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# Photosynthetic bark: Use of chlorophyll absorption continuum index to estimate *Boswellia papyrifera* bark chlorophyll content

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# ABSTRACT

Ouantification of chlorophyll content provides useful insight into the physiological performance of plants. Several leaf chlorophyll estimation techniques, using hyperspectral instruments, are available. However, to our knowledge, a non-destructive bark chlorophyll estimation technique is not available. We set out to assess Boswellia papyrifera tree bark chlorophyll content and to provide an appropriate bark chlorophyll estimation technique using hyperspectral remote sensing techniques. In contrast to the leaves, the bark of *B. papyrifera* has several outer layers masking the inner photosynthetic bark layer. Thus, our interest includes understanding how much light energy is transmitted to the photosynthetic inner bark and to what extent the inner photosynthetic bark chlorophyll activity could be remotely sensed during both the wet and the dry season. In this study, chlorophyll estimation using the chlorophyll absorption continuum index (CACI) yielded a higher  $R^2$  (0.87) than others indices and methods, such as the use of single band, simple ratios, normalized differences, and conventional red edge position (REP) based estimation techniques. The chlorophyll absorption continuum index approach considers the increase or widening in area of the chlorophyll absorption region, attributed to high concentrations of chlorophyll causing spectral shifts in both the yellow and the red edge. During the wet season *B. papyrifera* trees contain more bark layers than during the dry season. Having less bark layers during the dry season (leaf off condition) is an advantage for the plants as then their inner photosynthetic bark is more exposed to light, enabling them to trap light energy. It is concluded that *B. papyrifera* bark chlorophyll content can be reliably estimated using the chlorophyll absorption continuum index analysis. Further research on the use of bark signatures is recommended, in order to discriminate the deciduous B. papyrifera from other species during the dry season.

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# 1. Introduction

Non-foliar plant parts may contain chlorophyll (Aschan and Pfanz, 2003) and carry out photosynthesis (Pilarski, 1995; Pfanz et al., 2002; Pfanz, 2008; Saveyn et al., 2010; van Cleve et al., 1993). Photosynthetic non-foliar parts include bark (Foote and Schaedle, 1978; Kharouk et al., 1995; Tausz et al., 2005), stem tissues (Nilsen, 1995; Pilarski, 2002; Pfanz, 2008), twigs (Aschan et al., 2001; Pfanz, 1999; Schmidt et al., 2000; Wittmann et al., 2001), fruits, and even roots (Aschan and Pfanz, 2003). Pfanz (2008) indicated that whenever light penetrates such plant organs photosynthesis occurs.

Hyperspectral remote sensing techniques allow nondestructive detection and quantification of chlorophyll content in plants (Botha et al., 2010; Chen and Chen, 2008; Cho et al., 2008; Darvishzadeh et al., 2008; Datt, 1999; Delegido et al., 2008; Joyce and Phinn, 2003; le Maire et al., 2004; Mutanga and Skidmore, 2007; Serrano, 2008; Sims and Gamon, 2002). For example, through the selection of a single wavelength band (Carter, 1994), simple band ratios (Datt, 1999; Gitelson and Merzlyak, 1994; Maccioni et al., 2001; Sims and Gamon, 2002), normalized differences (Rouse et al., 1973; Sims and Gamon, 2002), the analysis of spectral edges (yellow or red edge) (Cho and Skidmore, 2006), derivatives (Dawson and Curran, 1998; Guyot and Baret, 1988; le Maire et al., 2004), multivariate analysis (Darvishzadeh et al., 2008; Kokaly and Clark, 1999; Schlerf et al., 2003), or through parameterization of absorption features (Broge and Leblanc, 2000; Schlerf et al., 2003; Van der Meer, 2004). Indices derived from off chlorophyll absorption center wavebands are more sensitive to subtle variations in leaf chlorophyll than the center bands, because reflectance is more sensitive to high chlorophyll concentration than to other plant pigments (le Maire et al., 2004) and at chlorophyll absorption maxima absorption tends to saturate at a low chlorophyll content (Sims and Gamon, 2002). Due to this, many

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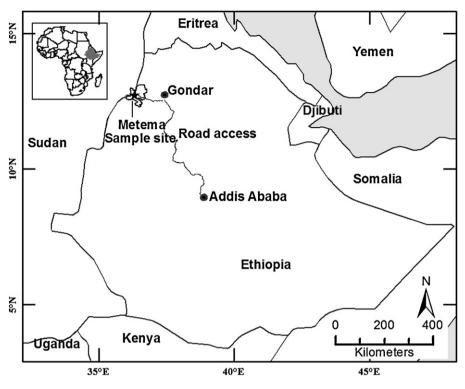


Fig. 1. Location of the sample site, Ethiopia.

leaf chlorophyll estimation techniques have been developed and widely tested.

