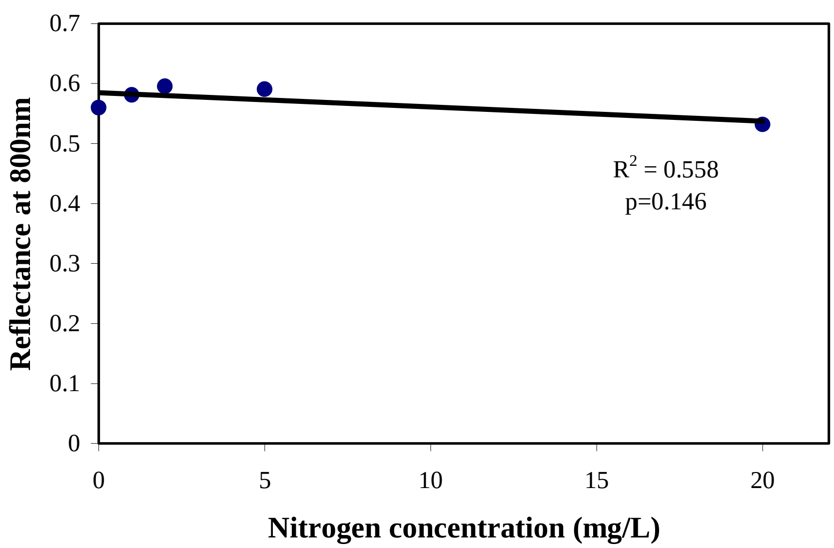
(c)

Figure 3.4. Mean reflectance of four species at the 680nm (red waveband) across five fertilization levels (0, 1, 2, 5, and 20 mg-N/L) on (a) July 15, 2004 (pre-fertilization). (b) August 9, 2004 (post-fertilization), and (c) September 1, 2004 (post-fertilization). Coefficient of determination and significance of slope (p-value) included.

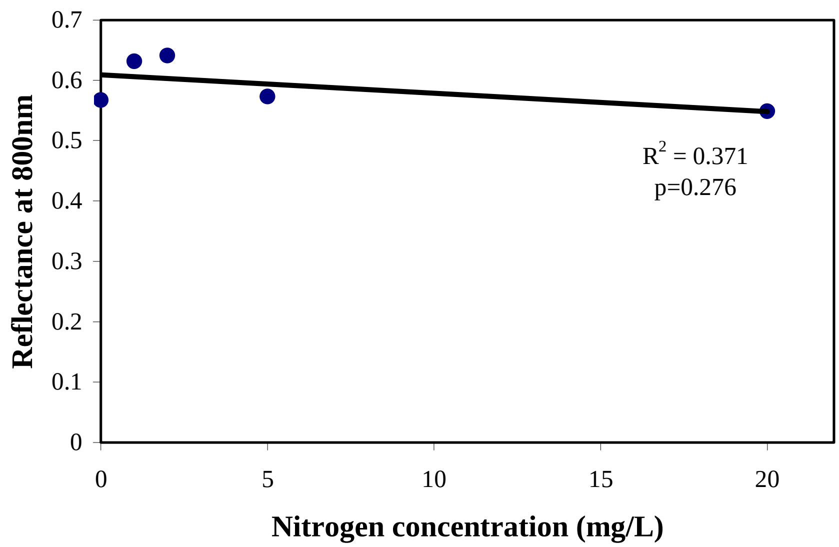
Figure 3.5 shows the effect of N treatment concentration on mean reflectance at 800 nm, which is a representative NIR waveband, when all four species were combine. There was no significant relationship between R_{800} and N availability when all plants were considered together.


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Reflectance at 800nm
R^2 = 0.353
p=0.291
0
5
10
15
20
Nitrogen concentration (mg/L)

(a)


0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Reflectance at 800nm
R^2 = 0.558
p=0.146
0
5
10
15
20
Nitrogen concentration (mg/L)

(b)

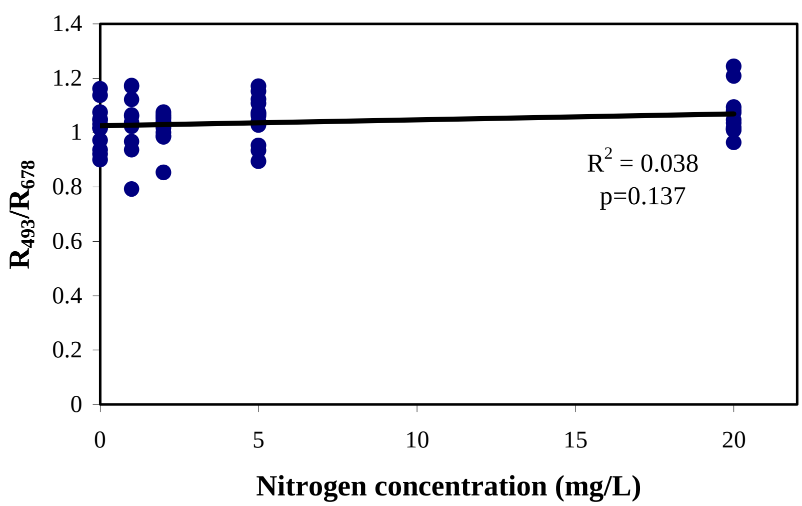


(c)

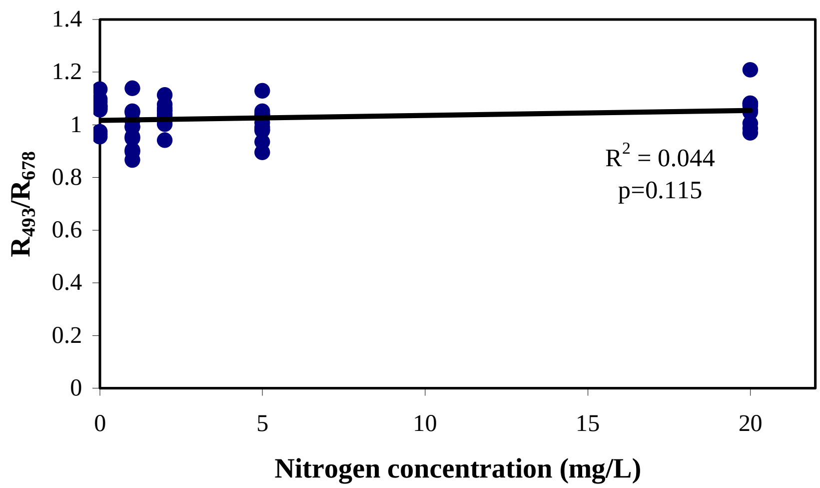
Figure 3.5. Mean reflectance of four species at the 800 nm (NIR waveband) across five fertilization levels (0, 1, 2, 5, and 20 mg-N/L) on (a) July 15, 2004 (pre-fertilization). (b) August 9, 2004 (post-fertilization), and (c) September 1, 2004 (post-fertilization). Coefficient of determination and significance of slope (p-value) included.

3.1.3 Effect of N on Reflectance Indices

Figure 3.6 shows relationship between N treatment level and the simple reflectance ratio R_{493}/R_{678} before and after fertilization.



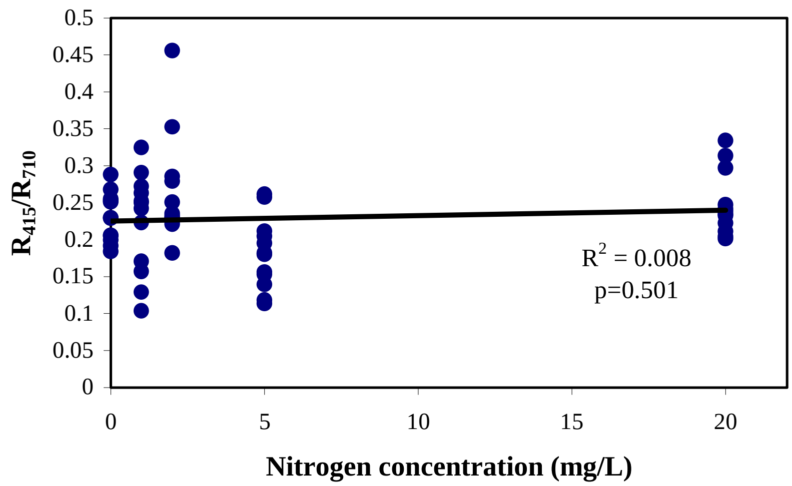
(a)



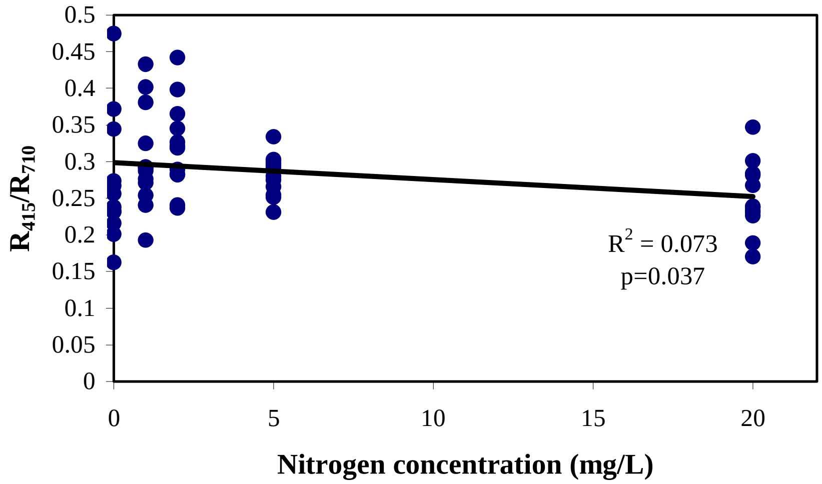
(b)

Figure 3.6. Relationship between reflectance index, R_{493}/R_{678}, and N treatment level (a) before and (b) after fertilization when all four species were combine.

Figure 3.7 shows relationship between N treatment level and the simple reflectance ratio R_{415}/R_{710} before and after fertilization. R_{415}/R_{710} exhibited a significant negative response to N availability after 49 d of N fertilization (Fig. 3.7b).



