Do seasonal profiles of foliar pigments improve species discrimination of evergreen coastal tree species in KwaZulu-Natal, South Africa?

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Abstract. Studies in the Northern hemisphere have shown the potential of foliar pigment seasonal profiles as a means of improving species discrimination. Remote sensing vegetation indices have been used to optimise absorption features presented by foliar pigments, as well as improve species discrimination. This study investigated the potential of seasonal pigment profiles (for foliar carotenoid and total chlorophyll) in improving species discrimination for trees using leaf spectral data. Our aims were to (i) determine whether species have unique seasonal profiles of carotenoids and chlorophyll; and (ii) whether these seasonal profiles can be used to improve species discrimination, compared to single season pigment concentrations. We sampled sunlit leaves of seven evergreen tree species in a sub-tropical region of South Africa, over four seasons during 2011-12. Parametric ANOVA classification was compared to similarity measures of shape (spectral angle mapper; SAM) and magnitude (sum of Euclidean Distance; ED). For both pigments, the parametric analysis of combined seasonal content improved species discrimination when compared to single season content and the similarity measures. ED outperformed SAM in species discrimination for both pigments. Multi-seasonal carotenoid and chlorophyll content information improved species discrimination of evergreen coastal tree species in KwaZulu-Natal, South Africa.

1. Introduction

Phytosynthetic pigments (carotenoids and chlorophylls) respond to environmental and climate conditions and hence reflect corresponding phenological changes in vegetation [1][2][3]. Deciduous species show an increase in carotenoid and chlorophyll concentration at the onset of spring, and then decline towards leaf fall [4][5]. A study focused on oak tree (evergreen) in Portugal showed an increase in carotenoids such as the xanthophyll cycle components, violaxanthin, antheraxanthin and zeaxanthin in spring [1], whereas other coniferous evergreen species in Canada showed peak carotenoid content during winter [5]. Chlorophyll concentration and content has been observed to increase in winter and spring times, with peaks in summer and a decline in autumn [2][5]. Contradictions and exceptions have however been reported for both pigments [6]. Regardless of similar foliar pigment phenological patterns, a number of studies have indicated that foliar profiles of pigments may be seasonally unique to species [2][7], as well as over a number of years [8]. The significance of differences in seasonal profiles of carotenoids and chlorophyll has yet to be tested for evergreen tree species, and whether or not the trends noted in the northern hemisphere prevail in the southern hemisphere too.

Foliar pigments have distinct absorption features in the visible range of the electromagnetic spectrum between 400 – 700 nm [9]. Vegetation indices have therefore been developed in order to quantify foliar pigment content in leaves as observed in the leaf reflectance [10]. A number of studies investigated the ability to discriminate species using spectral features that use foliar pigments over a number of seasons [11][12][13][14]. Most species were found to be spectrally unique when using a number of season of vegetation indices for these pigments [14][15][16], there were however exceptions at the genus level [17].

While a number of studies have investigated seasonal changes in foliar pigments, few have focused on the seasonal profile characterization over four seasons and determined the uniqueness of these profiles [11][12][13][14]. There is a need to understand how species exhibit unique seasonal profiles in their carotenoid and chlorophyll contents, and whether or not these can be used in remote sensing to improve species discrimination and mapping. Understanding the seasonal profiles of carotenoid and chlorophyll contents for each species can contribute to the choice of pigments and season(s) to use in species discrimination, as well as identifying regions of the electromagnetic spectrum to target for sensor development [18]. We set out to investigate the foliar carotenoid and chlorophyll content of a number of evergreen tree species to determine whether (i) species have unique foliar carotenoid and chlorophyll profiles across seasons; and (ii) whether these seasonal profiles of foliar pigments can be used to improve species discrimination.

