

Article

Spectral Reflectance Characteristics and Chlorophyll Content Estimation Model of *Quercus aquifolioides* Leaves at Different Altitudes in Sejila Mountain

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Abstract: Quercus aquifolioides is one of the most representative broad-leaved plants in Qinghai-Tibet Plateau with important ecological status. So far, understanding how to quickly estimate the chlorophyll content of plants in plateau areas is still an urgent problem. Field Spec 3 spectrometer was used to measure hyperspectral reflectance data of Quercus aquifolioides leaves at different altitudes, and CCI (chlorophyll relative content) of corresponding leaves was measured by a chlorophyll meter. The correlation and univariate linear fitting analysis techniques were used to establish their relationship models. The results showed that: (1) Chlorophyll relative content of Quercus aquifolioides, under different altitude gradients, were significantly different. From 2905 m to 3500 m, chlorophyll relative content increased first and then decreased. Altitude 3300 m was the most suitable growth area. (2) In 350~550 nm, the spectral reflectance was 3500 m > 3300 m > 2905 m. In 750~1100 nm, the spectral reflectivity was 2905 m > 3500 m > 3300 m. (3) There were 4 main reflection peaks and 5 main absorption valleys in the leaf surface spectral reflection curve. While, 750~1400 nm was the sensitive range of leaf spectral response of Quercus aquifolioides. (4) The red edge position and red valley position moved to short wave direction with the increase of altitude, while the yellow edge position and green peak position moved to long wave direction first and then to short wave direction. (5) The correlation curve between the original spectrum and the CCI value was the best between the wavelengths 509~650 nm. The correlation between the first derivative spectrum and CCI value was the best and most stable at 450~500 nm. The green peak reflectance was most sensitive to the relative chlorophyll content of *Quercus aquifolioides*. The estimation model R^2 of green peak reflectance was the highest ($y = 206.98e^{-10.85x}$, $R^2 = 0.8523$), and the prediction accuracy was 95.85%. The research results can provide some technical and theoretical support for the protection of natural Quercus aquifolioides forests in Tibet.

Keywords: reflection spectrum; chlorophyll content estimation Model; Quercus aquifolioides; altitude

1. Introduction

Altitude is one of the most important site factors that affects plant growth and its physiological ecology [1–3]. Many studies have shown that the average temperature and pressure of the ambient atmosphere decrease and the solar radiation increases with the increase in altitude. These factors



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have an important impact on the morphological structure and physiological structure of plants [3–7]. Therefore, in order to ensure their normal growth, plants at different altitudes have gradually formed their own adaptive mechanism to resist adversity [7–9]. Southeast Tibet is one of the largest primitive forest regions in China, and is the key research area of plateau ecosystem in China. Quercus aquifoliodes is a unique tree species in northwestern Yunnan, southeastern Tibet and western Sichuan, which irreplaceable in water conservation, soil and water conservation and biodiversity protection [10–13]. In particular, after the "two barriers and three belts" (the Qinghai-Tibet plateau ecological barrier, the loess plateau-Sichuan-Yunnan ecological barrier and the northeast forest belt, the northern sand prevention belt and the southern hilly mountain belt form an overall green development ecological contour) as the main body of China's ecological security strategic pattern was put forward, the ecological barrier function and position of Quercus aquifoliodes in Tibet area have become increasingly prominent [14]. At present, the research on this species mainly focuses on litter decomposition characteristics, soil respiration, genetic diversity, population spatial pattern characteristics, etc. [9–13,15]. However, there is still a large gap in the research on spectral characteristics of Quercus aquifoliodes leaves at different altitudes. Therefore, based on the importance and particularity of the region, understanding how to monitor and quickly obtain the growth status of Quercus aquifoliodes in different altitude environments is the key to its ecological service function and value.

