Response of total phenols, flavonoids, minerals, and amino acids of four edible fern species to four shading treatments

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Total phenols, flavonoids, minerals content and amino acids content were investigated in four fern species grown under four shading treatments with different transmittance of 35% full sunlight (FS), 13% FS, 8% FS and 4% FS. The four fern species contain high levels of total phenols and flavonoids, as well as abundant mineral elements and amino acids. The total phenols, flavonoids, minerals and amino acids content were strongly affected by transmittance, the total phenols and flavonoids content were significantly positively correlated with transmittance, and minerals and total amino acids content were significantly negatively correlated with transmittance. Higher light intensity can effectively stimulate the synthesis of phenols and flavonoids, and proper shading can stimulate the accumulation of amino acids. In addition, Matteuccia struthiopteris (L.) Todaro (MS) had the highest total phenols content, Athyrium multidentatum (Doll.) Ching (AM) showed the highest total amino acids, total essential amino acids content, Osmunda cinnamomea (L) var. asiatica Fernald (OCA) exhibited the highest total non-essential amino acids content and flavonoids content, and Pteridium aquilinum L. Kuhn var latiusculum (Desy.) Underw. ex Heller (PAL) exhibited the highest minerals content. This will provide a scientific basis for the cultivation and management of four fern species.
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\textbf{Abstract}

Total phenols, flavonoids, minerals and amino acids content were investigated in four fern species grown under four shading treatments with different transmittance of 35\% full sunlight (FS), 13\% FS, 8\% FS and 4\% FS. The four fern species contain high levels of total phenols and flavonoids, as well as abundant mineral elements and amino acids. The total phenols, flavonoids, minerals and amino acids content were strongly affected by transmittance, the total phenols and flavonoids content were significantly positively correlated with transmittance, and minerals and total amino acids content were significantly negatively correlated with transmittance. Higher light intensity can effectively stimulate the synthesis of phenols and flavonoid, and proper shading can stimulate the accumulation of minerals and amino acids. In addition, \textit{Matteuccia struthiopteris} (L.) Todaro (MS) had the highest total phenols content, \textit{Athyrium multidentatum} (Doll.) Ching (AM) showed the highest total amino acids, total essential amino acids content, \textit{Osmunda cinnamonnea} (L) var. \textit{asiatica} Fernald (OCA) exhibited the highest total non-essential amino acidsand flavonoids content, and \textit{Pteridium aquilinum} L. Kuhn var \textit{latiusculum} (Desy.) Underw. ex Heller (PAL) exhibited the highest minerals content. This research will provide a scientific basis for the cultivation and management of four fern species.

\textbf{Keywords}
edible fern species; shading; total phenols; flavonoids; minerals; amino acids

\textbf{Introduction}

There are about 12000 fern species in the world distributed in various environment. In China, approximately 2300 fern species are recorded, and 300 species can be used as traditional Chinese medicine and some species are very popular as wild vegetables (Xiao 2017; Zhang et al. 2012). Fern species are widely distributed in the Northeastern China, and up to eight fern species can be eaten, while \textit{Matteuccia struthiopteris} (L.) Todaro (MS), \textit{Athyrium multidentatum} (Doll.) Ching
(AM), Osmunda cinnamomea (L) var. asiatica Fernald (OCA) and Pteridium aquilinum L. Kuhn var latiusculum (Desy.) Underw. ex Heller (PAL) are well known and rich in nutrients (Liu & Li 1995; Liu & Wang 2018).

Previous studies reported that the four fern species are rich in nutrients including antioxidants, minerals, amino acids, vitamins, etc (Dong et al. 1993; Liu et al. 2011; Qi et al. 2015; Yao 2003; Yao et al. 2003; Zhao et al. 1991). MS and AM have multiple pharmacological effects such as heat-clearing, detoxifying, regulation of blood pressure and pain relief (Han et al. 2018; Zhu et al. 2016). Secondary metabolites extracted from OCA showed high antioxidant activity and a broad antibacterial spectrum (Qi et al. 2015). PAL also has some bioactivities like anti-inflammatory, anti-cancer, anti-viral and antibacterial (Wang & Wu 2013).

