Response To Light Intensity of Sun And Shade On Two Cool Season Turfgrass

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Research Article

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Abstract

Background: Obtaining superior performance of SupraNova and Lark C3 turfgrasses under shade conditions is a challenging task. Both durability and performance of turfgrass are significantly affected by shade. In particular, morphological and physiological adaptation to low light is critical for maintaining quality and overall performance in turfgrass plants.

Results: The purpose of this study was to study the response of SupraNova and Lark turfgrass morphology and photo-physiological potential to shading. The plants of 'SupraNova' and 'Lark' were collected from the lawn plots of the Horticulture Research Center of Northeast Agricultural University of China for 2 years and treated with gradient shading 35.62% after 2 months of culture, normal light intensity 70.79 % and 93.45% with full sun as the contrast represented by CK for comparison. Lark showed TQ and TCI in shady stage compared with SupraNova. Lark showed strong resistance to MDA, H_2O_2, O_2-, SOD, POD, CAT and AsA, indicating that the antioxidant system of C3 turfgrass at 35.6% shade level. Under 70.79% shade treatment, MDA, H_2O_2, O_2-, SOD, POD, CAT and AsA of the two cultivars decreased the most, and the longer the shading time, the average daily growth of the two turfgrasses increased first and then decreased. Lark outperformed SupraNova throughout the shading treatment, with 70.79% and 93.45%, respectively. Lark showed increased Chl A and Chl (A/B) in response to different shading levels, while SupraNova had the highest concentrations of Chl B and total Chl. Chlorophyll fluorescence qP, ETR, and Fv/Fm decreased significantly when shaded at 93.45%;

Conclusion: The results of this study proved that decrease was more significant in SupraNova than Lark, and shading caused more severe changes in leaf morphology and anatomical structure than Lark turfgrass has the highest negative tolerance than SupraNova turfgrass, which is due to the better photosynthetic product transport capacity of Lark plants. In its anatomical structure, and" vascular bundle sheath structure, which enables it to have higher photosynthetic efficiency to adapt to negative stress. SupraNova and Lark first increased and then decreased with the increase of shade degree

Background

Plants use photosynthesis to synthesize organic matter to supply their own growth, development, and reproduction. Light is a signal factor for plant morphogenesis that can induce, promote, and regulate plant growth, development, differentiation, and other processes that are crucial for determination of overall plant productivity [1-3]. Plant components are very sensitive to changes in their surrounding light environments, and have the ability to change morphological attributes such as leaf area index and leaf are duration, and their nutrient uptake rates, to adapt to different light environments [4-6]. Numerous studies have shown that plants have developed appropriate countermeasures to cope against light environmental changes in their long-term evolution [7]. As a result, when plants are stressed by low light intensity, they are capable to adjust morphological and physiological characteristics to improve their ability to capture and assimilate more light and reduce the damages caused in these environments [8-10].

The light energy absorbed by plants is mainly consumed through photosynthetic electron transfer, chlorophyll fluorescence, and heat dissipation, among other processes [11]. Chlorophyll fluorescence is closely related to

various processes in the photosynthetic reaction, and reflects the photosynthetic situation and efficiency of the plant [12]. Therefore, studying chlorophyll fluorescence is an effective way to study plant photosynthesis [13]. The maximum photochemical quantum yield of PS□ and maximum photochemical efficiency (Fv/Fm) usually reflects the potential maximum photochemical efficiency of the plant and the maximum light energy conversion rate of the PS□ reaction center [14]. Although the chlorophyll fluorescence parameters of different plants have different responses to light intensity, the value of Fv/Fm increases as the degree of shading increases [15]. However, recent studies have shown that shade could reduce the value of Fv/Fm [16, 17]. Photochemical quenching coefficient (qP) can reflect the opening degree of the PS□ reaction center and the electron share of light energy absorbed by the photosynthetic pigments [18]. The electron transfer rate (ETR) is closely related to leaf stomatal conductance, and thus, to the net photosynthetic rate of plants [19, 20]. While some studies have shown that the value of ETR will first increase and then decrease as the degree of shading increases [7], others concluded by saying that shading can actually reduce the value of ETR [21]. To present, research on the fluorescence parameters and photosynthetic characteristics of different turfgrass has mainly focused on the effects of nitrogen fertilizer application, heavy metal ion effects, drought stress, and waterlogging, but there are few studies on shading conditions. Thus, it is particularly important to explore the changes in fluorescence parameters and photosynthetic characteristics of turfgrass under shading conditions.

Osmotic regulation of plant cells is an important metabolic regulation mechanism in plant life [22, 23]. One of the mechanisms by which plants respond to changes in their environment is through the modification in the soluble protein (SP), soluble sugar (SS), and free proline (Proline, Pro) contents [24]. When plants face stressful environments, they can adjust the content of SP and SS to maintain the osmotic pressure in the plant cell, both key products of plant carbon metabolism [25]. Soluble sugars have two important tasks in plants, serving as a photosynthetic product to supply for plant growth and development and an osmotic regulator to balance the osmotic potential in cell [26]. Pro is a component of plant protein and usually exists in a free state in plants [27]. When plants are subjected to different environmental stresses, Pro will accumulate in large quantities to regulate the osmotic pressure in the cytoplasm, thereby maintaining the normal plant growth. Although is known that shading can change plants content of Pro in plants, the specific mechanisms by which this occurs are not yet clear [28].

Under stress conditions, the balance of the enzymatic system in the plant is affected, and excess reactive oxygen species produced in the cells aggravate the peroxidation of the cell membrane. In response to this, plants use their enzymatic and non-enzymatic antioxidant defense systems to remove excess reactive oxygen species (ROS) and protect their photosynthetic organs and cell membranes from injury [29]. Non-enzymatic antioxidants such as reduced ascorbic acid (AsA) and reduced glutathione (Glutathione, GSH) are utilized to scavenge harmful substances such as free radicals, peroxides, and reactive oxygen species. Likewise, antioxidant enzymes are one of the main means for plants to cope against environmental stresses [30]. Among these, Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) are the main enzymes in the plant protection and defense system. SOD is the first line of defense of the antioxidant system. It can remove the damage of superoxide anions to plants. CAT mainly removes excess hydrogen peroxide in plants. POD can remove the toxic effects of hydrogen peroxide and phenols and amines on plants [31]. Under shading

conditions, the interaction between these enzymes can ensure the normal operation of the plant's antioxidant defense mechanism, thus minimizing plant damage to ROS [32].

Malonaldehyde (MDA) is a typical product of plant membrane peroxidation, and its content can indicate the degree of membrane damage. Both optimal and sub-optimal light conditions can cause an increase in MDA (cite). An abundant body of literature has shown that the active oxygen content in plants will change under conditions such as senescence, damage, and environmental stresses such as drought, salinity, shading, and others. Moreover, there are many ways to produce ROS. The electron transport on chloroplasts, mitochondria, and plasma membranes can produce toxic and active ROS. Some enzyme-catalyzed processes and the auto-oxidation process of certain biological substances can produce superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (-OH), and others. If ROS excess cannot be eliminated in time, it could severely damage plant biofilms, photosynthetic organs and molecules with important biological functions [33]. Studies have shown that shade can significantly reduce the O_2^- content in plants, but there is currently no strong agreement in this sense [34]. Research regarding the antioxidant system and active oxygen of turfgrass under shading conditions has been adequately addressed, but the comparative research between cold-season turfgrass and warm-season turfgrass under shade is still relatively rare. The current study aimed to explored this research gap.