In recent years, more researchers have begun to study scientific issues of agriculture and forestry, based on hyperspectral technology, with the rapid development of hyperspectral technology. Research has shown that, compared with conventional research methods, hyperspectral technology has the advantages of simple data acquisition and rich information. Spectral data contain a lot of information, such as chlorophyll content, plant stress, water content, photosynthetic rate, etc. [16–20]. Extracting these information through various methods can effectively solve many ecological problems. Using reflectance spectroscopy to detect the physiological and ecological characteristics of plants has gradually become one of the research hotspots in forestry remote sensing. At present, scholars have carried out a large number of researches and pioneering applications using spectral reflectance, including the correlation between plant pigments and spectral reflectance, physiological adaptation of plants to changes in altitude, adaptation countermeasures of plants to drought conditions and other harsh environments, estimation of green plant productivity, monitoring seasonal changes of plant communities, biodiversity research, detection of plant diseases and insect pests and biological invasion patterns, fire monitoring, monitoring applied to carbon cycle and water cycle, environmental pollution monitoring, etc. [17–28]. However, research on the effects of different altitudes on plants, based on hyperspectral analysis are rarely reported, especially on *Quercus aquifoliodes*, which are relatively limited. The forest community in Tibet's Sejila Mountain changes obviously with altitude gradient, and the change of plant spectral reflectance with altitude is a problem to be revealed. Quercus aquifoliodes, as an important broad-leaved tree species in Sejila Mountain, has important economic value and ecosystem service value. Therefore, in order to compare the spectral variation characteristics and laws of *Quercus aquifoliodes* leaves under different altitude gradients, explore how to infer the photosynthetic capacity of Quercus aquifoliodes leaves, under different altitude gradients, based on hyperspectral parameters, and further analyze its sensitive band to altitude, in order to provide theoretical basis for further understanding its growth characteristics and strategies for adapting to the environment.

2. General Situation and Research Methods of the Research Area

2.1. Survey of Research Area

The study area is located in the Tibetan autonomous region of Tibet's Sejila mountain, between east longitude $94^{\circ}19'-94^{\circ}53'$, north latitude $29^{\circ}33'-30^{\circ}00'$. This region is a typical sub-alpine temperate semi-humid climate zone with distinct dry and wet seasons. The altitude range of the study area is 2900–3500 m, the annual average temperature is about -0.73 °C, the average temperature in the highest month (July) is 9.23 °C, the average temperature in the lowest month (January) -13.98 °C,

the extreme minimum temperature -31.6 °C, the extreme maximum temperature 24.0 °C, the average annual sunshine hours 1150.6 h, the sunshine percentage 26.1%, and the maximum sunshine hours are December (151.7 h). The sunshine percentage is 40%, the average annual relative humidity is 78.83%, the average annual precipitation is 1134.1 mm, and the evaporation amount is 544.0 mm, accounting for 48.0% of the average annual precipitation. June–September is the rainy season, accounting for 30% of the annual rainfall, of which August has the most rainfall, averaging 294.2 mm, accounting for 30% of the annual rainfall. The soil is mainly mountainous brown soil and acidic brown soil, with a pH value of 4–6, an average thickness of 60 cm, and no obvious degree of humus. The stand structure of *Quercus aquifolioides* in Sichuan-Yunnan region is relatively single, mostly distributed in pure forests, sometimes mixed with Pinus densata Mast to become dominant species in secondary forest.

2.2. Leaf Reflectance Spectrum Collection and Chlorophyll Determination

We selected 60 functional leaves in the middle of *Quercus aquifolioides* plants at each altitude gradient. Spectral data were collected using an ASD FieldSpec3 portable near infrared spectrometer (Analytical Spectral Device, Almero, Netherlands). The band range of ASD spectrometer is $300 \sim 2500$ nm. The sampling interval is 1.4 nm (350~1000 nm band) and 2 nm (1000~2500 nm band) respectively in different band ranges. The spectral reflection curve was set to 10 repetitions, and the final output spectral data was the average value of the 10 data. The operation procedures of the instrument were as follows: optimizing spectrometer \rightarrow dark scanning \rightarrow white board scanning \rightarrow adjusting transmission mode \rightarrow aiming optical fiber at the sample \rightarrow saving the value after reading is stable (Figure 1) [16].



Figure 1. Flow chart of leaf spectrum measurement based on ASD spectrometer [16].

The relative Chlorophyll content index (CCI) was measured by CCM-200 plus chlorophyll meter (OPTI-Science, Tyngsboro, MA, USA), with the resolution of ± 0.1 CCI and the measurement range of 0.71 cm^2 . The principle of this instrument is to measure the relative content of chlorophyll in the measured object by the optical absorptivity when the light source is excited in red light and infrared light [19].