Phenols and flavonoids are common secondary metabolites in plants, which not only regulate the growth and development of plants (Agati & Tattini 2010; Manoj & Murugan 2012), but also have important health benefits for human (Andrae-Marobela et al. 2013; Chen et al. 2018). Phenols play vital roles in plants, including defending against herbivores, weeds and pathogens, in addition, phenolic compounds also serve as structural support in plants (Jones & Hartley 1999; Otálora et al. 2018). Phenolic compounds are of great importance for the sensory, nutritional, medicinal and commercial value of edible and medicinal plants (Otálora et al. 2018; Wahle et al. 2010). Similarly, flavonoid compounds have important physiological and ecological functions for plants. Flavonoids protect plants from UV radiation by scavenging reactive oxygen species (ROS) due to their cytotoxicity and ability to interact with enzymes (Heim et al. 2002; Treutter 2006; Vaknin et al. 2005). Flavonoids in many foods play an important role in human health, such as preventing diseases associated with oxidative stress (Pourcel et al. 2007; Williams et al. 2004).

Mineral elements play an important role in plants and humans and participate in various physiological activities (Hänsch & Mendel 2009; Mir-Marqués et al. 2016). Copper (Cu) is essential for photosynthesis and mitochondrial respiration, carbon and nitrogen metabolism, oxidative stress protection, and is required for cell wall synthesis. Iron (Fe) is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis, production and elimination of ROS, osmotic protection and pathogen defense. In addition, Fe is involved in the synthesis of proteins such as iron-sulfur proteins, hemoglobin and other proteins. Manganese (Mn) is essential for plant metabolism and development and is involved in the formation and reaction of approximately 35 enzymes. Zinc (Zn) is an important component of enzymes for protein synthesis and energy production and maintains the structural integrity of cell membranes. More than 1,200 proteins contain, bind or transport zinc ions (Maathuis 2009; Mir-Marqués et al. 2016). Magnesium (Mg) is involved in the synthesis of various enzymes and biochemical reactions in plants and human organisms. In addition, potassium (K), calcium (Ca), and sodium (Na) all have important physiological effects on living things (Hänsch & Mendel 2009; Mir-Marqués et al. 2016), and the lack of mineral elements in the diet causes serious problems in the human body, especially children and pregnant women (Paiva et al. 2017b).
Amino acids involve a range of physiological activities in plants and human body, which are mainly used to synthesize proteins, maintain nitrogen balance, constitute various materials for enzymes, antibodies and certain hormones in body (Hildebrandt et al. 2015; Sonawala et al. 2018; Zhao et al. 2018).

Light is the important factor influencing plant growth, and light intensity have different impacts on plant growth and physiology (Chen et al. 2017; Shao et al. 2014). In addition, light intensity affects the accumulation of some secondary metabolites and nutrition. Higher light intensity stimulates the synthesis of phenols and flavonoids to protect plants (Liu et al. 2018; Riachi et al. 2018). Similarly, studies have shown that light intensity can affect the accumulation of amino acids and mineral elements (Riga et al. 2019; Stagnari et al. 2015; Zrig et al. 2016). However, the effects of light intensity on the secondary metabolites and nutrient accumulation of edible fern species have not been reported. In this study, total phenols, flavonoids, minerals and amino acids content were investigated in four edible fern species (MS, AM, OCA, and PAL) grown under four shading treatments with different transmittance of 35% full sunlight (FS), 13% FS, 8% FS and 4% FS to understand the effects of light intensity on the secondary metabolites and nutrient content of the four edible fern species.

**Materials & Methods**

**Experimental site**

The study was conducted in an open and unshaded area of the Arboretum of the Institute of Applied Ecology, Chinese Academy of Sciences (41°46’N, 123°27’E), which has a mean elevation of 45 m above sea level.