2. Materials and Methods

2.1. Study area

The iSimangaliso Wetland Park (28°S, 32°30’E) is located on the east coast of South Africa in the KwaZulu-Natal province. The park experiences sub-tropical climate conditions. Mean Annual Precipitation (MAP) is listed as 1 000 – 1 500 mm on the coast, and decreases inland to below 1 000 mm (Middleton & Bailey, 2008). Mean temperatures during summer range from 23 – 30°C, and can decrease to approximately 10°C during winter periods [19]. Elevation ranges from 10 m to 20 m above mean sea level (a.m.s.l.). The Park was extended from the original Ramsar site boundaries in 2000 and declared a World Heritage Site (WHS), primarily due to the high biodiversity of fauna and flora in the region [20].

A number of the Park’s evergreen tree species are found to grow in their natural habitat, which provides the opportunity for investigating pigment content in a natural environment. The iSimangaliso Wetland Park hosts the highest number of wetland habitat types (thirteen listed for Ramsar) for its size in Southern Africa [20]. A variety of wetland tree species are found in the park (Table 1); ranging from estuarine, swamp, riverine woodland and groundwater-fed depression wetland types.

Table 1. Tree species sampled for each season.

Tree species	Common name	Acronymn	Trees Winter (*n* =)	Trees Spring (*n* =)	Trees Summer (*n* =)	Trees Autumn (*n* =)
Avicennia marina	White mangrove	AM	23	23	22	22
Bruguiera gymnorrhiza	Black mangrove	BG	20	19	20	20

Ficus sur	Broom cluster fig	FSUR	6	10	11	11
Ficus sycamores	Sycamore fig	FSYC	16	16	16	16
Ficus trichopoda	Swamp fig	FT	12	11	11	11
Hibiscus tiliaceus	Lagoon hibiscus	HT	31	31	30	30
Syzigium cordatum	Waterberry	SC	17	17	17	17

2.2. Pigment data collection

We sampled sun exposed canopies of mature trees that were approximately 2 x 2 m in size. Predictive equations for the carotenoid and chlorophyll content for each tree were derived from laboratory chemical analysis and leaf spectral measurements. Five fresh sunlit leaves were sampled across the canopy of 17 trees (*Avicennia marina*, *Barringtonia racemosa*, *Bruguiera gymnorrhiza*, *Ficus sur*, *Ficus sycamores*, *Ficus trichopoda*, *Hibiscus tiliaceus* and *Syzigium cordatum*) for laboratory analysis. Carotenoids and chlorophylls were extracted using 100 % acetone and absorbance measured at 470 nm for carotenoids, 644.8 nm for chlorophyll *b* and 661.2 nm for chlorophyll *a*. Total chlorophyll content were computed using equations from Lichtenthaler and Buschmann [21].

Spectral measurements of each of the five leaves were collected using an Analytical Spectral Device spectroradiometer (FieldSpec Pro FR, Analytical Spectral Device, Inc, USA.). The ASD covers the spectral range between 350 to 2500 nm with a 1.4 nm sampling interval between 350-1050 nm range, and a ±2 nm between 1 050 – 2 500 nm.

Vegetation indices, which have previously been proven to be robust across species [14], were calculated using the collected leaf spectra (Table 2). An iterative bootstrap process (1 000 iterations) using R software divided the data randomly into a training (2/3) and test (1/3) data set. A linear model was fit to the training data set between pigment concentration and each vegetation index, and then applied to the test data set as well. The root mean square error (RMSE) was then calculated for both the training and test data set and recorded, before each new reiteration. The vegetation index with the lowest RMSE was considered the best predictive index and was then used to predict the pigment content from the spectral data.

Table 2. Vegetation indices used in predicting foliar pigment content from leaf spectra [9] [14].