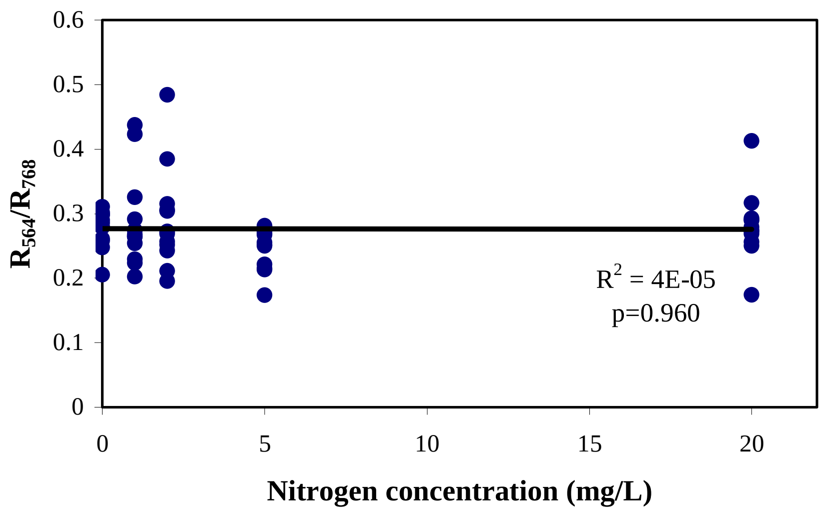
(a)



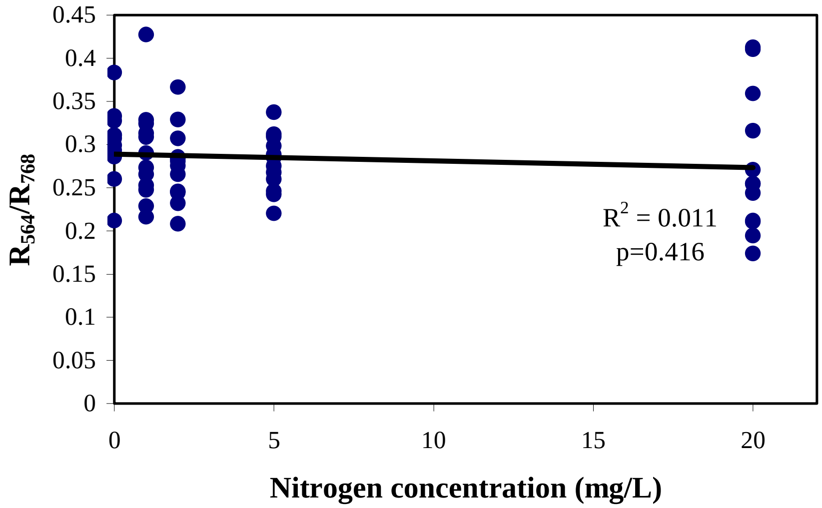
(b)

Figure 3.7. Relationship between reflectance index, R_{415}/R_{710}, and N treatment level (a) before and (b) after fertilization when all four species were combine.

Figure 3.8 shows relationship between N treatment level and the simple reflectance ratio R_{564}/R_{768} before and after fertilization. R_{564}/R_{768} did not exhibit a significant relationship with N availability after 49 d of N fertilization (Fig. 3.8b).



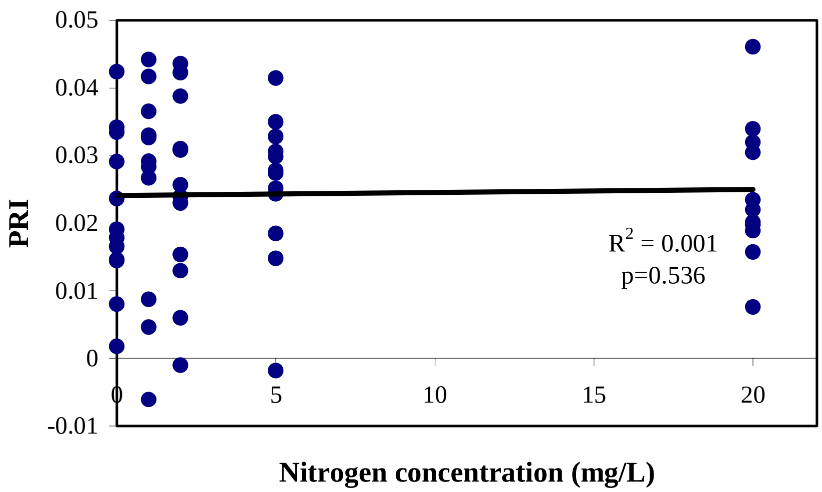
(a)



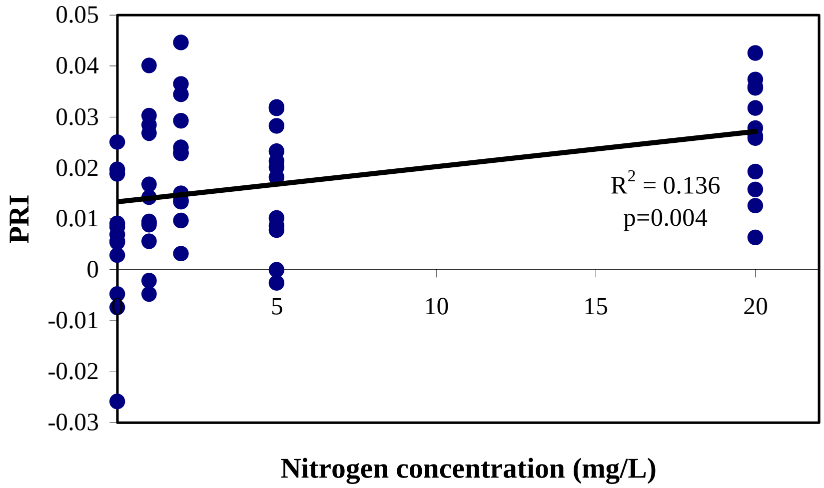
(b)

Figure 3.8. Relationship between reflectance index, R_{564}/R_{768}, and N treatment level (a) before and (b) after fertilization when all four species were combine.

Figure 3.9 shows relationship between N treatment level and the simple reflectance ratio PRI before and after fertilization. PRI exhibited a significant positive response to N availability after 49 d of N fertilization (Fig. 3.9b).



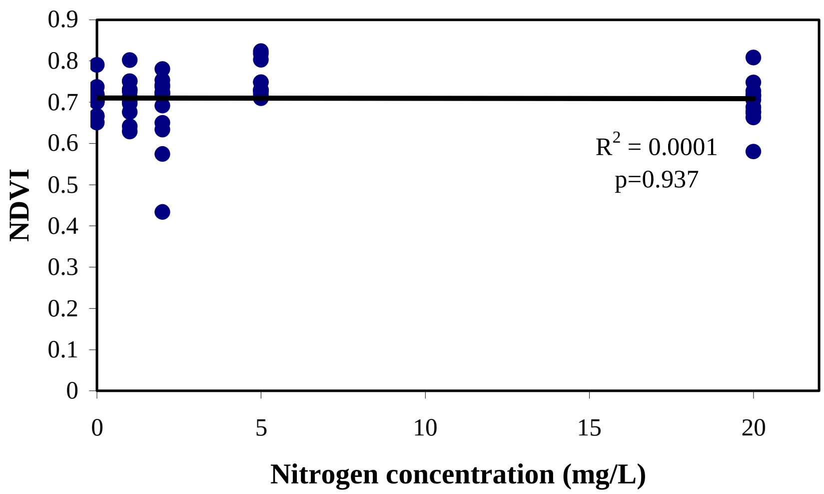
(a)



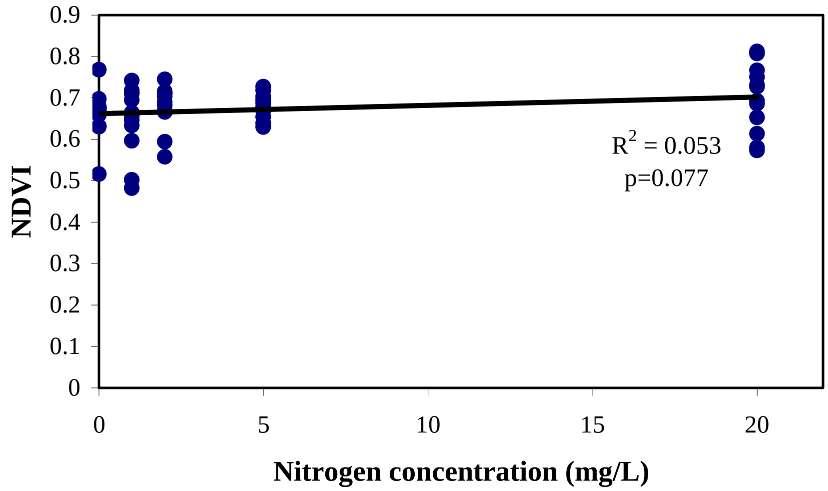
(b)

Figure 3.9. Relationship between reflectance index, PRI, and N treatment level (a) before and (b) after fertilization when all four species were combine.

Figure 3.10 shows relationship between N treatment level and the simple reflectance ratio NDVI before and after fertilization. NDVI exhibited a significant positive response to N availability after 49 d of N fertilization (Fig. 3.10b).



(a)



(b)

Figure 3.10. Relationship between reflectance index, NDVI, and N treatment level (a) before and (b) after fertilization when all four species were combine.

Table 3.1 identifies whether chosen reflectance indices were significantly affected by N treatment level according to species. Only R_{493}/R_{678} for *Typha* exhibited a significant relationship to N treatment level before fertilization. All five reflectance indices were significantly affected by N level for at least one species. Likely the sensitivity of the experiment could have been improved by a higher number of replicates for each treatment (species x N level).

Table 3.1. Relationship (R^2 (p-value)) between chosen reflectance indices to N treatment level for each of four species (ns—not significant at p=0.05).

Reflectance Index	*Acorus*	*Phragmites*	*Peltandra*	*Typha*
		Before N fertilization		
R_{493}/R_{678}	ns	ns	ns	0.32 (0.03)
R_{415}/R_{710}	ns	ns	ns	Ns
R_{564}/R_{768}	ns	ns	ns	Ns
PRI	ns	ns	ns	Ns
NDVI	ns	ns	ns	Ns
		After N fertilization (45 d)		
R_{493}/R_{678}	ns	ns	0.39 (0.01)	Ns
R_{415}/R_{710}	ns	ns	0.66 (0.00)	Ns
R_{564}/R_{768}	0.28 (0.04)	ns	ns	Ns
PRI	0.40 (0.01)	ns	ns	Ns
NDVI	0.29 (0.04)	ns	ns	Ns

Figure 3.11 highlights the differences among N treatment levels in the visible waveband after samples were fertilized for 49 d. The highest N treatment had the lowest mean visible reflectance, which supported the contention that higher N availability increases absorption of visible radiation (i.e., photosynthetically active radiation, PAR) by taking up N to increase chlorophyll concentration.