2.3. Data Processing Method

Analysis and processing of spectral data were based on ViewSpecPro software, and correlation analysis of spectral parameters and chlorophyll was based on Origin 2019b software and Excel 2019 software.

3. Results and Analysis

Photosynthesis accumulates and stores important energy and substances for plant growth. Chlorophyll, as the basic pigment in plants, plays an important role in the absorption, transmission and transformation of light energy. As can be seen from Figure 2, the relative values of chlorophyll content of Quercus aquifolioides under different altitude gradients were significantly different. From 2905 m to 3500 m, the relative chlorophyll content of Quercus aquifolioides increased first and then decreased, reaching a peak at 3200 m and decreasing at 3500 m. This showed that Quercus aquifolioides has lower photosynthetic capacity at 2905 m compared with 3200 m. At the same time, under high altitude and strong radiation environment, Quercus aquifolioides at 3500 m reduced its chlorophyll content. This can reduce its absorption of light and weaken the pressure of the light system, thus avoiding its peroxidation. The chlorophyll content, in this study, showed a rising trend first and then falling with the elevation. Similar results have been reported in the study of chlorophyll content in Acer ginnala and the study of the forest structure of *Phyllostachys heterocycle* [29,30]. The increase of chlorophyll content to a certain extent can accumulate more photosynthetic products for the growth of plants, and the synthesis is affected by light intensity. As the light intensity at low altitude is weaker, and the light intensity at middle altitude increases, it is more conducive to chlorophyll synthesis [30,31]. With the further increase in altitude, environmental factors, such as air humidity reduction, ultraviolet radiation and low temperature at high altitude become more serious. This may, in turn, cause the chlorophyll synthesis to be blocked or the chlorophyll decomposition to be accelerated, causing the chlorophyll content in the leaves to decrease [32,33]. Therefore, with the increase in altitude, chlorophyll showed first a rising trend and then falling, which also showed that the middle altitude was more conducive to the photosynthesis and growth of plants.



Figure 2. Relative chlorophyll content of *Quercus aquifolioides* at different altitudes. ** indicates that the parameters are significantly different at p < 0.01 level. CCI (chlorophyll content index).

3.2. Spectral Characteristics of Quercus aquifolioides with Different Altitude Gradient

As shown in Figure 3, the trend changes of leaf spectral reflectance curves of *Quercus aquifolioides* were generally consistent under different altitude gradients. In the visible light band range (350~700 nm), there are obvious differences in spectral reflectance. In the range of 350~700 nm, the reflectivity of each band of 3500 m is greater than 3300 m and the reflectivity of 2905 m. As shown in Figure 4, their maximum reflectance ratios were 1.39, and 1.26, respectively. In the range of 350–500 nm, the reflectance of 3300 m was greater than that of 2905 m, and the maximum ratio of reflectance was 0.95. In the range of 500~700 nm, the reflectivity of 2905 m was greater than that of 3300 m, and the reflectivity ratio was 1.14 at the maximum. In the near infrared band (750~1100 nm), the reflectivity of 2905 m was higher than that of 3500 m and 3300 m, and their maximum reflectivity ratios were 1.56, and 1.75, respectively. The closer the reflectance ratio was to 1, the smaller the difference between them was.

In the range of 350~1800 nm, there were 4 main reflection peaks and 5 main absorption valleys in the leaf surface spectral reflection curve of *Quercus aquifolioides*, and their positions were basically the same. The reflection peaks were located at 550 nm, 1096 nm, 1273 nm, and 1656 nm, respectively, and the absorption valleys were located at 350–500 nm, 600–750 nm, 950–1050 nm, 1200~1250 nm, and 1400~1500 nm, respectively.

As shown in Figures 3 and 4, the spectral reflectance of *Quercus aquifolioides* leaves at different altitudes in the visible light to near infrared band (350–1800 nm) was the most distinguishable in the range of 750–1400 nm, indicating that this band was the sensitive range of spectral response of plants to different altitudes. In addition, we can see that the slope of the spectral reflectance curve of *Quercus aquifolioides* leaves has a sharp increase trend in the range of 700~780 nm. Studies have shown that the phenomenon of plants increasing suddenly in this wave band belongs to the typical "red edge effect" characteristic of plants [16,33,34].



Figure 3. Reflectance spectral characteristics of Quercus aquifolioides leaves at different altitudes.



