Nondestructive assessments of carotenoids content of broadleaved plant species using hyperspectral indices

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Abstract

Carotenoids play important roles regarding photoprotection as well as light harvesting during the process of photosynthesis, resulting in the opportunity of quantifying carotenoids content to evaluate the productivity of vegetation. The traditional approaches such as ultraviolet and visible (UV-VIS) spectroscopy are destructive and hence do not allow to determine the temporal dynamics of carotenoids content over time. As a promising alternative, hyperspectral remote sensing provides a way to evaluate carotenoid content changes over time and at multiple scales. Furthermore, it is easier to expand such approaches for large scale monitoring. However, to identify a generally applicable hyperspectral index sensitive to carotenoids remains a big challenge. In this study, we have evaluated thirteen available hyperspectral indices to quantify carotenoids, based on four independent datasets including two field datasets from Japan and two publicly available datasets (LOPEX and ANGERS). We attempted to develop a new generally applicable hyperspectral index for broadleaved plant species using the original and first derivative reflected spectra of the four datasets. We found that dND (516,744), a normalized differences type index using reflectance derivatives at 516 and 744 nm ($(D_{516} - D_{744}) / (D_{516} + D_{744})$), had the highest robustness among all datasets and also was the best index when all data were combined (R^2=0.475, WAIC= 2430.1, and RPD=1.45 for all datasets), suggesting its potential for general applications. Further extensive evaluations of the proposed index in other types of plants is required to test whether it can also be applied in other than broadleaved species.

Keywords: carotenoids; broadleaved species; first-order derivative; hyperspectral reflectance; ratio of performance to deviation (RPD)

1. Introduction

Plant pigments are significant factors in the biosphere forming essential elements of the photosynthetic apparatus of plants and their nutritional functions (Blackburn 2007). Among diverse pigments, chlorophylls and carotenoids are the two principle types. While chlorophylls are primarily involved in collecting light energy, carotenoids also contribute to harvesting light energy for the photosynthesis (Sims and Gamon 2002). Furthermore, carotenoids play a key role in the photoprotection of the photosynthetic apparatus by their ability to dissipate excessive light energy to protect unsaturated fatty acids, phospholipids and galactolipids against cellular damages (Demmig Adams and Adams 1996; Edge et al. 1997). This makes the carotenoids content an indicator for assessing plants physiological status (Young and Britton 1990). In addition, carotenoid content is also known to be closely correlated with plant stress and photosynthetic capacity (Hernandez-Clemente et al. 2012). Indeed, it increases under high irradiance levels and high temperature environments (Siffel et al. 1996) or at the onset of leaf senescence (Munne-Bosch and Penuelas 2003; Penuelas et al. 1994).

Traditional techniques including ultraviolet and visible (UV-VIS) spectroscopy and high performance liquid chromatography (HPLC) (Thayer and Bjorkman 1990) have been widely used for quantifying carotenoids based on spectral absorption features with peaks in the blue spectral range between 400 and 500 nm (Wellburn 1994). However, such approaches are time consuming and expensive and they require destructive sampling of leaf tissue and thus do not allow catching changes in pigments over time in a single leaf (Gitelson et al. 2006), nor do they allow for monitoring over large extents.

As a non-destructive method, remote sensing offers one of the most attractive alternative options for this purpose. There are two main approaches to use hyperspectral reflectance: the first approach is through the numerical inversion of radiative transfer models (RTM), while the other one relies on hyperspectral indices. Although several studies have reported that the model inversion methods using RTM have successfully estimated leaf optical properties (Dawson et al. 1998; Di Vittorio 2009; Féret et al. 2008; Fukshansky et al. 1993; Ganapol et al. 1998; Jacquemoud and Baret 1990; Zarco-Tejada et al. 2005), the approach has apparent drawbacks. For instance, Di Vittorio (2009) pointed out that the existing RTMs such as LIBERTY (Dawson et al. 1998), PROSPECT (Féret et al. 2008; Jacquemoud and Baret 1990), four-flux model (Fukshansky et al. 1993) and LEAFMOD (Ganapol et al. 1998), do not accurately represent leaf cellular structure. Furthermore, the well-known "ill-posed" problem of the approach also makes it difficult to estimate leaf structural and biochemical parameters with acceptable accuracy (Li and Wang 2013).

Compared with the inversion approach, the application of hyperspectral indices is another convenient choice and has been widely examined. For quantifying carotenoids content, several studies have also been undertaken to develop hyperspectral indices based on the ratios of reflectance bands (Blackburn 1998; Chappelle et al. 1992; Datt 1998; Gitelson et al. 2002; Sonobe and Wang 2017). However, less indices explicitly focusing on carotenoids were reported than indices focusing on chlorophyll. One major reason for this is the fact that chlorophyll and carotenoids have largely overlapping absorption features in the visible domain, which makes it difficult to retrieve information about each pigment type independently. As chlorophyll typically strongly dominates in healthy leaves, capturing a carotenoids-specific signal is challenging. Among the reported hyperspectral indices, a small peak of reflectance around 470 – 530 nm has been widely used for assessing carotenoids content (Blackburn 1998; Chappelle et al. 1992; Gitelson et al. 2002). In addition, indices based on normalized differences (ND type) (Blackburn 1998; Sonobe and Wang 2017) or modified types were proposed (Datt 1998). Furthermore, inverse reflectance differences (ID) such as the carotenoids reflectance index (CRI), based on reflectance at 510 nm and 550 nm or 770 nm, have been developed based on experiments using juvenile, mature and senescent leaves of Norway maple (*Acer platanoides*) and horse chestnut (*Aesculus hippocastanum*) (Gitelson et al. 2002). After having extensively evaluated indices of Chappelle, PRI, Blackburn1, Blackburn2 and Datt, Gitelson et al. (2002) proposed three carotenoids reflectance indices for assessing carotenoids. They are, namely, R_{NIR}/R_{510}, where NIR is the mean reflectance between 760 nm and 800 nm, CRI550, and CRI700, with the reflectance longer than 700 nm being used to minimize the effect of chlorophylls (Gitelson et al. 2002). Gitelson et al. (2006) conducted a further experiment using anthocyanin-free juvenile, mature and senescent leaves collected from 1992 to 2005 and developed two improved indices, including $(1/R_{510\text{-}520}-1/R_{560\text{-}570})*R_{NIR}$ and $(1/R_{510\text{-}520}-1/R_{690\text{-}710})*R_{NIR}$. Recently, Fassnacht et al. (2015) proposed three angular vegetation indices (Fassnacht et al. 2012) and merged vegetation indices, which they claimed to have advantages over reported indices in the assessment of carotenoids. However, few hyperspectral indices for assessing carotenoids have undergone extensive evaluations through various independent datasets and have yet to reach consensus.