Kharouk et al. (1995) reported chlorophyll concentrations in Aspen bark, twigs and stems to be similar to concentrations in leaves. Pfanz et al. (2002) tabulated measured chlorophyll content of stripped bark layers of nineteen deciduous and coniferous trees. They found the lowest chlorophyll content ( $0.27 \text{ mg g}^{-1}$ ) in *Betula pendula* and the highest chlorophyll content ( $3.97 \text{ mg g}^{-1}$ ) in *Quercus robur*, which had a chlorophyll content in its bark similar to that in its leaves. Studies estimating non-foliar pigment or chlorophyll content based on remote sensing techniques are much less common than those estimating leaf chlorophyll content (Levizou and Manetas, 2007). *Boswellia papyrifera* (Del.) Hochst, is a deciduous tree occurring in dry woodlands. It has flaky and papery outer bark layers (Fichtl and Admasu, 1994) and a greenish photosynthetic inner bark layer. In this paper the chlorophyll content of the bark of *B. papyrifera* is assessed and an appropriate bark chlorophyll estimation technique is provided using hyperspectral remote sensing. Hyperspectral analysis techniques were chosen to assess the *B. papyrifera* bark chlorophyll content because they are non-destructive and quick, and can be applied in remote areas. Measurement of chlorophyll content in the laboratory demands cutting (destructing) of the branches or stems of the *B. papyrifera* tree and scrapping the barks on a wide surface. This activity can damage or kill the tree if it mainly conducted on the standing main tree stem.



Fig. 2. Left: B. papyrifera during the dry season with relatively few bark layers compared to the wet season. The few bark layers are an advantage for Boswellia to access more light energy. Right: the papery bark layers of the tree during the wet season. The bark layers are labeled A, B, C, and D from the outside to the inside layer. The major part of the inner bark layer is greenish; the random yellowish spots represent opaque bark spots.

As the inner most photosynthetic bark layer of *B. papyrifera* is masked by several outer bark layers, this study also focused on how much energy was transmitted through successive outer bark layers to the inner photosynthetic bark during both the wet and the dry season.

#### 2. Materials and methods

#### 2.1. Study area and sampling

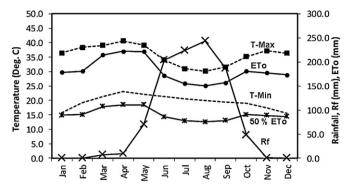
The study area (Fig. 1) is located in Metema woreda, Ethiopia  $(12^{\circ}17' \text{ to } 13^{\circ}6'\text{N}, 36^{\circ}00' \text{ to } 36^{\circ}47'\text{E})$  near Lemlem Terara village. This is about 916 km northwest of the capital city Addis Ababa and 225 km west of Gondar-town. The woreda (an administrative unit equivalent to a county) covers an area of 3795 km<sup>2</sup> and shares a 60 km border with Sudan. The elevation of the study area ranges between 600 and 850 m. The major farming system within the area is based on sesame, cotton, sorghum and livestock production. The woreda has a high coverage of Acacia dominated woodland (*Acacia seyal* and *Acacia polyacantha*) with several gum and resin producing tree species such as *B. papyrifera*. The soil types found where the sampled trees are located are Leptosols and Regosols. The Metema woreda was selected mainly due to easy access, availability of untapped and undisturbed *B. papyrifera* natural stands.

One hundred and fourteen (114) *B. papyrifera* trees were randomly selected and sampled for their bark and leaf spectra during both the wet (mid-June to early September) and the dry (December to April) season of 2008/2009.

#### 2.2. Boswellia papyrifera

*B. papyrifera* (Del.) Hochst is a deciduous tree species that produces the widely traded aromatic olio-gum resin called frank-incense or olibanum (Fichtl and Admasu, 1994; Goettsch, 1991; Lemenih et al., 2007; Tucker, 1986). This product is mainly used as incense for burning (Azene, 2007; Lemenih and Teketay, 2003), for perfumery (Adamson, 1969; Gebrehiwot et al., 2003; Tucker, 1986), and for the preparation of certain medicines (Atta-ur-Rahman et al., 2005; Huang et al., 2000; Khan et al., 1997; Lemenih and Teketay, 2003). Additionally, the tree has several household uses (Eshete, 2002; Tilahun, 1997) as well as ecological functions (Gebrehiwot et al., 2003; Lemenih and Teketay, 2003). In Ethiopia and Eritrea, it is known to grow well on shallow soils and in rocky areas (Fichtl and Admasu, 1994; Gebrehiwot, 2003; Gebrehiwot et al., 2003; Ogbazghi et al., 2006a,b).