Stomata is a special leaf structure composed of a pair of guard cells and a gap between these guard cells [35]. Plants exchange gas and water with the atmosphere through stomate, and their size directly determines the photosynthesis and transpiration rates of plants. Studies have shown that the number and distribution of stomata on plant leaves undergoes adaptive changes when plants are subjected to environmental stress, and this greatly affects their gas exchange rates [36]. Stomata can use guard cells to sense the external environment and also the internal plant signals, make countermeasures to environmental stress, reduce and even improve resistance against environmental damage to plants [37]. Other plant structures, such as the root system can also synthesize plant hormones, organic acids, and amino acids and transmit stress signals that can induce the opening and closing of stomata [38]. The light intensity, photoperiod, and the growth and development of the above-ground parts are closely related to the morphology, growth and development, and physiological characteristics of the root system [39]. In general, moderate light is conducive to photosynthesis, and it will promote the accumulation of more photosynthetic products in the aboveground biomass to transfer to the root system, which is conducive to root growth [40]. The study on leaf stomata and root system characteristics of turfgrass leaves is helpful to reveal the morphological and physiological change mechanism of turfgrass to adapt to different stresses, and provide basis for production. The morphology and physiological functions of plant organs are inseparable from the surrounding environmental conditions [41]. Leaves are particularly sensitive to environmental changes as a plant with high plasticity, and they can adapt to various environmental stresses by modifying their morphology and internal anatomical structure. [42]. Therefore, structural characteristics of plants can reflect the impact of environmental factors and their ability to adapt to these changes [43]. For example, leaves and other structures of plants will generally be thicker under strong light, and thinner under weak light conditions [44]. The aim of this study was to investigate the effects of turfgrass on two species, i.e., C3 plant SupraNova (*P. supina*), and Lark (Lolium Perenne L.) Kuntze for shade performance, growth, leaf anatomy and photophysiological response a C4

plant). Both grass species are widely used in playgrounds and are generally considered the most shade-tolerant of their respective C3 and C4 groups.

Materials And Methods

Plant material and experimental site

A total of two typical cold-season turfgrasses species were selected as test materials, including SupraNova' (*P. supina*) is a European-bred variety provided by

British Seed Houses, UK, and Lark (*Lolium perenne L.*) from Beijing, China provided by Topgreen Turf Company. Poa SupraNova is a stoloniferous variety that has strong shade tolerance, while Lark ryegrass has erect stems that better thrive under strong light conditions.

Cultivation And Processing Of Test Materials

The pot experiment was carried out in the greenhouse (126°68'E, 45°72'N) of the Horticulture Experimental Station of Northeast Agricultural University, Harbin. The organic matter content in the potted substrate was 16.8g kg^{-1}, and the pH was 7.0. Pots had 15-cm in diameter and 20-cm in height. 'SupraNova' is propagated by cuttings, and the stems of the seedlings were cut and inserted in the pots, each with 60 plants. Lark's propagation method consisted in 60 seeds per pot. The two grass species were trimmed as needed to maintain the height at 4.5cm. Two months after planting, gradient shading was applied to the two varieties.

The field experiment adopted a random block design with an area of $1m^2$, and 4 plots of each species were planted, for a total of 32 plots. 'The solely factor under analysis consisted of different shading treatments applied two months after planting for both grass species. Shading gradient included: 1) control treatment, normal light treatment without shading; 2) shading treatment ((35.6% shading resulting from covering the pots with a layer of shading net). The light intensity was measured with an illuminance meter (Spectrum 3413F, USA), and each treatment was repeated 4 times for a total of 32 pots. The appearance quality of the two turfgrass was measured every 15 days since treatments were imposed, and morphological indicators, anatomical structures, physiological indicators, and photosynthetic indicators were measured after 45 days from treatment allocation. Following 15 days of shading, an ASD portable field spectrometer (ASD Field-Spec Handheld, USA) was used to measure the canopy spectroscopy of the two varieties.

Index Measurement

The appearance quality and color of the lawn

The nine-point evaluation scale is used to measure and analyze turf quality (TQ) and turf color intensity (TCI), as follows: 9 points indicates the highest, 6 an acceptable, and 1 the lowest TQ and TCI. The evaluation was conducted every 15 days starting from the planting time until. As a result, the higher the score, the better the appearance quality of the lawn and the higher its turf value.

Shade Treatment

The soluble protein content was determined by the Coomassie Brilliant Blue method [45]. Briefly, 320μL of supernatant was, repeated 4 times, and the absorbance was measured at 595nm. The soluble sugar content was determined by the anthrone colorimetry method [45]. Each time, 320 μL of supernatant was drawn, repeated 4 times, and the absorbance was measured at 630 nm. Determination of free proline content was performed using the sulfosalicylic acid method [46]. Here, 320μL of toluene layer were extracted 4 times and color compared at 520nm. Malondialdehyde (MDA) content was determined by the thiobarbituric acid method [45], 320μL of supernatant was drawn for colorimetry at 450nm, 532nm, and 600nm each time. Determination of SOD activity was performed by using NBT photochemical reduction method [47]. Each time, 320μL of the mixed solution was drawn, repeated 4 times, and the absorbance was measured at 560nm. The guaiacol method was used to determine the POD activity [48], by extracting 320 μL of the mixed solution 4 times, and then measuring the absorbance at 470 nm every 60 seconds. CAT activity was determined by the ultraviolet absorption method [48], by extracting 320 μL of the mixed solution 4 times, and the absorbance value was measured at 240 nm every 60 seconds. AsA content determination, using the spectrophotometric method to determine AsA content [49], each time 320μL of the mixed solution was drawn, repeated 4 times, and the absorbance was measured at 534nm. The H_2O_2 content was determined using the spectrophotometric method [50], Here, 320μL of the mixed solution were removed 4 times, and then the absorbance measured at 415nm. The O_2^- content was determined using the hydroxylamine oxidation method [51]. Each time 320μL of the mixed solution was drawn, repeated 4 times, and the absorbance was measured at 530nm.

The chlorophyll fluorescence imaging system (IMAGING-PAM, Germany) was used to determine the chlorophyll fluorescence parameters. After 45 days of shading treatment, 4 pots of each shade gradient were taken from each test material. After 30 minutes of dark treatment, the plants were placed on the chlorophyll fluorescence IMAGING system instrument to measure the photochemical quenching coefficient (qP). Electron transfer efficiency (ETR) and maximum photochemical efficiency (Fv/Fm).Moved up. Here was disconnected from previous and following sentences.

Determination Of Morphological Indicators

After 45 days of shading treatment, 4 plants of each test material were randomly selected from each shade gradient, and the root morphology was determined. After washing with distilled water, the plant roots were cut and laid on the test instrument's glass plate with an alcohol-sterilized blade above, being cautious not to overlap the roots. Use a root scanner (LA-S Series Plant Root Analyzer, China) to scan the roots and get the average value.

Stomatal density was measured. After 45 days of shading treatment, 4 plants were randomly selected from each shade gradient for each test material, and the 4th leaf from top to bottom was taken out, and 3.5cm long was cut from the middle position and placed in Suspension culture in MES-KCL buffer, the light at 25°C for 2h, then put the leaves in 5ml load buffer and add H2DCF-DA fluorescent probe to make the final concentration of H2DCF-DA 50μmol/L. Shake gently, incubate in the dark for 30-60 minutes, wash with

loading buffer 3 times to wash off excess fluorescent probes, and prepare and observe under a fluorescent inverted microscope.

Anatomical Structure Determination

After 45 days of shading treatment, 4 plants were randomly selected from each shade gradient of each test material. The fourth leaf was completely expanded from top to bottom, and a 1cm blade was cut from the middle part of leaf. It was processed according to conventional paraffin sectioning and photographed under an optical microscope (Nikon E200MV, Japan)

Data analysis.

The data uses Excel and SPSS software for correlation, significance analysis, variance, and average calculation, and comprehensive evaluation of the response of SupraNova bluegrass and Lark ryegrass to light intensity. Finally, Graph Pad Prism 7 software was used to graph the analysis results.

Results And Analysis

Visual turf quality (TQ) and turf color intensity (TCI)

Shading significantly affected the color and quality of the lawn of the two varieties (Table 1). For ‘SupraNova’, proper shade can improve TQ and TCL, Under 36% shading level, the TQ and TCI of grass species were the highest, although these values were not different to those observed in the control. This shows that ‘SupraNova’ has a certain degree of adaptability to short-term light shade. For ‘Lark’, all shade treatments reduced its TCI compared to the control, and 71% and 93% shading reduced the TQ. With the prolongation of shading time and the increase of shading intensity, the difference intolerance of the two plants to shading was extremely significant ($p < 0.05$). On July 16, 2018, at 70.79% and 93.45% shading, the TQ and TCI values of ‘Lark’ and SupraNova were both lower than 6 under the shade of 93.45%. After one month of shading, when the shading intensity is 70.79% and 93.45%, ‘SupraNova’ and ‘Lark’ will gradually turn yellow and even die. The shade tolerance of the two grass species is expressed as ‘SupraNova’>‘Lark’.