In this study, we have evaluated 13 published indices for estimating the carotenoids content of broadleaved plant species using a series of independent datasets with simultaneous measurements of hyperspectral reflectance and carotenoids content. This includes two field datasets, with one dataset obtained from natural beech (*Fagus creanata*) leaves in Mt. Naeba, (Niigata Prefecture, Japan) and another with a total of 32 different deciduous tree species in Nakagawane (Shizuoka Prefecture, Japan), as well as two publicly available datasets of LOPEX (Hosgood et al., 1994) and

ANGERS (Feret et al., 2008) both with 23 broadleaved species. The overarching goal of this study is to identify a generally applicable index to assess the dynamics of carotenoids in broadleaved plant species. Aside from the earlier indices proposed in the literature, we attempted to develop new hyperspectral indices that are applicable to all four datasets.

2. Materials and methods

2.1 Study sites

In Japan, deciduous forests are classified into three types within two climatic zones including cool temperature mixed deciduous broadleaf/conifer forest, cool temperature deciduous broadleaf forest and warm temperature deciduous broadleaf forest (Kira 1991; Nakashizuka and Iida 1995). Earlier studies have revealed the historical backgrounds responsible for the climatic and vegetation differences between the area adjacent to the Sea of Japan and that adjacent to the Pacific Ocean (Iwasaki et al. 2012; Takahara and Takeoka 1992). Beech (*Fagus crenata*) is almost a mono-dominant species of cool temperate deciduous forests in western Japan (Nakashizuka 1987), while dominant species in eastern Japan are now very fragmented and possess less beech (Nozaki and Okutomi 1990). In this study, two forests in alpine cold-temperate climate areas were selected as the study sites: the Naeba Mountains, Niigata (Japan; Lat. 36° 50'N and Long. 138°41'E, adjacent to the Sea of Japan). Nakagawane is a university forest associated with Shizuoka University (Japan; Lat. 35° 04'N and Long. 138°06'E, facing the Pacific Ocean coast).

2.2 Measurements and datasets

Leaf sampling was conducted using the detached branch method (Jiang et al. 2008; Rowan and Mars 2003) from June 2007 to August 2013 in Mt. Naeba, while it was carried out during the summer of 2014 at the Nakagawane site. For sampling, leafy branches were cut predawn, recut under water and stored under humid conditions in dim light until analyzed (Wang et al. 2008).

The spectral reflectance and carotenoids contents of leaves were measured within three days after sampling. Reflectance was obtained using a FieldSpec spectrometer (Analytical Spectral Devices

Inc., Boulder, CO, USA) that had been equipped with a leaf clip. Leaf discs for carotenoids content measurements were punched after spectral measurements and then frozen in liquid nitrogen before they were analyzed using dual-beam scanning UV-VIS spectrophotometers (Ultrospec 3300 pro, Biosciences, USA). Carotenoids contents were calculated using Wellburn's method (Wellburn 1994). The equations used in this method for quantifying Chlorophyll a (Ch_a, μg/ml), Chlorophyll b (Ch_b, μg/ml), and carotenoids (C_{car}, μg/ml) in 80% acetone extracts are:

$$C_{car} = (1000 * A_{470} - 1.82 * Ch_a - 85.02 * Ch_b)/198 \quad (1)$$

$$Ch_a = 12.25 * A_{663.2} - 279 * A_{646.8} \quad (2)$$

$$Ch_b = 21.5 * A_{646.8} - 5.1 * A_{663.2} \quad (3)$$

where *A* is absorbance and the suffixes are wavelength (nm).

While only leaves of beech (*Fagus crenata*) were investigated in Naeba, a total of 29 different deciduous species (Table 1) were examined in Nakagawane. Additional data of broadleaf species were obtained from two experimental campaigns including LOPEX (Hosgood et al. 1994) and ANGERS (Féret et al. 2008), both containing measurements of 23 broadleaf species (Table 1). In the LOPEX dataset, five repetitions of spectral measurements were done for each physical and biological measurement (Hosgood et al. 1994). The averaged reflectance values were adopted for further analysis.

Table 1. Summary of datasets used in this study.

Naeba		Nakagawane		LOPEX		ANGERS	
Species	Number of samples	Species	Number of samples	Species	Number of samples	Species	Number of samples
Fagus crenata	135	*Acer argutum*	6	*Acer pseudoplatanus*	2	*Acer negundo*	2
		Acer capillipes	5	*Alnus glutinosa*	1	*Acer pseudoplatanus*	181
		Acer micranthum	6	*Betula pendula*	1	*Alnus glutinosa L.*	2
		Acer mono	6	*Castanea sativa*	2	*Calicarpa bodinieri*	2
		Acer nipponicum	5	*Corylus avellana*	2	*Castanea sativa*	2
		Acer rufinerve	6	*Fagus sylvatica*	1	*Cercis siliquastrum*	2
		Acer sieboldianum	6	*Ficus carica*	1	*Cornus alba*	6
		Carpinus japonica	3	*Fraxinus excelsior*	2	*Corylus maxima*	3
		Clethra barbinervis	1	*Hedera helix*	1	*Cotinus coggygria*	1
		Enkianthus campanulatus	2	*Juglans regia*	1	*Hydrangea macrophylla*	2
		Euptelea polyandra	3	*Morus alba*	1	*Liquidambar styraciflua*	2
		Ilex macropoda	3	*Morus nigra*	1	*Liriodendron tulipifera*	2
		Kalopanax septemlobus	3	*Populus tremula*	1	*Parthenocissus tricuspidata*	2
		Lindera praecox	6	*Populus x canadensis*	1	*Populus alba*	2
		Lindera umbellata	3	*Prunus armeniaca*	2	*Quercus palustris*	2
		Magnolia obovata	6	*Prunus serotina*	1	*Rhus typhina*	2
		Pourthiaea villosa	5	*Quercus pubescens*	2	*Robinia pseudoacacia*	6
		Prunus buergeriana	3	*Quercus rubra*	1	*Salix atrocinerea*	2
		Prunus maximowiczii	3	*Robinia pseudoacacia*	2	*Syringa vulgaris*	2
		Pterostyrax hispida	5	*Salix alba*	1	*Tilia tomentosa*	2
		Quercus crispula	3	*Tilia platyphyllos*	2	*Viburnum plicatum*	2
		Rhododendron dilatatum	3	*Ulmus glabra*	1	*Vitis vinifera*	2
		Rhododendron quinquefolium	3	*Vitis vinifera*	3	*Weigela florida*	2

