Detection of the photosynthesis protective mechanisms of C3 and C4 Crops from hyper spectral data

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Abstract: The classification of C3 and C4 plant functional types has become an important issue in global change research, but the mechanism of using remote sensing data remains unclear. In this paper, a diurnal variation experiment was designed for soybean (C3 plant functional type) and maize (C4 plant functional type) to acquire canopy spectra under different illumination and temperature conditions, and chlorophyll fluorescence (ChlF) signals and photochemical reflectance index (PRI) were extracted from the canopy spectra. The results showed that: (1) the amplitudes of the diurnal variation in ChlF between soybean and maize were significantly different, as the relative ChlF of soybean increased rapidly under heat and high irradiance stresses in the afternoon, but the increasing trend was not found in maize; (2) there was also an apparent increase in the ratio f_{688}/f_{760} of ChlF intensities of soybean in the afternoon, but this did not appear in maize; (3) the midday photosynthetic depression occurred in C3 crops owing to the stressed light and temperature, with a rapid increase in PRI. However, there was no apparent noon depression in C4 crops. Based on the different characteristics between the responses of C3 and C4 crops to high irradiance and temperature stressws, we proposed a potential method to discriminate C3 and C4 plants by multitemporal and hyper spectral remote sensing data.

Key words: C3 and C4 plant functional types, hyper spectral, chlorophyll fluorescence (ChlF), photochemical reflectance index (PRI)

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1 INTRODUCTION

Photo inhibition can occur in natural situations (Anderson, et al., 1997), thus photosynthetic organs of plants have evolved a variety of photo protective mechanisms to minimize light-induced oxidative damage. One of the mechanisms is to reemit the excessive energy as chlorophyll fluorescence (ChlF), and the other is the non-radiative energy dissipation as heat related with the xanthophylls cycle (Bolhar-Nordenkampf, et al., 1989; Anderson, et al., 1997; Niyogi, 1999).

Part of energy dissipation was reemited as ChlF with a longer wavelength in the photosynthetic process (usually less than 1% of the incident energy) (Liu, et al., 2005). The apparent vegetation reflectance spectra consist of contributions from both the reflected and fluoresced radiations. Although ChlF signal is inevitably superimposed on reflectance signatures under natural illumination condition, many researchers prove that it is possible to separate solar-induced ChlF radiation from observed apparent vegetation reflectance based on the Fraunhofer-line principle. McFarlane, et al., (1980) and Carter, et al., (1990,1996) measured solar-induced fluorescence in citrus canopies using the H-a Fraunhofer line at 656 nm. According to Zarco-Tejada, et al., (2000) and Zhang (2007), ChlF effects could be detected at the leave-level under controlled illumination conditions. They found that the contribution of ChlF to apparent reflectance accounted from 10% to 25% at 685 nm and from 2% to 6% at 740 nm. Subsequent studies demonstrated quantitatively that ChlF signals could be detected at canopy level at 688 nm and 760 nm based on Fraunhofer line principle (Moya, et al., 2004; Liu, et al., 2005; Liu, et al., 2006; Alonso, et al., 2008; Amoros-Lopez, et al., 2008). Along with the development of sensors, Zarco-Tejada, et al., (2004) and Perez-Priego, et al., (2005) used a narrow-band spectrometer (0.065 nm FWHM) to measure the spectral reflectance of tree canopies and found a characteristic peak superimposed on the reflectance at 760 nm, which was due to in-filling of the atmospheric oxygen absorption band (the Fraunhofer lines) at 760 nm by chlorophyll fluorescence, and this caused

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a reflectance difference between 760.59 nm and 759.5 nm ranging from 1% to 3%.

Heat dissipation of excessive light energy plays an important role in controlling energy absorption and electron transport, and protecting photosynthetic organs from photoinhibition or even photooxidation. When the proton gradient across the thylakoid membrane increases under highlight conditions, LHC Ⅱ can be triggered to dissipate the excess light energy through enhanced heat loss. Plants can consume more than half of the absorbed energy in the antenna system via this way (Demmig-Adams, et al., 1996). Heat dissipation of excess light energy is related to the activity of the xanthophyll cycle, which involves the enzymatic removal of epoxy groups from xanthophylls to create so-called de-epoxidised xanthophylls (Gamon, et al., 1992). The conversion could result in a decrease of reflectance at 531 nm, whereas reflectance at 570 nm is not affected. Therefore, Photochemical Reflectance Index (PRI) which is defined as a normalized ratio of 531 nm to 570 nm by Gamon, et al., (1992) and Penuelas, et al., (1997), could monitor the heat dissipation in the xanthophyll cycle. Some studies proved that PRI was closely related to xanthophyll cycle (Demmig-Adams & Adams, 1994; Peguero-Pina, et al., 2008). Additionally, many scholars found there was a reliable negative relationship between PRI and non-photochemical quenching (NPQ) which was caused by xanthophyll changes (Evain, et al., 2004; Nichol, et al., 2006; Zhang, et al., 2006; Gaalen, et al., 2007).

Plants can be divided into three categories based on photosynthetic pathways: C3, C4 and crassulacean acid metabolism (CAM) (Taiz & Zeiger, 2006). As C4 plants have higher light, water and nitrogen use efficiencies than C3 plants, and perform more tolerant of high temperature, aridity and salinity, there is increasing concern of C4 plants in the global change research (Han, et al., 2006), but the mechanism of monitoring C3 and C4 plants using remote sensing remains unclear. In this paper, a diurnal change experiment was designed for soybean (C3) and maize (C4) to understand the photoprotective mechanisms between C3 and C4 crops under stresses. Based on the different spectral characteristics of diurnal variation in ChlF effects and heat dissipation, we explored a feasible classification method to discriminate C3 and C4 plants under stressed conditions from hyperspectral data.

2 MATERIALS AND EXPERIMENTS

2.1 Study area and data collection

The diurnal change experiment was performed on a sunny, clear and windless day (July 21st, 2010) at Huailai site belonging to the Institute of Remote Sensing Applications of Chinese Academy of Science. The soybean was at the end of the branching stage and the spring maize was at the big trumpet period. The experimental soybean plot was adjacent to maize plot with north-south ridges, and the plant growth was homogenous under normal fertilizer and water management.