Carotenoid Index	Chlorophyll index
Carotenoid red edge [22][23]	Carter$_4$ [24]
Carotenoid Reflectance Index using reflectance at 550 nm (CRI_550) [22]	Datt$_1$ [25]
Carotenoid Reflectance Index using reflectance at 700 nm (CRI_700) [22]	Maccioni [26]
Datt1998U [27]	Modified Normalised Difference Vegetation Index (NDVI) - (mND$_{705}$) [28]
Datt1998SA [27]	
Photochemical Reflectance Index (PRI) [29]	Modified Ref-Edge Inflection Point (mREIP) or Inverted Gaussian fit on reflectance (IG_REP) [30]
Photochemical Reflectance Index x Chlorophyll Index (PRI_CI) [31]	
Pigment Specific Simple Ratio using the reflectance at 470 nm (PSSR_470) [9]	MERIS Terrestrial chlorophyll index (MTCI) [32]
Pigment Specific Simple Ratio using the reflectance at 500 nm (PSSR_500) [9]	
Pigment Specific Normalised Difference using the reflectance at 470 nm (PSND_470) [9]	Normalised Difference Vegetation Index (NDVI$_2$) [33]
Pigment Specific Normalised Difference using the reflectance at 500 nm (PSND_500) [9]	
Reflectance at 470 nm (R_{470}) [18]	Optimised Soil-Adjusted Vegetation Index (OSAVI$_2$) [34]
Reflectance at 500 nm, adjusted from Blackburn 1998b (R_{500}) [18] adjusted	Red-edge Inflection Point (REIP) [35]

Ratio analysis of reflectance spectra for carotenoids (RARS_c) [36]	Red-edge Position Linear Extrapolation (REP_Le1) [37]
Structure Insensitive Pigment Index (SIPI) [38]	Vogelman$_1$ [39]
Yellowness Index (YI) [40]	Vogelman$_3$ [39]

2.3. Analysing seasonal variance of foliar pigments per species

Significant differences between species' carotenoid and chlorophyll content were assessed with a one-way Analysis of variance (ANOVA) using a Tukey Honest Significant Difference (HCD) multiple comparisons test. Secondly, similarity measures were used to assess whether shape (using Spectral Angle Mapper (SAM)) or magnitude (Sum of Euclidian Distances (ED)) presented the best description of the seasonal profiles. An iterative validation process (10 x) involved splitting the data into a 1/3 training data and 2/3 validation data set respectively. Mean seasonal profiles were calculated from the training data set and each tree of the test data set compared to these. Average user and producer's accuracies were calculated for each species, pigment, and similarity measure using the ten iterations.

3. Results

3.1. Seasonal profiles of pigments per species

The Datt1998 index for untransformed spectra had the lowest RMSE for carotenoids while the Vogelman3 index had the lowest RMSE for chlorophyll (Tables 2 and 3).

Table 2. Results of the bootstrap process of the best predictive vegetation index for carotenoids. Values are sorted for the test data set by increasing mean RMSE.

