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A Dataset of *in situ* SPAD Readings and *in vitro* Chlorophyll Concentration Analyses from *Quercus variabilis Bl.* in Mt. Funiu, China

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Abstract: Chlorophyll concentration (Chl) in plant leaves is a key indicator for monitoring plant health and dynamics, but direct chlorophyll concentration measuring (Chl) in laboratory (*in vitro*) is both expensive and laborious, which makes it prohibitive to conduct large scale Chl studies. An alternative approach is to develop a statistical relationship by measuring optical readings in field (*in situ*) and meanwhile measuring Chl in laboratory (*in vitro*). This dataset consists of three parts for a type of oak tree (*Quercus variabilis Bl.*), a dominant species in Mt. Funiu, Henan province, China. The first part is *in situ* SPAD readings in oak leaves, an optical measure with Minolta SPAD-502 meter, while the second part is *in vitro* chlorophyll concentration measurement (Chl) of the same oak leaves, with a two-step extraction and determination method for chlorophyll presented by Qiu *et al*. (2016). Totally, there were 31 samples for *in situ* SPAD readings, and 93 measurements for *in vitro* Chl (three Chl repeats for each SPAD). Finally, these 93 SPAD-Chl pairs data were used to develop various statistical relationships and the best-fit correlation was an exponential correlation (Chl ($\mu g/cm^2$) = $8.712e^{0.035 \times SPAD}$), with R^2= 0.927. This dataset is consisted of three files, with the data size of 31.6 KB.

Keywords: chlorophyll concentration; SPAD; *in situ*; *in vitro*; relationship; oak; *Quercus variabilis Bl.*

1 Introduction

Chlorophyll concentration (Chl) in plant leaves is a key indicator for monitoring plant health, but direct chlorophyll concentration measuring (Chl) in laboratory (*in vitro*) is both time- and labor-intensive, which makes it less applicable to large scale Chl studies. An alternative approach is to develop a statistical equation by measuring optical readings in the field (*in situ*) and meanwhile measuring Chl in the laboratory (*in vitro*).

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SPAD-502 is one of the optical chlorophyll meters and its reading is a unitless number between 0 and 100[1]. The earliest literature of SPAD application in China came into being in 1991[2], and many publications followed covering diverse plants from crops to vegetables, mostly in the field of intensive agriculture. However, the application of SPAD to forest study came relatively late and rare. The first literature about SPAD application in forest did not appear until 2005[3]. Trees from *Fagaceae* dominate most of forests and distributed very broadly in China (with an area of 161,271 km^2, accounting for 9.80% of the forest in China), but the study on these trees based on SPAD is just started recently and focuses on Northeast China[4–6]. The present dataset is the first case on SPAD-Chl of Fagaceae trees in middle of China.

2 Metadata of Dataset

Table 1 shows the metadata summary for the dataset of *in situ* SPAD readings, *in vitro* chlorophyll concentration measuring, and their relationship: *Quercus variabilis Bl.* in Mt. Funiu, China[7].

Table 1 Metadata summary of the dataset of *in situ* SPAD readings, *in vitro* chlorophyll concentration measuring, and their relationship: *Quercus variabilis Bl*. in Mt. Funiu, China

Items	Description
Dataset full name	Dataset of *in situ* SPAD readings, *in vitro* chlorophyll concentration measuring, and their relationship: *Quercus variabilis Bl.* in Mt. Funiu, China
Dataset short name	Chlorophyll_Quercus variabilis *Bl*._Mt.Funiu
Authors	Wang, Z. X. L-5255-2016, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, wangzx@igsnrr.ac.cn Li, F. L-3424-2018, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, lif@igsnrr.ac.cn
Geographic region	Luanchuan county, Henan province (in Mt. Funiu region), 33°52′34.16″N, 111°45′10.79″E
Sampling date	September 19, 2018
Data format	.shp, .kmz, .xlsx
Data size	31.6 KB (28.4 KB after compression)
Data files	(1) Site location: 1.shp file, 1 .kml file; (2) SPAD and Chlorophyll measurements: 1 .xlsx file
Foundation	Ministry of Science and Technology of P. R. China (2016YFA0600201)
Data publisher	Global Change Research Data Publishing & Repository, http://www.geodoi.ac.cn
Address	No. 11A, Datun Road, Chaoyang District, Beijing 100101, China
Data sharing policy	***Data*** from the Global Change Research Data Publishing & Repository includes metadata, datasets (data products), and publications (in this case, in the *Journal of Global Change Data* & *Discovery*). ***Data*** sharing policy includes: (1) ***Data*** are openly available and can be free downloaded via the Internet; (2) End users are encouraged to use ***Data*** subject to citation; (3) Users, who are by definition also value-added service providers, are welcome to redistribute ***Data*** subject to written permission from the GCdataPR Editorial Office and the issuance of a ***Data*** redistribution license; and (4) If ***Data*** are used to compile new datasets, the 'ten percent principal' should be followed such that ***Data*** records utilized should not surpass 10% of the new dataset contents, while sources should be clearly noted in suitable places in the new dataset[8]