The canopy spectral measurements were taken from the canopy using an ASD FieldSpec Prospectrometer, which was fitted with a 25° field-of-view bare fiber-optic cable, and operated in the 350—2500 nm spectral region with a sampling interval of 1.4 nm between 350 nm and 1050 nm, and 2 nm between 1050 nm and 2500 nm. The spectral resolution of the spectrometer (or FWHM) was 3 nm@VNIR(350—1050 nm), 10 nm@SWIR(1050—2500 nm). A $BaSO_4$ panel with 0.5 m×0.5 m was also selected as a calibration reference to measure the spectrum of incident radiation. All the canopy and panel radiance spectra were taken every 30 min from 10:00 to 18:00. After 18:00, the incident PAR decreased rapidly from 200 $\mu\cdot mol\cdot m^{-2}\cdot s^{-1}$, and it was not suitable to measure reflectance spectra at so low illumination. During spectrum collection, radiance observation pattern was adopted for the field spectrometer, and the fiber optics was kept vertically downward and about one meter above the canopy. Each spectrum was averaged from ten individual measurements of the detective targets which were kept basically the same during the diurnal experiment.

2.2 Separation of ChlF signal based on the Fraunhofer lines in-filling method

When green plants are excited by short-wavelength light, there are three obvious fluorescence maxima between 400 nm and 800 nm (near 440 nm, 685 nm and 740 nm). In this spectral region, the fluorescence from 650 nm to 780 nm is emitted by chlorophyll, and the emission peaks were observed near 685 nm and 735 nm. Many studies demonstrated that these special fluorescence bands and the ratio of bands could provide some information on the health state and growth condition of the plants, especially the early diagnosis of stress status (Subhash, 1999; McMurtrey, 2003).

The apparent vegetation reflectance spectra consist of contributions from both the reflected and fluoresced radiations. However, the amount of chlorophyll fluorescence emitted by a leaf under natural sunlight only accounts for up to 1% or 3% of the absorbed light in the visible part of the spectrum. Therefore, it is difficult to quantify and separate the tiny ChlF signal from the reflected light. However, at certain wavelengths where the solar spectrum is attenuated (Fraunhofer lines), the fluorescence signal may be quantifiable. Due to the atmospheric absorption of the sun and the earth, there are tiny dark absorption lines, called Fraunhofer lines, in the spectrum of the sun, with the widths range from 0.1 nm to 10 nm. As the central intensity of some lines is less than 10% of the adjacent continuum, it may provide a feasible method to detect ChlF signals under natural sunlight illumination conditions. The plant fluorescence excited by short-wavelength light can fill the Fraunhofer "well" to a certain degree. Therefore, vegetation ChlF radiance can be extracted by comparison of incident solar radiance and vegetation reflected radiance at certain wavelengths (Louis, et al., 2005; Liu, et al., 2006).

Fraunhofer lines are dark absorption lines in the solar spectrum, including those at 486 nm, 527 nm, 589 nm, 656 nm, 688 nm and 760 nm. All these bands can be detected if the signal to noise ratio (SNR) and spectral resolution of the spectral instrument are sufficiently high. According to Moya, et al. (2004) and Liu, et al. (2006), it is reliable to monitor solar-induced ChlF signals at 688 nm and 760 nm using ASD FieldSpec NIR spectrometer. As shown in Fig. 1, λ_0 is the central wavelength of Fraunhofer lines, and R is the constant vegetation reflectance near λ_0. Parameters a and b represent the detected irradiance from the reference panel in and out of the oxygen absorption feature, respectively. Similarly, c and d represent the detected radiance from the target at the border and at the bottom of the band. The fluorescence flux (f) can be calculated as following Eq. (1) to Eq. (4).

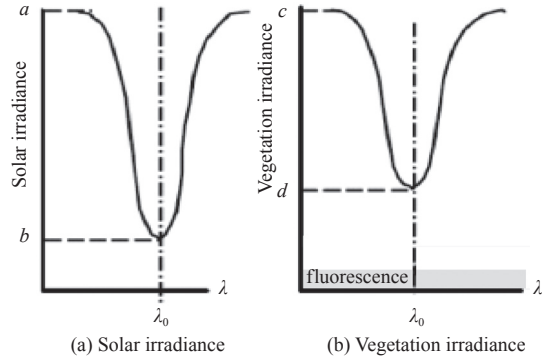
$$c = R \cdot a + f \quad (1)$$

$$d = R \cdot b + f \quad (2)$$

$$R = (c-d)/(a-b) \quad (3)$$

$$f = d - R \cdot b \quad (4)$$

In this experiment, the solar-induced fluorescence at 688 nm and 760 nm was calculated according to Eq. (4). For the 688 nm O_2 Fraunhofer line, the bands in and out of the oxygen absorption feature were set at 684 nm and 688 nm sampling bands of the ASD spectrometer, where *a* and *b* were the detected irradiances from the reference panel at the 684 nm and 688 nm sampling bands of the ASD spectrometer, and *c* and *d* were the reflected radiances from the canopy at those same bands. Similarly, the bands in and out of the 760 nm oxygen-absorption feature were set at 756 nm and 761 nm sampling bands of the ASD spectrometer.



(a) Solar irradiance (b) Vegetation irradiance

Fig. 1 Detection of chlorophyll fluorescence using Fraunhofer lines

As ChlF signals are extracted from apparent spectrum, they are directly related to the photosynthetic active radiation. The light use efficiency (LUE) was defined as defined as the ratio of gross primary production (GPP) to absorbed photo synthetically active radiation. To track the LUE more sensitively, the relative ChlF is defined as the ratio of ChlF radiation to the canopy irradiance at the border band of the Fraunhofer line. According to Eq. (4), the relative ChlF, f^*, can be calculated as below.

$$f^*(\%) = \frac{f}{a} \times 100 = (ad - cb)/(a^2 - ab) \times 100 \quad (5)$$

2.3 Heat dissipation detection based on PRI

Heat dissipation of excess light energy is related to the activity of the xanthophyll cycle, which involves the enzymatic removal of epoxy groups from xanthophylls to create so-called de-epoxidised xanthophylls (Gamon, et al., 1992). There are three carotenoid pigments in the xanthophyll cycle (violaxanthin, antheraxanthin and zeaxanthin). During light stress, violaxanthin is converted to zeaxanthin and antheraxanthin, which can protect the plants from photodamege by dissipating the excessive light energy (Demmig-Adams & Adams, 1994; Peguero-Pina et al., 2008). The conversion could result in a decrease of reflectance at 531 nm, whereas reflectance at 570 nm is not affected. Therefore, the higher LUE would lead to lower heat dissipation but a higher normalized ratio of 531 nm to 570 nm, and this indicates that LUE is positively related to the normalized ratio.

PRI was proposed by Gamon in the study of biochemical components of sunflower, and was named Physilogical Reflectance Index in the early 1990s (Gamon, et al., 1992). Subsequently, Peñuelas, et al., (1995) renamed it to Photochemical Reflectance Index. PRI is expressed as below.

$$\mathrm{PRI} = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} \quad (6)$$

where R_{531} and R_{570} represent the narrow-band (±2 nm) reflectance centered on the stated wavelength.