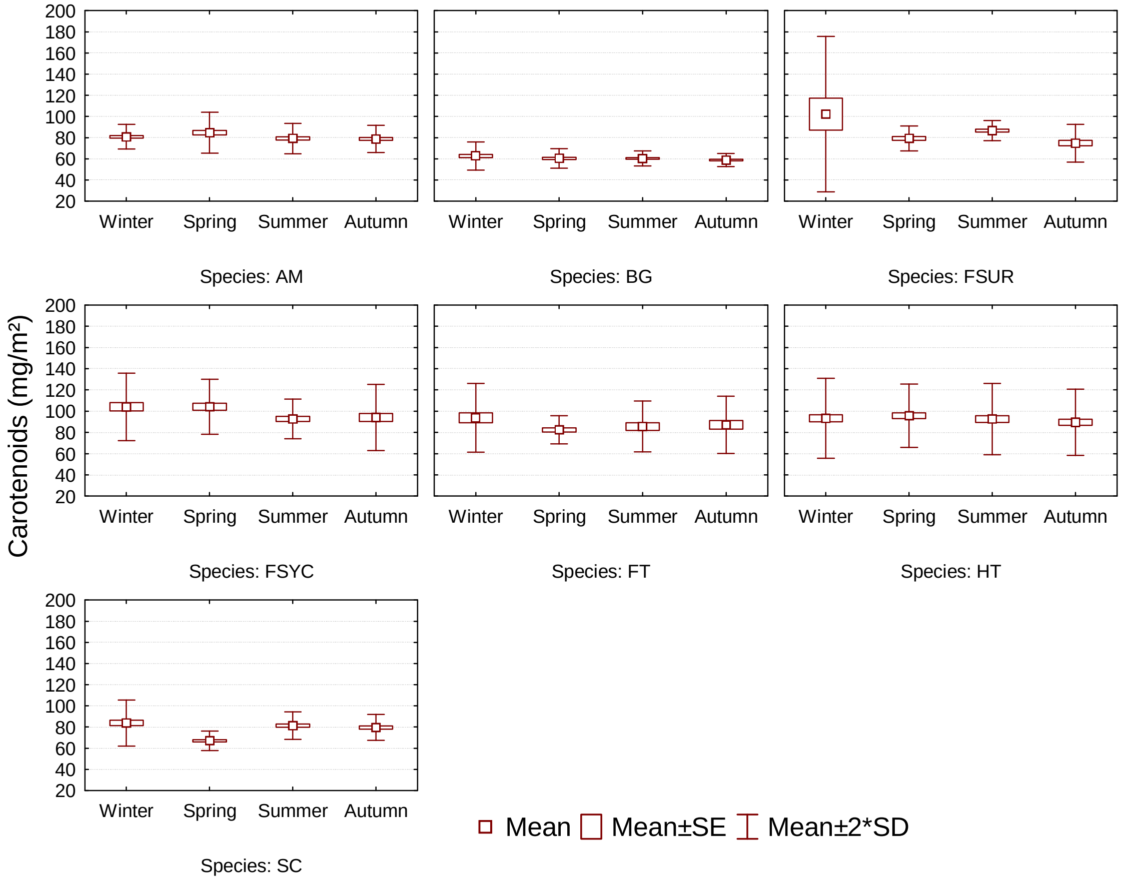
Carotenoid vegetation index	Training data set							Test data set						
	Min	1st Qu	Median	Mean	3rd Qu	Max	SD	Min	1st Qu	Median	Mean	3rd Qu	Max	SD
Car_rededge	29.21	34.91	36.55	36.38	38.03	41.92	2.15	10.93	16.51	18.03	18.03	19.57	23.26	2.07
CRI_550	39.80	48.10	50.19	50.00	52.21	56.47	2.90	17.11	22.64	24.80	24.71	26.64	32.82	2.79
CRI_700	41.22	49.48	51.35	51.17	53.21	58.51	2.85	16.31	23.31	25.05	25.10	26.84	33.85	2.77
Datt1998U	29.66	34.07	35.51	35.33	36.61	40.03	1.82	11.75	16.16	17.35	17.41	18.71	22.08	1.79
PRI (Gamon)	40.31	48.20	49.96	49.85	51.63	56.13	2.57	17.14	22.99	24.70	24.61	26.31	32.19	2.55
PRI_CI	34.44	43.64	45.52	45.30	47.09	52.35	2.57	13.54	20.96	22.62	22.66	24.29	31.86	2.56
PSSR_470	43.24	50.18	51.94	51.90	53.81	58.65	2.72	17.86	24.07	25.99	25.82	27.59	33.57	2.65
PSSR_500	40.22	46.81	48.84	48.59	50.46	55.03	2.53	16.07	22.16	23.81	23.81	25.65	30.66	2.48
PSND_470	40.73	49.2	51.31	51.09	52.99	58.34	2.69	17.21	23.46	25.29	25.29	27.08	33.47	2.59
PSND_500	38.65	45.96	47.6	47.52	49.35	53.24	2.51	17.04	21.70	23.39	23.34	24.97	31.22	2.42
R470	40.96	50.4	52.26	52.12	54.05	58.90	2.65	17.27	23.70	25.55	25.51	27.27	33.93	2.59
R500	40	47.38	49.14	49.03	50.82	54.99	2.47	17.33	22.54	24.22	24.17	25.85	32.17	2.41
RARS_c	39.34	47.51	49.14	49.01	50.74	55.80	2.49	15.53	22.38	23.97	23.97	25.55	32.01	2.44
SIPI	37.36	44.65	46.5	46.36	48.18	52.60	2.60	15.19	21.39	23.04	23.10	24.96	31.65	2.68
YI	40.29	47.11	49.05	48.92	50.79	55.00	2.50	16.55	22.27	24.01	23.96	25.69	31.24	2.51