Styrax japonica	3
Styrax shiraianus	5
Symplocos coreana	5
Tilia japonica	3
Viburnum furcatum	6
Viburnum wrightii	6

3

2.3 Hyperspectral indices for assessing carotenoids

In this study, thirteen earlier reported hyperspectral indices (Table 2) were evaluated for their correlations with carotenoids contents based on the aforementioned four datasets. In previous studies, Chappelle, PRI, Blackburn1, Blackburn2, Datt, Giltson1, CRI550 and CRI700 were evaluated based on the carotenoids contents from 1.6 to 25.1 nmol/cm^2 (Gitelson et al. 2002), while CARrededge and CARgreen were assessed from 30 to 190 mg/m^2 (Gitelson et al. 2006). Furthermore, Chappelle, CARrededge, AVIcar, Fassnacht1 and Fassnacht2 have been examined under the contents of 15.96 to 137.20 mg m^{-2} (Fassnacht et al., 2015).

Besides these earlier suggested indices, all band combinations of six common types of indices (Cao et al. 2015; Gitelson et al. 2001; Gitelson et al. 2002; le Maire et al. 2008; Sonobe and Wang 2016; Wang and Li 2012) based on either original reflectance or derivative spectra were screened to identify applicable indices for all datasets. These indices were evaluated by regression analysis with carotenoids contents.

In addition, angular vegetation indices (AVIs), which are calculated as angles within a triangle based on three points of a reflectance spectrum (Fassnacht et al. 2012), were also examined. In this study, the wavelength was expressed in nanometers, while reflectance values (0–1) were multiplied by 10000 according to Fassnacht et al. (2015). The best combinations of AVI indices were searched from all possible combinations with steps of 5 nm.

Moreover, all indices were further evaluated with different spectral resolutions at 10 nm, which is almost equal to the spectral resolution of Earth Observing-1 Hyperion (Kruse et al. 2003) in order to clarify the potential for efficient large extent monitoring.

Table 2. Hyperspectral indices evaluated in this study. The $R_{wavelength}$ indicate the reflectance at this wavelength. The use of scaled index values from 0-1(scale) and the angular vegetation index (AVI) were proposed by Fassnacht et al. (2015). Gitelson et al. (2006) defined NIR as the reflectance at 760-800 nm.

Index	Formula	Reference
Chappelle	R760/R500	(Chappelle et al. 1992)
PRI	(R530-R570)/ (R530-R570)	(Gamon et al. 1992)
Blackburn1	R800/R470	(Blackburn 1998)
Blackburn2	(R800-R470)/(R800+R470)	
Datt	0.0049[R672/(R550*R708)]0.748	(Datt 1998)
Gitelson1	R_{NIR}/R_{510}	(Gitelson et al. 2002)
CRI550	$1/R_{510}-1/R_{550}$	
CRI700	$1/R_{510}-1/R_{700}$	
CARrededge	$(1/R_{510\text{-}520}-1/R_{690\text{-}710})*R_{NIR}$	(Gitelson et al. 2006)
CARgreen	$(1/R_{510\text{-}520}-1/R_{560\text{-}570})*R_{NIR}$	
AVIcar	$AVI2(R_{410}, R_{530}, R_{550})$	
Fassnacht1	scale(AVIcar) + scale(Chappelle)	(Fassnacht et al. 2015)
Fassnacht2	scale(AVIcar) + scale(CARred-edge)	
R	R_{λ_1}	This study
D	$R_{\lambda_1} - R_{\lambda_2}$	
SR	$R_{\lambda_1}/R_{\lambda_2}$	
ND	$(R_{\lambda_1} - R_{\lambda_2})/(R_{\lambda_1} + R_{\lambda_2})$	
DDn	$2R_{\lambda_1} - R_{\lambda_1-\lambda_2} - R_{\lambda_1+\lambda_2}$	
ID	$1/R_{\lambda_1} - 1/R_{\lambda_2}$	
dR	dR_{λ_1}	
dD	$dR_{\lambda_1} - dR_{\lambda_2}$	
dSR	$dR_{\lambda_1}/dR_{\lambda_2}$	
dND	$(dR_{\lambda_1} - dR_{\lambda_2})/(dR_{\lambda_e} + dR_{\lambda_2})$	
dDDn	$2dR_{\lambda_1} - dR_{\lambda_1-\lambda_2} - dR_{\lambda_1+\lambda_2}$	
dID	$1/dR_{\lambda_1} - 1/dR_{\lambda_2}$	
AVI1	$a\cos\left(\frac{-c^2+a^2+b^2}{2ab}\right)$	
AVI2	$a\,cos\left(\frac{-b^2+a^2+c^2}{2ac}\right)$	
AVI3	$a\,cos\left(\frac{-a^2+b^2+c^2}{2bc}\right)$	

2.4 Statistical criteria

Both linear and exponential regression models were used for assessing the published and newly developed indices. Details are as follows,

Linear regression model:

$$C_{car} = a * x + b \qquad (4)$$

Exponential regression model:

$$C_{car} = ae^{bx} \qquad (5)$$

where a and b are coefficients and x refers to a given hyperspectral index

The ratio of performance to deviation (RPD) (Williams 1987) was applied to evaluate the performances of the hyperspectral indices, and the indices were classified into three categories (Chang et al. 2001): Category A (RPD > 2.0), Category B (1.4 ≤ RPD ≤ 2.0) and Category C (RPD < 1.4). RPD was calculated using equation (6):

$$\mathrm{RPD} = {}^{\mathrm{Sd}}/_{\mathrm{SEP}} \qquad (6)$$

where SEP is standard error of prediction, which is calculated as the root mean squared error, and Sd is standard deviation of the carotenoids content. The indices ranked as Category A and B possess the potential to quantify carotenoids content, while indices assigned to Category C can not reliably predict carotenoid content (Chang et al., 2001).