3 RESULTS AND ANALYSIS

The plants with three types of plant photosynthesis (C3, C4 and CAM) have different responses to water, heat and light conditions by the plant leaf structure, physiological function, ecologic adaptation, and geographic distribution; the application of those responses is taken as an ideal classification approach of plant functional types (Han, et al., 2006). A significant relation between plant ChlF and photosynthesis was confirmed under natural illumination, which revealed the plant physiological responses with the different environmental stresses. C3 and C4 plants have different LUE because of their different photosynthesis pathways, as well as the responses of their photoprotective mechanisms (ie, ChlF and heat dissipation) to heat and light conditions.

In this study, the diurnal change experiment was carried out under the cloudless weather, and the photosynthetically active radiation (PAR) reached its maximum (about 1800 $\mu \cdot mol \cdot m^{-2} \cdot s^{-1}$) around 13:00. The lowest and highest temperature was 26.2ºC, and 33.2ºC, respectively, and the highest temperature appeared around 15:00 (Fig. 2). C4 plants have higher LUE compared to C3 plants. Therefore, under the conditions of high light and temperature stresses, the variation characteristics of ChlF and heat dissipation should be significantly different between C3 and C4 plants.

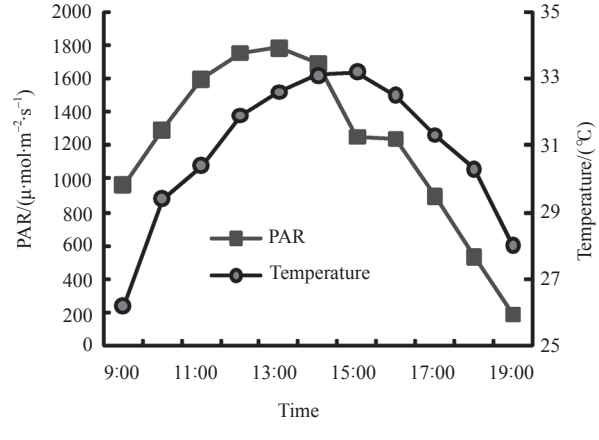
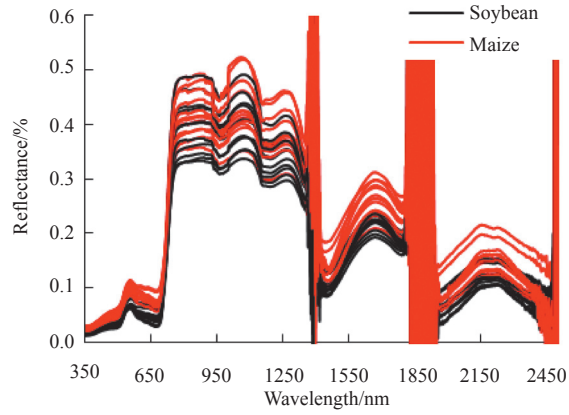


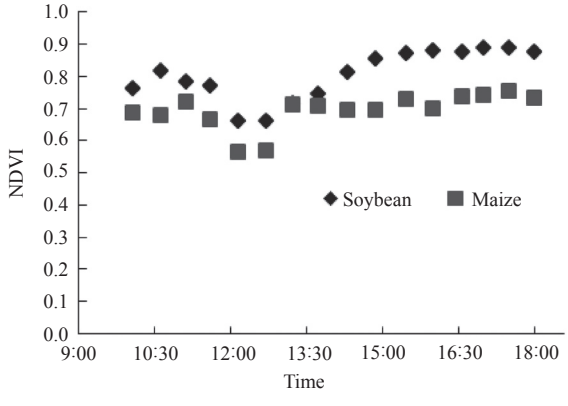
Fig. 2 Diurnal change curves of PAR and temperature

3.1 Diurnal change of C3 and C4 crops canopy spectra

Fig. 3 showed the diurnal change curves of canopy spectra and NDVI of soybean and maize. In this experiment, the leaves of maize had a surface wax layer. These properties caused a higher canopy spectra reflectance of soybean than that of maize in the visible band, while two corps had a similar reflectance in the near-infrared band (Fig. 3 (a)). It was observed from Fig. 3 (b) that both NDVI of soybean and maize had a relative low value at noon due to the soil effects, and a high value during the period of morning and dust. Therefore, we may conclude, that al-



(a) Diurnal change curves of canopy spectra



(b) NDVI of Soybean and maize

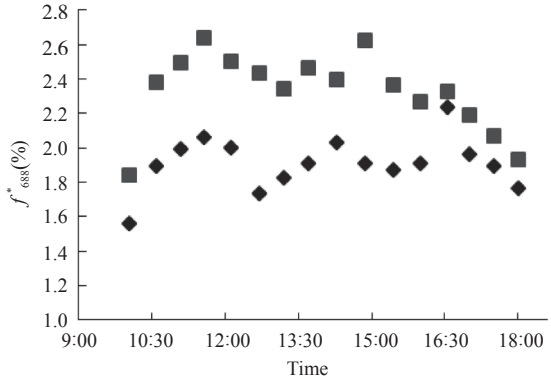
Fig. 3 Diurnal change curves of canopy spectra (a) and NDVI (b) of soybean and maize

though C3 and C4 plants had some differences in physiological and ecological responses under the high light and temperature stresses, it is difficult to detect C3 and C4 plants according to the differences in canopy spectra reflectance, although the diurnal changes of the canopy spectral reveal some differences in vegetation canopy structure.

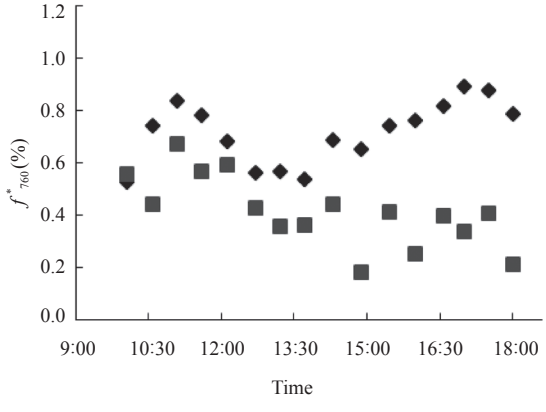
3.2 Stress responses of C3 and C4 crops photosynthesis ChlF and their difference analyses

ChlF is sensitive to photosynthesis change. In this experiment, the stress response of photosynthesis ChlF was detected according to the differences in environmental factors, such as light, temperature, and humidity.