3 Methods

3.1 *In Situ* Sampling and Optical Measurement of Leaf Chlorophyll: SPAD

(1) Site and Date of sampling: The oak leaf sampling was conducted nearby the New South Village Reservoir, Luanchuan county, Henan province (in Mt. Funiu region), on September

19, 2018 (Table 1). It should be made clear that the purpose of this sampling is to build a SPAD-Chl relationship; therefore, an ideal sampling is to collect as diverse leaves as possible for oak. Once leaves leaving crown, the rest of processing of leaves should be conducted in shade to minimize the exposure of leaves under sunshine.

(2) Pre-processing of leaves: Leaves were rinsed with tap water to clean dust.

(3) Ranking and classification: Rank all leaves according to greenness, and classify them into 31 groups (samples). Each sample consists of 4–6 leaves.

(4) Measurement of SPAD: Each leaf was measured at ten locations (except midrib) using SPAD-502 meter. The leaves with large SPAD deviation from group average (>1.5) were reclassified into relevant groups. After adjustments, we got 31 *in situ* samples.

3.2 Preservation of Samples

The key to preserve the freshness of leaf is to keep it in dark, in low temperature, and wet. It is ideal to use liquid nitrogen or dry ice, but both are constrained materials due to their high volatility. After trials and errors, we used wet ice to reserve the samples: wrap plastic sample bag with wet towel; and wrap wet ice (small bottle) with dry towels to prevent frost resulted from direct contact of leaves and ice. It took three hours to send samples (Luanchuan, Henan) to the laboratory in Henan University of Science and Technology (Luoyang, Henan).

3.3 *In Vitro* Measurement of Absolute Leaf Chlorophyll Concentration: Chl

(1) Unit of Chl: Leaf Chlorophyll concentration (Chl) can be represented on a mass basis (μg/g), or on an area basis (μg/cm^2). While the former still dominates the literature in Chinese journals, we chose the latter for its two merits: area-based Chl can minimize the uncertainties resulted from moisture changes after leaves' departure from crown[9]; and area-based Chl can be directly integrated with Leaf Area Index (LAI, cm^2/cm^2) to facilitate large scale Chl research.

(2) Collection of Chl Samples: A puncher with 9 mm in diameter was used to collect discs in the same location of sample leaves. The number of discs was determined according to pre-set sample mass (0.05 g).

(3) Pigment extraction and quantification: Although it is fundamental, there is no standard procedure to extract and quantify Chl. Here we chose the two-step extraction method presented by Qiu *et al.*[10]. First, the discs (or filaments) were dropped into 2 mL DMSO in 65 ^{o}C until became white or transparent; then, 8 mL 80% acetone was added to cooled DMSO. Some samples may be further diluted to meet the requirement of spectrophotometer. The absorbance of the extract was measured with spectrophotometer, and the Chl was calculated using following formulas:

$$Chl\text{a (mg/L)} = 12.27A_{663.6} - 2.52A_{646.6} \quad (1)$$

$$Chl\text{b (mg/L)} = 20.10A_{646.6} - 4.92A_{663.6} \quad (2)$$

$$Chl\text{ (mg/L)} = Chl\text{a} + Chl\text{b} = 7.35A_{663.6} + 17.58A_{646.6} \quad (3)$$

where *Chl* (mg/L) is the leaf chlorophyll concentration, $A_{663.6}$ and $A_{646.6}$ are absorbance of the extract in 663.6 nm and 646.6 nm, respectively. *Chl* (mg/L) can be transformed into *Chl* (μg/cm^2) using discs area and dilution data. There are 3 *in vitro* analysis repeats for each *in situ* sample, thus there are totally 93 SPAD-Chl pairs.