Aside from RPD, the widely applicable information criterion (WAIC) (Watanabe 2010), the root mean square errors (RMSE) and the coefficient of determination (R^2) were also calculated to evaluate the fit between the index values and carotenoids content. And the final selection of the best indices was based on the WAIC.

3 Results

3.1 Carotenoids content of each dataset

Table 3 summarizes the main characteristics of the four datasets used in this study. The range of leaf carotenoids content of all datasets varied between 0.00 (two samples of *Cornus alba*) and 25.28 μg/cm² (*Acer pseudoplatanus*), with an average value of 7.97 μg/cm². The averaged values and standard deviations of carotenoids contents were 7.13 ±1.79, 7.28±1.70, 10.28±2.91 and 8.50±4.91 μg/cm², respectively for measurements of Naeba, Nakagawane, LOPEX and ANGERS. The highest mean value was observed in LOPEX, while the highest diversity was observed in ANGERS. According to their skewness, the measurements obtained for Naeba and LOPEX have better fits with normal distributions than those obtained for Nakagawane and ANGERS.

Table 3 Main characteristics of the datasets used in this study.

	Dataset				
	Naeba	Nakagawane	LOPEX	ANGERS	All
Number of samples	135	123	33	233	524
Mean value (μg/cm²)	7.13	7.28	10.28	8.50	7.97
Maximum value (μg/cm²)	12.37	13.49	16.16	25.28	25.28
Minimum value (μg/cm²)	2.44	3.63	3.45	0.00	0.00
Standard deviation (μg/cm²)	1.79	1.70	2.91	4.91	3.67
Median value (μg/cm²)	7.15	7.09	10.52	7.28	7.30
Skewness	0.16	0.86	-0.18	1.48	1.95

As expected, we observed high correlations between carotenoids and chlorophyll content(Figure 1). The determination coefficients (R^2) ranged from R^2 = 0.620 for the LOPEX site to R^2 = 0.922 for the ANGERS site, suggesting that all measurements contained in the four datasets were generally taken under non-stressful conditions. Such linear correlations between chlorophylls and carotenoids typically do not occur for stressful conditions, as in these situations the concentrations of carotenoids would generally increase with concomitant decrease in chlorophyll concentrations.

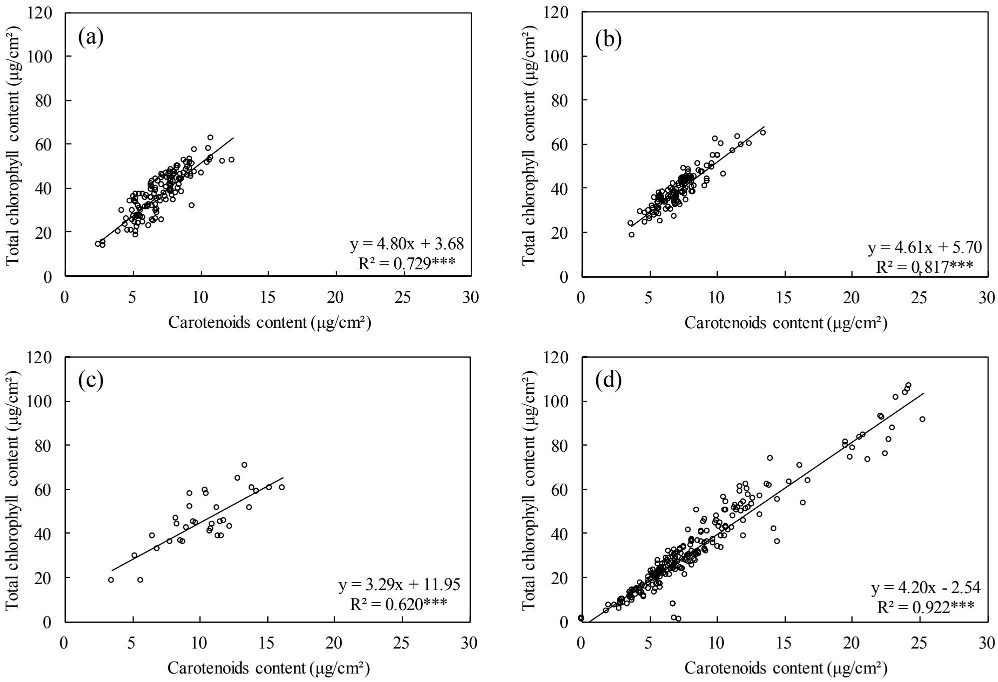

Figure 1. The relationship between chlorophyll and carotenoids content.

3.2 Correlations between reflectance and carotenoid contents

Figure 2 shows the correlations of each wavelength of reflectance or first-order derivative spectra with carotenoids. The features of the relationships between carotenoids content and reflectance were similar among all datasets, with apparent inflection points (negative correlations) near 540 and 720 nm and one peak near 680 nm. However, the magnitudes of correlation coefficients varied greatly, e.g., the coefficients near the first inflection point changed from -0.725 at 535 nm (ANGERS) to -0.554 at 544 nm (Nakagawane), while those near the second inflection points fluctuated from -0.849 at 714 nm (ANGERS) to -0.539 at 706 nm (LOPEX). Similarly, coefficients near the peaks ranged from -0.261 at 673 nm (ANGERS) to 0.130 at 676 nm (LOPEX). In contrast, several peaks (positive correlations) and troughs (negative correlations) were identified in the relationships between carotenoids content and first-order derivative spectra. These correlations proofed to be more stable across the datasets as compared to those of reflectance.

The differences or the normalized differences of the first-order derivative spectra at 516 – 517 nm and 744 – 750 nm were particularly interesting. The wavelengths of 516 – 517 nm correspond to the green peaks of deciduous species, indicating that the green peak is also useful for estimating the carotenoids content of deciduous species, similar to the wavelengths at 744 – 750 nm, which correspond to the ends of the red edge for the examined broadleaved species.