The B. papyrifera tree is reported to be in decline, which has become an ecological concern (Gebrehiwot et al., 2003; Ogbazghi et al., 2006a,b; Tilahun, 1997). Reasons for this decline are: overtapping (Ogbazghi et al., 2006a,b; Rijkers et al., 2006), insect pest attacks, as well as clearing and burning of the undergrowth (Abiyu et al., 2010; BoANR, 2003, 2004a,b; Eshete, 2002; Eshete et al., 2005; Gebrehiwot et al., 2003; Lemenih et al., 2007; Ogbazghi et al., 2006a,b; Rijkers et al., 2006). B. papyrifera has a weak regeneration capacity (Abiyu et al., 2006, 2010; Eshete, 2002; Gebrehiwot et al., 2003; Tilahun et al., 2007). In addition, detail scientific knowledge on the proper management, quality (vitality) and quantity of the resource is not available in Ethiopia. Considering this, the FRaME (The FRAnkincense, Myrrh and gum Arabic: sustainable use of dry woodland resources in Ethiopia) program was initiated, under which umbrella this research was conducted. To properly manage the resource (monitor and restore the declining population), knowledge on the forest tree quality and quantity is very important. In this study, understanding of the chlorophyll content of B. papyrifera bark will help to understand the physiological performance or health of the plant. This research identified the presence



**Fig. 3.** Rainfall, temperature and reference evapotranspiration (ETo) in Metema (based on 13 years of monthly data from the National Meteorological Agency of Ethiopia).

and estimated the amount of chlorophyll in the *B. papyrifera* bark. The annual carbon gain of *B. papyrifera* was recently analyzed based on the leaf analysis alone and the role or contribution of the bark to the annual carbon gain was not captured (Mengistu et al., 2012).

Leaves of the *B. papyrifera* usually stay on the tree for 3 months of the year, from early June to the end of August, mainly depending on the onset and end of rains. In Metema woreda, *B. papyrifera* sheds its leaves earlier than any other tree species present in the landscape, usually from the end of August to mid September. Almost all other tree species will be devoid of leaves by mid November. The tree has several papery bark layers (Fig. 2 right, labeled A–D from outside to inside). The top, sun exposed, branches have fewer bark layers with the greenish inside bark layer becoming exposed during dry season (left).

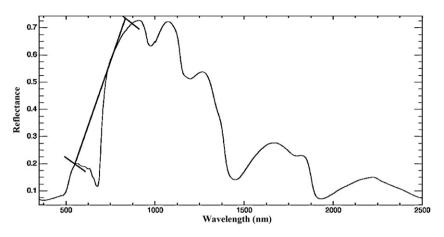
# 2.3. Climate

Thirteen years of meteorological data on temperature and rainfall were obtained from the National Meteorological Agency of Ethiopia. Rainfall in Metema is unimodal with an annual average of 1000 mm (Fig. 3). The annual average, maximum, and minimum temperatures are 28, 40, and 15 °C, respectively. Reference potential evapotranspiration (ETo) was also computed (Hargreaves et al., 1985). The long term rainfall data indicate that the dry season covers more than eight months of the year.

#### 2.4. Reflectance measurement

One hundred fourteen *B. papyrifera* plants were randomly selected from the vicinity of Lemlem Terara village of Metema woreda. Reflectance measurements of the bark and leaves of the same 114 plants in the dry and in the wet season were recorded. The wet season data were collected during August and September; the dry season field data were collected during March and April when the trees were leafless.

An analytical spectral device (ASD) FieldSpec FR spectroradiometer, with a spectral resolution of 1 nm, with a contact probe was used to capture bark and leaf reflectance of *B. papyrifera*. The reflectance measurements were calibrated against a white reference panel (SPECTRALON, Labsphere Inc.) for each reflectance measurement taken. Ten reflectance values from the same target were averaged (set in the ASD instrument) to obtain one representative signature value during each bark and leaf spectral measurement. The size of the measured area for reflectance is the same in size as the ASD contact probe ( $\sim$ 3 cm<sup>2</sup> in area) replicated three times and then averaged. The reflectance measurements of bark layers were conducted on the sun facing side of the branches sampled for laboratory chlorophyll analysis.



**Fig. 4.** Bark chlorophyll region parameter extraction (area, band depth, position, asymmetry, and width) after continuum removal, from green peak maximum (554 nm) to shoulder maximum (886 nm), indicated by the reddish line, for *Boswellia papyrifera*'s inner bark layer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

*B. papyrifera* trees shed their outer bark layers to expose their inner greenish-photosynthetic layer. When several bark layers cover the inner photosynthetic layer, the inner layer becomes masked and its chlorophyll absorption signature captured through the ASD device becomes blurred. This happens during the rainy season when there are several bark layers as opposed to the dry season (few bark layers). When the top layer is dry and opaque the reflectance spectrum will be dominated mainly by the spectrum of the top layer.

When taking the reflectance measurements (1) the reflectance of the bark layers (intact, with all bark layers) was recorded with ASD contact probe by bringing the probe in contact with the bark to restrict light energy. Hence, the illumination was coming only from the ASD light source. The spectral measurement was done immediately after the layer was peeled-off. The size of the measured area is the same as the size of the ASD contact probe ( $\sim$ 3 cm in diameter). (2) The top bark layer was peeled off and (3) reflectance of the remaining bark layers was recorded as above with a contact probe. (4) The procedure was repeated until the last bark layer (inside) which is not removable is measured for reflectance. All bark layers were coded (numbered) from the inside (number 1) to the outer most layer (with the maximum number during the wet season being 7). The reflectance measurement of the leaves was recorded also with a contact probe. A minimum of ten leaves were stacked during each leaf reflectance measurements.