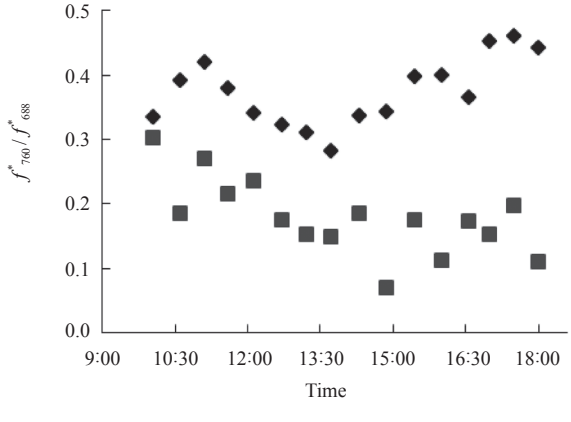
Fig. 4 showed the diurnal change curves of solar-induce ChlF of soybean and maize, and the following results were obtained. (1) ChlF of soybean was lower than that of maize at 688 nm, and an inverse relationship was observed at 760 nm. (2) The relative ChlF of soybean had a rapid increase until 4:30 PM. Under the stress of high temperature, maize (C4 plants) had a high light saturation point, as indicated by the observations that ChlF had a decrease at 688 nm (Fig. 4(a)) while no significant change at 760 nm (Fig. 4(b)). This indicated that C4 plants had a growth advantage under the high light and temperature conditions. (3) A large difference of relative ChlF calculated by the ChlF at 688 nm and 760 nm was observed between soybean and maize (Fig. 4(c)). In this experiment, soybean and maize had a similar growth status, and had small difference of relative ChlF in the morning from 9:30 to 12:00. However, in the afternoon from 12:00 to 18:00, because the differences of stress responses of temperature and light were existed between soybean and maize, a rapid increase was observed for the relative ChlF of soybean, while no significant change for maize. Therefore, it is potential to detect soybean and maize by their relative ChlF.



(a) The relative ChlF at 688 nm (f^*_{688})



(b) The relative ChlF at 760 nm (f^*_{760})



(c) The ratio of the relative ChlF at 760 nm to 688 nm (f^*_{760}/f^*_{688})

Fig. 4 Diurnal change curves of the relative ChlF signals of soybean and maize

3.3 Stress responses of C3 and C4 crops PRI and their difference analyses

Fig. 5 showed the diurnal change curves of PRI of soybean (C3 plant) and maize (C4 plant) acquired on 25 July 2010 at Huailai site. PRI had a similar trend with NDVI (Fig. 3(b)), such as a relative low value at noon and a high value during the period of morning and dust. However, larger change range of PRI was observed, especially in the afternoon, a more rapid increase was observed in the PRI of soybean than that of maize. This phenomenon indirectly confirmed that C3 plants had a "lunch break" due to the high light restraint at noon, leading to an increase in photosynthetic efficiency, and was shown as low heat dissipation and PRI rapid decrease, while C4 plant had no "lunch break" and no significant increase in PRI afternoon.

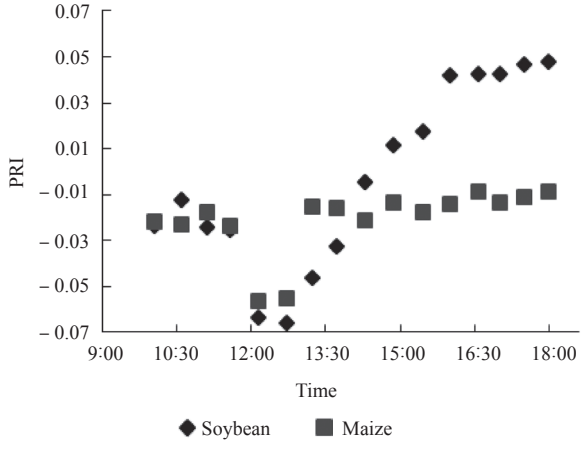


Fig. 5 Diurnal change curves of PRI of soybean and maize

4 CONCLUSIONS AND DISCUSSIONS

Although C3 and C4 plants had some differences in physiological and ecological responses under the high light and temperature stresses, it is difficult to detect C3 and C4 plants according to their canopy spectra reflectance characteristics, although the diurnal changes of canopy spectra can reveal some spectral differences due to vegetation canopy structure.

C4 plants always have much higher photosynthetic efficiency than C3 plants under the conditions of high light and temperature stresses, and they show the different photoprotective mechanisms. In this experiment, soybean (C3 plant) and maize (C4 plant) were selected, and their ChlF (at 688 nm and 760 nm) and PRI were acquired. Based on the analyses of ChlF and PRI diurnal changes, we found that the variation characteristics of ChlF and PRI of C3 and C4 plants under the stress of high light and temperature at the time around noon.

ChlF (688 nm and 760 nm) and PRI can be obtained by the way of hyperspectral remote sensing. According to the characteristics of C3 and C4 crops with the high light and temperature stress in this experiment, we can design the methodology to monitor C3 and C4 plants by hyperspectral remote sensing technology. For example, based on the natural diurnal changes of light and temperature, we can collect multitemporal photosynthesis ChlF and PRI by the hyperspectral remote sensing data at the scale of airborne or satellite, and then analyze their characteristics under the conditions of high light and temperature stress, to classify C3 and C4 plants.

In this study, our findings on C3 and C4 crops characteristics of ChlF and PRI are consistent with their well-established physiology knowledge. However, due to the plant diversity, growth conditions complexity, and long-term evolution adaptability, we need more field experiments to test our method of detecting C3 and C4 plants based on the differences in environmental stress response and hyperspectral remote sensing technology. A better understanding on the stress response physiological mechanism of C3 and C4 plants is needed to clarify the remote sensing photosynthetic parameters and universal quantitative characteristics for ChlF and PRI of C3 and C4 plants under the conditions of high light and temperature stresses.

Furthermore, the upward travelling flux of the emitted chlorophyll fluorescence would be strongly absorbed by oxygen at the 760 Fraunhofer line. Therefore, the atmosphere correction is indispensible, if the method is applied to airborne or space borne hyperspectral image.