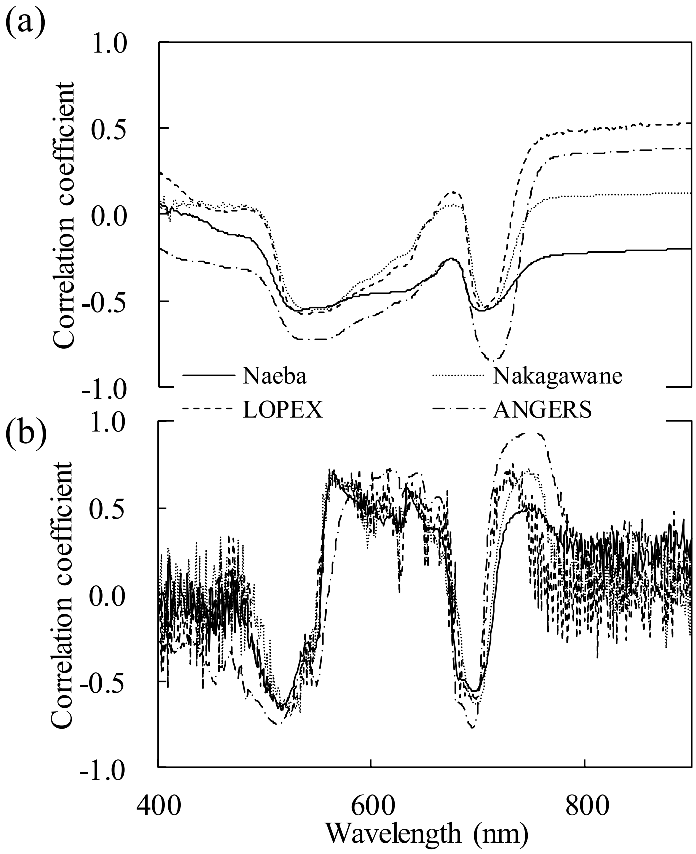

Figure 2. Correlations between carotenoids content and (a) reflectance or (b) first-order derivative.

3.3 Performances of hyperspectral indices

The results of the 13 earlier suggested indices including the R^2, RMSE, WAIC, RPD and category assignment are presented in Table 4. Chappelle, Datt, Gitelson1, AVIcar, Fassnacht1 and Fassnacht2 showed good performances for the ANGERS dataset and were categorized as 'B'. Especially, indices marked as Datt, Fassnacht1 and Fassnacht2 were significantly correlated with the measurements of all datasets. Figure 3 has further illustrated the relationships between the three published indices and carotenoids content, which were significantly correlated with the measurements of the four datasets.

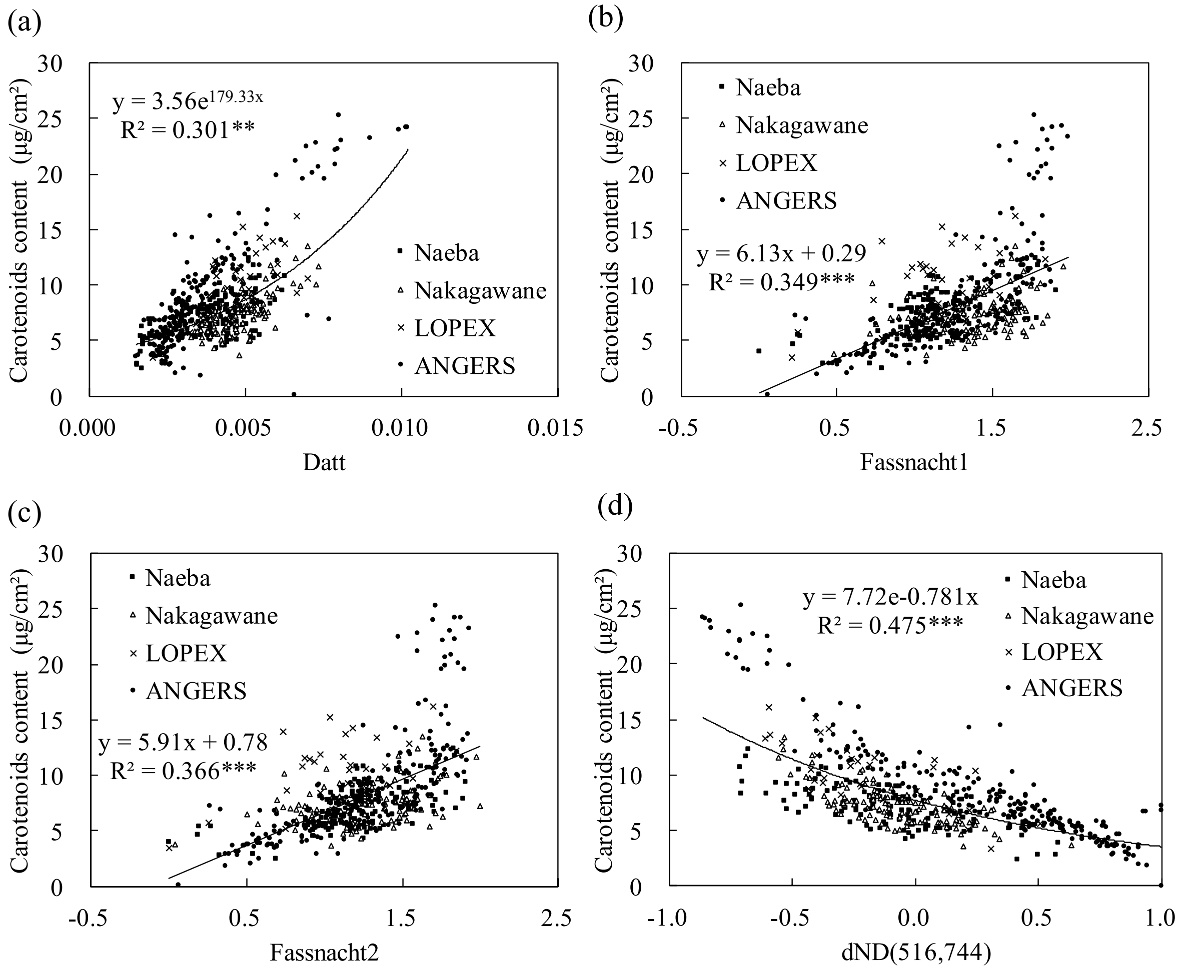

Figure 3. The relationship between hyperspectral indices and carotenoids content.

However, no indices were categorized as ‘A’ or ‘B’ when applying the datasets from Naeba, Nakagawane or LOPEX. Furthermore, no indices showed good performances when all datasets were merged. All indices were categorized as “C”, even though Datt, Gitelson1, AVIcar, and Fassnacht1 had significant correlations with carotenoids ($P < 0.001$).