The data was analyzed using STATISTICA software (http://www.statsoft.com). Analysis was carried out in two ways: (1) considering the average of three spectral regions, i.e. region (a) from 400 to 680 nm, region (b) from 730 to 1380 nm, and region (c) from 1380 to 2500 nm, excluding the area below 400 nm from the analysis due to noise; (2) using NDVI ( $(R_{920} - R_{680})/(R_{920} + R_{680})$ ).

# 2.5. Bark layers transmittance measurement

During the dry season thirty out of the 114 plants (sampled for reflectance measurement) each with three to four bark layers, were sampled for transmittance measurement. The dry papery bark layers were carefully peeled off in large pieces and measured for transmittance using an ASD instrument in a dark-room, layer by layer. The transmittance of a bark layer is the ratio of the intensity of the light energy that has passed through the bark layer to the initial intensity of the light when it reached the sample  $(T = I_{out}/I_{in})$ . The transmittance measurement was conducted first by calibrating the ASD sensor against a white reference panel (SPECTRALON, Labsphere Inc.), then by placing the bark layer in between the ASD light source and the ASD sensor. The energy that was measured was the transmitted energy. We used this approach to have relative comparisons between the lower, middle and top bark layers.

#### 2.6. Bark chlorophyll measurement

A temporary laboratory was established in the study area to measure the chlorophyll content of *B. papyrifera* bark. Daily, around six tree branches (1 m in length each) were sampled from a total of 30 samples drawn from the 114 plants considered for the wet and dry season bark layer analysis. The sun facing sides of the branches were marked and sampled only. The sampling and analysis was always carried out between 8:00 and 9:00 a.m. During this period there was neither cloud cover nor a significant variability in temperature ( $\sim 20 \circ C$ ). Ice cubes were used to maintain freshness of the sample branches until analysis.

The green (inner) layer of bark was thinly peeled off and chopped using a mortar and pestle. Four grams of it was weighed out and dissolved in 10 ml 80% acetone for 2–3 min. The impurities were filtered out, with fourfold clean polyester cloth. This was centrifuged at 6000 revolutions per minute for 20 min. One ml of the pure liquid (the supernatant) was siphoned off with finnepipette digital. The supernatant was mixed with 10 ml of acetone again. The spectrophotometer (JUNWAY 6305 UV/vis) was calibrated with distilled water at 645 nm. Then, the extract was put inside the cuvette and to the spectrometer. Absorbance reading was taken at both 645 nm and 663 nm the total chlorophyll content was calculated using the method of Arnon (1949) and Mackinney (1941).

# 2.7. Bark chlorophyll estimation

Several leaf chlorophyll estimation methods based on remote sensing and described in the literature were tested and compared. The available leaf chlorophyll estimation methods can be grouped as follows, based on their mode of operation (see also Table 1):

- a. *Single band* (*SB*): measured chlorophyll data are compared to single band hyperspectral remote sensing data.
- b. *Band region (BR)*: measured chlorophyll data are compared to one or more band regions. This is common when multispectral remote sensing data are used.
- c. *Simple ratio* (*SR*): when chlorophyll estimation is based on the ratio relationship between two or more spectral bands. This may involve calculations such as additions or subtractions (Datt, 1999; Gitelson and Merzlyak, 1994).
- d. *Normalized differences* (*ND*): considers single or band region differences normalized (divided) by their sums. The most common

#### Table 1

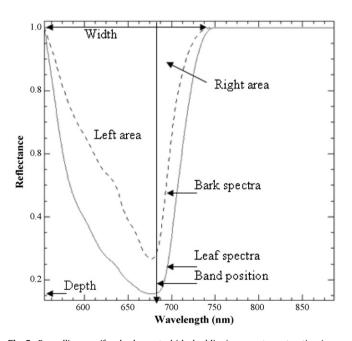
Bark chlorophyll regression analysis results based on commonly used leaf spectral indices. R<sup>2</sup> values in bold (above 0.5) indicate values significant at p < 0.00.