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C3、C4作物的光保护机制差异的光谱探测研究

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摘　要：本文设计了大豆(C3作物)和玉米(C4作物)日变化光谱探测试验，提取了太阳光照条件下叶绿素荧光信号和光化学指数PRI。结果表明，首先大豆和玉米叶绿素荧光强度的日变化特征有较大差异，上午大豆的叶绿素荧光比例呈现快速增加的趋势，而玉米的叶绿素荧光未出现增加趋势；其次，下午高温胁迫条件下C3、C4叶绿素荧光光谱比值的变化差异显著，大豆在760 nm和688 nm波段的叶绿素荧光比值快速增加，而玉米未出现显著变化；最后，研究证实了C3作物中午受到强光抑制出现光合作用“午休”现象，即午后光合恢复后其光合效率快速增加，表现为C3作物的热耗散降低、PRI快速增加，而C4作物无“午休”现象，午后PRI增加较小。本文研究表明，强光和高温环境胁迫下C3、C4作物的叶绿素荧光和热耗散变化特征有较大差异，利用胁迫条件下的多时相、高光谱遥感数据，提取C3、C4作物的叶绿素荧光和PRI，有可能实现C3、C4作物的遥感分类目标。

关键词：C3、C4作物功能类型，高光谱，叶绿素荧光，光化学指数

中图分类号：TP701　　**文献标志码**：A

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1　引　言

植物在自然条件下可能发生光抑制(Anderson 等，1997)，植物的光合器官在进化过程中形成了各种各样的光保护机制来最大限度地减少强光可能造成的潜在伤害。这些光保护机制包括：以叶绿素荧光方式释放，避免叶绿体吸收光能超过光合作用的消化能力；通过天线系统非辐射耗散将过剩的光能以热能的形式消耗掉等(Bolhar-Nordenkampf 等，1989；Anderson 等，1997；Niyogi，1999)。

光合作用过程中有一部分光能损耗以较长波长的叶绿素荧光方式释放(通常不到入射能量的1%)(Liu 等，2005)。遥感探测的植被反射的光谱信号既包括植被反射的光谱信号，也包括植被光合作用发射的叶绿素荧光信号。尽管自然太阳光照条件下叶绿素荧光信号与植被反射光谱信号是叠加在一起的，但已有很多学者的研究发现，可以利用植被反射光谱中的Fraunhofer线，设计叶绿素荧光的光谱分离算法。McFarlane等人(1980)及Carter等人(1990，1996)利用太阳光谱的H-a Fraunhofer线(656 nm)测量柑橘等植物冠层的光合作用荧光。Zarco-Tejada等人(2000)及张永江等人(2007)研究表明，通过控制光源照明条件，可以探测叶片水平的荧光发射光谱，其中685 nm波长处表观反射率中荧光发射光谱比例占10%—25%，740 nm波长处表观反射率中荧光比例占2%—6%。Moya等人(2004)、Liu等人(2005)、刘良云等人(2006)、Alonso等人(2008)及Amoros-Lopez等人(2008)发现利用植被冠层和太阳入射的辐亮度光谱(光谱采样间隔1 nm左右)，采用Fraunhofer线原理，可以定量计算和探测688 nm和760 nm波长的冠层叶绿素荧光。Zarco-Tejada等人(2004)及Perez-Priego等人(2005)利用高光谱分辨率 (半高宽FWHM为0.065 nm)的光谱仪测

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定树冠反射率光谱，结果表明，由于叶绿素荧光对地球大气的氧气吸收Fraunhofer线的填充效果，760 nm波长处表观反射率中有明显尖峰，760.59 nm与759.5 nm反射率差值为1%—3%。

过剩光能的热耗散对于调节光能的吸收和电子传递起关键作用，而且对于防止光合器官的光抑制甚至光氧化起至关重要的作用。强光条件下，类囊体膜两侧的质子梯度加大，触发光捕获天线蛋白LHCⅡ将过剩的激发能以热的形式无危害地耗散掉，植物可以通过热耗散消耗掉天线系统所吸收光能的一半以上(Demmig-Adams 等，1996) 。光能的热耗散是叶黄素从环氧化状态转变为脱环氧化状态导致的结果，部分研究发现这种色素形态的变化会导致531 nm 处反射率的下降，但对570 nm处的反射率却几乎没有影响，通过计算570 nm和531 nm波段反射率的归一化比值植被指数，就能够监测叶黄素循环的热耗散。Gamon等人(1992)及Peñuelas等人(1997)将该归一化比值指数定义为光化学植被指数PRI (Photochemical Reflectance Index)。Demmig-Adams和Adams(1994)以及 Peguero-Pina等人(2008) 研究发现PRI与叶黄素循环存极显著的相关关系。此外，许多学者研究了PRI与叶黄素变化导致的非光化学荧光猝灭，NPQ(可用于反映热散失的大小)之间的关系，发现PRI与NPQ存在可靠的负相关关系(Evain 等，2004；Nichol 等，2006；张永江 等，2006；van 等，2007)。

根据植物的光合途径，可以分为C3、C4和景天酸CAM类型(Taiz和Zeiger，2006)。由于C4型比C3型作物对光、水分和N素的利用效率更高，对高温、干旱和盐碱的抗逆性更强，在全球变化研究中备受关注(Han 等，2006)，但C3、C4作物的遥感分类的机理研究几乎空白。本文以大豆(C3作物)和玉米(C4作物)为例，通过光谱探测日变化实验，研究C3、C4作物两种光保护机制(叶绿素荧光和热耗散)的环境胁迫响应机制，提出C3、C4两种作物叶绿素荧光和热耗散敏感光谱信号的日变化特征及其差异，分析胁迫条件下的C3、C4作物的多时相高光谱遥感分类的可行性。

2 实验与方法

2.1 实验区域与数据获取

本次实验于2010-07-21在中国科学院遥感应用研究所怀来实验基地进行。当日天气晴朗、无云、无风。实验对象包括春玉米(C4)和大豆(C3)。此时大豆和春玉米都已经封垄，大豆处于分枝末期，玉米处于大喇叭口期。挑选正常肥水管理，长势均匀大豆和春玉米种植区域设计日变化试验。试验挑选的大豆和春玉米小区位于同一地块，相互毗邻，皆为南北垄向，方便准同步地测量两种作物的冠层光谱。

实验采用ASD FieldSpec光谱仪获取植被冠层光谱，从10：00到18：00每隔半小时测定一次，下午18：00时光合有效辐射约为200 $\mu\cdot mol\cdot m^{-2}\cdot s^{-1}$，此后光强迅速下降，测量信号信噪比不能满足测量要求。光谱测量时，光谱仪探头垂直向下观测，光谱仪探头距植被冠层高度为1.0 m左右，通过20次水平移动的重复测量平均得到冠层光谱，移动光谱测量冠层范围为1 m × 1 m范围的均匀冠层。在整个日变化测量过程中始终保持观测目标基本不变。该光谱仪采样间隔为1 nm，光谱范围为350—2500 nm，光谱分辨率为3 nm@VNIR(350—1050 nm)、10 nm@SWIR(1050—2500 nm)，视场角FOV为25°。实验采用0.5 × 0.5 m^2灰板作为参考板，以测量太阳入射辐射光谱。