Table 3. Results of the bootstrap process of the best predictive vegetation index for chlorophyll. Values are sorted for the test data set by increasing mean RMSE.

Chlorophyll vegetation index	Training data set							Test data set						
	Min	1st Qu	Median	Mean	3rd Qu	Max	SD	Min	1st Qu	Median	Mean	3rd Qu	Max	SD
Vogelman3	38.41	51.15	54.95	54.19	57.81	63.60	4.76	29.54	48.91	55.49	55.87	62.89	84.18	9.75
REP_Le1	38.77	51.78	56.46	55.35	59.37	64.40	4.94	31.59	48.37	55.10	56.27	64.60	82.43	9.80
Vogelman1	40.81	54.21	57.78	57.17	60.68	66.72	4.67	33.53	52.98	59.62	60.07	67.00	89.62	9.68
NDVI2	38.59	54.96	59.32	58.76	62.67	68.66	5.07	33.28	52.89	60.40	60.31	68.73	88.29	10.23
mND705	42.65	54.91	60.35	59.21	63.69	68.90	5.44	34.57	51.95	59.69	60.65	70.14	87.17	10.80
Carter4	45.56	56.96	61.46	60.85	64.99	71.36	5.13	34.93	55.58	63.77	63.69	72.18	87.42	10.29
Maccioni	48.22	59.94	63.98	63.33	67.18	74.52	4.97	33.44	57.63	64.79	65.09	72.93	90.97	10.08
Datt1	46.70	60.63	64.67	64.00	67.81	75.26	4.93	34.29	58.77	65.94	66.16	73.57	96.36	9.98
mREIP/IG_REP	97.89	133	142.64	141.56	151.93	167.81	13.14	34.94	60.85	70.44	69.89	78.86	99.90	12.42
OSAVI2	49.1	67.45	71.22	70.96	74.92	83.19	5.29	41.03	66.56	74.79	74.27	82.37	107.92	10.90
REIP	113.1	141.8	149.30	148.80	156.50	173.20	10.33	45.80	67.43	74.88	74.42	81.44	102.95	9.92
MTCI	40.46	61.47	66.72	65.42	70.98	78.35	7.25	38.52	62.53	72.28	75.02	82.34	131.30	16.91

The average carotenoids and chlorophyll content increased between winter and spring for the two species AM and HT only (Figure 1). For all other species, both carotenoids and chlorophyll decreased over the same time, except for species FSYC, which showed an increase in chlorophyll while carotenoids remained the same. Average carotenoid and chlorophyll values dropped between spring and summer for species AM, FSYC and HT; increased for FSUR and SC; whereas species BG's carotenoids remained the same while its chlorophyll decreased. For species FT, the average carotenoid values increased between spring and summer while chlorophyll decreased.

With the changeover from summer to autumn, average carotenoid levels dropped for all species, except FSYC and FT. For the same time period, average chlorophyll levels increased for all species, except FSUR and SC. Average carotenoid and chlorophyll levels peaked in winter for species BG, FSUR, FT and SC, while species FSYC and HT had the highest average carotenoid and chlorophyll levels in spring. Average carotenoid levels for species AM peaked in spring, while average chlorophyll levels peaked in autumn. Species BG showed distinctly low levels of carotenoids and chlorophyll compared to all the other species.