**Plant materials and experiment design**

Four fern species commonly found in the Northeast China were studied, namely *Matteuccia struthiopteris* (L.) Todaro (MS), *Athyrium multitentatum* (Doll.) ching (AM), *Osmunda cinnamomea* (L.) var. *asiatica* Fernald (OCA) and *Pteridium aquilinum* L. Kuhn var. *latiusculum* (Desy.) Underw. ex Heller (PAL), which were used for the outdoor pot experiment. The rhizomes of the four fern species (three-year-old) were planted in pots (caliber 21 cm × depth 14 cm), the pots were filled with turfy soil and sand mixed at a volume ratio of 3:1 (v/v), the organic matter content was 52%, and the N:P:K was 23:4:8, which were fully watered for cultivation. After the plants growth traits were stable, the plants with consistent height and good growth were used for experimental studies. The experiment consisted of 4 treatments, every treatment had 4 fern species and 3 repetitions. The four fern species were placed under four black shading nets of different specifications. The light transmittance of the 4 shading nets was determined to be 35% full sunlight (35% FS), 13% full sunlight (13% FS), 8% full sunlight (8% FS) and 4% full sunlight (4% FS) by measuring the light intensity under full sunlight and shade. After 60 days of shading, the relevant indicators were determined.

**Sample processing and preparation**

The leaves of four fern species under four shading treatments were collected. The leaves were washed and placed in envelopes and dried at 105°C for 30 min, then dried at 60°C to constant
weight, and the dried leaves were ground with a grinder for the detection of total phenols, flavonoids, mineral elements and amino acids content.

**Determination of total phenols content**

The total phenols content in the plants were tested by the total phenols test kit (Solarbio). About 0.1 g of leaves dried powder was weighed, 2.5 mL of extract solution was added, and total phenols were extracted by ultrasonic extraction. The ultrasonic power was 300W, broken for 5s, intermittent 8s, and then extracted at 60°C for 30 min. The mixture was centrifuged at 12000 rpm, 25°C for 10 min, the supernatant was taken and diluted to 2.5 mL with the extract solution, the absorbance of the extraction was measured at wavelength of 760 nm using a Microplate reader (InterMed, South Portland, ME, USA). The standard curve was tested with 1 mg mL⁻¹ tannic acid standard solution.

**Determination of flavonoids content**

The flavonoids content in the plant was tested by the flavonoid test kit (Solarbio). About 0.1 g of leaves dried powder was weighed, 1.0 mL of extract solution was added, and total phenols were extracted by ultrasonic extraction. The ultrasonic power was 300W, broken for 5s, intermittent 8s, and then extracted at 60°C for 30 min. The mixture was centrifuged at 12000 rpm, 25°C for 10 min, the supernatant was taken and diluted to 1.0 mL with the extract solution, the absorbance of the extraction was measured at wavelength of 470 nm using a Microplate reader (InterMed, South Portland, ME, USA). The standard curve was tested with 10 mg mL⁻¹ tannic acid standard solution.

**Determination of minerals content**

Approximately 0.5 g of leaves dried powder was analyzed for the content of K, Ca, Mg, Fe, Mn, Cu, Zn, Na. The mineral elements were extracted by nitric-perchloric acid digestion (Bystriakova et al. 2011). The leaf samples were placed into clean beakers, and then 20 mL of nitric acid (65%) with 5 mL of perchloric acid (70%) were added, the mixture was stayed overnight. After nitric-perchloric acid digestion, 2% nitric acid was added into the beaker and diluted to 25 mL. The absorbance of solution was measured at wavelength 766.5 nm (K), 317.9 nm (Ca), 279.6 nm (Mg), 238.2 nm (Fe), 257.6 nm (Mn), 327.4 nm (Cu), 213.9 nm (Zn), 589.6 nm (Na) by using an ICP-OES (Agilent, America). The standard solutions (1000 μg mL⁻¹) used for calibration were purchased from Tianjin Guangfu Fine Chemical Research Institute.

**Determination of amino acids content**

The amino acids content was measured according to Yang et al. (2002). Approximately 1.0 g leaves dried powder was placed into digestion bottle, 20 mL of 6 mol L⁻¹ HCl was added, and the mixture was digested at 110°C for 24 h. After digestion, the mixture was filtered and diluted to 100 mL with ultrapure water. Solution (2 mL) was added into a beaker and evaporated in a 60°C water bath, then the sample was dissolved with 0.02 mol L⁻¹ HCl and filtered to a volume of 2 mL, which was used to measure the amino acids content by using an amino acids analyzer (Hitachi Japan).

**Statistical analysis**
All data were analyzed with Microsoft Excel 2016 and SPSS 22.0 software, graphs were edited with GraphPad Prism 5.0 software. Analysis of variance (ANOVA) and correlation analysis (Pearson) were performed using SPSS 22.0 software, and means were compared based on Duncan’s test at $P \leq 0.05$.