Table1. Changes of TQ (1-9) and TCI (1-9) of 2 species under different shade treatment

Species	Shading level (%)	15d		30d		45d	
		TCl	TQ	TCl	TQ	TCl	TQ
SupraNova	CK	7.1a	7.6a	7.3a	7.5a	7.1a	7.6a
	35.62	8.1a	8.6a	7.8b	7.9a	7.5a	7.7a
	70.79	6.0b	7.2a	3.2e	3.5e	2.1f	2.8f
	93.45	4.8d	4.4d	2.2f	3.0e	1.3g	1.7g
Lark	CK	8.3a	8.0a	9.0a	8.2a	9.0a	8.2a
	35.62	6.2b	7.2a	4.7d	3.5e	3.5e	4.2d
	70.79	4.6d	4.7d	2.7f	3.2e	1.5g	2.3f
	93.45	2.7f	3.1e	2.5f	1.7g	1.3g	1.7g

Note: within the same breed, different lowercase letters in the same column indicate significant differences under different shading treatments at the same time level. Different uppercase letters in the same column indicated significant differences among different varieties under the same time treatment ($p < 0.05$)

Analysis Of Changes In Physiological Indicators

Analysis of changes in soluble protein content

The SP content of the two grass species under different shading treatments is shown in Figure 2 (A). There are significant differences between each grass species and each treatment ($p<0.05$). In the control treatment, the SP and SS content of 'Lark' was significantly higher than that of 'SupraNova'. Under the 93.45% shading treatment, the SP and SS content of the two grass species were at the lowest level. For 'Lark', the SP content under the control treatment is higher, and then it will gradually decrease as the shading intensity increases. Compared with CK, 'Lark' had the most significant decline, with a decline of 56.99%. As a shade-tolerant grass species, the SP content of' SupraNova' reached a maximum value of 35.62% during shading treatment, which increased by 16.43% and 22.89% compared with CK, respectively, and then decreased with the increase of shading intensity. The SP content of the two grass species under the shading treatment of 70.79% and 93.45% was significantly ($p<0.05$) lower than that of the control treatment. In the 93.45% treatment, compared with the 35.62% shading treatment, the SP and SP content of' SupraNova' were reduced by 46.23% and 32.65%, respectively. Figure 1 (B), in the control treatment, the SS content of 'Lark' was higher, while the content of' SupraNova' was lower. With the increase of shading intensity, the SS accumulation of 'Lark' gradually decreased, reaching a minimum value under 93.45% shading treatment, and the difference between treatments was significant ($p<0.05$). Compared with CK, when the shading treatment was 93.45%, 'Lark' had the largest decline, down 67.42%. With the increase of shading intensity, the SS content of'SupraNova' showed a trend of increasing first and then decreasing, reaching its maximum value under 35.62% shading treatment. The degree of variation of the two grass species is different. Compared with the control treatment, under 35.62% shading,' SupraNova' SS content increased by 0.33 times treatment. At after 93.45% treatment,

the SS content of'SupraNova' decreased by 30.19%. In Figure 1 (C), under CK, the Pro content of the two grass species is at a relatively low level. For "Lark", the shading stress gradually increased the Pro content in the test grass, reaching the maximum value when the shading treatment was 93.45%, which was significantly higher than other treatments ($p<0.05$). The range of variation of different grass species is different. Compared with CK, 'Lark' increased by 2.79 times when the shading treatment was 93.45%. 'SupraNova' will show a trend of decreasing first and then increasing with the increase of shading intensity, reaching a minimum of 35.62% shading treatment. Compared with CK, the Pro content of' SupraNova' was reduced by 23.44% under the shading treatment of 35.62%. In 93.45% treatment, compared with 35.62% shading treatment, the Pro content of'supraNova' increased by 1.77 times Figure 1 (D), with the increase of shading treatment, the trend of ASA content varies between different varieties. As the shadow intensity increases, the ASA content of'SupraNova' will first increase and then decrease. 'SupraNova' reached its highest value at 70.79% shading treatment, an increase of 0.67 times compared with the control treatment, and then began to decline at 93.45% shading treatment, a decrease of 60.00% compared with 70.79% shading treatment. The ASA content of "Lark" will continue to decrease as the shade of the tree increases, with a decrease of 50.00%

MDA, H_2O_2, O_2^-content change

The trend of MDA content varies between different varieties as the shading treatment increases in Figure 2 (A).the MDA content of 'Lark' will continue to increase compared with the control treatment, the MDA content of 'Lark' in 93.45% shade increased by 1.62 times. For 'SupraNova', as the shading intensity increases, the MDA content will show a trend of "V" shape change, reaching a minimum at 35.62% shading. Compared with the control treatment,'SupraNova' reduced shading by 35.62% by 10.80%. At 93.45% treatment, the MDA content of' SupraNova' increased almost the same, which was 1.13 times higher than that of 35.62% shading treatment. AS shown in Figure 2 (B), with the increase of shading treatment, the change trend of H_2O_2 content between different varieties is different, but the change trend is roughly similar to that of MDA. For 'SupraNova', as the shading intensity increases, the H_2O_2 content will decrease under the 35.62% shading treatment, but when the shading intensity continues to increase, the H_2O_2 content will increase, and when the shading intensity is 35.62%, it will drop to the lowest value. Compared with the control treatment, the decrease was 64.45%. When the shading intensity increased to 93.45%,'SupraNova' increased by 1.52 times over the 35.62% treatment. The H_2O_2 content of 'Lark' will increase as the shade of the tree increases, eventually increasing by 1.63 times. However in Figure 2 (C), with the increase of shading treatment, the change trend of O_2-content between different varieties is different, but the change trend is roughly similar to that of MDA. With the increase of the shading intensity in 'SupraNova', the O_2-content in the 35.62% shading treatment decreased. Compared with the control treatment, the decrease of' SupraNova' was 11.75%, but as the degree of shading continues to increase, the O_2-content will increase, reaching its maximum value when the shading intensity increases to 93.45%. Compared with the 35.62% shading treatment,'SupraNova' has increased by 1.11 times. The O_2-content of 'Lark' will increase with the increase of shading degree, and finally increased by 78.47% compared to the control treatment. The O_2-content of 'Lark' has a more significant tendency to increase with the increase of shading intensity.

SOD、POD、CAT activity

The SOD activity of' Lark' and 'SupraNova' will show an upward trend at 35.62 %, as shown in Figure 3 (A), but the increase will be different. Compared with the control treatment, the highest increase rate of SOD activity of' SupraNova' was 19.54%, and the increase rate of 'Lark' was 16.31%. The SOD activity of both grass species will begin to decrease when treated with 70.79%. Compared with 35.62% shading treatment, the reduction rate of' SupraNova' was 40.61%, and the reduction rate of 'Lark' was 55.57%, in Figure 3 (B), with the increase of shading intensity, there are significant differences in the activity of different varieties and treated pods ($p<0$. POD activity increased by 52.72 % compared to the control treatment and began to decrease. The POD activity of 'SupraNova' was reduced by 56.02 % to the 70.79 % shading treatment. The activity of the 'Lark' pod will decrease sharply with the increase of shading intensity, and its downward trend is particularly obvious, with a final decline of 75.31%. As shown in Figure 3 (C), with the increase of shading treatment, there are differences in the changes in cat activity between different breeds.05). The POD activity of'SupraNova' first increased and then decreased sharply as the shading intensity increased, peaking at 70.79% of the shading intensity with the increase of shading intensity, the CAT activity of'SupraNova' first increased and then decreased, and the difference between treatments was significant ($p<0.05$). Compared with the control treatment, the cat activity of ' SupraNova ' after 70.79% shading treatment will first increase by 48.35%, and when the shading luminosity is 93.45%, compared with 70.79% shading treatment, it will decrease by 19.22%. 'Lark' will show a continuous downward trend with the increase of shade, which is 41.25% lower than the control treatment.