观测采用辐亮度模式(Radiance)进行，实验中每次对目标采样十次，取平均值作为冠层辐照度光谱。并在测定目标前后及时测定参考板反射辐亮度值，利用参考板测定的辐亮度值来计算到达冠层的太阳辐照度光谱。测定的辐亮度单位为$W\cdot m^{-2}\cdot nm^{-1}\cdot sr^{-1}$，其中sr为立体角，辐亮度乘以π得到辐照度光谱。

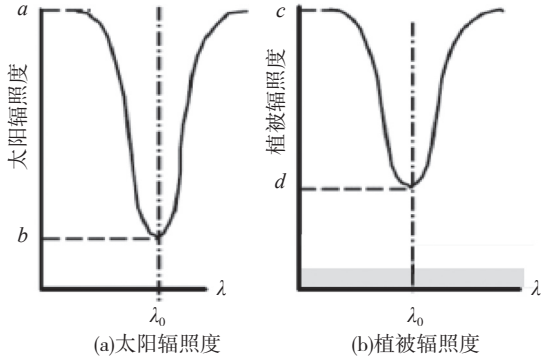
2.2 光合作用叶绿素荧光光谱探测方法

通常绿色植物受短波光激发后，会在400—800 nm之间产生3个较为明显的荧光峰值，主要在440 nm、685 nm和740 nm左右。在该区域中，仅650—780 nm范围内荧光是由叶绿素发出，其发射峰值在685 nm和735 nm左右。研究表明，这些特殊荧光发射波段和波段比可以反应植物健康状况和生长条件，特别是胁迫状况的早期诊断上(Subhash 等，1999；McMurtrey 等，2003)。

在自然光照条件下，叶片或冠层测量的表观反射率光谱，既包括叶片对入射光的反射光谱又包括叶绿素荧光发射光谱。由于荧光发射能量只占到叶片吸收总能量的1%—3%，因此很难直接从反射光谱中直接分离出来。由于太阳和地球大气的吸收，到达地表的太阳辐射存在许多细小的暗线，称为夫琅和费(Fraunhofer)暗线。这些暗线的宽度在0.1—10 nm之间，其

中有些暗线的中心强度比其相邻谱区低10%以上，这为自然光照条件下的植被荧光探测提供了可能的弥补方法。来自于短波激发的植被荧光将这些夫琅和费“井”填充到一定程度，通过比较入射和反射光谱中夫琅和费“井”中心区以及其相邻谱区的强度就可以计算出某些特定波段的荧光效应(Louis 等，2005；刘良云 等，2006)。

荧光发射光谱和植被冠层反射光谱是混合在一起的，且由于光谱仪的光谱分辨率和信噪比的限制，所以利用夫琅和费暗线探测植被荧光的波段选择十分重要。夫琅和费暗线的波长主要有486 nm、527 nm、589 nm、656 nm、688 nm和760 nm，如果探测器的光谱分辨率和信噪比足够高，是有可能探测到夫琅和费暗线的。文献(Moya 等，2004；刘良云 等，2006)等研究表明利用ASD FieldSpec NIR光谱仪探测的688 nm和760 nm波长的太阳光激发的叶绿素荧光是可靠的。如图1所示，λ_0为夫琅和费暗线的中心波长，假定植被在中心波长附近反射率恒定为R。其中a、c为夫琅和费暗线临近区域的入射和反射的光谱强度，b、d为夫琅和费暗线位置的入射和反射的光谱强度，f为植被冠层发射的荧光强度。根据式(1)—式(4)就可以得到植被荧光发射光谱。



(a)太阳辐照度 (b)植被辐照度

图1 夫琅和费线探测探测荧光原理

(图1(b)中的阴影表示荧光)

$$c = R \cdot a + f \quad (1)$$

$$d = R \cdot b + f \quad (2)$$

$$R = (c-d)/(a-b) \quad (3)$$

$$f = d - R \cdot b \quad (4)$$

由于荧光发射光谱是从植被反射光谱中直接提取，与植被接收到的光合有效辐射直接相关。因此，利用反射光谱中的荧光强度与入射光谱的比值定义为荧光相对强度f^*，能够在一定程度上消除这种影响，更加敏感的追踪植被光能利用率变化情况。结合式(1)—式(4)可以得到最终荧光相对强度表达式(式5):

$$f^*(\%) = \frac{f}{a} \times 100 = (ad - cb)/(a^2 - ab) \times 100 \quad (5)$$

本文中，根据ASD光谱仪测量的辐亮度光谱曲线特征，688 nm夫琅和费暗线和相邻谱区的波长为688 nm与684 nm，760 nm夫琅和费暗线和相邻谱区的波长为761 nm与756 nm。

2.3 热耗散光谱探测方法

光能的热散失是叶黄素从环氧化变成脱环氧化的结果(Gamon 等，1992)，这种色素形态的变化也称为叶黄素循环。叶黄素具有3种成分：紫黄质(violaxanthin)、花药黄质(antheraxanthin)和玉米黄质(zeaxanthin)。紫黄质经过两次脱环化能够形成花药黄质和玉米黄质。当植物在暗中或者弱光情况下，叶黄素循环的主要组分是紫黄质，当叶片吸收的光能超过光化学利用时，产生的过剩光能会将紫黄质转化成花药黄质和玉米黄质，从而完成了光能转换成热能向外耗散的过程(Demmig-Adams和Adams，1994；Peguero-Pina 等，2008)。当这种色素形态的转变发生时会导致531 nm处的反射率下降，而570 nm处的反射率几乎不受影响。因此，光能利用率越高热耗散越低，531 nm与570 nm归一化比值越高，两者应呈正相关关系。

光化学指数最早由Gamon等人(1992)在研究向日葵生化组分时提出，并命名为Physilogical Reflectance Index。后来由Peñuelas等人(1995)对其进行了修正并将名称改为Photochemical Reflectance Index(PRI)。它可以通过式(6)计算得到。

$$\mathrm{PRI} = \frac{R_{531} - R_{570}}{R_{531} + R_{570}} \quad (6)$$

式中，R_{531}、R_{570}分别代表531 nm和570 nm处的植被光谱反射率。

3 结果与分析

植物不同的光合途径(C3、C4和CAM)能够从叶片结构到生理功能、从生态适应到地理分布均表现出对不同水、热、光条件的响应，是一种理想的植物功能型分类方法(韩梅 等，2006)。自然光照条件下植被发射的荧光与光合作用状况密切相关，能够揭示植物在各种环境胁迫下的生理响应。由于光合途径的差异，在不同温度、光照胁迫条件下，C3和