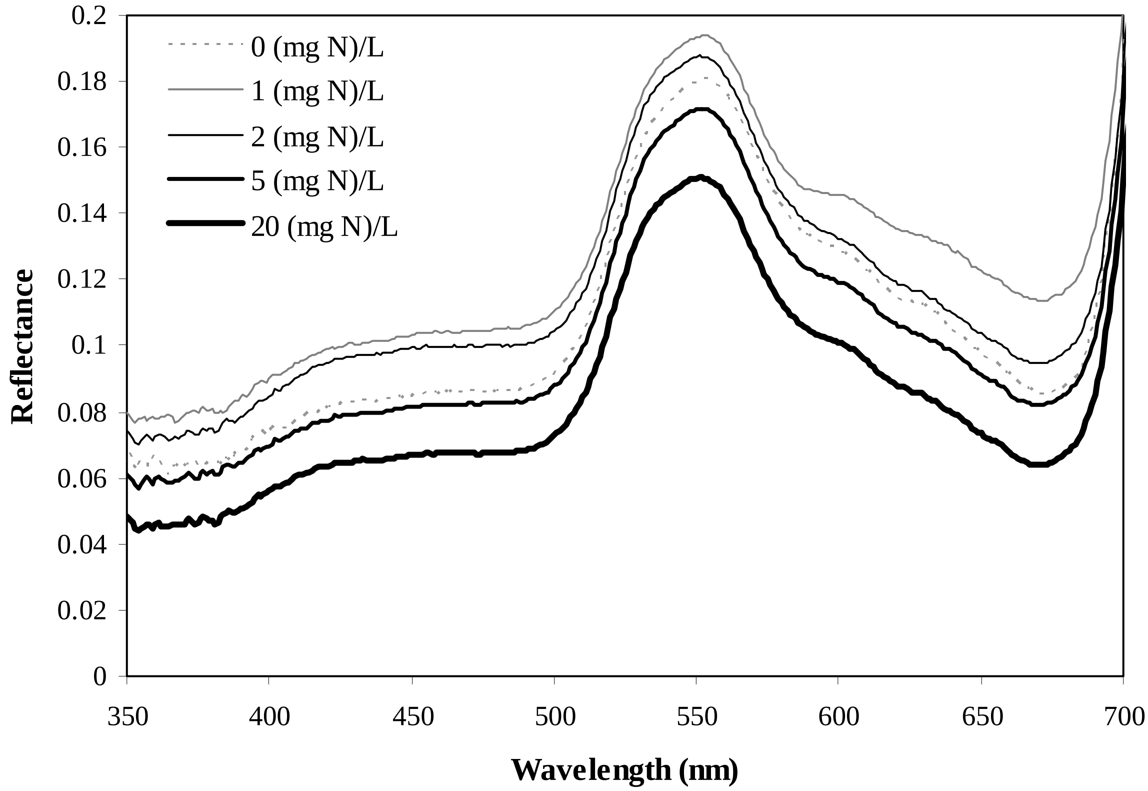


Figure 3.11. Mean visible reflectance on September 1, 2004 of four species for each nitrogen level.

3.2 Species Differences before N fertilization

Fig. 3.12 shows the mean spectral reflectance of the four species on July 15, 2004 after growing in the greenhouse for 21 d without any fertilization. Species had a significant effect on reflectance in all spectral bands except in the UV-blue waveband from 374 to 456 nm (Fig. 3.13). Multiple comparison tests of the species effect revealed that one species (*Acorus*) was responsible for all of the effect (Fig. 3.14a,b,c). The contrast of *Acorus* with *Peltandra* and *Phragmites* exhibited the highest number of significantly different spectral bands, while the *Acorus/Typha* comparison had only a few different bands (Fig. 3.14c). In the 497-658 nm waveband, *Acorus* was 2.7 to 5.5% greater than *Peltandra*, while the difference in the red-edge centered 685-712 nm waveband ranged from 2.8 to 7.1% (Fig. 3.14a). A large portion of the NIR was also greater for *Acorus* compared to *Peltandra*, averaging around 12% (Fig. 3.14a). The significant difference in spectral response between *Acorus* and *Phragmites* covered the entire portion of the spectrum from 492 nm to 1075 nm (Fig. 3.14b). *Acorus* reflected around 15% more in the NIR, 3.1% more in the red, 5.3% more in the green and 2.7% more in the blue than *Phragmites*. Pairwise comparisons between *Peltandra*, *Phragmites*, and *Typha* exhibited no significantly different spectral bands (Fig. 3.14d,e,f).

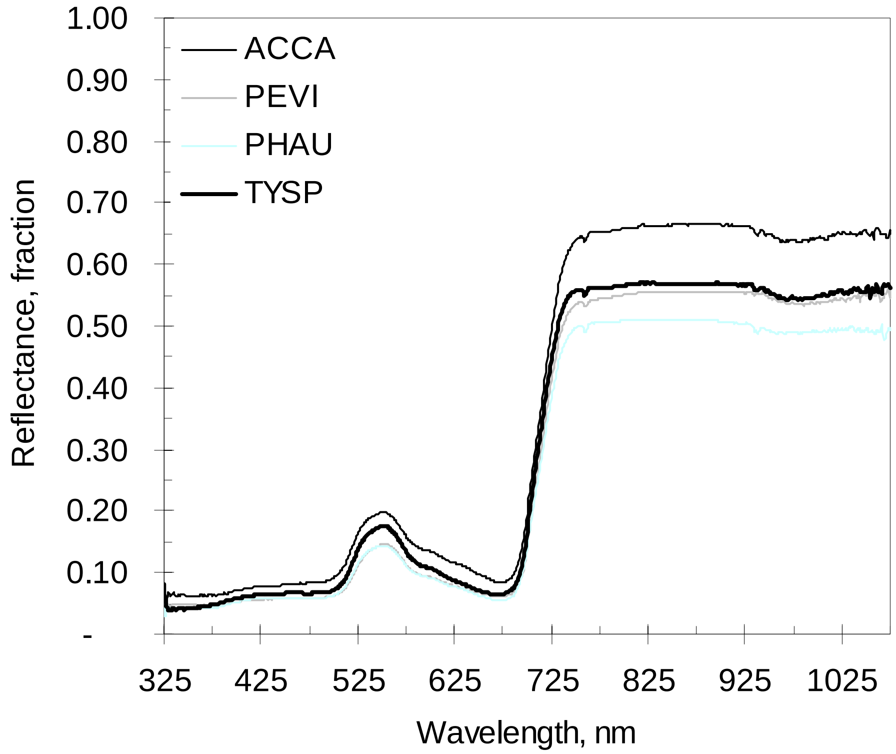


Figure 3.12. Mean reflectance of four emergent freshwater macrophytes prior to N-fertilization (7/15/04). n=15 per species.

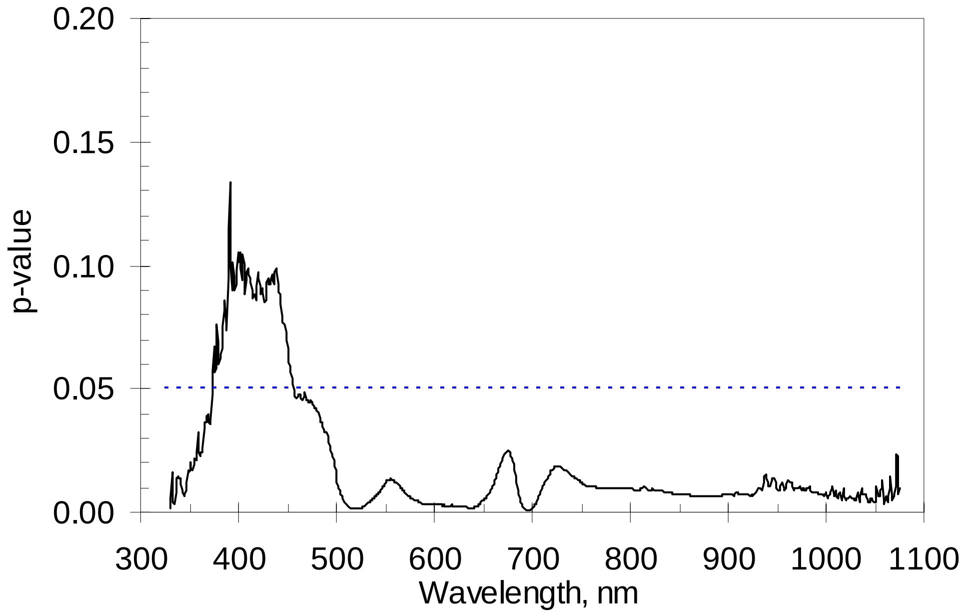
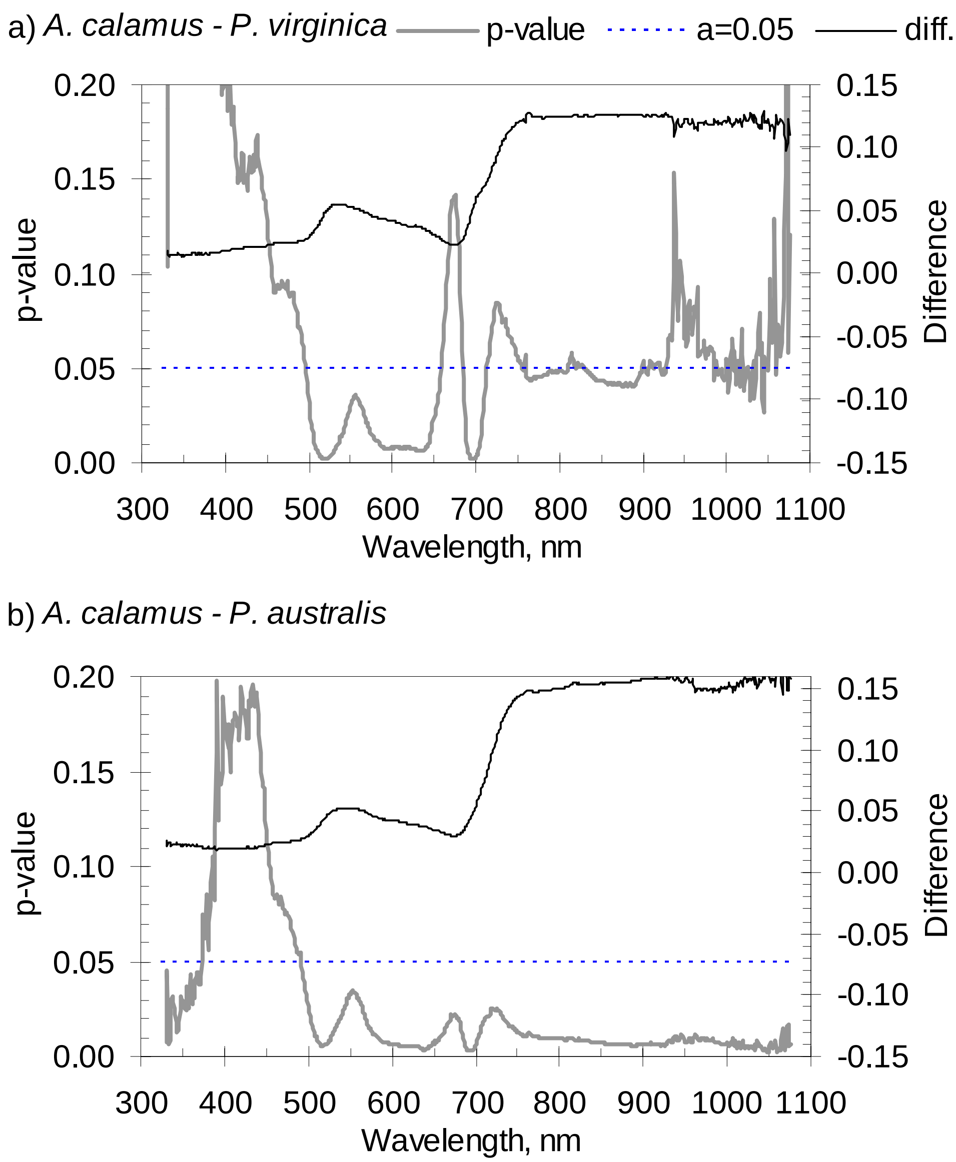
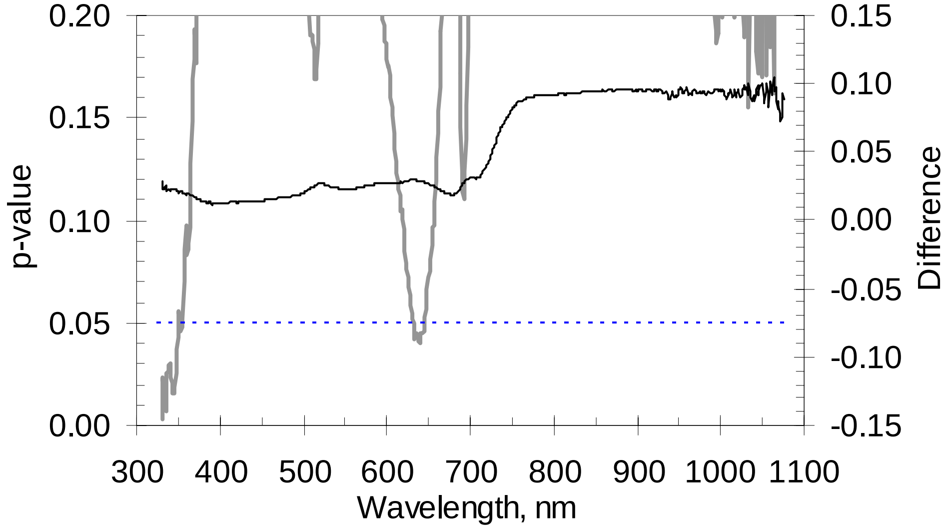