Analysis of changes in chlorophyll fluorescence Parameters

The effect of shade on qP may be observed in the graph below (Fig. 4a, b). The qP value under shade treatment is usually lower than the control treatment for 'Larks,' although the changing trend different among grass species. The qP value of 'Larks' is significantly affected by shading. The qP value of 'Larks' will show a significant gradual decline as the intensity of shading increases. Light shading will increase the qP value of 'SupraNova,' but raising the shading intensity will have a negative impact; as demonstrated in (Figure 4c,d),under the shade treatment, the ETR value of ‘Lark’ is lower than that of the control treatment,However, the ETR value of ‘SupraNova’shows a trend of first increasing and then decreasing with the increase of shading intensity, which is the same as the changing trend of its qP; As shown in (Figure 4e), the Fv/Fm of the two grass species are affected by shading, In the control treatment, the Fv/Fm of ‘Lark’ is higher than that of ‘SupraNova’, which is 0.71. But as the shade intensity increases, the light shade will increase the Fv/Fm value of ‘SupraNova’ and will be higher than that of ‘Lark’ Under the moderate and heavy shade, the Fv/Fm of all grass species will decrease, but the degree of decline is different. Compared with light shade, the decline of ‘Lark’ and'SupraNova' is 47.62%, 35.62%.

Daily Variation Spectrum Analysis

The diurnal variation spectrum and normalized vegetation index (NDVI) of two grass species are shown in Figures (5). The reflectance of the leaves in 'Lark' and 'SupraNova' is lower in the shade than in normal light, and the reflectance of 'Lark' is higher in the visible light band than that of 'SupraNova'. The NDVI index of the two test materials has a comparable changing trend, decreasing first and then rising with time. Except with 'SupraNova,' the NDVI index will be slightly higher in the shade than in the control treatment.

Analysis Of Morphological Indicators

Root morphology analysis

In the normal condition of the two types of grass, the root system of 'Lark' is more developed and the root system length is longer, while the root system of 'SupraNova' has more fibrous roots, as shown in Figure (6). With increasing shade intensity, the root system of 'Lark' will show a trend, but with increasing shading intensity, the root system length of 'SupraNova' will first rise, then decline. It can be seen in Table 2 that the shading treatment has a significant effect on the root morphology of two grass species. In terms of total root length, effect of shading on the ‘Larks’ was more significant, and the total root length decreased by 73.39% compared with the control treatment. ‘SupraNova’s’ total root length increased by 33.91% in the 35.62% treatment compared to the control treatment, and then began to decrease in the 70.79% shading treatment, and decreased by 65.07% compared to the 35.62% treatment. For the two grass species, in terms of total root length, the influence of shade on ‘SupraNova’ is always greater than that of ‘Lark’ the changing trend of root surface area is same as that of total root length. Under shading treatment, the root surface area of ‘Lark’ will be significantly lower than the control treatment ($p < 0.05$). As the shade intensity increases, the root surface area of ‘SupraNova’ will first increase and then decrease.

Table 2
Changes of root morphology of two species under different shade treatment

Species	Shading level (%)	Root length (cm)	Root surface area (cm^2)
SupraNova	0	401.83±5.24bC	52.79±3.41bC
	35.62	674.99±8.67aA	70.69±2.43aB
	70.79	222.45±3.20cE	41.84±4.21cC
	93.45	103.45±7.39dE	24.69±1.23dC
Lark	0	1162.36±17.51aA	111.24±7.32aA
	35.62	512.86±6.77bC	52.46±4.78bC
	70.79	354.82±3.37cB	56.23±4.45bB
	93.45	309.28±4.29dA	53.56±1.41bA

Analysis of skin stomata changes

The stomata size of 'SupraNova' is relatively large in compared to the two grass species, as seen in Fig. (7).The arrangement of the stomata of ‘Lark’ is more orderly than that of ‘SupraNova’. The epidermis of ‘SupraNova’ is relatively smooth, without other appendages, while ‘Lark’ is not. There will be silicone papillae on the surface of ‘Lark’, and the density of silicone papillae of ‘Lark’ is higher. Compared with ‘SupraNova’, the leaves of ‘Lark’ will be severely damaged in 93.45% shading. It can be seen from Table 3 that in terms of

stomatal density (SD), the stomatal density of ‘Lark’ will be greater than that of ‘SupraNova’, which may be related to the size of the stoma. With the change of shading intensity, the two grass species will show the same change trend. As the degree of shading increases, the stomata density gradually decreases, but the magnitude of the decrease is different, The decline of ‘Lark’ was relatively large, at 55.57%, and the decline of ‘SupraNova’ was relatively small at 12.87%. Although under the control, 35.62% and 70.79% treatments, the order of the stomatal density of the two turfgrass is ‘SupraNova’ and ‘Lark’, However, under 93.45% shading, the order of arrangement will change to ‘SupraNova’ and ‘Lark’.

Table 3
Changes of the stomatal density of two species under different shade treatment

Shading level(%)	Stomatal density(number/mm^2)	
	SupraNova	Lark
0	112.32±7.37aD	199.32±12.49aC
35.62	105.09±3.64bD	163.86±9.22bC
70.79	100.92±3.22cD	128.82±7.43cC
93.45	97.86±2.11dC	88.56±8.22dD

Analysis Of Anatomical Structure Changes

The difference in anatomical structures between the two grass species can be noticed in Figures (Fig. 8a,b)."Lark" and "SupraNova" have obvious C3 plant structural characteristics, and their vascular bundles are only composed of xylem, phloem, and vascular bundle sheath cells. It can be seen from (Table 4) that the anatomical structures under the control and the shade treatment are significantly different. For all grass species, shading significantly reduces leaf thickness ($p < 0.05$). Different grass species have different leaf thickness, upper and lower skin thickness, number of vascular bundles, and diameter of vascular bundles. Shading significantly reduces the thickness of plant epidermal cells ($p < 0.05$), including the upper and lower epidermis. Shading will reduce the vascular bundle diameter of grass seeds, but the effect is not significant in ‘SupraNova’ ($p > 0.05$), In ‘Lark’, significant differences ($p < 0.05$) will occur, and the diameter of the vascular bundle will gradually decrease as the degree of shading increases.

Table 4
Changes of the anatomical structure of two species under different shade treatment

Species	Shading level (%)	Leaf thickness (μm)	Thickness of upper epidermis (μm)	Thickness of lower epidermis (μm)	Vascular bundle numbers (Entire blade)	Vascular bundle diameter(μm)
SupraNova	CK	129.28±2.76b	42.10±1.21a	53.92±1.61a	15.00±0.00a	104.83±5.91a
	35.62	118.12±1.42b	40.36±1.13a	32.52±1.12b	15.00±0.00a	100.64±3.84a
	70.79	106.92±4.69b	26.92±2.24b	21.41±0.82d	15.00±0.00a	98.52±2.89b
	93.45	97.19±5.96c	20.98±1.49c	24.98±1.90c	15.00±0.00a	94.56±5.31c
Lark	CK	183.76±5.89a	25.77±1.76a	23.22±2.25a	12.54±0.76a	146.99±5.76a
	35.62	140.31±6.43b	23.48±1.89b	21.01±1.78b	10.27±0.97a	124.85±6.95b
	70.79	128.09±6.73c	21.25±1.28c	19.86±1.19c	10.57±0.45a	106.86±7.34c
	93.45	103.12±6.45d	19.72±0.89d	17.98±1.26d	10.10±0.89a	78.25±6.27d

Discussion

Light is the most important factor affecting plant growth [52]. Plant growth requires suitable light intensity, too high or too low light intensity may inhibit plant growth [53]. The adaptability of plants to shaded environments will first be reflected in the appearance of plants [54]. The results of this study show that different shades and different light intensities will have a significant impact on the appearance quality of C3 turfgrass. In the same period, under different light intensities, the adaptability of each tested grass species was different. As the shading intensity increases, the quality of ‘SupraNova’ turf will first increase significantly and then decrease significantly. The main reason may be that the light intensity during the control treatment will have a photoinhibition effect on the grass seeds, Light shade will make the two grass species under more suitable light conditions, [19] which will make the leaves slender, the grass species will grow vigorously, and the greenness of lawn will be deepened, thereby improving the texture, color, uniformity, and density of the lawn, finally, the appearance quality score of lawn is increased with gradual increase in shading level, the lawn quality of ‘Lark’ has decreased significantly. Ryegrass is a grass species that is not shade-tolerant among the two turf types of grass. Shading will reduce the light intensity, which will inhibit the growth conditions of the tested grass species and reduce the color, uniformity, and density score of the turf texture, finally, the appearance quality score of the lawn is reduced [55]. For the same light intensity and different treatment times, the turf quality of each grass species responds differently to its response.