C4植物光合作用的光能利用各不相同，两种光保护机制(即叶绿素荧光与热耗散)的胁迫响应应该有所差异。

在本次日变化实验中，天气晴朗无云，光合有效辐射PAR在13：00左右达到最大值，约1800 μ·mol·m^{-2}·s^{-1}；温度最低26.2℃，最高33.2℃，最高温出现在15：00左右，如图2所示。本次试验中的强光和高温对C3作物会形成一定的生长抑制的胁迫条件，出现典型的“光合午休”现象；而C4作物的光饱点和适宜生长温度要大于C3作物，不会出现“光合午休”现象。因此，实验中午和下午出现的强光、高温胁迫条件下，C3、C4作物的叶绿素荧光和热耗散变化特征应该能够呈现显著差异。

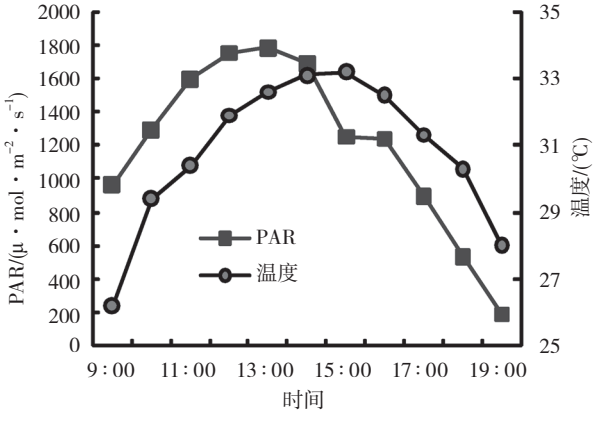
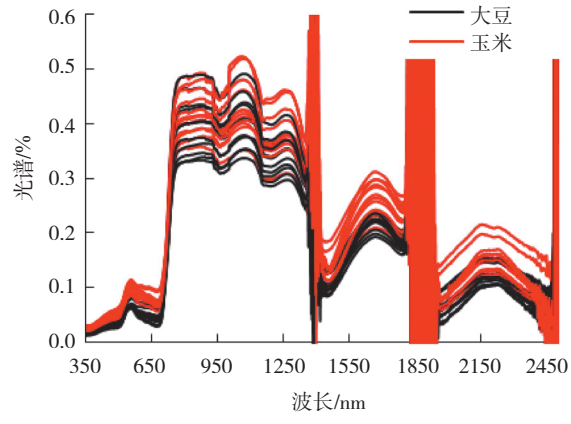
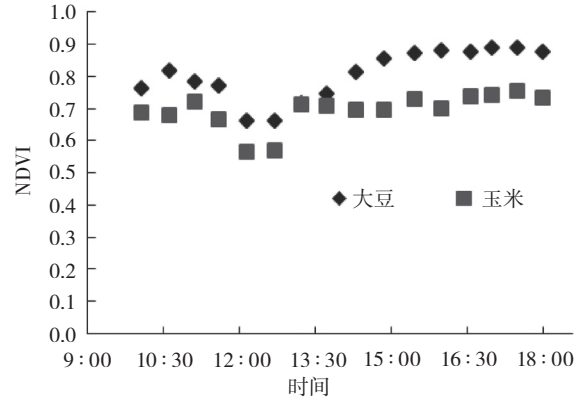


图2 光合有效辐射和温度的日变化响应曲线



(a) 大豆和玉米日变化光谱



(b) 大豆和玉米NDVI日变化曲线

图3 大豆和玉米光合的日变化光谱与NDVI响应曲线

3.1 C3、C4作物的日变化光谱

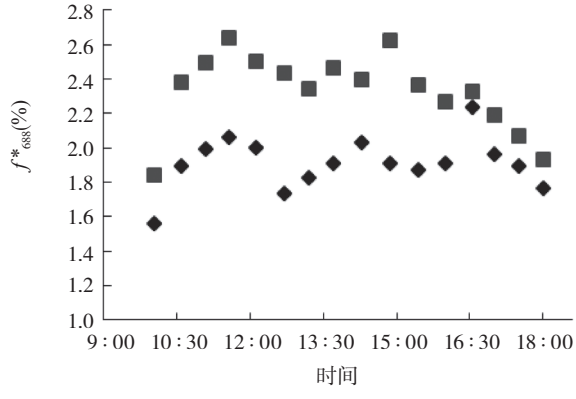
图3为实验获取的大豆和玉米日变化冠层光谱和归一化植被指数(NDVI)。由于实验大豆的植被覆盖优于玉米冠层，玉米叶片因叶绿素浓度相对较低、表面蜡质层等原因，玉米叶片反射率比大豆要高些。所以图3(a)表现的玉米冠层光谱反射率在可见光波段要高于大豆，在近红外波段与大豆光谱相当。但从图3(b)表现的NDVI日变化曲线中可以发现，玉米和大豆的光谱日变化趋势是一致的，即中午土壤背景贡献相对较大，呈现出NDVI中午低、早晚高的相同变化趋势。因此，即便存在强光、高温胁迫，C3、C4作物尽管存在部分生理、生态响应差异，但由于植被冠层光谱日变化代表地物二向反射特征，而二向反射光谱特征主要反映植被冠层结构信息，因此难以从光谱二向反射特征差异去识别C3、C4作物。

3.2 C3、C4作物的光合作用叶绿素荧光的胁迫响应与差异分析

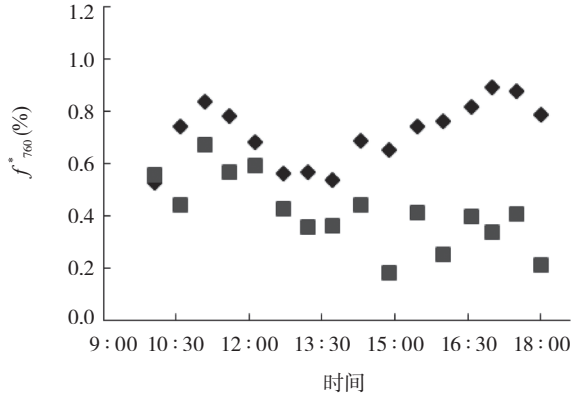
植被叶绿素荧光作为光合作用探针，能够敏感跟踪光合作用变化。在日变化实验中，由于光照、温度、湿度等环境因素差异，导致植被光合作用叶绿素荧光的环境胁迫响应差异。