Figure 4. Spectral reflectance ratio of Quercus aquifolioides leaves at different altitudes.

3.3. Dynamic Changes of Spectral Characteristic Parameters of Quercus aquifolioides at Different Altitudes

Research shows that the original spectrum can eliminate the influence of background noise on the spectrum, and reduce the scattering and absorption of light by atmosphere in the process of spectrum acquisition, after first-order differential processing [35–37]. Representative hyperspectral characteristic parameters selected in this study were shown in Table 1 [38]. The "trilateration" parameter is an important in the first derivative spectrum, and is one of the hot spots and focuses in hyperspectral research in recent years. Among them, the red edge is the interval in which the reflection spectrum of plants suddenly changes obviously in the red light band (680~750 nm), which is the most significant mark in the spectrum curve, and is widely used as the characterization parameters of plant growth, leaf water content, nutritional status, chlorophyll content and other indicators [39]. Red edge includes two important concepts: the position of red edge and the other is the slope of red edge. The red edge position is the wavelength position corresponding to the maximum reflectance in the red wavelength band. It represents the degree of stress of plants to the external environment well [39–41]. For example, under the conditions of environmental pollution, disease threat, drought stress and so on, its position tends to shift to a certain extent. Generally speaking, there are two main directions for the shift of the red edge position, one is to shift towards the long wave direction, while the other is to shift towards the short wave direction. The red edge slope is the maximum reflectance in the red band, which can better reflect the chlorophyll content of plants and is often closely related to the photosynthetic rate of plants [42].

Spectral Parameters	Definition
Red edge position, REP	The wavelength position corresponding to RES
Red edge slope, RES	The largest first-order derivative value in the red edge (680~750 nm)
Blue edge position, BEP	The wavelength position corresponding to BES
Blue edge slope, BES	The largest first-order derivative value in the blue edge (490~530 nm)
Yellow edge position, YEP	The wavelength position corresponding to YES
Yellow edge slope, YES	The largest first-order derivative value in the yellow edge (560~640 nm)
Red valley position, RVP	The wavelength position corresponding to RRV
Reflectance of red valley, RRV	Minimum reflectance in the wavelength range of 640~700 nm
Green peak position, GPP	The wavelength position corresponding to RGP
Reflectance of green peak, RGP	Maximum reflectance in the wavelength range of 510~580 nm
Reflectance of water stress band, RWSB	Maximum reflectivity in the wavelength range of 1550~1750 nm
Red edge area, REA	Sum of first derivative in red edge (680~750 nm)
Yellow edge area, YEA	Sum of first derivative in yellow edge (490~530 nm)
Blue edge area, BEA	Sum of first derivative in blue edge (560~640 nm)
Leaf chlorophyll index, LCI	$(R_{850}-R_{710})/(R_{850}-R_{680})$

Table 1. Description of spectral parameters.

From Figures 5 and 6, it can be seen that the general trend of the first derivative spectra of Quercus aquifolioides leaves, at different altitudes, was basically the same, but there were some differences in values. As shown in Figure 6a, the red edge position and the red valley position of the leaf surface spectral curve move toward the short wave direction as the altitude increases. This showed that, with the increase in altitude, the effect on the position of red edge and red valley on leaf surface became more severe. However, the yellow edge position and the green peak position move to the long wave direction first and then to the short wave direction with the elevation. As shown in Figure 6b, with the elevation increasing, the water stress band reflectance and green peak reflectance first increased and then decreased, while the red valley reflectance increased. The slope of red edge, yellow edge and blue edge all decreased with the elevation. As can be seen from Figure 6c, with the elevation increasing, the red edge area first decreases and then increases, the yellow edge area decreases and the blue edge area increases. Previous studies generally believed that chlorophyll is one of the important parameters in determining the characteristics of plant spectral reflectance curves. When the vegetation is in a healthy growth state and has a high chlorophyll content, the red edge position generally moves towards the long wave direction. However, when vegetation is subjected to external environmental stress, such as drought stress, high temperature stress or pest damage, the red edge position tends to move towards short wave direction [41,42]. In this study, the red edge of Quercus aquifolioides showed a decreasing trend with the elevation. This just proves the indication effect of red edge position on chlorophyll content.



Figure 5. The first derivative spectral curves of the *Quercus aquifolioides* leaves at different altitudes.