Shading affects the photosynthetic characteristics and chlorophyll fluorescence parameters of plants [56]. For the 'Larks', shading significantly reduced its qP value, which shows that shading will decrease the electron transport activity of PS□ reaction centers. Heavy shading can enhance adaptability of plant components to shading environments, but heavy shading will inhibit efficiency and activity [57]. Shading affects the photosynthetic characteristics and chlorophyll fluorescence parameters of plants. For the 'Larks', shading

significantly reduced its qP value [12], which shows that shading will decrease the electron transport activity of PS□ reaction centers. Heavy shading can enhance adaptability of plant components to shading environments, but heavy shading will inhibit efficiency and activity.Full light and high-intensity shading will have a photoinhibition effect on the two plants, and will inactivate PS□ or damage the photosynthetic machinery. Light shade will promote the activity of the PS system and reduce phot inhibited, and increase photochemical efficiency [58]. The value of Fv/Fm is unchanged under non-stress conditions and will decrease only under conditions of Photoinhibition. The ETR reflects the apparent electron transfer efficiency under actual light intensity, and its value will decrease when stressed [59]. In this experiment, the ETR value of 'Lark' was lower than the control treatment, while the 'SupraNova' showed a trend of rising and then falling. The study shows that full light and high-intensity shading will have a photoinhibition effect on the two plants, and will inactivate PS□ or damage the photosynthetic machinery.the light shade will promote the activity of the PS□ system, reduce photoinhibition, and increase photochemical efficiency. The Fv/Fm value of ‘Lark’ under the shading treatment was lower than that of the control treatment, and with the increase of the shading intensity, the stress effect gradually increased. It shows that when the ‘Lark’ is under the stress of shade, their PS□ activity is harmed, which in turn affects photosynthesis.

The reflectance of ‘SupraNova’ under the control treatment is higher than that under the shading treatment, this may be because the chlorophyll level of ‘SupraNova’ under the shading treatment is higher, and the green is denser, so the reflectance is lower, it also shows that shading improves the spectral reflectance of ‘SupraNova’ in the near-infrared band (750-1000nm), avoids damage to the cell structure of leaves by strong light, and reduces leaf senescence. ‘Lark’ is different from ‘SupraNova’ because its leaf texture is glossy and leathery, so it cannot be evaluated only from the level of leaf color, it may be due to the best leaf quality and the best gloss in the control treatment, and the shading affects its normal development, making the leaf gloss and texture poor, resulting in higher reflectance than the shading treatment light can affect the production and accumulation of primary and secondary metabolites of plants [60]. SS, SP, and Pro have the functions of maintaining stomata opening, cell turgor, cell growth, and protecting enzyme activity [61]. In this experiment, the content of SS and SP in 'SupraNova' showed a trend of first increasing and then decreasing with the increase of shading intensity. Pro can be transformed into small molecular organic solutes when plants are under adversity stress, thereby increasing cytosolic concentration, reducing osmotic potential, maintaining cell turgor, and maintaining normal cell function. In this experiment, the Pro content of 'SupraNova' first decreases and then increases with the increase of shading intensity [62, 63]. This is the same as the research result that stress can increase the content of Pro. The continued increase of shading will cause the decrease of SOD activity, the possible reason is that the plant's ability to resist stress is limited, too strong shading will exceed the upper limit of its tolerance, resulting in a decrease in SOD activity. The stress resistance of plants can be judged by measuring POD activity [64, 65]. In this experiment, the activity of ‘SupraNova’ POD will first increase and then decrease with the increase of shading intensity, and ‘Lark’ will continue to decrease. The reason may be that, within the tolerance range of plants, the increase in active oxygen caused by shading stress will activate the defense mechanism in the plant and induce an increase in the activity of antioxidant enzymes in the cell, eliminate excess reactive oxygen species in cells and reduce the damage suffered by plants, however, the tolerance range of plants has a certain threshold [66, 67]. This study found that with the increase of shade intensity, the CAT activity of ‘SupraNova’ will first increase and then decrease, and the CAT activity of ‘Lark’ will continue to decrease. When subjected to shading stress, the plant can increase enzyme

activity through self-regulation to reduce damage, indicating that ‘SupraNova’ has a strong ability to regulate and adapt to moderate shading [68]. under stress, a large number of reactive oxygen species will be produced in the cells, which will increase the oxidation of unsaturated fatty acids on membrane lipids, thereby producing the peroxidation product MDA [69, 70]. In this study, the changes in MDA content of the two grass species were different. O2- belongs to oxygen-containing free radicals, and when plants are exposed to external stimuli, they will cause the accumulation of O2- in cells [71, 72]. This may be because, for plants, too strong or weak light has a stress effect on the plants, which increases the amount of active oxygen in the plant, and reduces the activity of the antioxidant enzyme system, which is caused by the inability to clean up the excess active oxygen in time [73].

AsA is an antioxidant, which acts as an electron donor in the reaction of PS□, and has the function of scavenging active oxygen [74-76]. It can be seen that shading can induce the production of reactive oxygen species, regulate gene expression, increase the activity of antioxidant enzymes, and increase the synthesis of AsA [77], thereby enhancing the ability of the two to cooperate to remove reactive oxygen species, but too strong shade will destroy the growth and development of the plant [78, 79]. The stomata are the main organs for plants to exchange CO_2 and water with the external environment [61]. The density and function of the stomata are extremely sensitive to environmental factors, in this study, the stomata density will continue to rise with the increase of the shading level. It shows that the plant will adjust the stomata density per unit area to adapt to the shading stress. The changes in stomatal density may have the following two reasons: one is that shading directly affects the occurrence, differentiation, and development of stomata, and the other is that shading increases the leaf area, thereby regulating the stomata density [80, 81].

In this experiment, as the shading intensity increases, the vascular bundle diameters of the two grass species will gradually decrease, and the vascular bundle diameters of different species vary significantly. The number of vascular bundles in ‘SupraNova’ does not respond significantly to changes in light, while the number of vascular bundles in ‘Lark’ will continue to decline. It shows that light has a certain effect on the development of vascular bundles, and the development of vascular bundles affects the transportation of water and water-soluble nutrients. The more vascular bundles, the higher the water transport efficiency [82]. Moreover, the increase in the number of vascular bundles effectively increases transpiration, promotes the transport of carbohydrates and minerals, thereby increasing the net photosynthetic rate [52], so the ‘Lark ‘respond more significantly to light intensity.

Conclusion

Morphological and physiological attributes of both SupraNova and Lark become diminished under shading conditions, yet the reductions were more pronounced in SupraNova. Since the shading of 35.62 % had a positive effect on the quality, biomass accumulation, growth rate, and chlorophyll fluorescence characteristics of SupraNova and Lark, this shading intensity could be used as the optimum condition for turfgrass cultivation and management. Lark had better shade tolerance than SupraNova, which may be due to the different anatomical structures of the two turfgrass species. The result indicated ‘Lark’ had higher shade tolerance than ‘SupraNova’ turfgrass.

Declarations

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Ethics approval and consent to participate

The research on leaf lodging in the manuscript has been conducted under the guidance of international ethical standards. All research protocols were conducted with the approval of the Northeast agricultural university, China.

Consent to publish

All authors are agree and gave their consent to publish this manuscript.

Availability of data and materials

It will be available on request

Competing Interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Authors' Contributions

All authors read and approved the manuscript. L.D., L, X., and Y.C initiated and designed the research, F.X., Q.Z., and L.D, L.X performed the experiments, X.S., Z.G C.Y. and analyzed the data and wrote the manuscript, and R.Y., W.D, revised and edited the manuscript and provided advice on the experiments.