Index	Formulae	Reference	$R^2$	р
Selected indices (linear)				
Simple ratio (SR-1)	$R_{750}/R_{550}$	Gitelson and Merzlyak (1994)	0.34	0.00
(SR-2)	$R_{750}/R_{700}$		0.78	0.00
(SR-3)	$(R_{850} - R_{710})/(R_{850} - R_{680})$	Datt (1999)	0.30	0.00
mSR	$(R_{780} - R_{710})/(R_{780} - R_{680})$	Maccioni et al. (2001) after Datt (1999)	0.03	0.29
NDVI (greenness)	$(R_{800} - R_{680})/(R_{800} + R_{680})$	Rouse et al. (1973)	0.77	0.00
mND <sub>705</sub>	$(R_{750} - R_{705})/(R_{750} + R_{705} - 2 \times R_{445})$	Sims and Gamon (2002)	0.65	0.00
Red edge				
Red edge inflection point (REIP) <sub>linear</sub>	$R_{\text{red-edge}} = (R_{670} - R_{780})/2$ 700 + 40[( $R_{\text{red-edge}} - R_{700}$ )/( $R_{740} - R_{700}$ )]	Guyot and Baret (1988)	0.48	0.00
Linear extrapolation <sup>a</sup>	$-(c_1-c_2)/(m_1-m_2)$	Cho and Skidmore (2006)	0.65	0.00
Savitzky-Golay 1st order		Svitzky and Golay (1964)	0.65	0.00
2nd order			0.63	0.00
REIPLagrangian		Dawson and Curran (1998)	0.66	0.00
PLSR bands (linear)				
R <sub>652</sub>			0.68	0.00
R <sub>677</sub>			0.67	0.00
R <sub>695</sub>			0.52	0.00
Continuum removal				
Width		Van der Meer (2004)	0.79	0.00
Depth (CRCWD)		Broge and Leblanc (2000) and Van der Meer (2004)	0.78	0.00
Area (CACI)			0.87	0.00
Asymmetry		Van der Meer (2004)	0.05	0.18

<sup>a</sup> c<sub>1</sub> and c<sub>2</sub>, and m<sub>1</sub> and m<sub>2</sub> represent the intercepts and slopes of the far-red and NIR lines of the first derivative spectra, respectively.

index of this type is NDVI (normalized difference vegetation index, greenness index) (Rouse et al., 1973; Sims and Gamon, 2002).

e. *Curve fitting or use of derivatives* (*D*): several reflectance derivative approaches that estimate curve edges and positions are implemented, such as Savitzky–Golay 1st and 2nd order derivatives (Svitzky and Golay, 1964), REIP<sub>Lagrangian</sub> (Dawson and Curran, 1998), REIP<sub>linear</sub> (Guyot and Baret, 1988) and REP linear extrapolation (Cho and Skidmore, 2006). The linear extrapolation technique developed by Cho and Skidmore (2006) was developed to determine the red edge position (REP) through



**Fig. 5.** *Boswellia papyrifera* bark spectral (dashed line) parameter extraction (area, band width, depth and asymmetry) in the chlorophyll absorption region in comparison to its leaf spectra (solid line) after the continuum is removed. Leaves have a larger absorption depth and absorption area than the photosynthetic inner bark layer.

extrapolation of two straight lines fitted on first derivative spectra flanks of the far-red (680–700 nm) and the NIR (725–760 nm). This technique resolves the problem of the double peak featuring in between the aforementioned regions.

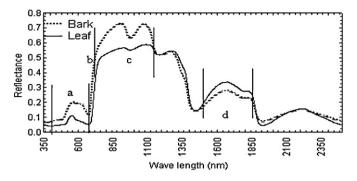
f. Multivariate analysis: in addition to the aforementioned techniques, Partial least square regression (PLSR) analysis is used to find out which wavelength bands or regions correlate best with bark chlorophyll content. The analysis was done using the Unscrambler v10.0.1 CAMO software (http://www.camo.com). Wavelengths from 400 to 850 nm were selected for this analysis. PLSR tries to predict a set of dependent (y) variables from a set of independent (x) variables. When there are large numbers of predictors and response variables, PLSR is less affected by the problem of multicollinearity than multiple linear regression (MLR) analysis is. Also, in cases of noisy data, MLR tends to overfit. PLSR is also closely related to principal component regression (PCR) analysis (Darvishzadeh et al., 2008). The advantage of PLSR over PCR is that it uses decomposition on both the spectral data and the response simultaneously, while PCR relies solely on spectral data analysis. More information on PCR, MLR and PLSR can be found in Darvishzadeh et al. (2008), Geladi and Kowalski (1986) and Schlerf et al. (2003). The Akaike information criterion (AIC) is used to decide the optimal number of factor loadings and for the selection of a PLSR model representing the variability in the data without over fitting, rather than using lower RMSE (root mean square of error) value directly. This is defined by the following formula (Li et al., 2002):

# $AIC(m) = N\log(a) + 2m$

where "*a*" is the maximum likelihood estimate of the variance of the response variable, "*N*" is the sample size and "*m*" is the number of model parameters.

Secondly, a 2D correlation plot was used to illustrate the coefficient of determination ( $R^2$ ) between chlorophyll and NDVI of the band combinations for the *B. papyrifera* inner bark samples.

g. Parameter extraction after continuum is removed (CR): Continuum removed spectral analysis of the absorption band depth or chlorophyll well depth (CRCWD), as well as of



**Fig. 6.** Leaf (solid line) and inner bark (dashed line) average spectra for *Boswellia papyrifera* (n = 114). Four significant/separable regions are evident: a (400–673 nm), b (673–720 nm), c (720–1167 nm), and d (1475–1873 nm). Except for region d, where inner bark reflectance is significantly lower, the other regions depict the inner bark reflectance to be significantly higher than the leaf reflectance at  $\alpha > 0.00$ .

the area (chlorophyll absorption continuum index, CACI), the band position, and the width were calculated. The hull is applied from the area of maximum green peak reflectance at 554 nm to maximum NIR reflectance at 886 nm for all samples (Figs. 4 and 5). Details on continuum removal are explained by Clark et al. (2003). The analysis was executed using IDL-ENVI (http://www.ittvis.com) software with the DISPEC 3.2 IDL spectral analysis program developed by Harald van der Werff (2007–2010) (http://www.itc.nl/personal/vdwerff/). This technique is most commonly used by geologists to identify subtle differences between mineral spectrum absorption features.