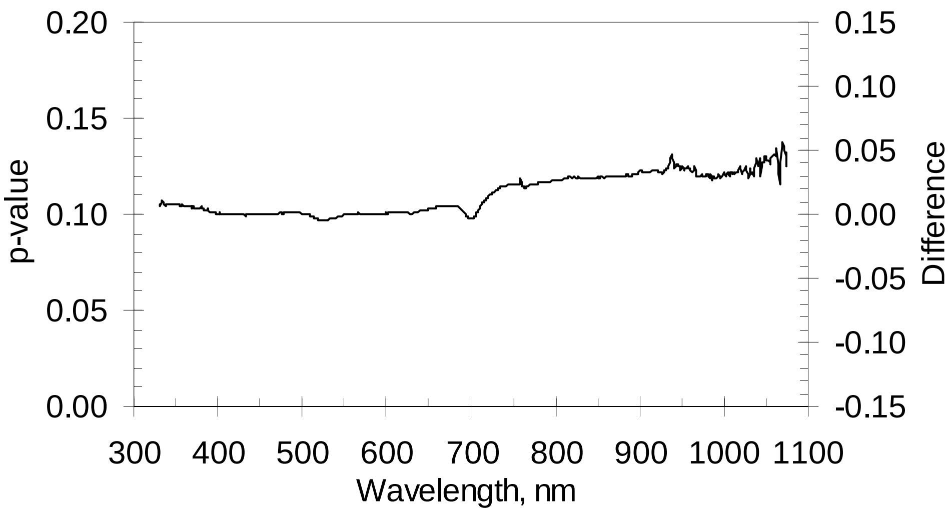
Figure 3.13. Significance of effect of species on spectral reflectance of four wetland species prior to fertilization (7/15/04). Species as fixed factor ANOVA


a) A. calamus - P. virginica
p-value
a=0.05
diff.
p-value
Difference
0.20
0.15
0.10
0.05
0.00
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
300
400
500
600
700
800
900
1000
1100
Wavelength, nm
b) A. calamus - P. australis
p-value
Difference
0.20
0.15
0.10
0.05
0.00
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
300
400
500
600
700
800
900
1000
1100
Wavelength, nm

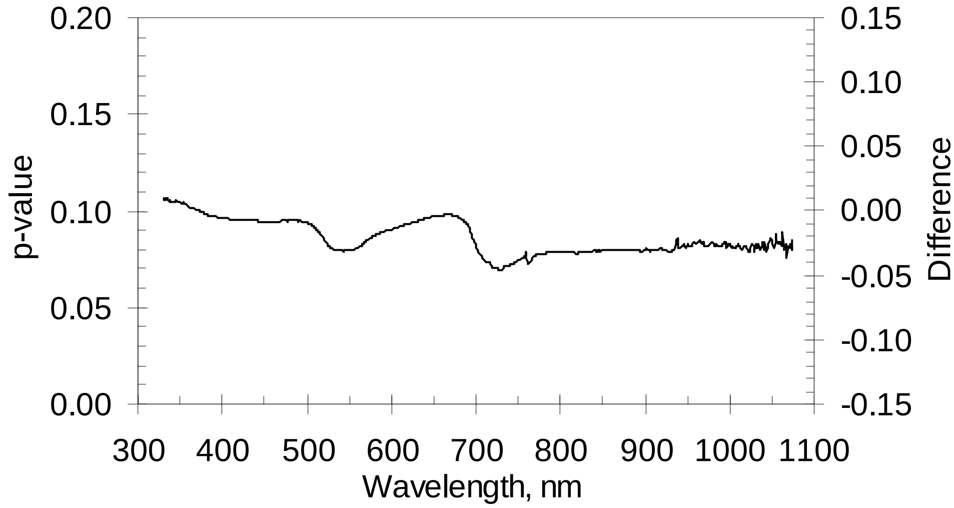
c) *A. calamus - Typha* spp.



d) *P. virginica - P. australis*



e) *P. virginica - Typha* spp.



f) *P. australis - Typha* spp.

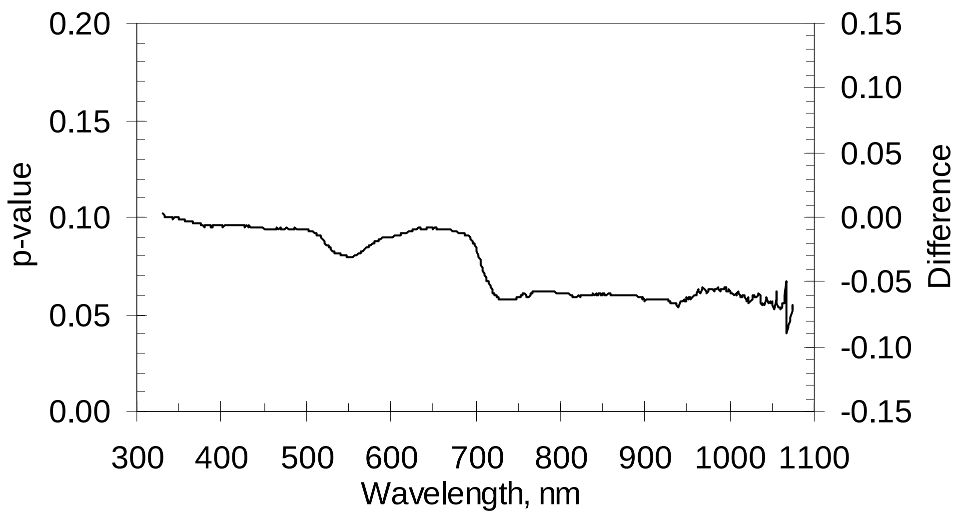
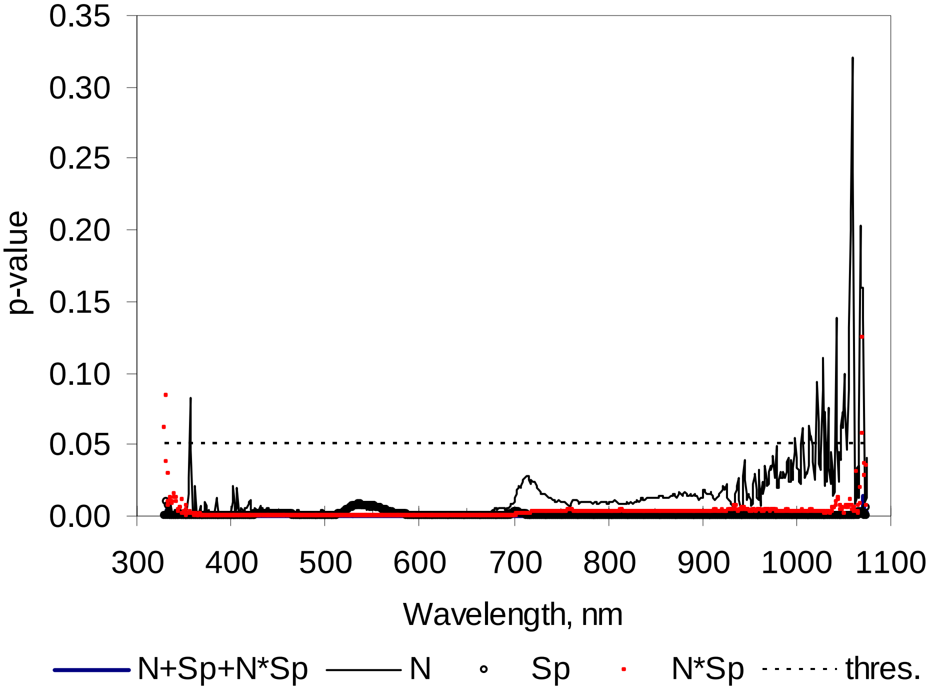


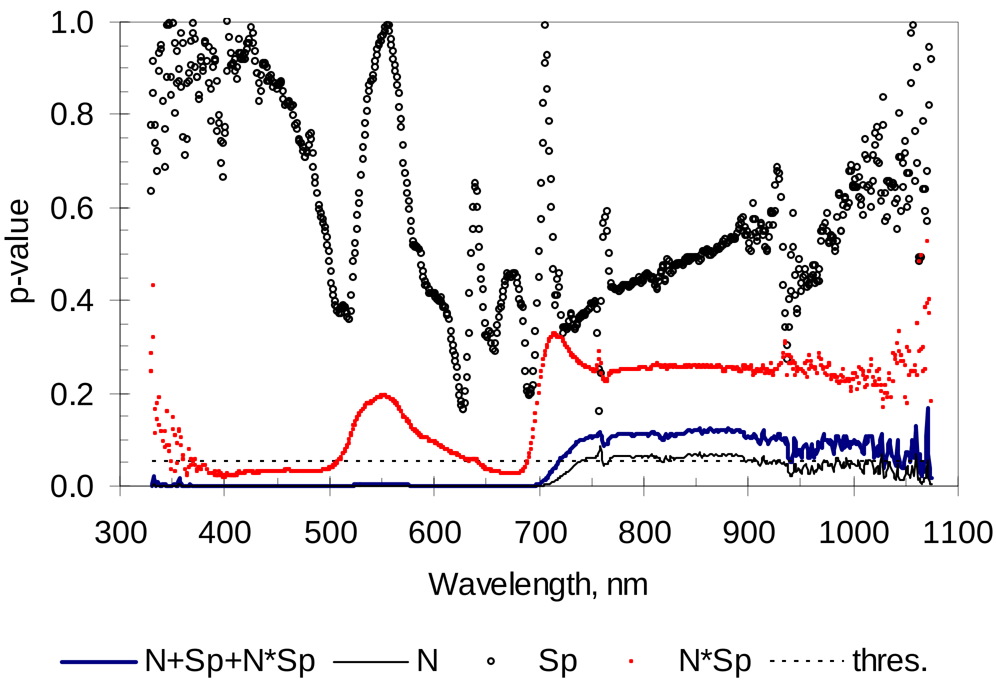
Figure 3.14. Mean difference in spectral reflectance for each two-way species comparison and its significance as estimated with Bonferroni's multiple comparison test for the four wetland species prior to fertilization (7/15/04).