Table 4 Performance of hyperspectral indices in different datasets (*: $p<0.001$, **: $p<0.01$, *: $p<0.05$).**

	Naeba						Nakagawane						LOPEX					
	R^2		RMSE	WAIC	RPD	Category	R^2		RMSE	WAIC	RPD	Category	R^2		RMSE	WAIC	RPD	Category
Chappelle	0.021		1.760	540.8	1.01	C	0.006		1.69	482.5	1.01	C	0.076		2.76	164.9	1.06	C
PRI	0.015		1.770	541.4	1.01	C	0.001		1.69	482.5	1.00	C	0.027		2.83	166.2	1.03	C
Blackburn1	0.001		1.780	543.2	1.00	C	0.000		1.69	482.7	1.00	C	0.051		2.80	165.5	1.04	C
Blackburn2	0.000		1.780	543.5	1.00	C	0.000		1.69	481.7	1.00	C	0.062		2.78	165.6	1.05	C
Datt	0.260	***	1.530	503.1	1.17	C	0.447	***	1.26	409.7	1.35	C	0.346	***	2.32	153.7	1.26	C
Gitelson1	0.114	***	1.680	527.1	1.07	C	0.097		1.61	470.4	1.06	C	0.222	**	2.53	159.6	1.15	C
CRI550	0.043		1.740	537.1	1.03	C	0.071		1.63	473.9	1.04	C	0.024		2.83	167.0	1.03	C
CRI700	0.042		1.740	537.5	1.03	C	0.017		1.68	480.4	1.01	C	0.006		2.86	167.0	1.02	C
CARrededge	0.098		1.690	529.3	1.06	C	0.052		1.65	475.7	1.03	C	0.125	*	2.68	162.7	1.09	C
CARgreen	0.045		1.740	537.8	1.03	C	0.047		1.65	476.3	1.03	C	0.010		2.86	166.5	1.02	C
AVIcar	0.400	***	1.380	477.2	1.30	C	0.222	***	1.49	452.0	1.14	C	0.331	***	2.35	154.2	1.24	C
Fassnacht1	0.213	***	1.580	510.9	1.13	C	0.111	***	1.60	469.4	1.07	C	0.248	***	2.49	157.6	1.17	C
Fassnacht2	0.305	***	1.490	493.8	1.20	C	0.150	***	1.56	462.6	1.09	C	0.280	***	2.43	156.4	1.20	C
dD(516,750)	0.524	***	1.260	447.1	1.43	B	0.499	***	1.21	392.8	1.44	B	0.518	***	1.99	142.7	1.46	B
dD(517,750)	0.518	***	1.270	450.2	1.41	B	0.508	***	1.19	387.9	1.47	B	0.523	***	1.98	142.7	1.47	B
dD(516,747)	0.498	***	1.260	450.3	1.42	B	0.518	***	1.18	385.0	1.48	B	0.501	***	2.03	143.9	1.44	B
dD(517,747)	0.490	***	1.270	452.5	1.41	B	0.525	***	1.16	382.8	1.50	B	0.501	***	2.03	144.1	1.44	B
dD(516,744)	0.487	***	1.280	453.5	1.40	B	0.516	***	1.18	387.2	1.48	B	0.513	***	2.00	143.3	1.46	B
dND(516,744)	0.492	***	1.270	451.8	1.41	B	0.514	***	1.19	389.9	1.46	B	0.494	***	2.04	145.6	1.43	B
dND(516,741)	0.490	***	1.270	451.6	1.41	B	0.504	***	1.21	393.0	1.44	B	0.655	***	1.84	137.2	1.58	B

	ANGERS						All					
	R^2		RMSE	WAIC	RPD	Category	R^2		RMSE	WAIC	RPD	Category
Chappelle	0.554	***	3.48	1232.6	1.40	B	0.072		3.51	2796.1	1.04	C
PRI	0.201	***	4.34	1339.1	1.12	C	0.049		3.55	2808.1	1.03	C
Blackburn1	0.453	***	3.85	1278.8	1.26	C	0.031		3.59	2817.7	1.02	C
Blackburn2	0.530	***	4.11	1296.7	1.19	C	0.064		3.52	2800.8	1.03	C
Datt	0.728	***	2.53	1091.0	1.92	B	0.301	***	2.63	2497.5	1.38	C
Gitelson1	0.640	***	2.72	1120.4	1.79	B	0.177	***	3.30	2734.0	1.10	C
CRI550	0.112	***	4.58	1362.3	1.06	C	0.008		3.63	2829.7	1.01	C
CRI700	0.107	***	4.59	1363.6	1.06	C	0.008		3.63	2830.5	1.01	C
CARrededge	0.453	***	3.59	1250.6	1.36	C	0.086		3.48	2787.9	1.05	C
CARgreen	0.164	***	4.44	1347.7	1.10	C	0.011		3.62	2829.3	1.01	C
AVIcar	0.613	***	2.84	1130.0	1.72	B	0.227	***	3.20	2700.2	1.14	C
Fassnacht1	0.622	***	2.98	1157.7	1.64	B	0.349	***	2.94	2610.5	1.24	C
Fassnacht2	0.598	***	3.16	1188.6	1.54	B	0.366	***	2.90	2597.0	1.26	C
dD(516,750)	0.746	***	2.45	1075.2	1.99	B	0.408	***	2.60	2452.7	1.40	B
dD(517,750)	0.743	***	2.46	1078.1	1.98	B	0.397	***	2.61	2452.6	1.40	B
dD(516,747)	0.758	***	2.39	1063.3	2.04	A	0.417	***	2.57	2443.1	1.42	B
dD(517,747)	0.756	***	2.40	1065.5	2.03	A	0.405	***	2.58	2443.4	1.41	B
dD(516,744)	0.769	***	2.34	1054.1	2.08	A	0.423	***	2.57	2442.3	1.42	B
dND(516,744)	0.841	***	1.94	966.6	2.51	A	0.475	***	2.51	2430.1	1.45	B
dND(516,741)	0.804	***	2.15	1015.0	2.27	A	0.472	***	2.59	2462.8	1.41	B