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Figures

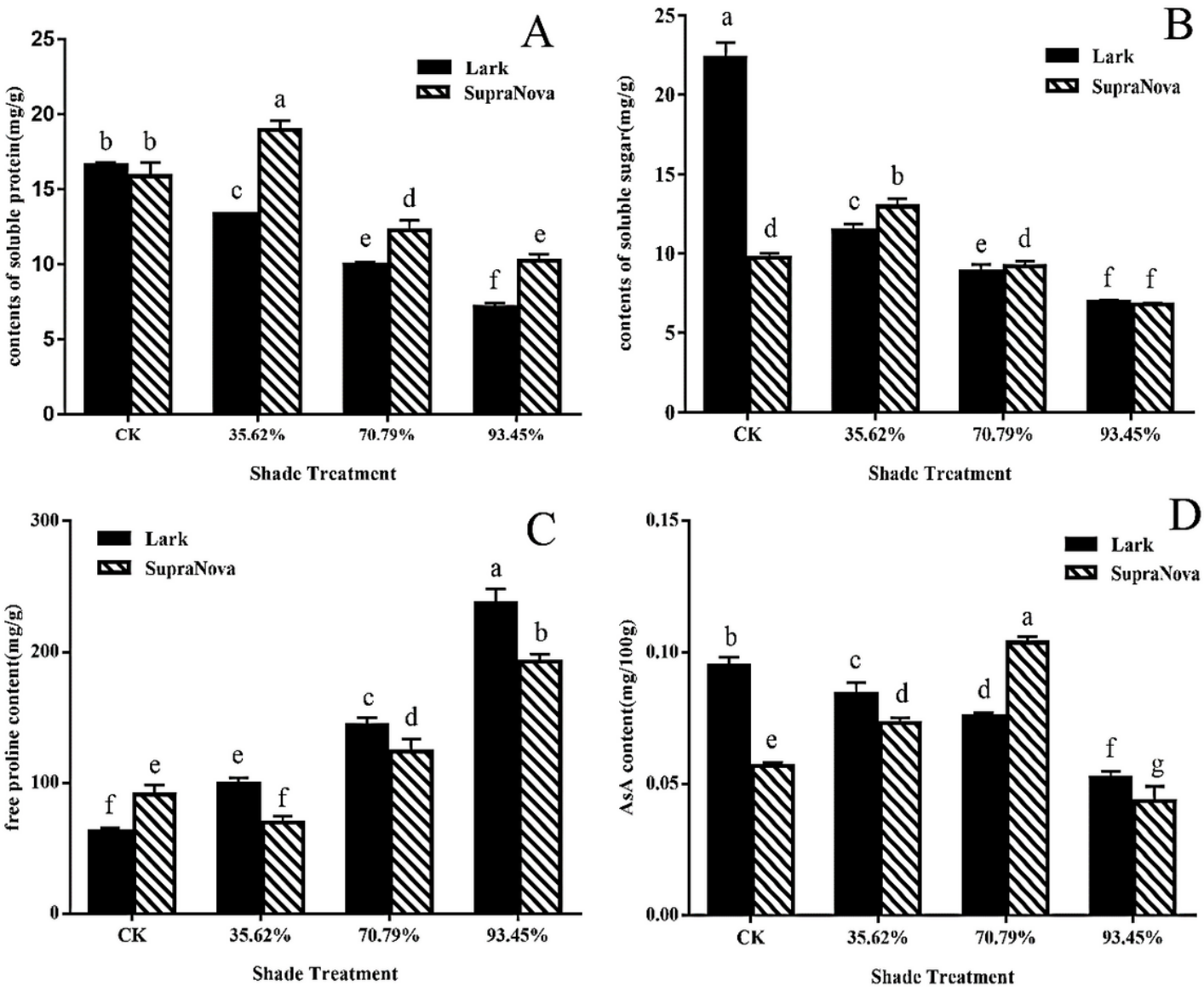

Figure 1

A: Changes of contents of soluble protein of two species under different shade treatment; B: Changes of contents of soluble sugar of two species under different shade treatment; C: Changes of free proline content in two species under different shade treatment; D: Changes of AsA content in two species under different shade treatment.

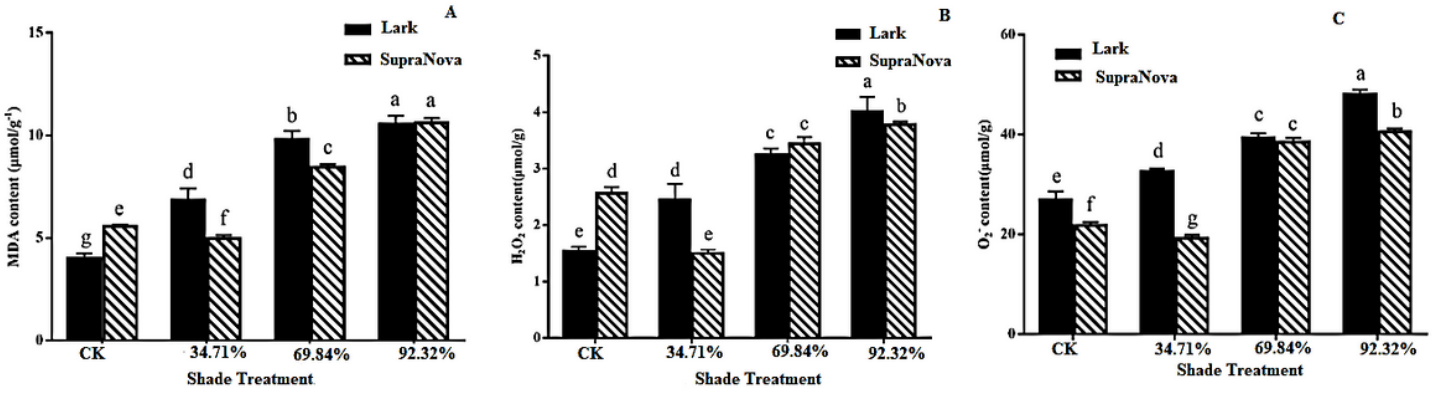

Figure 2

A: Changes of MDA content in two species under different shade treatment; B: Changes of H2O2 content in two species under different shade treatment; C: Changes of O2- content in two species under different shade treatment.

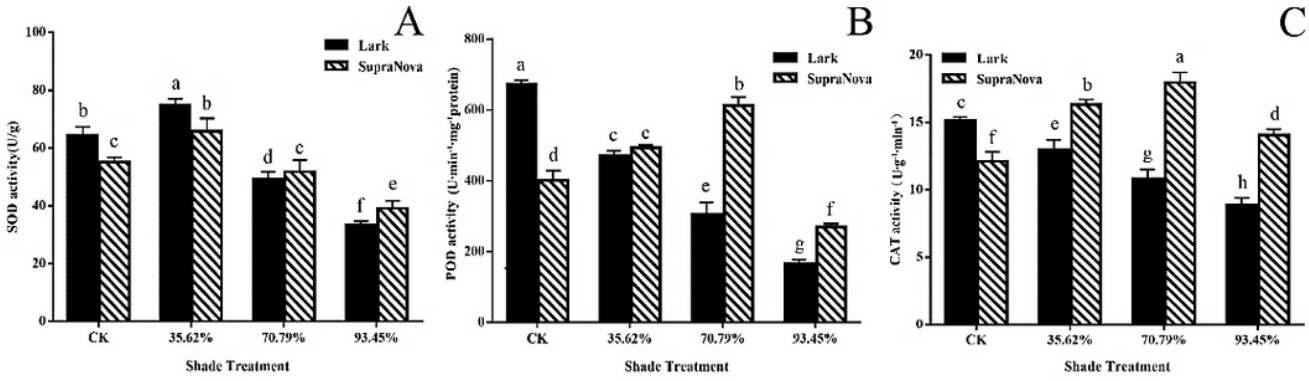

Figure 3

A: Changes of SOD activity in two species under different shade treatment; B: Changes of POD activity in two species under different shade treatment; C: Changes of CAT activity in two species under different shade treatment.