**Results**

**Total phenols and total flavonoids content**

The total phenols and flavonoids content of four fern species were significantly affected by transmittance (Fig. 1A and B). MS, AM and OCA exhibited the highest total phenols and flavonoids content in 35% FS, but PAL showed the highest total phenols and flavonoids content in 13% FS and 4% FS, respectively. In addition, the lowest total phenols and flavonoids content of MS appeared in 4% FS, the lowest total phenols and flavonoids content of AM and OCA appeared in 4% FS and 8% FS, respectively, while PAL showed the lowest total phenols and flavonoids content in 35% FS and 13% FS, respectively.

Regardless of fern species, total phenols content was decreased with the decrease of transmittance, while the change order of flavonoids content was: 8% FS $< 13\%$ FS $< 4\%$ FS $< 35\%$ FS (Fig. 1C). Within four fern species, the total phenols and flavonoids content were largely different in different fern species (Fig. 1D). The content of total phenols of MS was higher than other fern species, while the flavonoids content of MS was lower than other fern species, the flavonoids content of AM, OCA and PAL were 1.89, 5.06 and 4.24 times of MS, respectively.

**Minerals content**

The minerals content of four fern species was affected significantly by transmittance (Fig. 2). In this study, the highest content of majority of minerals of MS, AM and OCA appeared in lower transmittance (8% and 4% FS). Conversely, the lowest content of most mineral elements appeared in 35% FS. However, the minerals content’s change of PAL was complicated. The PAL exhibited the highest K and Mg content in 13% FS and 35% FS, the highest Ca, Fe, Na content in 4% FS, the highest Mn, Cu and Zn content in 8% FS. The lowest K, Mg, Cu and Zn content was recorded in 4% FS, the lowest Ca and Na content was recorded in 8% and 13% FS, respectively, and the lowest Fe and Mn content was recorded in 35% FS. Similarly, the total minerals content of MS, AM and OCA appeared in 4% FS, and the lowest total minerals content was recorded in 35% FS. But PAL exhibited the highest total minerals content in 13% FS, the lowest total minerals content in 4% FS.

Across the transmittance of shading nets, K, Fe, Mn, Cu, Zn, Na and total minerals content were increased with the decrease of transmittance, while the change order of Ca and Mg was similar (8% FS $< 35\%$ FS $< 13\%$ FS $< 4\%$ FS) (Fig. 3A). In general, the highest mineral elements content was observed in the lowest transmittance (4% FS).

Within four fern species, the content of each mineral element varies greatly among the four ferns (Fig. 3B). The highest K and total minerals content were observed in PAL, the highest Ca and Fe content were recorded in MS, and AM had the highest Mg and Cu content, while OCA had the highest Mn, Zn and Na content.
Amino acids content

The amino acids content of four fern species was significantly affected by four shading treatments with different transmittance. 16 amino acids including 7 essential amino acids and 9 non-essential amino acids were detected (Fig. 4A). Among the essential amino acids, the leucine content is the highest and the methionine content is the least; while among the non-essential amino acids, the glutamic acid content is the highest and the cysteine content is the least.

In addition to cysteine, the amino acid content of the four fern species increased with decreasing transmittance, while the highest cysteine content of four fern species was observed in 8% FS, and MS, AM and PAL had the lowest cysteine content in 4% FS, however, OCA had the lowest cysteine content in 35% FS (Supplemental Fig. S1 and S2). The highest total amino acid, total essential amino acids and total non-essential amino acids content of MS were recorded in 8% FS, the highest total amino acid and total non-essential amino acid content of AM content also appeared in 8% FS, but the highest total essential amino acid content was recorded in 13% FS.

OCA exhibited the highest level of total amino acid, total essential amino acid and total non-essential amino acid in 13% FS, PAL exhibited the highest total amino acid and total non-essential amino acid content in 4% FS, the highest total essential amino acid content in 13% FS (Fig. 4B, 4C and 4D).