3.3 Effects of N and species on reflectance post-fertilization

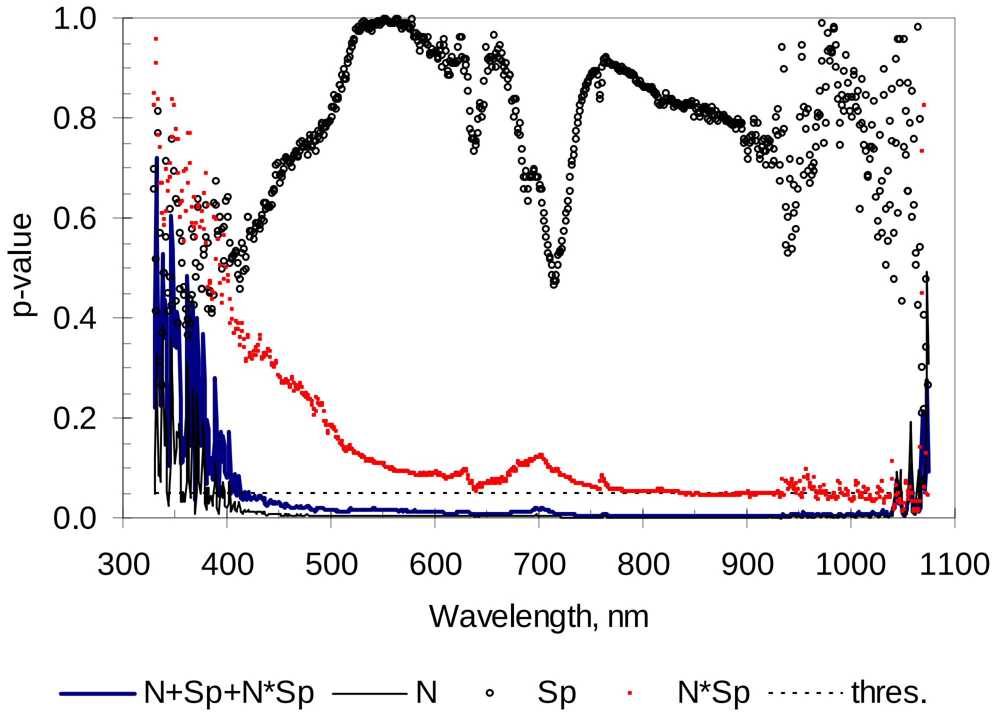
Fig. 3.15a shows that nitrogen, species, and their interaction had a significant effect on reflectance across the spectrum 49 d after initiating fertilization. The only exceptions were the effect of N on several NIR bands greater than 1000 nm and the 357 nm UV band, and the interaction effect at the extreme ends of the spectrum. Grouping *Acorus* and *Peltandra* together and *Phragmites* and *Typha* together reduced much of the effect of species and the nitrogen x species interaction (Fig. 3.15b,c). For the *Acorus+Peltandra* group, the main effect of species was non-existent with no spectral bands exhibiting a significant effect (Fig. 3.15b). The effect of the nitrogen x species interaction was reduced greatly with only the blue (400-500 nm) and a portion of red (650-680 nm) wavebands significantly affected (Fig. 3.15b). Nearly all spectral bands showed a significant response to N in the *Acorus+Peltandra* group with a less distinct response in the NIR. Similarly, the main effect of species for the *Phragmites+Typha* group was removed when the two species were analyzed separately and the nitrogen x species interaction was restricted to the NIR wavebands (Fig. 3.15c). All spectral bands between 400 and 1050 nm were affected by N treatments for the *Phragmites+Typha* group.



(a)



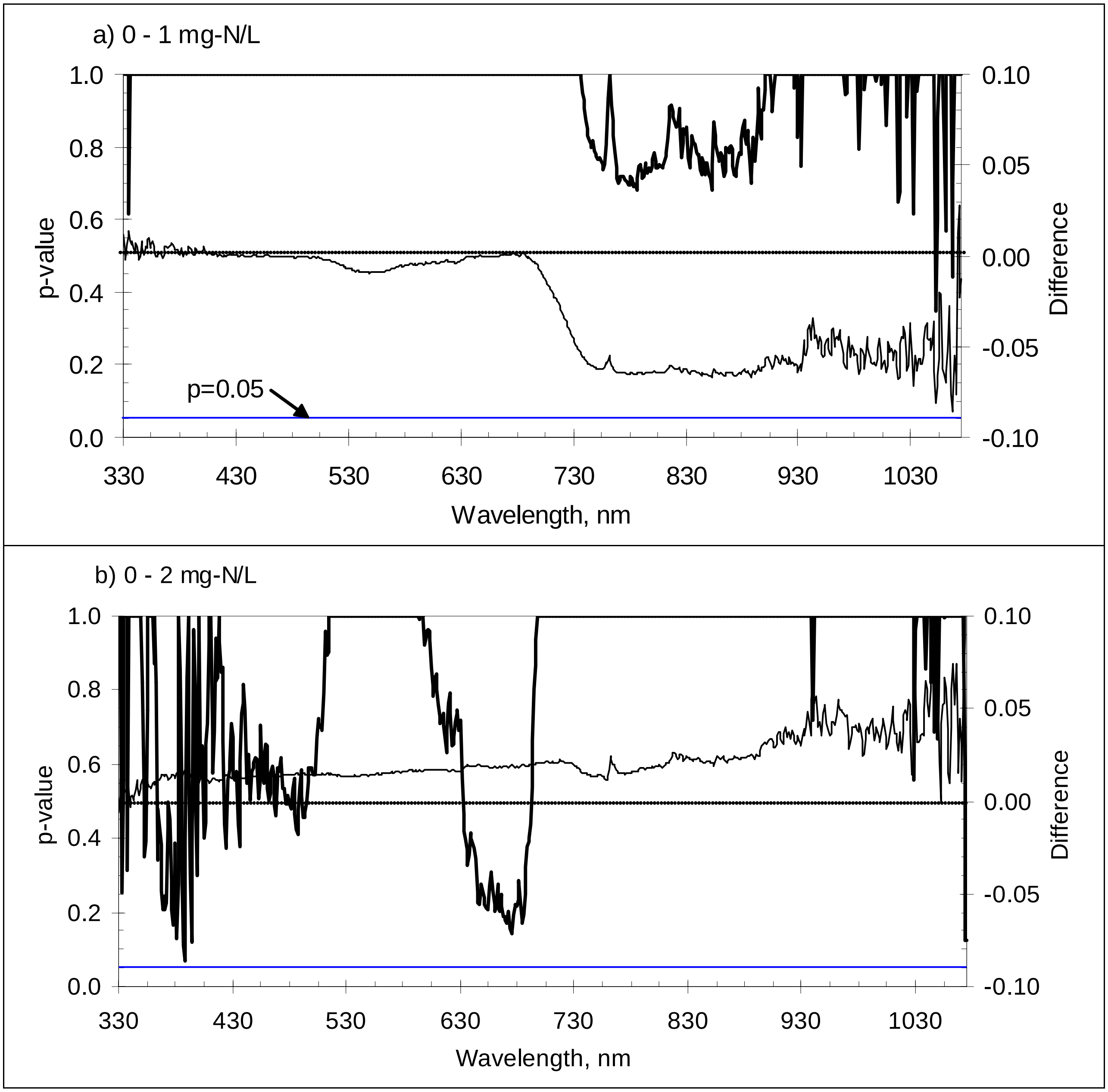
(b)

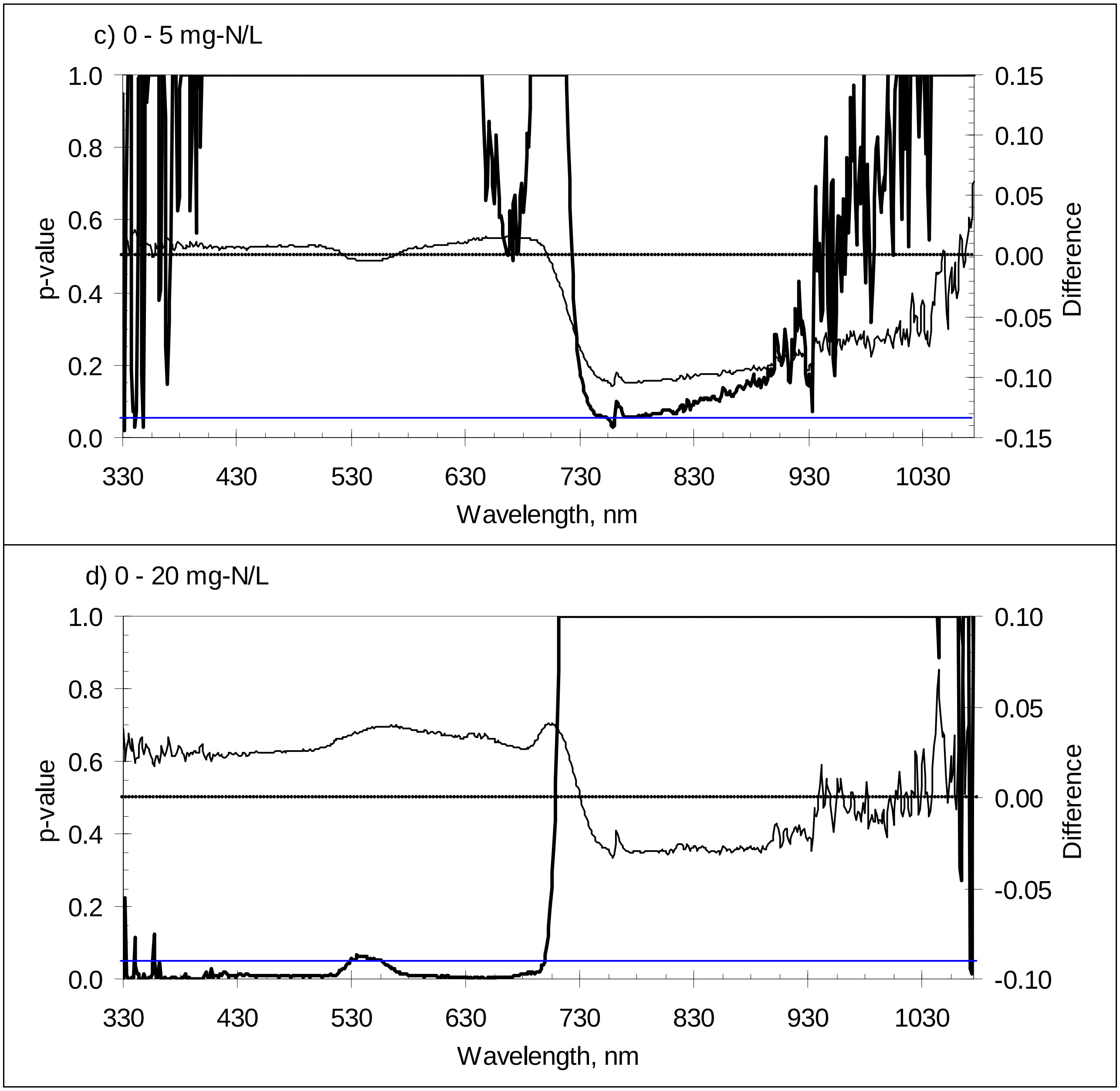


(c)