Figure 6. Spectral parameters of *Quercus aquifolioides* leaves at different altitudes. (**a**) spectral position, (**b**) spectral reflectance, (**c**) spectral area parameter. REP (red edge position), RES (red edge slope), BEP (blue edge position), BES (blue edge slope), YEP (yellow edge position), YES (yellow edge slope), RVP (red valley position), RRV (reflectance of red valley), GPP (green peak position), RGP (reflectance of green peak), RWSB (reflectance of water stress band), REA (red edge area), YEA (yellow edge area), BEA (blue edge area).

3.4. Correlation between Leaf Spectra and Derivative Spectra and Chlorophyll Content in Leaves

As shown in Figure 7, the leaf reflectance spectral data, and its first derivative spectral data, were subjected to single correlation analysis with chlorophyll relative content values, respectively. The correlation coefficient between the original spectral reflectance and CCI value, the first derivative spectrum and CCI value on each spectral channel was calculated. The correlation curve between the original spectrum of *Quercus aquifolioides* leaves and the CCI value of the relative content of chlorophyll has the best correlation between the wavelength of 509~650 nm, and this wavelength was just the strong absorption band of chlorophyll. The correlation was almost zero at 761 nm, i.e., the original spectrum hardly reflects chlorophyll content information after 761 nm. The correlation between the first derivative spectrum and CCI value was the best and most stable at 450~500 nm, and the correlation in other spectral channels fluctuates greatly.



Figure 7. Correlation analysis and comparison of leaf spectrum, first derivative spectrum data and CCI value of relative chlorophyll content of *Quercus aquifolioides*.

3.5. Correlation between Leaf Spectral Parameters and Chlorophyll Relative Values of Quercus aquifolioides at Different Altitudes

Spectral parameters can better reflect the spectral characteristics of green vegetation, and they are sensitive to changes in chlorophyll content [43]. It is pointed out that there is an exponential function relationship between chlorophyll content and the reflectance spectral characteristic parameters such as red edge slope, blue edge slope and green peak reflectance [44–48]. Therefore, we used 13 typical spectral characteristic variables of plants and chlorophyll content of leaves for correlation analysis. Table 2 showed that there was a certain correlation between spectral parameters and relative chlorophyll content. Among them, the correlation between RES, BES, YES, YEP, RGP, RWSB, REA, BEA, YEA, LCI and chlorophyll relative content value has reached significant level. The correlation degree of green peak reflectance is the largest among all spectral parameters, which showed that RGP

was the most sensitive to the relative chlorophyll content of *Quercus aquifolioides*. The change of this parameter can be used to characterize the change of chlorophyll content of *Quercus aquifolioides* at different altitudes.

Table 2. Relationships between chlorophyll content index and spectral feature parameters. * indicates that the correlation reaches a significant level at the level of 0.05, ** indicates that it is extremely significant at the level of 0.01. The number of samples n = 60.

Spectral Parameter	Correlation Coefficient	
REP	0.162	
RES	-0.292 *	
BEP	-0.031	
BES	-0.484 **	
YEP	-0.098	
YES	-0.270 *	
YEP	-0.421 *	
RGP	-0.892 **	
GPP	-0.023	
RWSB	-0.623 **	
REA	-0.318 *	
BEA	0.461 *	
YEA	-0.264 *	
LCI	-0.335 *	

3.6. Estimation Model of Chlorophyll Content in Plant Leaves Based on Spectral Parameters

From Table 2, it can be seen that the chlorophyll content of plant leaves has a good correlation with 10 spectral parameters (RES, BES, YES, YEP, RGP, RWSB, REA, BEA, YEA and LCI), which indicated that it was feasible to estimate the chlorophyll content of *Quercus aquifolioides* in Sejila Mountain by using these spectral characteristic variables. As shown in Table 3, this study takes 9 typical plant spectral characteristic parameters as independent variables and chlorophyll content as dependent variables, and conducts univariate regression analysis to establish an index, linear and quadratic polynomial chlorophyll content estimation model. Among them, the estimation model R^2 of RGP was the highest ($y = 206.98e^{-10.85x}$, $R^2 = 0.8523$), which showed that this model has high accuracy (Figure 8). After the establishment of the estimation model, in order to verify the reliability and applicability of the estimation model, the estimation model was evaluated with three general indexes of root mean square error (RMSE), relative error (RE) and determination coefficient (R^2) (Table 4), and the relationship diagram between the predicted value and the measured value was drawn (Figure 9) to visually show the fitting degree and reliability of the estimation model. The test results showed that the chlorophyll content estimation model based on the spectral characteristic parameters of RGP has achieved good test results. The determination coefficient (R^2) of the test fitting equation reaches above 0.8, and the root mean square error (RMSE) and relative error (RE) were both small, with higher fitting accuracy and smaller relative error. The estimation model of vegetation chlorophyll content has the largest determining coefficient ($R^2 = 0.8024$), the smallest root mean square error (RMSE = 3.0512), the smallest relative entropy (MRE = 9.1721) and the smallest relative error (RE = 6.13%), and the prediction accuracy reaches 95.85%.