Across four fern species, each single amino acid, total amino acids, total essential and total non-essential amino acids content of AM were lower. Except for methionine, MS had the highest essential amino acids content. In addition, MS also had the highest aspartic acid, alanine, tyrosine, proline and total amino acid and total essential amino acids content. OCA had the highest glycine, cysteine and total non-essential amino acids content, and PAL had the highest level of glutamic acid, histidine and arginine (Fig. 5A, 5B and 5C).

Considering only the transmittance of the shading nets, the content of various amino acids and total amino acids increased with the decrease of transmittance, except for cysteine, which decreased after reaching the maximum at 8% FS (Figure 5D, 5E and 5F).

Discussion

Environmental conditions affect the growth, development and distribution of plants, and the nutrients of plants are also affected by environmental factors (Siracusa & Ruberto 2014; Tounekti & Khemira 2015; Tounekti et al. 2010). As an important ecological factor, light not only affects the photosynthesis of plants, but also affects the chemical composition of plants (Fukuda 2019; Kyriacou et al. 2016; Rouphael et al. 2018). Previous studies have focused on the effects of light quality and light intensity on crop growth and quality (Fiutak et al. 2019; Frede et al. 2019; Kaiser et al. 2019; Ruangrak & Khummueng 2019; Shibuya et al. 2019), while few studies have involved under-forest economic crops such as wild vegetables. MS, AM, OCA and PAL are important wild vegetables in Northeast China (Liu & Li 1995; Liu & Wang 2018), but there is little research on the effects of light conditions on the quality of four edible ferns. Therefore, understanding the effects of light conditions on the quality of four edible fern species is critical to their cultivation, management and utilization.
There is a relationship between light intensity and the synthesis of plant secondary metabolites. Previous studies have shown that increased light can cause accumulation of flavonoids and total phenols in herbal medicines (Graham 1998), and can result in a decrease in phenolic compounds in the roots of Beta vulgaris var. conditiva Alef. and leaves of lettuce (Galieni et al. 2015; Perez-Lopez et al. 2018; Stagnari et al. 2014). In this research, the shading treatments affected the total phenols and flavonoids content significantly, and the content of total phenols and flavonoids was significantly positively correlated with the transmittance of shading nets \( r = 0.69 \) (\( P<0.0001 \) and 0.52 (\( P = 0.0002 \), respectively) (Supplementary Table S1), which indicates that light with high intensity contributes to the synthesis of phenols and flavonoids. The synthesis of total phenols by higher light intensity may be due to higher light-induced activation of phenylalanine amino lysis enzyme in the phenolic acid synthesis pathway (Kumari et al. 2009). Similarly, the synthesis of flavonoids induced by high light intensity is also associated with the expression of phenylalanine ammonia lyase (Graham 1998; Saito et al. 2013).

Furthermore, the light with high intensity can cause the damage of plant cells and the produce of ROS (Mullineaux et al. 2018; Pinto-Marijuan & Munne-Bosch 2014; Szymańska et al. 2017), plants can scavenge ROS by activating antioxidant systems (enzymatic antioxidant systems and non-enzymatic antioxidant systems). The non-enzymatic antioxidant systems include secondary metabolites such as ascorbic acid, carotenoids, and \( \alpha \)-tocopherol (Georgieva et al. 2017; Kataria et al. 2019; Soares et al. 2018). Similarly, phenols and flavonoids can be used as ROS scavengers to remove ROS in plants (Franzoni et al. 2019; Liao et al. 2019; Meini et al. 2019; Naikoo et al. 2019; Schenke et al. 2019; Xiang et al. 2019). In our study, across the shading treatment, the content of \( \text{H}_2\text{O}_2 \) (The data was shown in raw data) was significantly positively with the content of total phenols and flavonoids content \( r = 0.62 \) (\( P<0.0001 \)) and 0.41 \( (P = 0.0047) \), respectively) (Supplementary Fig. S3), which indicates that the four fern species can scavenge \( \text{H}_2\text{O}_2 \) by increasing the synthesis of total phenols and flavonoids. As the light transmittance of the shading net decreases, the total phenols and flavonoids content also decreases, indicating that the shading net can be used to reduce the light intensity, thereby alleviating the oxidative damage caused by strong light (Ilić & Fallik 2017; Rouphael et al. 2018).