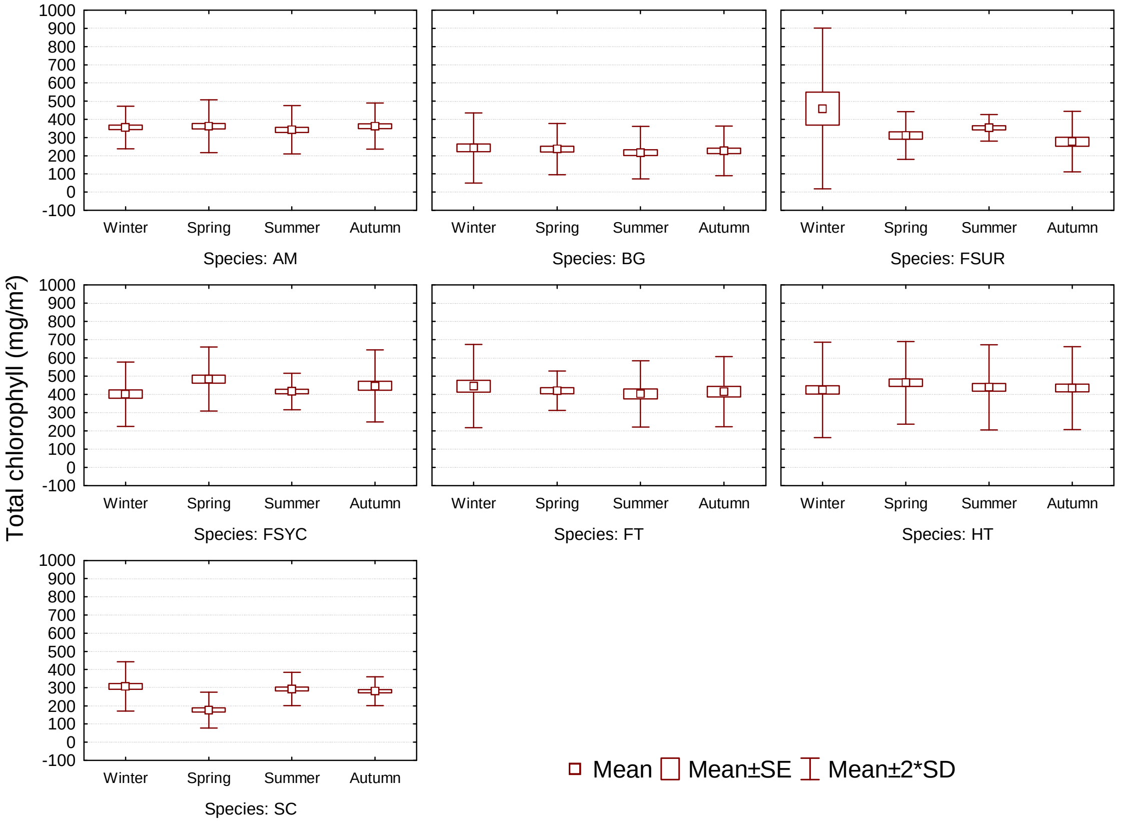


Figure 1. Seasonal foliar content per species for carotenoids (top) and chlorophyll (bottom).

3.2. Seasonal profile analysis: mean profiles, variance and similarity measures

3.2.1. *Mean seasonal profiles.* Mean seasonal profiles for carotenoids and chlorophylls are visually unique seasonal profiles per species (Figure 2). Species BG showed a distinctly low concentration of pigments over the four seasons compared to the other species. The mean seasonal profiles of other species overlap in variance.