Figure 8. Regression model between chlorophyll content and best spectral parameter. (**a**) RES (red edge slope), (**b**) YES (yellow edge slope), (**c**) BES (blue edge slope), (**d**) YEP (yellow edge position), (**e**) RGP (reflectance of green peak), (**f**) RWSB (reflectance of water stress band), (**g**) REA (red edge area), (**h**) YEA (yellow edge area), (**i**) BEA (blue edge area), (**j**) LCI (leaf chlorophyll index). CCI (chlorophyll content index).

Spectral Parameter	Regression Model	Decisive Coefficient	
RES	$y = 75.5e^{-37.56x}$ y = -2107.9x + 76.099	$R^2 = 0.0908$ $R^2 = 0.0852$	
	$y = 639,164x^2 - 15,453x + 140.95$	$R^2 = 0.1403$	
BES	$y = 23.476e^{-691x}$ $y = -46,608x + 13.537$	$R^2 = 0.2892$ $R^2 = 0.2357$	
	$y = -5E + 06x^2 - 55,192x + 10.031$	$R^2 = 0.2359$	
YES	$y = 87.435e^{-319.2x}$ y = -19,288x + 86.605 $y = -6E + 06x^{2} + 1654.4x + 69.045$	$R^2 = 0.0634$ $R^2 = 0.0690$ $R^2 = 0.0701$	
YEP	$y = 1E + 06e^{-0.019x}$ y = -0.9134x + 530.86 $y = -0.0474x^2 + 48.545x - 12,373$	$R^2 = 0.0147$ $R^2 = 0.0097$ $R^2 = 0.0097$	
RGP	$y = 220.99e^{-11.36x}$ y = -563.33x + 128.09 $y = 3124.9x^2 - 1411.9x + 181.94$	$R^2 = 0.8523$ $R^2 = 0.7368$ $R^2 = 0.7840$	
RWSB	$y = 92.18e^{-1.88x}$ y = -100.42x + 85.736 $y = 172.71x^2 - 220.49x + 103.77$	$R^2 = 0.4570$ $R^2 = 0.3885$ $R^2 = 0.4046$	
REA	$y = 80.529e^{-1.114x}$ y = -62x + 79.517 $y = 420.51x^2 - 403.98x + 144.75$	$R^2 = 0.1094$ $R^2 = 0.1009$ $R^2 = 0.1412$	
YEA	$y = 107.49e^{19.595x}$ y = 1160.1x + 98.23 $y = 72,497x^2 + 6679.1x + 198.72$	$R^2 = 0.2037$ $R^2 = 0.2129$ $R^2 = 0.2811$	
BEA	$y = 83.579e^{-13.29x}$ y = -793.36x + 83.563 $y = 5083.8x^2 - 1204.2x + 91.552$	$R^2 = 0.0657$ $R^2 = 0.0698$ $R^2 = 0.0700$	
LCI	$y = 2839.6e^{-5.298x}$ y = -240.95x + 236.02 $y = -5636.2x^2 + 8389.1x - 3064$	$R^2 = 0.1629$ $R^2 = 0.1121$ $R^2 = 0.1648$	

Table 3. Regression model of chlorophyll content and spectral parameters (sample number = 60).

Table 4. The fit evaluation indicators of the measured values (x) and predicted values (y).