3.4 Statistical Analysis

Four types of regression equations were developed based on 93 SPAD-Chl pairs, the best-fit

regression equation was chosen according to the Coefficient of Determination (R^2).

4 Results and Validation

The published dataset[7] consists of three files: one file in excel format (*in situ* optical measurements, SPAD; and *in vitro* absolute measurement, Chl); two files in .shp and .kml format, respectively (location of sampling). A brief analysis is as follows.

4.1 Basic Characteristics of Optical SPAD and Absolute Chl

According to Table 2, SPAD ranges from 10.6 to 56.6, with an average of 41.84; and Chl ranges from 8.64 to 72.17 μg/cm^2, larger than SPAD. Meanwhile, Coefficient of Variation (CV%) of Chl is also bigger than SPAD. Two factors may explain Chl's bigger CV%: the data range itself is bigger; there is a large uncertainty of Chl measurement because more factors involved.

Table 2 Statistics of SPAD and chlorophyll

Parameter	Statistics indexes						
	Samples	Minimum	Maximum	Mean	Median	Stdev	CV (%)
SPAD (unitless)	31	10.6	56.6	41.84	45.5	12.23	29.23
Chl (μg/cm^2)	93	8.64	72.17	42.63	42.77	15.84	37.16

4.2 Relationships between SPAD and Chl

Correlation relationships of SPAD and Chl were analyzed and four types of regression models were developed (Table 3). According to Coefficient of Determination (R^2), the best-fit of SPAD-Chl for total chlorophyll goes to exponential regressive model, with a R^2 of 0.927. Within Chl, the SPAD-Chl relationship for chlorophyll a (Chla) is better than that for chlorophyll b (Figure 1).

Table 3 Relationships between SPAD and Chl for Oak (*Quercus variabilis Bl.*)

Chlorophyll	$y=ax+b$	$y=ax^b$	$y=ae^{bx}$	$y=a\ln(x)+b$
Chla	$y = 0.903x-8.852$ $R^2 = 0.862$	$y = 0.265x^{1.246}$ $R^2 = 0.914$	$y = 3.815e^{0.045x}$ $R^2 = 0.948$	$y = 23.04\ln(x)-55.46$ $R^2 = 0.719$
Chlb	$y = 0.274x+2.197$ $R^2 = 0.661$	$y = 1.626x^{0.568}$ $R^2 = 0.621$	$y = 5.146e^{0.022x}$ $R^2 = 0.741$	$y = 6.759\ln(x)-11.07$ $R^2 = 0.514$
Chla+Chlb	$y = 1.177x-6.653$ $R^2 = 0.826$	$y = 1.150x^{0.962}$ $R^2 = 0.856$	$y = 8.712e^{0.035x}$ $R^2 = 0.927$	$y = 29.80\ln(x)-66.53$ $R^2 = 0.678$

x = SPAD (unitless); y = Chl (μg/cm^2)

4.3 Relationship of Chlorophyll Concentration and Chla/Chlb Ratio

Both Chlorophyll concentration and Chlorophyll a/Chlorophyll b ratio (Chla/Chlb) can be used as indicators of leaf health status. Figure 2(a) illustrates the relationship between chlorophyll concentration (Chl, μg/cm^2) and Chla/Chlb ratio: when Chl is bigger than 30 μg/cm^2, there is a broad and relatively stable plateau for Chla/Chlb ratio; while when Chl is less than 30 μg/cm^2, there is a linear relationship between Chl and Chla/Chlb ratio.

Figure 2(b) shows the relationship between SPAD and Chla/Chlb ratio: when SPAD is bigger than 35, there is a relatively stable plateau for Chla/Chlb ratio; while when SPAD is less than 35, there is a linear relationship between Chl and Chla/Chlb ratio. The prominent difference between SPAD and Chl occurs when Chla/Chlb ratio is bigger than 2. Under this

situation, a small change in SPAD may represent a relatively large change in Chl, indicating the accuracy of *in situ* SPAD measurement.