2

In addition to the earlier suggested indices, six types of indices, as well as AVIs that were calculated from the original reflectance spectra or first-order derivative spectra were examined for their applications for each dataset. From all examined indices, seven were identified to quantify carotenoids contents based on RPD, including five combinations of dD types (Figure 4a) and 2 combinations of dND types (Figure 4b). They are based on the first-order derivative at 516 – 517 and 744 – 750 nm. Among them, dD(516,750) (WAIC=447.1, RMSE=1.26 μg/cm²), dD(517,747) (WAIC=382.8, RMSE=1.16 μg/cm²), dND(516,741) (WAIC=137.2, RMSE=1.84 μg/cm²) and dND(516,744) (WAIC=966.6, RMSE=1.94 μg/cm²) were the best indices based on the WAIC（Table 4）. Their good performance was also confirmed by the lowest RMSE values for the datasets obtained from Naeba, Nakagawane, LOPEX and ANGERS, respectively (Table 4). When all the datasets were combined, the best performance was observed for dND (516,744) (see Figure 3d, R^2=0.474, WAIC=2430.1, and RPD=1.45) using exponential regression. The residuals of the model were normally distributed (p = 0.211, based on Kolmogorov-Smirnov test).

There were no AVIs applicable for all datasets, although a total of 86 combinations of AVI1, 4 of AVI2 and 791 of AVI3 were applicable for three datasets. In detail, AVI1 (555, 575, 780), AVI1 (545, 565, 775), AVI1 (545, 750, 775) and AVI1 (725, 750, 780) were the best indices for the datasets obtained in Naeba (R^2=0.518), Nakagawane (R^2=0.550), LOPEX (R^2=0.688) and ANGERS (R^2=0.873), respectively. Furthermore, the AVI3 (740, 760, 780) was the only well- performing angular vegetation index (categorized as ‘B’ when all data were considered together (determination coefficient of 0.521). However, its determination coefficients with the individual datasets were varied with 0.251, 0.463, 0.479 and 0.851 for Naeba, Nakagawane, LOPEX and ANGERS, respectively. Hence this index was applicable only for LOPEX and

ANGERS.

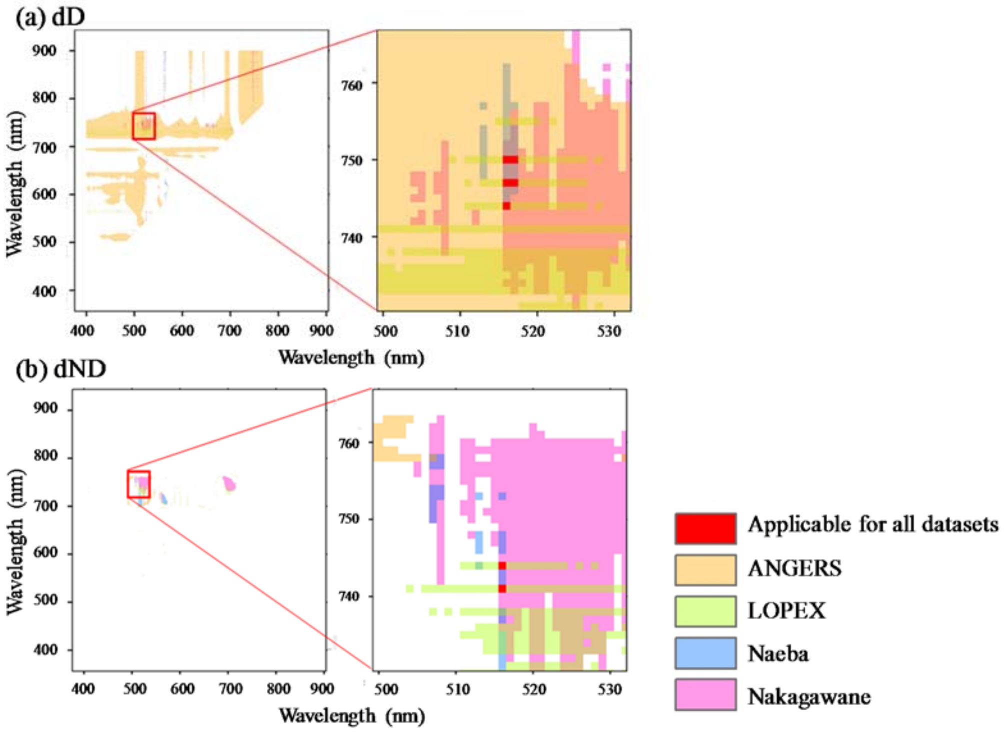

Figure 4. Categories based on RPD with different types of indices using first-order derivative spectra.

3.4 Evaluation of indices with down-scaled spectral resolution

Table 5 shows the results of the proposed indices with down-scaled spectral resolutions. The examined indices were categorized as 'B' for the datasets from Nakagawane, LOPEX and ANGERS, and were categorized as 'C' for those of Naeba (RPD=1.38–1.39 for dD-type indices and RPD=1.35 for dND-type indices). However, significant determination coefficients (p<0.001) were observed for these indices with R^2= 0.468–0.499 for dD-type indices and R^2=0.450 for dND-type indices. Thus, the earlier reported robustness of these indices was confirmed when testing the reflectance data with down-scaled resolution.

Table 5 Performance of selected indices for downscaled datasets (*: p<0.001).**

	Naeba						Nakagawane					
	R^2		RMSE	WAIC	RPD	Category	R^2		RMSE	WAIC	RPD	Category
dD(516,750)	0.499	***	1.29	454.14	1.39	C	0.461	***	1.21	398.898	1.40	B
dD(517,750)	0.499	***	1.29	454.21	1.39	C	0.461	***	1.21	398.916	1.40	B
dD(516,747)	0.468	***	1.30	457.89	1.38	C	0.502	***	1.15	387.027	1.47	B
dD(517,747)	0.468	***	1.30	457.85	1.38	C	0.502	***	1.15	387.78	1.47	B
dD(516,744)	0.468	***	1.30	456.93	1.38	C	0.502	***	1.15	387.005	1.47	B
dND(516,744)	0.450	***	1.32	462.78	1.35	C	0.527	***	1.13	382.957	1.50	B
dND(516,741)	0.450	***	1.32	462.81	1.35	C	0.527	***	1.13	382.836	1.50	B
	LOPEX						ANGERS					
	R^2		RMSE	WAIC	RPD	Category	R^2		RMSE	WAIC	RPD	Category
dD(516,750)	0.635	***	1.95	141.22	1.50	B	0.619	***	3.03	1183.51	1.62	B
dD(517,750)	0.635	***	1.95	141.75	1.50	B	0.619	***	3.03	1182.47	1.62	B
dD(516,747)	0.680	***	1.78	135.16	1.64	B	0.703	***	2.67	1124.71	1.84	B
dD(517,747)	0.680	***	1.78	135.27	1.64	B	0.703	***	2.67	1125.51	1.84	B
dD(516,744)	0.680	***	1.78	134.6	1.64	B	0.703	***	2.67	1125.97	1.84	B
dND(516,744)	0.594	***	1.83	137.14	1.59	B	0.889	***	1.63	895.156	3.01	A
dND(516,741)	0.594	***	1.83	137.52	1.59	B	0.889	***	1.63	895.676	3.01	A