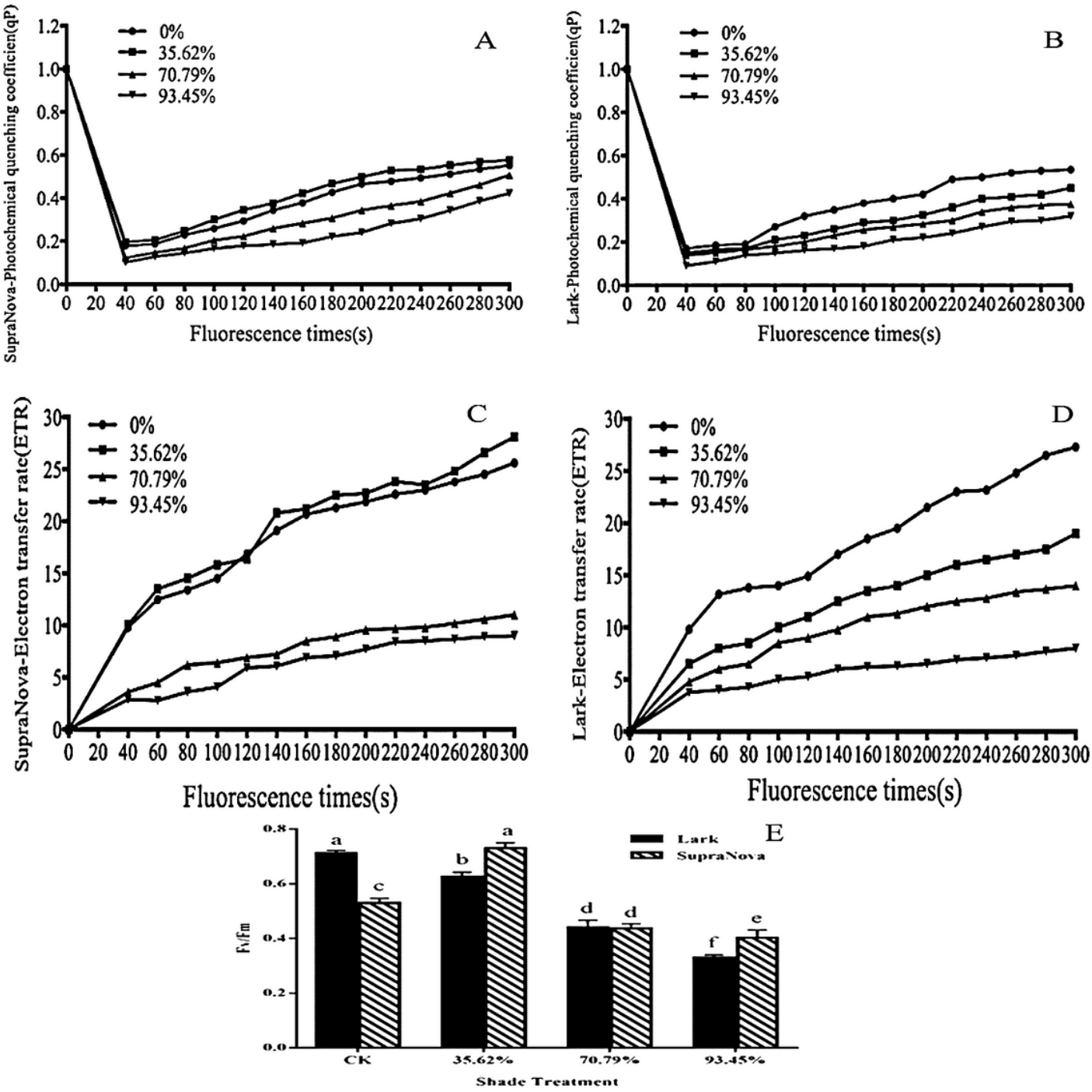

Figure 4

a,b Changes of qP of two species under different shade treatment,Fig.4c,d Changes of ETR of two species under different shade treatment,Fig.4e Changes of Fv/Fm of two species under different shade treatment Note: lowercase letters refer to the same breed, and the difference between different treatments is significant; uppercase letters refer to the same treatment, and the difference between different varieties is significant ($p<0.05$), the same as below.

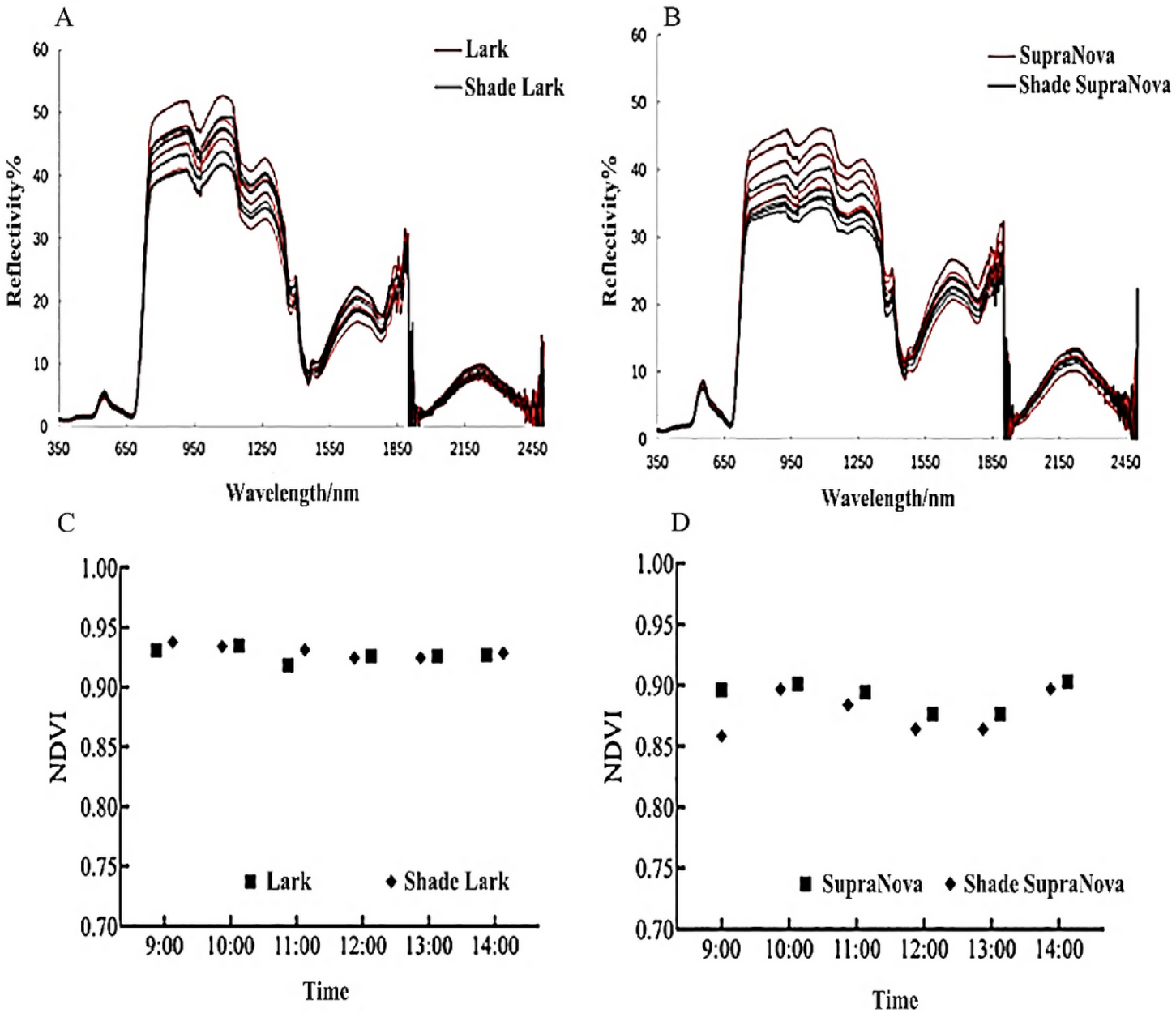

Figure 5

a,b Diurnal variation spectrum of two grass species, Fig.5c,d NDVI diurnal variation curve of two grass species

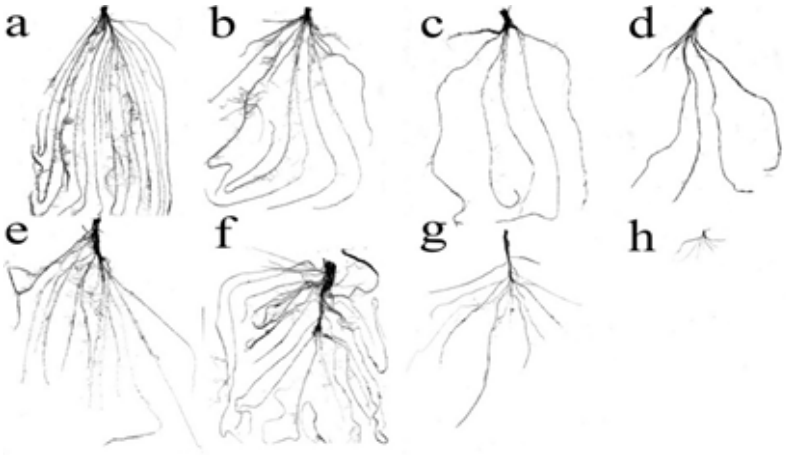

Figure 6

Root morphology of two grass species under different shade treatments Note: a-d for Lark in 0 %, 35.62 %, 70.79 % and 93.45 % respectively under the shade treatment of root morphology, e-h for SupraNova in 0 %, 35.62 %, 70.79 % and 93.45 % respectively under the shade treatment of root morphology.