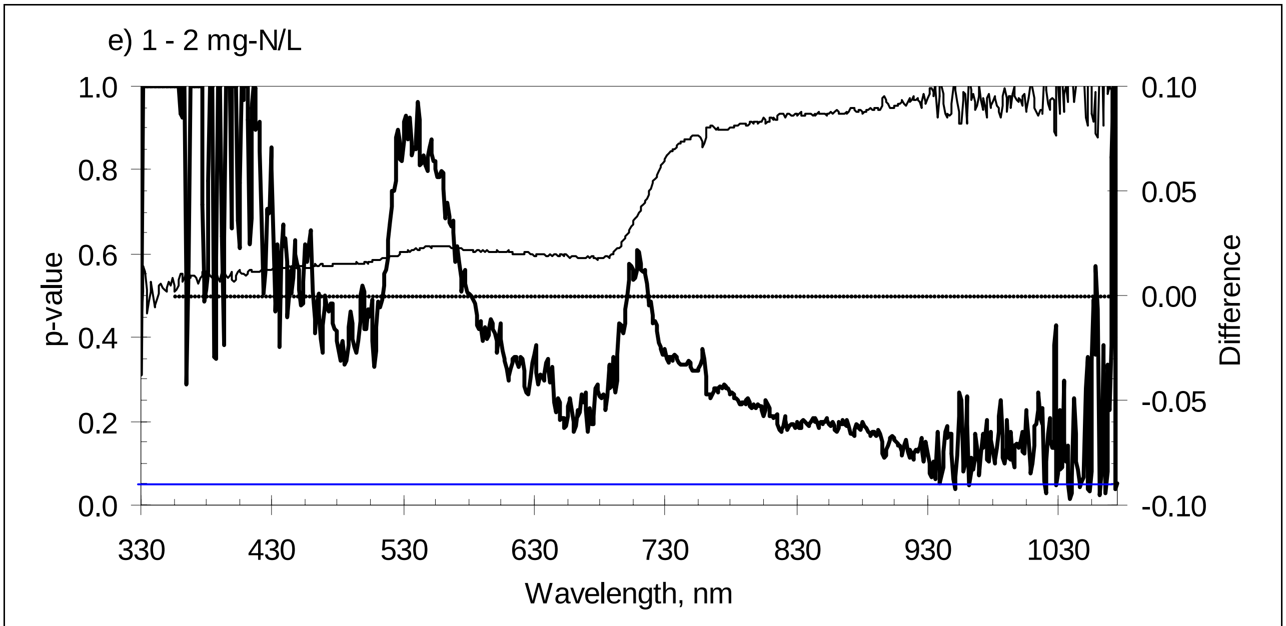
Figure 3.15. Significance of effects of nitrogen (N), species (Sp) and their interaction on reflectance 49 d after initiation of N fertilization for (a) all 4 species, (b) Acorus and Peltandra, and (c) Phragmites and Typha. (n=15 per species, except Typha n=14; thres = threshold of significance at p=0.05).

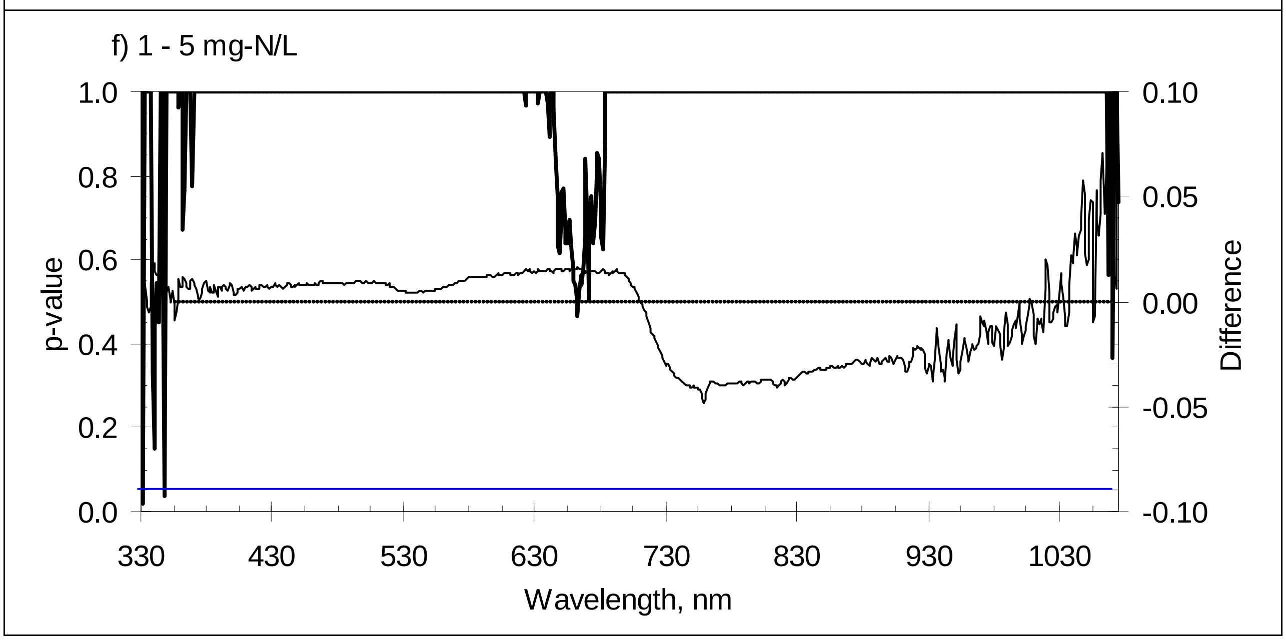
Fig. 3.16 shows the difference in spectrum reflectance between pair-wise nitrogen treatments for each 1 nm spectral band and the significance of the difference (Bonferroni's test). There were no significant differences across the spectrum for the 0N/1N comparison (Fig. 3.16a), but the mean NIR reflectance of the higher N treatment was 5 to 6% greater. Similarly, there was no significant difference between the 0N and 2N treatments (Fig. 3.16b), but there were faint indications of differences in the chlorophyll bands (400-460 and 610-680 nm) beginning to arise. The most significant difference was observed at 388 nm for the 0N/2N comparison. The contrast between 0N and 5N revealed a significant difference in the NIR at 756-760 waveband, but no difference in VIS wavebands (Fig. 3.16c). The UV bands at 341 and 348 nm were significantly different for the 0N/5N comparison, while the most likely different VIS bands were located around 671 nm, which is in the chl bands. All VIS bands, except for the green 533-556 nm waveband, reflected less for the 20N treatment compared to the 0N control, but there was no difference in NIR (Fig. 3.16d). The contrast between 1N and 2N treatments revealed significant differences only in a few NIR bands (951, 1020, 1038-40, 1046-47, 1061-62, 1066, 1074 nm) (Fig. 3.16e). The most likely VIS bands to be different were centered about 665 nm (p=0.17) and 487 nm (p=0.34), which are either in or adjacent to chl sensitive bands. There was no difference between the 1N and 5N treatment across the spectrum, except for 2 UV bands (332 and 348 nm, Fig. 3.16f), but the most likely different VIS band was centered about 663 nm (p=0.46). VIS bands for the 20N treatment reflected less than the 1N treatment (Fig. 3.16g). The majority of the UV bands (51 out of 70) also reflected less for the 20N treatment compared to the 1N, but the only NIR band to exhibit a difference was 1062-66 nm (Fig. 3.16g).