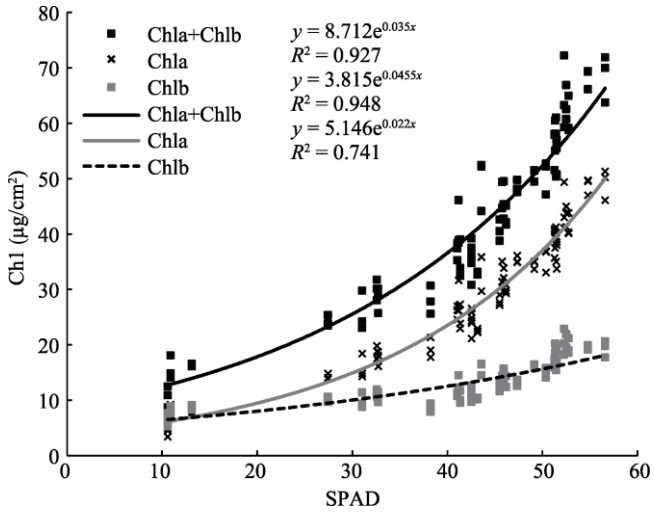

Figure 1 Exponential regression between SPAD and Chl for Oak

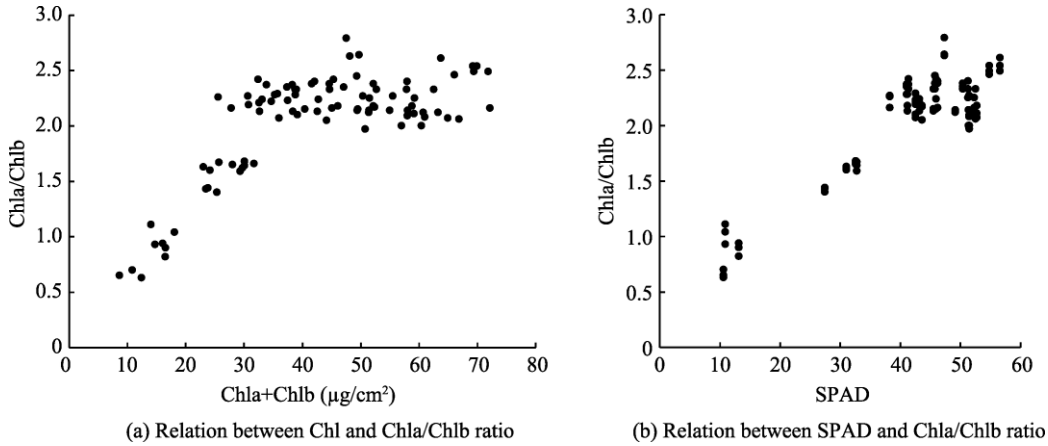

Figure 2 Relationship of Chlorophyll concentration and Chla/Chlb ratio for Oak

4.4 Validation and Accuracy Assessment

The design of the SPAD meter is based on the principle that increased leaf Chl increases the absorption of red and all leaves transmit a high fraction of NIR, thus theoretically, SPAD-Chl relationship can be regarded as a cause-effect relationship. The exponential regressive model is the best SPAD-Chl equation in terms of Coefficient of Determination (R^2), with a R^2 of 0.927, which means SPAD can determine 92.7% of the variance of Chl.

5 Discussion and Conclusion

(1) Reliability and Applicability of SPAD-Chl relationship: The SPAD-Chl relationship based on samples on September 19, 2018 in Mt. Funiu can be used to predict chlorophyll concentration (Chl) using SPAD readings, in this season and for this region at least, with a high confidence. Further study is on schedule to explore its broader applicability.

(2) Integration and inter-comparison of oak SPAD-Chl with other models: The oak SPAD-Chl model may be used for comparative studies among various plants[11]. In this respect, the digital publication of both *in situ* SPAD and *in vitro* Chl data offers a better opportunity.

(3) Refinement of SPAD-Chl Equation: The present SPAD-Chl equation may be modified in two aspects. First, for *in situ* sampling and SPAD measurement, one-leaf-one-sample may be better than many-leaf-one-sample, because there are variances among leaves in one sample and not every leaf is used for *in vitro* measurement. Second, when getting discs with puncher, a smaller puncher (5–6 mm in diameter) will offer more chances to keep the consistency between SPAD location and punching location in a leaf.

Author Contributions

Wang, Z. X. designed the development of dataset and wrote the data paper. Li, F. was responsible for *in situ* sampling, SPAD measurement, and sample preservation.

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