# 3. Results

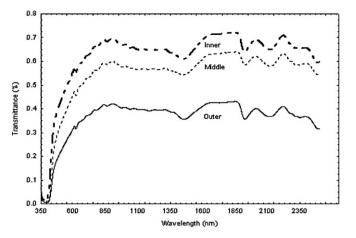
# 3.1. Leaf versus inner bark (photosynthetic) layer spectra

Fig. 6 shows the average spectra for the inner bark layer and leaves of 114 *B. papyrifera* trees. The greenish inner bark layer has an absorption spectrum similar to that of the leaves. There are four distinct regions (400–673, 673–720, 720–1167 and 1475–1873 nm) marking the differences in reflectance values.

For estimation of the chlorophyll content in the bark, the first three regions (Fig. 6a–c) usually are important. In part of region 'a' (from around 550 to 673 nm) the bark spectrum is convex, like a hump, with a double peak at 605 and 632 nm. The bark spectrum also exhibits a steeper slope than the leaf spectrum from 632 to 673 nm. In addition, its reflectance value is significantly higher ( $\alpha < 0.00$ ) than the leaf's. In the second region (b), the red edge position and inflection point of the bark are shifted more toward the blue region than in the leaf spectrum. In the regions 'b' and 'c' the reflectance of the inner bark spectrum. However, the bark spectrum in region d shows significantly lower reflectance ( $\alpha < 0.00$ ) than the leaf spectrum.

#### 3.2. Bark layer transmittance

Light energy is necessary to generate photosynthesis in plants. Transmittance was therefore measured for 30 *B. papyrifera* inner, middle, and outer bark layers and the analysis shown in Fig. 7. In the region 400–680 nm (visible region) the transmittance of the inner bark layer (mean = 0.44, SD = 0.15) was higher than that of the middle bark layer (mean = 0.35, SD = 0.14) (t(560) = 7.13, p < 0.00) and the middle bark layer had a higher transmittance than the outer bark layer (mean = 0.23, SD = 0.1) (t(560) = 11, p < 0.00). Less energy is transmitted below 400 nm, indicating that bark layers act as a shield protecting the inner photosynthetic bark from ultraviolet rays.



**Fig. 7.** Mean transmittance spectra of inner (44%), middle (35%) and outer (23%) bark layers of the frankincense tree *Boswellia papyrifera* that correspond to the first, second, and third bark layer from inside out.

### 3.3. Estimation of bark chlorophyll content

Because of the similarity in spectral signature between leaves and bark (Fig. 6) some of the commonly available, published leaf chlorophyll indices were tested (Table 1) for their applicability to chlorophyll content estimation of the B. papyrifera inner bark layer. The area approach (CACI) that takes into consideration the whole region ( $R^2 = 0.87$ , p < 0.00) estimates bark chlorophyll with a higher explained variance than other indices and methods. SR-2  $(R^2 = 0.78)$ , NDVI  $(R^2 = 0.77)$ , mND705  $(R^2 = 0.65)$ , as well as band width ( $R^2 = 0.79$ ) and depth ( $R^2 = 0.78$ ) all produced good results at p < 0.00. The coefficient of determination ( $R^2$ ) between chlorophyll and NDVI of the band combinations is shown in the 2D correlation plot (Fig. 9) for 36 *B. papyrifera* samples. The higher *R*<sup>2</sup> values are evident at the yellow and red edge positions. The regression plots of the continuum removed parameters extracted (area, depth, width, asymmetry) are shown in Fig. 8. Spectral derivatives, based on red edge inflection point (REIP) positions, using Savitzky-Golay 1st and 2nd order, and  $\text{REIP}_{\text{Lagrangian}}$ , estimated  $R^2$  values of 0.65, 0.63, and 0.66, respectively. The PLSR results on the predicted versus reference plot indicate 0.89 and 0.86 R<sup>2</sup>, respectively. The significant regions (p < 0.05) of the three factor loadings wavelength regions are approximately at 652 nm ( $R^2 = 0.68$ ), 677 nm ( $R^2 = 0.67$ ), and  $695 \,\mathrm{nm} \,(R^2 = 0.52).$ 

### 3.4. Bark reflectance by layer

The 114 plants sampled each had two to four bark layers during the dry season (mean 2.96, SD = 0.46), and three to seven layers during the wet season (mean 4.22, SD = 0.81). The difference is significant (t(114) = 11.56, p < 0.00).