	All					
	R^2		RMSE	WAIC	RPD	Category
dD(516,750)	0.385	***	2.88	2599.7	1.28	C
dD(517,750)	0.385	***	2.88	2599.6	1.28	C
dD(516,747)	0.428	***	2.77	2562.6	1.32	C
dD(517,747)	0.428	***	2.77	2561.5	1.32	C
dD(516,744)	0.428	***	2.77	2560.9	1.32	C
dND(516,744)	0.522	***	2.54	2467.9	1.45	B
dND(516,741)	0.522	***	2.54	2467.1	1.45	B

4 Discussion

The Datt index was developed based on 20 species including Eucalyptus and non-Eucalyptus species (*Angophora costata*) (Datt 1998) and significant correlations were observed for all datasets, suggesting a certain robustness. Hence, even though no specific absorption coefficient of carotenoids has been included in PROSPECT 5 for reflectance at wavelengths longer than 560 nm (Féret et al. 2008), the good performance of the Datt index, which includes reflectance at 672 and 708 nm, might suggest that the reflectance around the red edge has a robust correlation with carotenoids content. Furthermore, it was the only applicable index for ANGERS. Because most indices examined were derived from measurements of Norway maple (*Acer platanoides*) except for the indices of Chappelle (soybean), PRI ($\Delta F/F_m$' of 20 species), Datt, Blackburn1 and Blackburn2 (bracken), their performances were relatively high when evaluated with ANGERS, in which measurements of *Acer pseudoplatanus* and *Acer negundo* account for approximately 78.5 % of all measurements. Furthermore, the indices Gitelson1, AVIcar, Fassnacht1 and Fassnacht2 were significantly correlated with the carotenoids contents of the Nakagawane and ANGERS datasets which include samples of the *Acer* family. However, they were categorized as 'C' for all datasets except for ANGERS according to RPD, and thus their applications were restrictive.

Interestingly, significant correlations were also obtained between Naeba measurements and the AVIcar, Fassnacht1 and Fassnacht2 indices. One reason for this might be that these indices were also amongst other developed with a Beech dataset. However, despite their significant correlations, they were categorized as 'C' based on RPD values. When the regression line was applied for all data, the published indices showed a tendency to underestimate high carotenoid values in the ANGERS data, which is mainly composed of *Acer*, as compared to values for the measurements in Naeba, which is composed of beech leaves. This might make it difficult to monitor carotenoids contents in mixed deciduous forests using remote sensing data acquired from satellites or airborne sources.

Overall, extensive evaluations of published and newly developed hyperspectral indices in this study revealed that the differences or the normalized differences of the first-order derivative spectra at 516 – 517 nm and 744 – 750 nm delivered the best results. In a previous study, the reflectance of the green peak around 550 nm as well as the red edge position (REP) of rice leaves has been reported to be useful for measuring levels of pigments, including chlorophyll *a*, *b* and carotenoids (Chen et al. 2007). The wavelengths of 516 – 517 nm, identified in this study, correspond to the green peaks of broadleaved species, indicating that the green peak is also useful for estimating the carotenoids content of broadleaved species, similar to the wavelengths at 744 – 750 nm, which correspond to the ends of REP for the broadleaved species used in this study.

The robustness of newly identified hyperspectral indices has also been confirmed with downscaled resolutions (Table 5), suggesting that they may also be applicable if applied to satellite-borne hyperspectral data like Hyperion, which is an essential step towards large-scale monitoring. It has, however, to be considered that aside the reduced spectral resolution of spaceborne sensors, the reduced spatial resolution and a measurement at canopy instead of leaf level is likely to further complicate the retrieval of robust carotenoid content estimates with the suggested indices. This should be subject to further investigations.

Due to close linear relationships between chlorophyll contents and carotenoids of the datasets used in this study, all indices with a good potential to estimate carotenoids also showed high correlations with chlorophyll content. This might change under stress-condition where carotenoid content is typically increasing while chlorophyll content is decreasing (Dobrowski et al. 2005, Penuelas and Filella 1998). It is therefore recommended to conduct additional experiments during which the robustness of the suggested indices is evaluated with datasets that also contain samples obtained under various levels of stress.

5 Conclusions

In this study, robust indices for estimating carotenoids content were identified based on four independent datasets, including *in situ* measurements from two typical cold-temperate mountainous sites in Japan and the two well-knownLOPEX and ANGERS datasets. Besides 13 published indices, all band combinations of six common types of indices including reflectance or first-order derivative spectra at a given wavelength (R or dR), wavelength difference (D or dD), simple ratio (SR or dSR), normalized difference (ND or dND), double differences (DDn or dDDn) and Inverse reflectance differences (ID or dID) based on either original reflectance or derivative spectra were screened to identify applicable indices for all datasets.

Aside the earlier suggested indices we identified seven indices including dD (516,750), dD (517,750), dD (516,747), dD (517,747), dD (516,744), dND (516,744) and dND (516,741). The robustness of the hyperspectral indices across different datasets was evaluated using RPD values which were summarized in three quality classes A (best), B, and C (worst). While none of the earlier published indices were categorized as ‘A’ or ‘B’ when applying the datasets from Naeba, Nakagawane or LOPEX, our proposed indices reached good performances for all the datasets. In particular, dND (516,744) was the best index when all the datasets were combined (R^2=0.475, WAIC=2430.1 and RPD=1.45, Table 5). However, additional experiments are required to evaluate the robustness of these indices by using samples obtained under various levels of stress in future works.

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