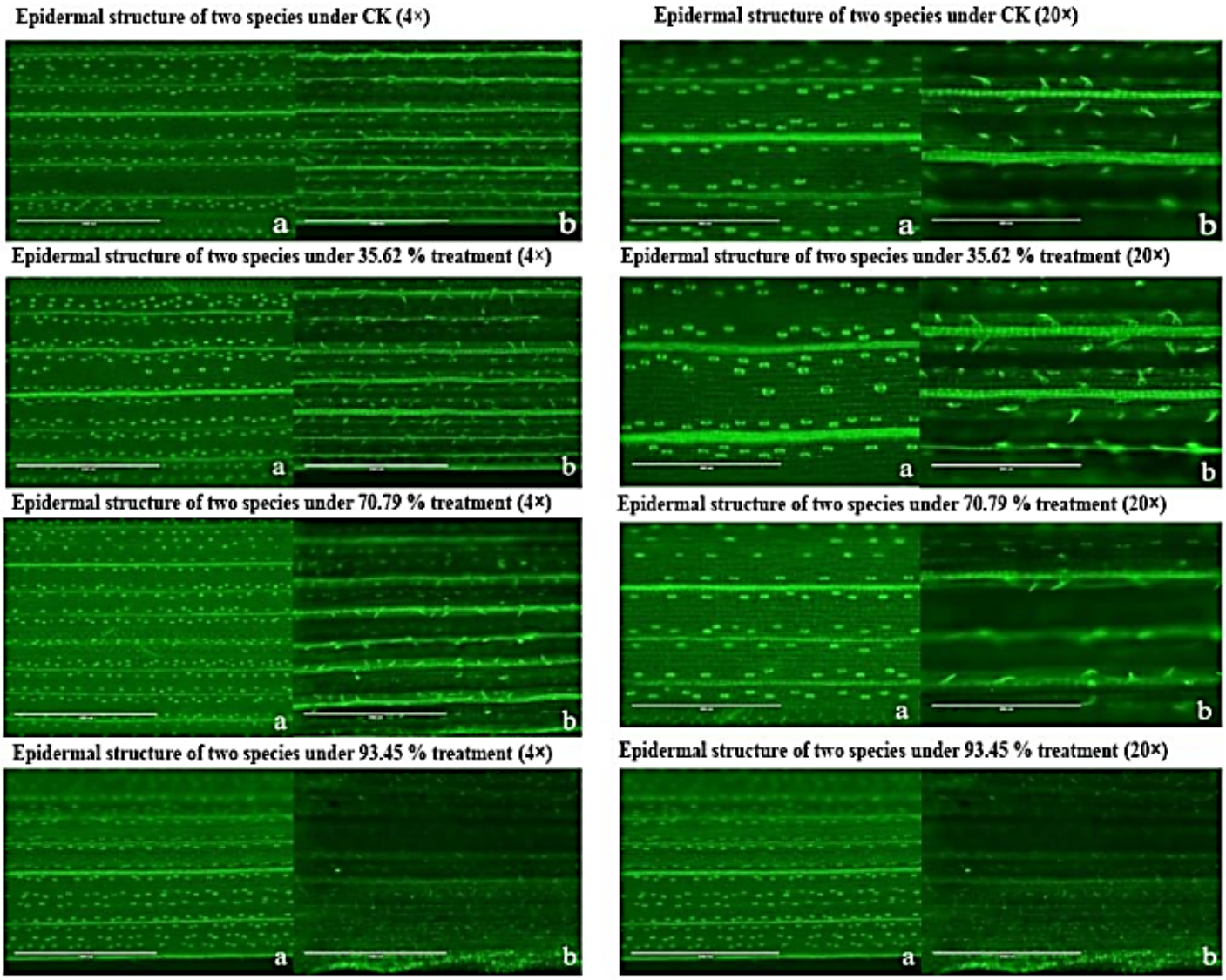

Figure 7

Epidermal structure of two species under different shade treatment Note: a-b is St. SupraNova and Lark respectively

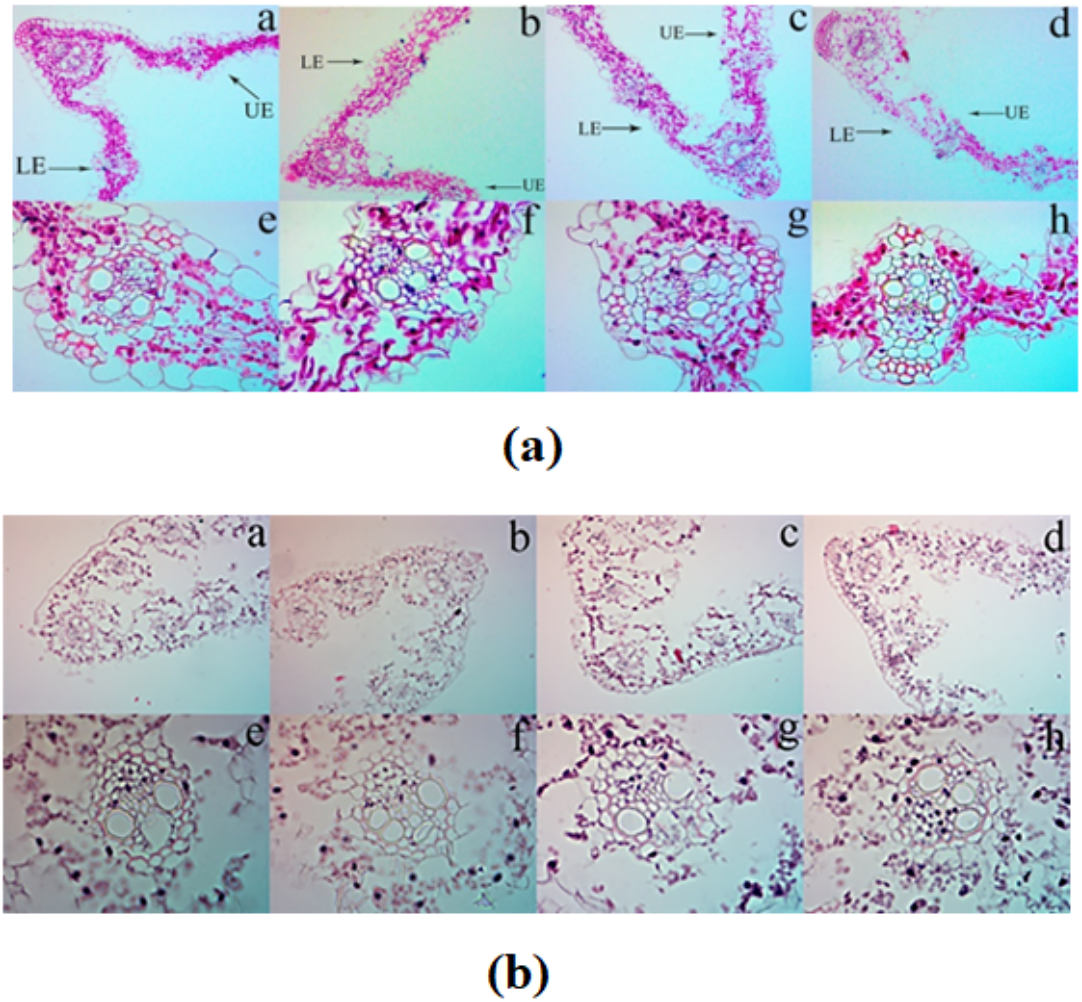

Figure 8

a, The cross-cutting structure of leaf of SupraNova under different shade treatments, b The cross-cutting structure of leaf of Lark under different shade treatment Note: a-d is the cross-cutting structure of blade under 0 %, 35.62 %, 70.79 % and 93.45 % shading treatment (4×), e-h is the cross-cutting structure of blade under 0 %, 35.62 %, 70.79 % and 93.45 % shading treatment(20×), same as below.