The average spectral signature of the different bark layers, from layer 1 to 6 (there were very few plants with 7 bark layers), is shown in Fig. 10. The analyzed spectral regions are: the visible (a), the near-infrared (b) and the middle-infrared (c). The mean reflectance value in the visible region increases until the fourth bark layer and then decreases. In the near-infrared region the mean reflectance value decreases from the inner to the outer bark layer, with the opposite occurring in the middle-infrared region. After bark layer 4, the decrease in signature mean is attributed to bark layer dryness and lower bark transmittance (opaqueness). This trend is similar in both the wet and the dry season (Figs. 10 and 11). The mean values of regions a, b, and c in the wet season have second order polynomial fits of  $R^2 = 0.96$ ,  $R^2 = 0.89$ , and  $R^2 = 0.95$ , respectively. In addition to the mean of these regions,

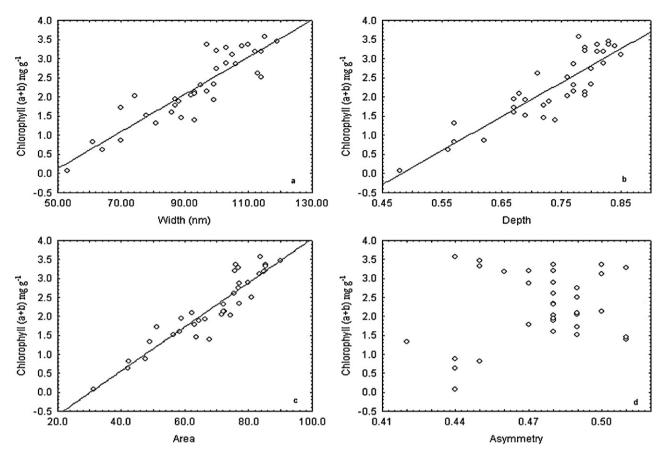
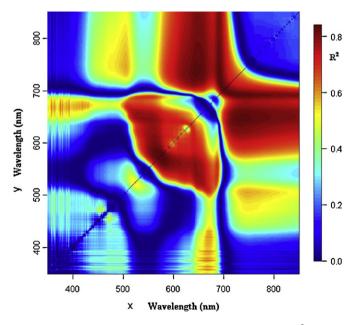


Fig. 8. Bark chlorophyll absorption region parameters: band width (a), depth (b), whole area (c) and asymmetry (d) correlated against measured total chlorophyll content after continuum is removed.

a comparison was made with NDVI (Fig. 12) for both the wet and the dry season. The same second order polynomial fit is also observed with NDVI for both the wet and the dry season ( $R^2 = 0.95$ , Fig. 12).



**Fig. 9.** 2D correlation plot illustrating the coefficient of determination ( $R^2$ ) between chlorophyll and NDVI of the band combinations for the *B. papyrifera* samples. The higher  $R^2$  values are shown in deep red (or black on a gray scale). *X* stands for the first and Y for the second band.

#### 4. Discussion

Chlorophyll estimation using (continuum removed) CACI analysis yielded the highest  $R^2$  compared to other indices and methods. This is due to an increase or widening in the area of the chlorophyll absorption region, attributed to high concentrations of chlorophyll causing spectral shifts in both the yellow and the red edge. The red edge position shift estimated by Savitzky-Golay (1st and 2nd order), REIP<sub>Lagrangian</sub>, REIP<sub>linear</sub>, and REP linear extrapolation were all directly related to an increase in chlorophyll content and can be used to some extent to estimate bark chlorophyll content. Similarly, the linear extrapolation technique based on 1st derivative spectra (Cho and Skidmore, 2006) that solves the double peak problem in the NIR, matched the 1st derivative spectra, due to the unimodal behavior of the signature in both leaf and bark of *B. papyrifera*. The REP estimation techniques analyze the right side (red-edge) of the chlorophyll absorption maxima only, and do not estimate the spectral shifts of the yellow edge.

The inner greenish bark layer of *B. papyrifera* contains chlorophyll and carries out photosynthesis. Information on chlorophyll content of bark is useful to understand the health of a plant (Haboudane et al., 2002) and for analyzing the carbon balance of a tree stand. Kharouk et al. (1995) indicated that the carbon balance of a tree stand should not be evaluated without taking bark pigment content into account. The chlorophyll content found in *B. papyrifera* bark ranged from 0.6 to  $3.5 \text{ mg g}^{-1}$ . This value is in line with reports by Pfanz et al. (2002) for bark chlorophyll, which showed higher values for *Q. robur* ( $3.97 \text{ mg g}^{-1}$ ), *Sorbus arcuparia* ( $3.92 \text{ mg g}^{-1}$ ), and *Fraxinus excelsior* ( $3.73 \text{ mg g}^{-1}$ ) and lowest values for *U. laevis* ( $0.12 \text{ mg g}^{-1}$ ) and *Picea abies* ( $0.19 \text{ mg g}^{-1}$ ). As the number of bark layers increases, the NDVI value decreases during