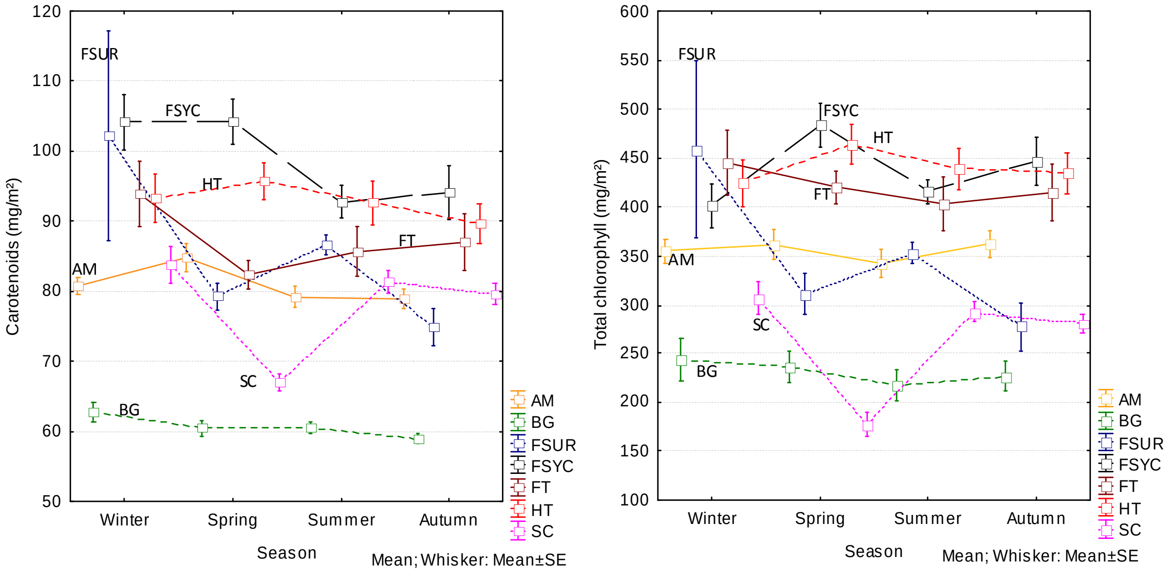


Figure 2. Mean seasonal profiles per species over four seasons for carotenoids (left) and chlorophyll (right).

3.2.2. *Analysis of variance.* Per season ANOVA shows that between 44 - 48 % of species can be distinguished from one another using single season information (Table 4). The season with the most significant differences is spring where 57 % of species are significantly different from one another for carotenoids, and 62 % significantly different for chlorophyll. Carotenoid pigments had higher overall significant differences between species, as compared to chlorophyll pigments.

Table 4. Number of significant differences between comparable species pairs base on a one-way ANOVA per season for carotenoids and chlorophyll.

Pigment	Number of pairs	Winter	Spring	Summer	Autumn	Overall accuracy (%) per pigment
Carotenoids	21	10	12	7	11	
Average accuracy (%):		47.6	57.1	33.3	52.4	47.6
Chlorophyll	21	7	13	8	9	
Average accuracy (%):		33.3	61.9	38.1	42.9	44.0

However, the ANOVA for the combined seasonal pigment content indicates there were more significant differences ($p < 0.04$) between 16 of the 21 (76 %) comparable pairs of species, for chlorophyll pigments and 15 of the 21 pairs for carotenoid pigments (71 %) (Table 5). Species BG and FSYC were significantly different to all other species for carotenoid pigments, whereas the differences in chlorophyll were not always significant.

Table 5. Analysis of Variance (ANOVA) for carotenoids and chlorophyll per species.

Pigment	Species	AM	BG	FSUR	FSYC	FT	HT	SC
Carotenoi-	AM							
	BG	0.000026						

	FSUR	0.907173^{ns}	0.000026				
	FSYC	0.000026	0.000026	0.000026			
	FT	0.068365^{ns}	0.000026	0.839097^{ns}	0.000080		
	HT	0.000026	0.000026	0.001786	0.032992	0.163236^{ns}	
	SC	0.755286^{ns}	0.000026	0.249034^{ns}	0.000026	0.001622	0.000026
Chlorophyll	AM						
	BG	0.000026					
	FSUR	0.939950^{ns}	0.000026				
	FSYC	0.000027	0.000026	0.000027			
	FT	0.001657	0.000026	0.000577	0.977359^{ns}		
	HT	0.000026	0.000026	0.000026	0.999977^{ns}	0.897125^{ns}	
	SC	0.000026	0.278164^{ns}	0.001911	0.000026	0.000026	0.000026

^{ns} not significant

3.2.3. *Similarity analysis.* SAM produced lower overall accuracies for the pigments compared to ED (Table 6).