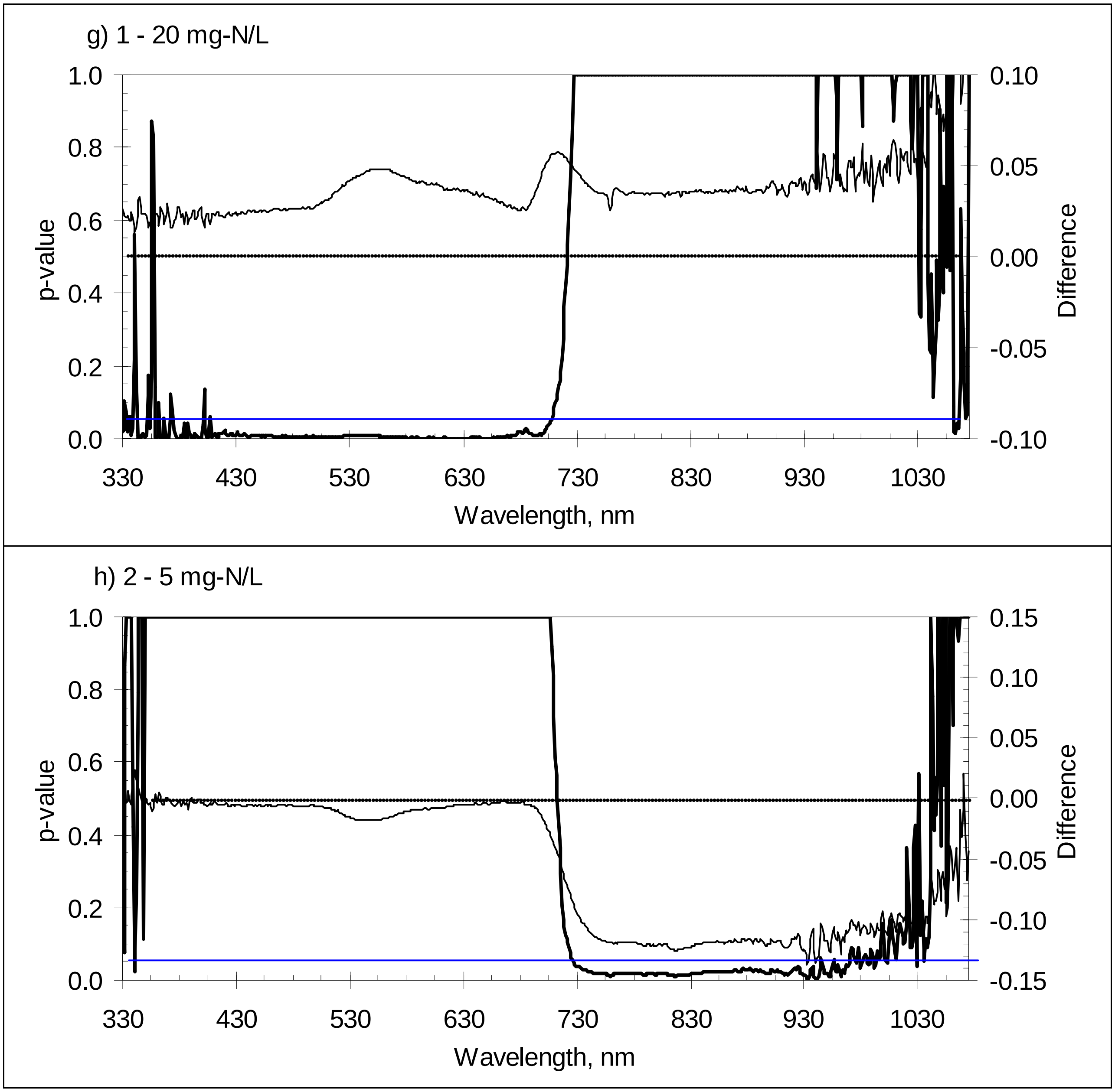

a) 0 - 1 mg-N/L
p-value
Difference
p=0.05
Wavelength, nm
b) 0 - 2 mg-N/L
p-value
Difference
Wavelength, nm



c) 0 - 5 mg-N/L
1.0
0.8
0.6
0.4
0.2
0.0
p-value
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
Difference
330
430
530
630
730
830
930
1030
Wavelength, nm
d) 0 - 20 mg-N/L
1.0
0.8
0.6
0.4
0.2
0.0
p-value
0.10
0.05
0.00
-0.05
-0.10
Difference
330
430
530
630
730
830
930
1030
Wavelength, nm


e) 1 - 2 mg-N/L
1.0
0.8
0.6
0.4
0.2
0.0
p-value
0.10
0.05
0.00
-0.05
-0.10
Difference
330
430
530
630
730
830
930
1030
Wavelength, nm


f) 1 - 5 mg-N/L
1.0
0.8
0.6
0.4
0.2
0.0
p-value
0.10
0.05
0.00
-0.05
-0.10
Difference
330
430
530
630
730
830
930
1030
Wavelength, nm


g) 1 - 20 mg-N/L
p-value
Difference
Wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
0.10
0.05
0.00
-0.05
-0.10
330
430
530
630
730
830
930
1030
h) 2 - 5 mg-N/L
p-value
Difference
Wavelength, nm
1.0
0.8
0.6
0.4
0.2
0.0
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15

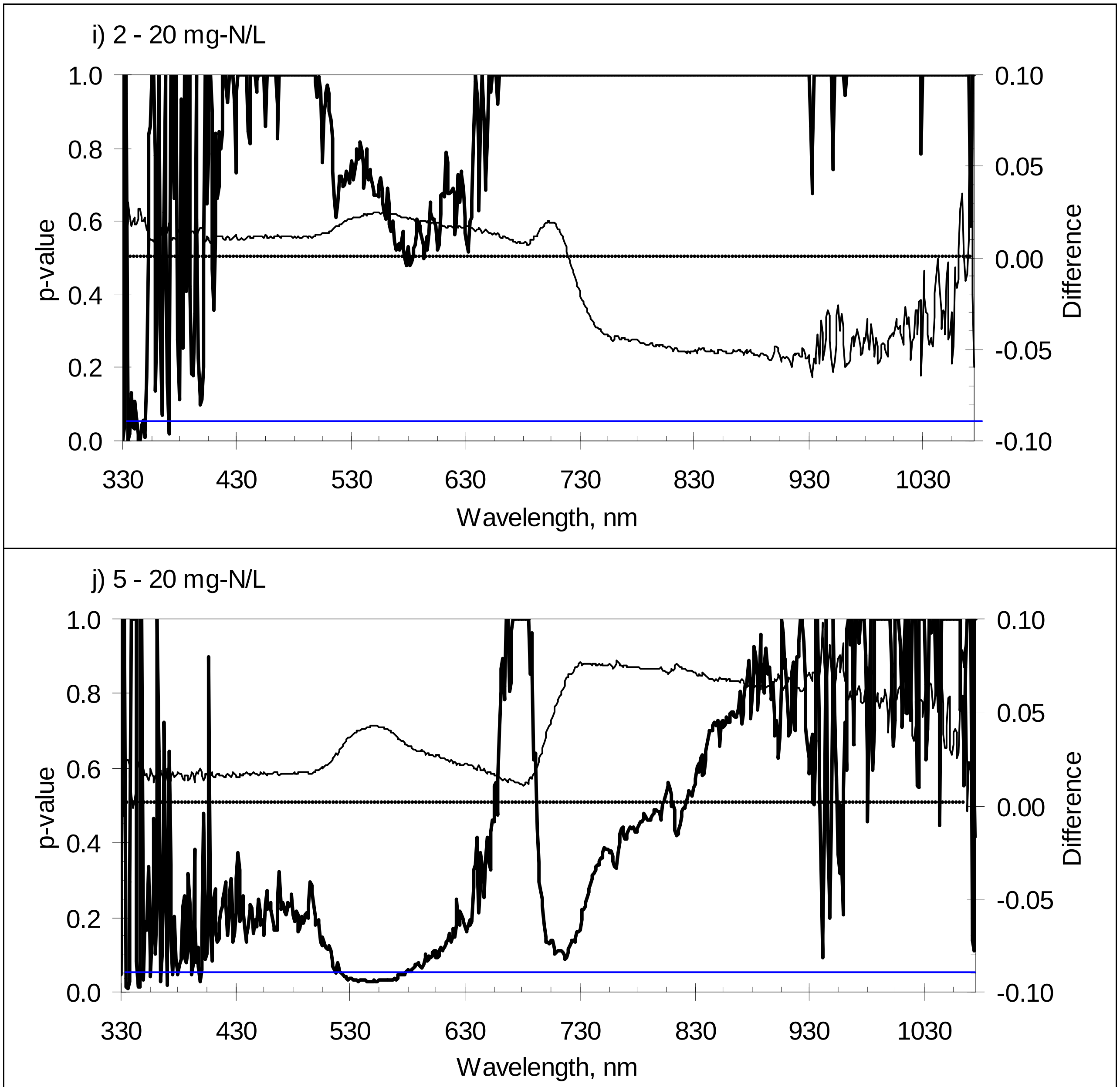


Figure 3.16. Detection of differences among pairwise contrasts of five N treatments using Bonferroni's multiple comparison test in a mixed effects two-factor ANOVA (main effects of species and N) for reflectance measured 35 d after starting fertilization. (a) 0N – 1N contrast, (b) 0N – 2N contrast, (c) 0N – 5 N, (d) 0N – 20N, (e) 1N – 2N, (f) 1N – 5N, (g) 1N –20N, (h) 2N – 5N, (i) 2N – 20N, and (j) 5N – 20N.

In general, plants supplied more N reflected less VIS, but left NIR unaffected (Fig. 3.16). A few NIR bands centered around 780 nm were different for the 0N/5N contrast (Fig. 3.16c) and a majority of NIR bands for the 2N/5N contrast were significantly affected by N (Fig. 3.16h). The VIS difference between the most extreme N treatments (0N and 20N, Fig. 3.16d) ranged from 2 to 4%. VIS differences between the lowest N level (0N) and the 1N, 2N and 5N treatment levels were not significant, but the spectral bands found most significantly different were centered around 670-680 nm (Fig. 3.16b,c).

The 1N level had no spectral bands different from either the 2N or 5N treatments (Fig. 3.16e,f), but reflected significantly more VIS than the highest N treatment (20N) (Fig. 3.16g).

Spectral bands that exhibited significant differences between the highest N treatment (20N) and the next highest (5N) were in the green waveband (530-580 nm) (Fig. 3.16j). A few spectral bands were moderately significantly different in the RE region (718 nm) (Fig. 3.16j).

Unexpectedly, the 10% difference in NIR reflectance (730-970 nm) between the 2N and 5N treatments was significant (Fig. 3.16h).

Fig. 3.17 shows that after N fertilization the reflectance response of the species formed two distinct groups (*Acorus+Peltandra* and *Phragmites+Typha*). That is, *Acorus* had no spectral bands different from *Peltandra* (Fig. 3.17a) and *Phragmites* had no bands different from *Typha* (Fig. 3.17f). However, nearly all of *Acorus*' spectrum exhibited more reflectance than *Phragmites* (Fig. 3.17b) and *Typha* (Fig. 3.17c). *Peltandra*'s reflectance spectrum was also less than that of *Phragmites* (Fig. 3.17d) and *Typha* (Fig. 3.17e), except for small wavebands centered about 530 nm and 700 nm. Notably the significant differences spanned the entire spectrum, including UV, VIS and NIR bands.