图4为大豆(C3作物)和玉米(C4作物)两种作物的叶绿素荧光比例的日变化曲线。结果表明大豆和玉米叶绿素荧光信号有较大差异，(1)大豆冠层在688 nm的叶绿素荧光低于玉米，而760 nm叶绿素荧光正好相反，大豆叶绿素荧光高于玉米；(2)下午受到高温胁迫的影响，大豆的叶绿素荧光比例呈现快速增加的趋势，直到下午4：30后才逐步降低，而玉米作为C4作物其光饱和点高，在高温胁迫下，688 nm波段叶绿素荧光比例总体呈下降趋势(图4(a))、760 nm波段叶

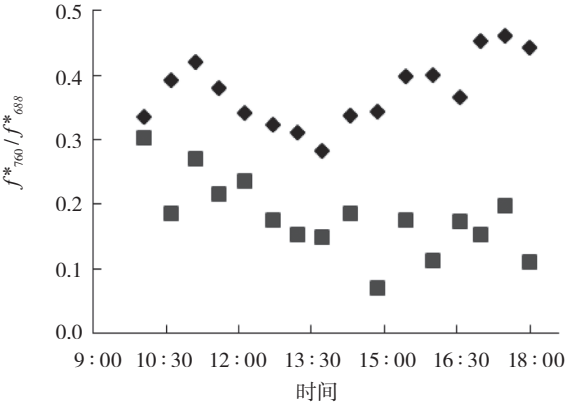
绿素荧光未出现显著变化趋势(图4(b))，说明了C4植物在强光、高温胁迫下的生长优势。(3)大豆和玉米在760nm和688nm波段的叶绿素荧光比值有很大差异(图4(c))，尽管试验对象的大豆和玉米的长势相近，上午760 nm和688 nm波段的叶绿素荧光比值差异相对较小，而下午时，由于在温度、光照等环境胁迫响应差异，大豆的叶绿素荧光比值快速增加，而玉米的叶绿素荧光比值未出现显著变化，因此，利用760 nm和688 nm波段的叶绿素荧光比值很容易区分大豆和玉米。



(a) 688 nm波段叶绿素荧光比例(f^*_{688})



(b) 760 nm波段叶绿素荧光比例(f^*_{760})



(c) 760 nm与688 nm波段叶绿素荧光的比值(f^*_{760}/f^*_{688})

图4 大豆和玉米光合作用叶绿素荧光的日变化响应曲线

3.3 C3、C4作物的PRI胁迫响应与差异分析

图5为2010-07-23怀来日变化中大豆(C3)、玉米(C4)的PRI的日变化曲线，结果表明大豆和玉米的PRI与NDVI有相似的日变化规律，即中午低、早晚高，但大豆PRI变化幅度更大，表现为中午下降幅度更大、午后增加幅度更突出。对比图3的大豆和玉米的NDVI日变化曲线，可以发现PRI作为一种光谱指数，与NDVI一样存在相似的日变化特征，但大豆和玉米的PRI日变化幅度差异要远大于NDVI的日变化幅度差异。特别是，午后大豆PRI指数快速增加，而玉米午后PRI增加很小。该现象间接证实了C3作物中午受到强光抑制出现光合作用“午休”现象，而午后光合恢复后C3作物光合效率快速增加，表现为C3作物的热耗散降低、PRI快速增加，而C4作物无“午休”现象，午后PRI无显著增加。

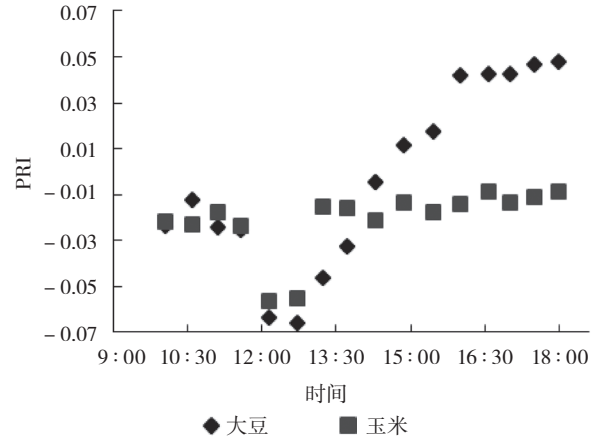


图5 大豆和玉米的PRI的日变化曲线

4 结论与讨论

C3、C4植物对强光、高温等日间胁迫变化存在部分生理、生态响应差异，但由于植被冠层光谱日变化更多反映植被冠层结构差异，冠层光谱对光照、温度等日变化差异的响应呈现相似变化趋势，因此难以从传统光谱胁迫变化差异角度识别C3、C4植物。

在光合作用生理过程方面，C4作物在强光、高温胁迫条件下，光合效率要远高于C3作物，因此，在不同温度、光照胁迫条件下，C3和C4光保护机制会呈现不同的变化规律。论文通过日变化光谱实验，提取了688 nm和760 nm两个波段的光合作用叶绿素荧光以及光化学指数PRI，观测了大豆(C3)、玉米(C4)的叶绿素荧光和光化学指数PRI的日变化规律，发现并证实了中午和午后强光、高温胁迫下的C3、C4作物的光合作用叶绿素荧光和PRI的变化特征。

由于可以通过高光谱遥感方式，获取688 nm、760 nm两个氧气吸收线的光合作用叶绿素荧光和PRI光谱指数。基于本实验研究证实的C3、C4作物的强光和高温环境胁迫响应差异特征，可以设计日变化胁迫响应差异的C3、C4作物高光谱遥感监测方法。如利用上午、中午和下午光照和温度条件的天然差异，通过获取上午、中午和下午等不同时间段的高光谱航空或卫星高光谱遥感数据，提取不同时间段的光合作用叶绿素荧光和PRI光谱指数，根据叶绿素荧光和PRI指数在强光和高温胁迫下的差异特征，实现C3、C4作物的高光谱遥感分类目标。

论文实验观测的C3、C4作物在强光、高温胁迫下的叶绿素荧光和PRI光谱指数变化特征与传统C3、C4作物生理研究认识一致。但论文由于实验条件的限制，只挑选了玉米和大豆两种生育进程相近的作物，代表C3、C4作物类型，且只获得了一个生育期的观测资料，研究存在有一定的局限性。由于自然条件中植物类型、生长条件的复杂性和长期进化出的适应性，还需要更多、更全面的观测实验研究来证实，并需要结合更深入的C3、C4作物胁迫响应生理研究基础，阐明C3、C4作物在强光、高温胁迫下的叶绿素荧光、PRI等可遥感光合参数的普适与定量差异特征。

此外，若将论文方法应用到航空、卫星遥感影像时，由于植被冠层发射的叶绿素荧光在上行辐射传输过程中，会受到大气吸收等强烈干扰，因此，在应用前还需要对成像过程中的大气辐射传输进行定量校正。

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