Table 6. User and producer accuracies (%) for the similarity analysis of pigments compared to the mean seasonal profiles. Values are given for the average of 10 iterations.

Pigment:	Carotenoids				Chlorophyll			
Similarity measure:	SAM (n = 80.5)		ED (n = 80.5)		SAM (n = 79.8)		ED (n = 80)	
Species	Producer accuracy	User accuracy	Producer accuracy	User accuracy	Producer accuracy	User accuracy	Producer accuracy	User accuracy
AM	43.5	42.67	51.3	16.6	27.4	16.6	44.7	16.6
BG	32.9	22.30	99.2	16.4	17.3	18.0	68.8	16.4
FSUR	24.8	9.17	22.0	40.0	21.8	35.8	34.1	40.0
FSYC	14.9	25.05	40.2	33.0	21.7	30.6	33.2	33.0
FT	13.7	28.14	27.3	7.4	9.9	9.9	13.5	7.4
HT	25.3	20.53	46.9	19.6	29.8	20.9	40.0	19.6
SC	63.7	63.25	71.4	68.2	78.7	67.7	71.1	68.2
Overall Accuracy (%)	31.26		51.2		29.49		43.6	

4. Discussion

From the literature on evergreen tree species, it was apparent that seasonal profiles of carotenoid and chlorophyll pigments would be unique on a per species basis. The seasonal profiles of evergreen tree species in the iSimangaliso Wetland Park were not unique for carotenoid and chlorophyll pigments. The tree species did show high intra-species variability. Seasonal carotenoid profiles for species BG and SC were however highly separable from the other species, with species BG consistently showing low carotenoids and chlorophyll content over the four seasons.

Parametric classification (ANOVA) of combined carotenoid or chlorophyll seasonal data improved species discrimination compared to single season ANOVA and non-parametric similarity analysis. The parametric classification accounts for the high intra-species variability observed in these evergreen trees. The combined seasonal pigment information improved species discrimination to above 70 % for both pigments. Further investigation is required to see if parametric classifiers such as Discriminant Analysis and Maximum Likelihood could improve classification accuracies.

Carotenoid and chlorophyll pigments do contribute to species discrimination, even though the foliar pigment seasonal profiles are not unique per species, which is as a result of intra-species variability. These results concur with a study on tropical evergreen trees in the Amazon where the similarity of photosynthetic pigment content was observed between various species [41]. Other studies have indicated that taxonomic differences in evergreen tropical trees are described by a number of leaf chemicals [41], hence further investigation is required into the effectiveness of using additional foliar chemicals or leaf structure in species discrimination.

Seasonality was represented in our study by only four seasons, and therefore not fully representative of a continuous seasonal phenology. A better understanding is required of how the pigment content of these particular species changes over the full phenological cycle. As has been noted by studies in Mexico [8], the pigments changes that occur over a number of years could also be of importance.

5. Conclusion

For the seven evergreen trees we studied in this sub-tropical environment, we found species BG to have unique seasonal profiles for carotenoid and chlorophyll pigments. Species SC was significantly different from the other species in spring however pigments showed similar ranges compared to other species over the other three seasons. Parametric classification could account better for intra-species variability than when compared to non-parametric similarity measures. Combined seasonal data did however show improvements to species discrimination. Further research into using seasonal pigment profiles and additional (untested) parametric classifiers is required. The use of alternative foliar chemicals or leaf structural components should also be included in future research efforts to improve species discrimination. We used data over four seasons, whereas a more continuous representation of seasonality may yield more unique phenological patterns between different species.

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