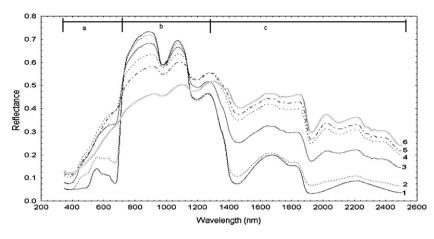


Fig. 10. Spectral signatures of all bark layers in the wet season from the outer bark layer (6) to the inner photosynthetic bark layer (1) as one bark layer at a time is peeled off. The legend of each layer is shown on the right side of the signatures.

both the wet and the dry season. The decrease in NDVI is due to an increase in the number of bark layers and the opacity (less transmittance) of the outer bark layers. As *B. papyrifera* has less bark layers during the dry season, higher mean NDVI values (above 0.4) were recorded then. These higher NDVI values are comparable to literature based forest and grass NDVI values (Zhu et al., in press; Ouyang et al., 2012).

Having fewer bark layers during the dry season is an advantage for the plants as their inner photosynthetic bark becomes more exposed to light and can more easily trap light energy. Bark layers strongly differ in light transmittance, which is attributed to the age of the bark and its dryness. The inner, mid, and outer bark layers, corresponding to the first, second, and third layer from the inside out transmit 44, 35, and 23% of the incoming radiation in the visible region. The continuous shedding of old or opaque bark layers exposes the inner photosynthetic bark layer, enabling the tree to acquire more light energy.

The shape of a healthy *B. papyrifera* inner bark layer spectral curve resembles that of a typical leaf spectrum. The leaf spectrum in the visible, near-infrared, and middle-infrared portion of electromagnetic energy spectrum is dominantly influenced by pigments (mainly absorption by chlorophyll), the internal structure of the leaves (reflectance due to scattering), and total moisture content (water absorption), respectively (Swain and Davis, 1978). In addition, other foliar chemicals are known to cause absorption in some

specific regions mainly in the near- and middle-infrared regions (Curran, 1989). *B. papyrifera* bark contains several chemicals (Attaur-Rahman et al., 2005). Tapping of the bark during the dry season induces frankincense exudate rich in primary and secondary metabolites. In the visible and near-infrared, the reflectance of the inner bark layer is higher than for leaves, but in the shortwave-infrared it is lower. The low reflectance in the shortwave-infrared could be attributed to a high total moisture and chemical content of the inner bark compared to the leaves.

The study was conducted in Metema woreda (less than 900 m a.s.l.). Other high altitude areas (900–1800 m a.s.l.) of Ethiopia were not covered in this study. In addition, the study was conducted only during end of the rainy season (September) and the dry season (April). Intermediate (during December) dry season data was not collected. Thus, complete conclusion for the entire dry season cannot be reached.

To conclude, bark chlorophyll content can be reliably estimated using continuum removed area analysis (CACI), which gives better results than single band, simple ratio, normalized difference, and REP based estimation techniques. In addition, it was demonstrated that the spectral properties of the deciduous *B. papyrifera* tree stem and branches have similar signatures to its leaves. The presence of chlorophyll in the bark provides valuable information for the discrimination of this species from others during the dry season (dry season mapping) using higher resolution imagery. Moreover,

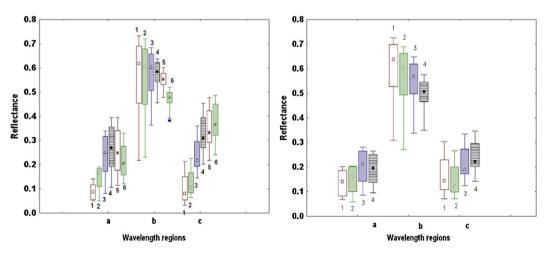
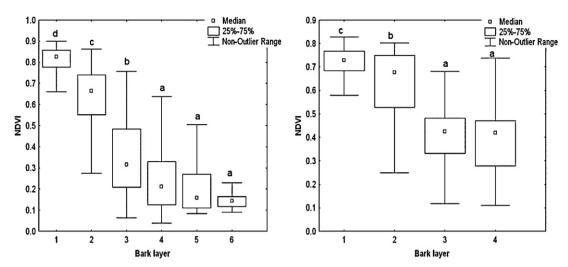


Fig. 11. The wet (A) and dry (B) season average bark layer reflectances. The numbers indicate bark layers for outer (6) to inner layer (1) for the regions 400–680 nm (a), 730–1380 nm (b) and 1380–2500 nm (c) for *Boswellia papyrifera*.



**Fig. 12.** NDVI by bark layer during the wet (A) and the dry (B) season for *Boswellia papyrifera*. The inner bark layer (1) shows higher NDVI values than the outer bark layer (6 in the wet season and 4 in the dry season). Different letters (per bark layer, ANOVA) indicate significant differences between the layers (*p* < 0.05).

this research has shown that *B. papyrifera* tree contains chlorophyll in the bark, hence, the *B. papyrifera* tree carbon gain analysis should also consider the bark chlorophyll content.

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