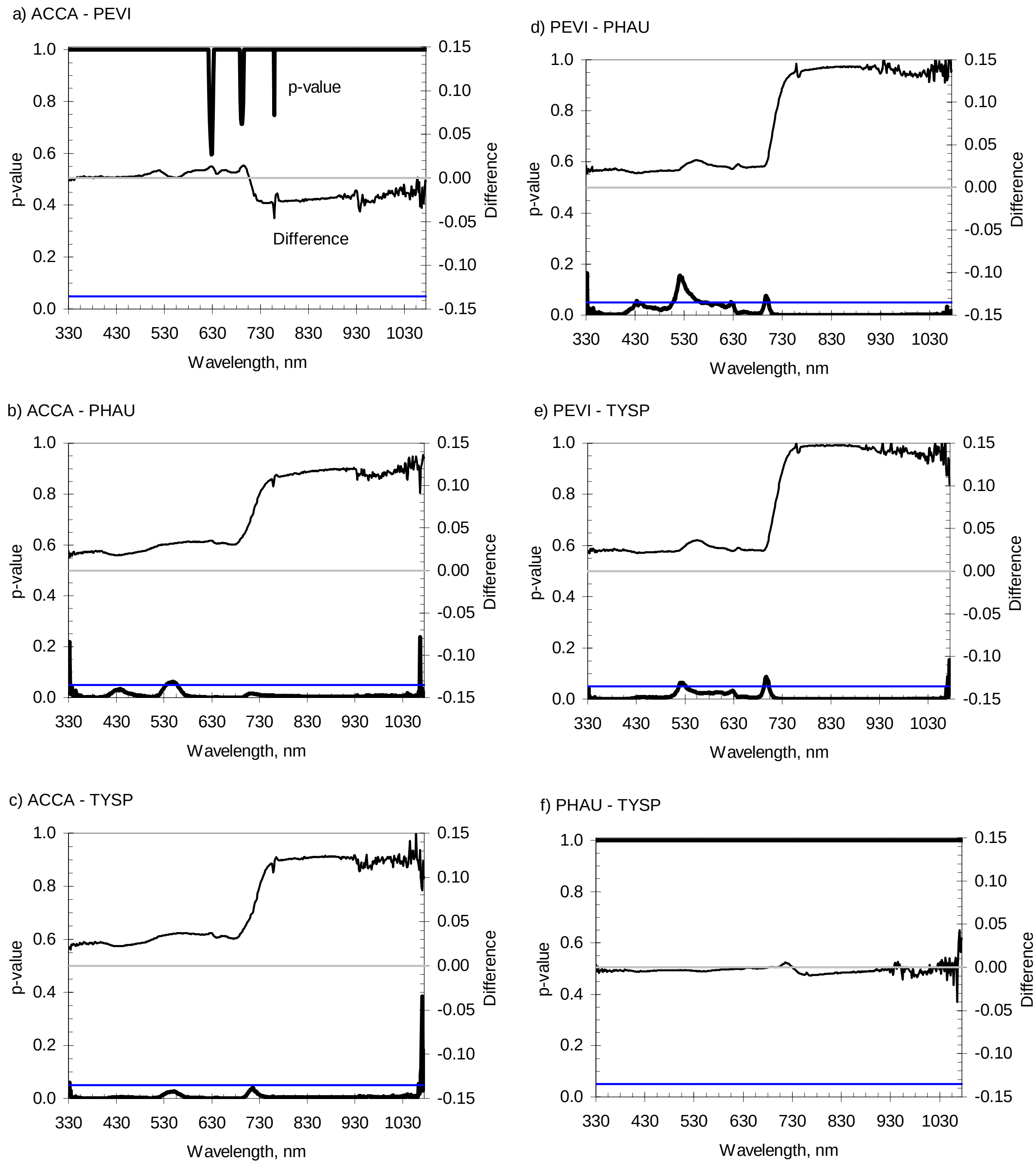


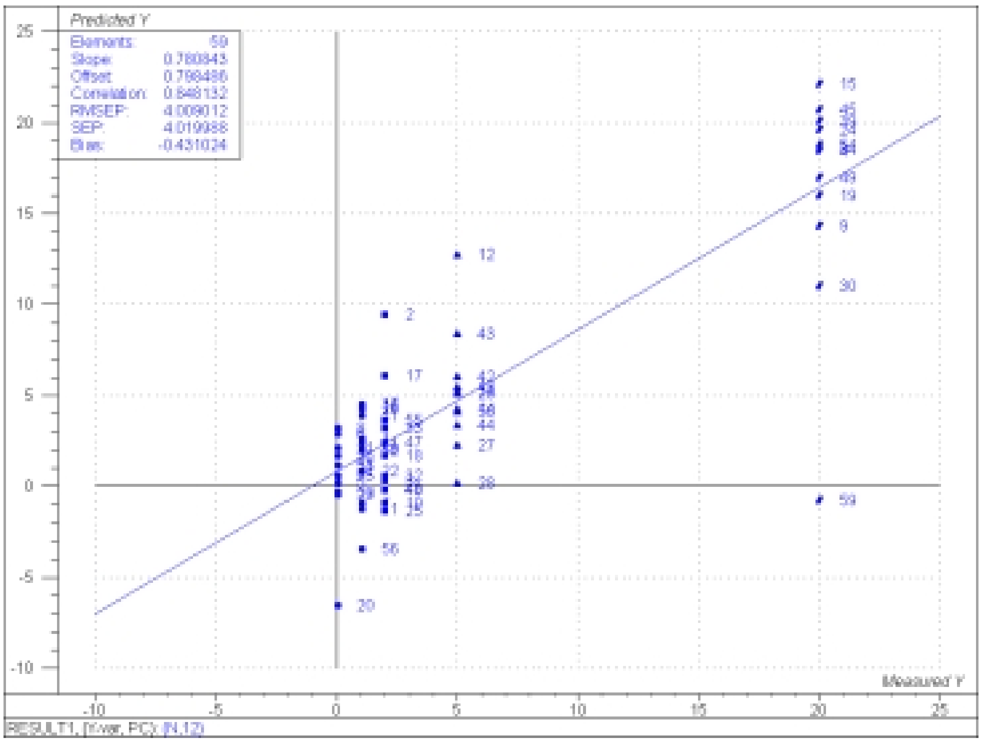
Figure 3.17. Significance of mean difference in spectral reflectance between species after nitrogen fertilization based on mixed effects two-factor ANOVA (main effects of species, nitrogen) and Bonferroni's test with n=15 for each species except Typha with n=14. (ACCA-Acorus, PEVI-Peltandra, TYSP-Typha, PHAU-Phragmites)

Phragmites and *Typha* are generally considered invasive wetland plants that tend to dominate vegetative composition in high nutrient or disturbed marshes. Their reflectance patterns, which were distinctly similar (Fig. 3.17f), showed that they reflected 2.5 to 4% less VIS irradiance and from 10 to 15% less in the NIR than the two non-invasive wetland species (*Acorus* and *Peltandra*). By reflecting less solar radiation after fertilization, *Phragmites* and

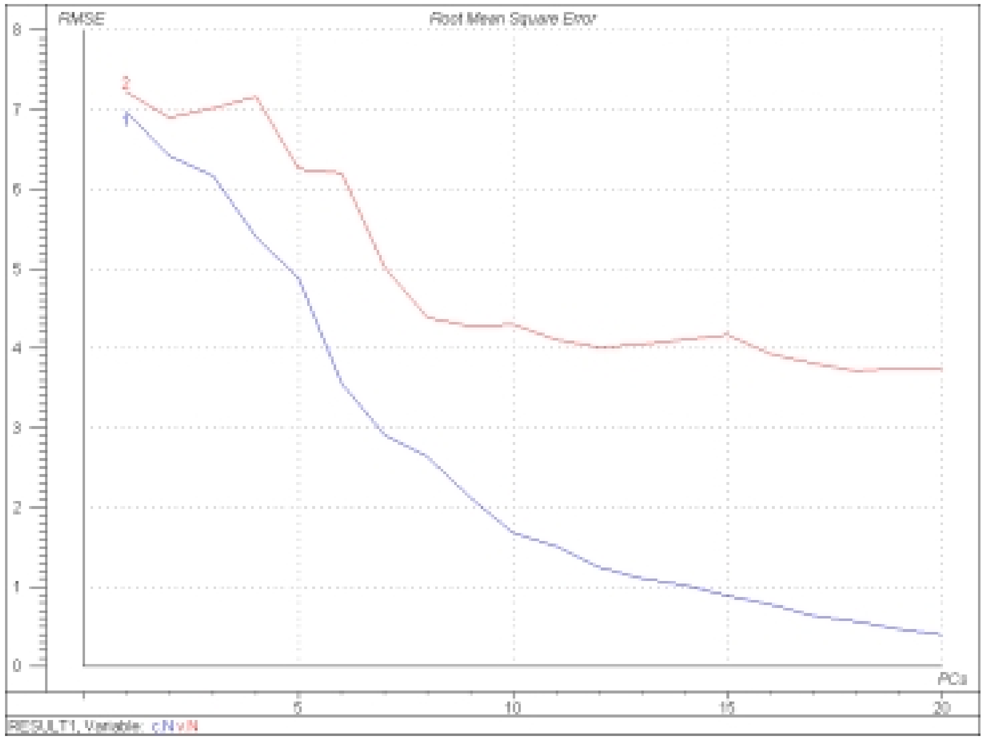
Typha are exhibiting a greater ability to capture and, presumably, transform energy, which likely contributes greatly to their superior ability to compete against other species under high N availability.

3.4 Partial Least Squares regression model of N Availability

Fig. 3.18a shows ability of PLS regression model to predict N treatment level. Correlation between prediction and observation was 0.848. Fig. 3.18b compares the RMSE of prediction (i.e., validation) and calibration as a function of the number of latent variables (i.e., PLS-components) included. RMSEC decreases towards 0.0, while RMSEP exhibited a local minimum at 12 PLS-components, which was the number of components included for the model shown in Fig. 3.18a.



(a)



(b)

Figure 3.18. Predictive vs. observed N treatment level based on PLS of difference in reflectance between before and after fertilization for 59 samples (1 sample was lost) using full cross validation to select 12 PLS-components (a) and RMSE of prediction (line 2) and calibration (line 1) as a function of PLS components.

4 Ancillary Project Benefits

Our proposed research project supported the program objectives of the MWRRC by (1) exploring new ideas in wetland remote sensing and wetland water quality monitoring, (2) fostering the research of a new scientists (Tilley is an assistant professor), (3) training graduate and undergraduate students in the new technology and (4) exposing the public to wetland radiometry. Our research developed information helpful toward protecting and enhancing the water quality and habitat of natural marshes, especially marshes of the Chesapeake Bay watershed, Mid-Atlantic region and the nation.

A benefit of developing the reflectance models was to form a solid foundation for scaling the radiometric technique to canopy scale applications which can eventually lead to aerial or satellite remote sensing specifically focused on cost-effective monitoring and assessment of emergent marsh N availability. Use of a hypespectral wetland radiometry for assessing elevated N concentrations in emergent marshes may provide a valuable tool for detecting non-point source (NPS) pollution. Because the position of wetlands in the landscape is often between farms and rivers, wetlands frequently receive surface runoff and groundwater discharge containing excess nitrogen from agricultural operations. Remote sensing would allow rapid screening of large areas of wetlands, potentially identifying “hot-spots” where wetlands are in a eutrophic state due to agricultural runoff. Identifying these major sources of NPS, which has traditionally been very difficult, would allow for directed application of environmental management techniques to reduce nitrogen in runoff and groundwater. This capability will benefit society by improving science-based management of agricultural operations to reduce impacts on coastal resources. Locally, this is important since agriculture has been implicated as the predominant cause of eutrophication in the Chesapeake Bay (Jaworski et al. 1992; Chesapeake Bay Program 1995).

By 2012 States will be required by the U.S. Environmental Protection Agency to report on the water quality conditions and ecological health of wetlands. Thus, development of the proposed sensor technology is timely and will have a significant impact on state monitoring strategies and capabilities.

MWRRC grant funds supported one Master's graduate research assistant (GRA) and partially-funded one undergraduate assistant. In addition, students enrolled in our Wetlands Ecology (ENBE 450) and Restoration Ecology (NRMT 489F) courses were given demonstrations of the technology. The public was introduced to the technology at the University's annual Maryland Day event in April 2005, which resulted in about 100 people seeing the technology. Thus, a total of about 25 college students were introduced to the technology. We are planning to demonstrate the radiometer system to state environmental managers this summer. We have received additional funding from the Washington, D.C. chapter of The Nature Conservancy and from the University of Maryland Agricultural Experiment Station.

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