Regression Equation	<i>R</i> ² (Determination Coefficient)	RMSE (Root Mean Square Error)	MRE% (Min Relative Entropy)	RE% (Relative Error)
y = 0.7812x + 9.1464	0.8024	3.0512	9.1721	6.13
140	y = 0.7812x + 9.	1464	_	



Figure 9. Comparison between the measured value and the predicted value (sample number = 60). CCI (chlorophyll content index).

4. Conclusions and Discussion

In this study, hyperspectral reflectance data of *Quercus aquifolioides* leaves measured by portable spectrometer and chlorophyll content measured synchronously were used. The spectral characteristics of *Quercus aquifolioides* leaves, at different altitudes, were analyzed, and the statistical analysis methods of correlation analysis and stepwise regression analysis were used. Through systematic analysis of the selected typical spectral characteristic parameters, the optimal spectral characteristic parameters for monitoring the chlorophyll content of *Quercus aquifolioides* were determined, and the estimation model of the chlorophyll content of *Quercus aquifolioides* was established. The estimation model was tested and verified by using 3K-CV method in cross-examination. The conclusion was as follows.

- (1) The relative values of chlorophyll content of *Quercus aquifolioides* at different altitude gradients were significantly different. From 2905 m to 3500 m, the relative chlorophyll content of *Quercus aquifolioides* showed a trend of increasing first and then decreasing, which indicated that the environment at lower altitude or higher altitude was not conducive to chlorophyll synthesis and accumulation. At a low altitude of 2905 m, due to insufficient light and human destruction, its chlorophyll content was reduced. 3300 m above sea level was the most suitable growth area for *Quercus aquifolioides*. However, with the further increase in altitude (3500 m), due to the aggravation of unfavorable environment such as low temperature, thin atmosphere or strong ultraviolet radiation, the plant's resistance ability is limited, which also limits the growth and development of *Quercus aquifolioides*.
- (2) The trend changes of leaf spectral reflectance curves of *Quercus aquifolioides* were generally consistent under different altitude gradients. In the visible light band (350~550 nm), the spectral reflectance was 3500 m > 3300 m > 2905 m. In the range of 550~700 nm, the reflectivity was 3500 m > 2905 m > 3300 m. In the near infrared band (750~1100 nm), the reflectivity was 2905 m > 3500 m > 3300 m.
- (3) In the range of 350~1800 nm, there were 4 main reflection peaks and five main absorption valleys in the leaf surface spectral reflection curve of *Quercus aquifolioides*, and their positions were basically the same. Visible light band was difficult to reflect the damage of host plants, while near infrared band (750~1400 nm) has the greatest degree of spectral reflectance discrimination. This band was the sensitive range of leaf spectral response of *Quercus aquifolioides*. At the same time, such variation characteristics were universal under different altitude conditions.
- (4) The red edge position and red valley position of leaf surface spectral curve move towards short wave direction with the elevation. This showed that, with the increase in altitude, the effect on the position of red edge and red valley on leaf surface becomes more severe. However, the yellow edge position and the green peak position move to the long wave direction first, and then to the short wave direction with the elevation. With the increase of altitude, the red edge area first decreases and then increases, the yellow edge area decreases and the blue edge area increases.
- (5) The correlation curve between the original spectrum of *Quercus aquifolioides* leaves and chlorophyll relative content was the best between the wavelengths 509~650 nm, and the original spectrum hardly reflects the chlorophyll content information after 761 nm. The correlation between the first derivative spectrum and CCI value was the best and most stable at 450~500 nm, and the correlation in other spectral channels fluctuates greatly.
- (6) The correlation between red edge slope, blue edge slope, yellow edge slope, yellow edge position, green peak reflectance, water stress wave band reflectance, red edge area, yellow edge area and blue edge area and chlorophyll relative content value reached significant level. The correlation degree of green peak reflectance is the largest among all spectral parameters, which showed that green peak reflectance is most sensitive to the relative chlorophyll content of *Quercus aquifolioides*. The estimation model R^2 of green peak reflectivity was the highest (y = 206.98e^{-10.85x}, R^2 = 0.8523), and the prediction accuracy was 95.85%.

Author Contributions: J.Z. conceived and designed the study. J.Z., Q.Y., W.H., C.X., and H.H. contributed materials and tools. J.Z., W.H., C.M.B.J. and J.Y. performed the experiments. J.Z. contributed to data analysis, paper preparation and revision. All authors have read and agreed to the published version of the manuscript.

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