Environmental factors also have an impact on the accumulation of mineral elements in plants (Paiva et al. 2017a; Sarker & Oba 2018). Light intensity affects the accumulation of minerals in plants. Colonna et al., (2016) reported that ten leafy vegetables accumulated more K, Ca and Mg under low light intensity. Lettuce (Lactuca sativa L.) exhibited higher K, Ca, Mg, Fe, Mn and Zn under low light intensity (Stagnari et al. 2015). The mineral content of four fern species also showed similar change. The lowest mineral content was recorded at 35% FS, and the K, Fe, Mn, Cu, Zn and Na content reached the maximum at 4% FS, which was 2.01, 2.82, 1.56, 2.83, 1.82 and 1.98 times that of 35% FS. The highest Ca and Mg content appeared at 8% FS. Among them, the content of K, Fe, Cu, Zn and Na were significantly negatively correlated with the transmittance of shading nets, and the correlation coefficients were -0.45 \( (P = 0.0012) \), -0.68 \( (P<0.0001) \), -0.70 (\( P<0.0001 \)), -0.58 (\( P<0.0001 \)) and -0.36 (\( P = 0.0125 \)) (Supplementary Table S1), respectively. The total minerals content was negatively correlated with transmittance with
correlation coefficients of -0.55 ($P<0.0001$) (Supplementary Table S1). The change of minerals indicates that moderate shading stimulates the accumulation of mineral elements. However, the mechanism by which low light causes accumulation of mineral elements has not yet been discovered.

Due to the important role of amino acids in protein synthesis and their role as precursors of many metabolites, plant amino acids have attracted more and more attention (Häusler et al. 2014; Less & Galili 2008). Previous studies reported that the free amino acids content can be affected by light (Riga et al. 2019; Zrig et al. 2016). There were significantly differences in free amino acids content between thyme plants (*Thymus vulgaris*) grown in different light environment, and the amino acids content in shading condition was higher than in open-field after four weeks (Zrig et al. 2016). Similarly, the free amino acids content of lettuce was affected by light intensity and global radiation (Riga et al. 2019). However, to the best of our knowledge, the effect of light intensity on the total amino acids (protein amino acids and free amino acids) content of plants has not been reported. In our study, there were significantly differences in amino acids content between four fern species grown under different shading nets. The content of most amino acids increased with the decrease of transmittance of shading nets. In addition to cysteine, other single amino acid, total amino acids, total essential amino acids and total non-essential amino acids content were all significantly negatively correlated with transmittance of shading nets ($P<0.0001$) (Supplementary Table S1), which indicates that lower light levels contribute the accumulation of amino acids in the four fern species, this may be due to the decrease in photosynthetic capacity of plants at low light intensities, and thus the rate of carbohydrate synthesis decreased, resulting in a relative increase in amino acid content (Song 2009; Zhen et al. 2010).

**Conclusions**

In the present study, the total phenols, flavonoids, minerals and amino acids content of four fern species in Northeast China under four shading treatments were compared. The four fern species can synthesis more total phenols and flavonoids to adapt to higher light environment. The four fern species are rich in mineral elements and amino acids, lower light can contribute the accumulation of minerals and amino acids of four fern species. In addition, regardless of the transmittance of shading nets, MS has higher total phenols, AM has higher total amino acids content, OCA has higher flavonoids content and PAL has total minerals content. Based on the current research results, future studies may attempt to explain the mechanism of changes in the content of minerals and amino acids of four fern species in different light environments.

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Yanlin Wang, Wei Chen and Xingyuan He conceived the study; Yanlin Wang and Shanshan Gao performed the experiment; Yan Li and Yue Zhang contributed reagents and materials; Yanlin Wang and Shanshan Gao analyzed the data; Yanlin Wang led the writing of the paper with substantial input from all co-authors.

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**Figure 1 (on next page)**

Effect of shading on total phenols and flavonoids content (mg g\(^{-1}\) DW) in four fern species.
Figure 1  Effect of shading on total phenols and flavonoids content (mg g\(^{-1}\) DW) in four fern species. (A) Total phenols content; (B) Flavonoids content. Changes of total phenols content and flavonoids content (mg g\(^{-1}\) DW) under four shading treatments (percentage relative to 35% FS) (C) and among four fern species (percentage relative to MS) (D). MS = M. struthiopteris, AM = A. multidentatum, OCA = O. cinnamomea (L.) var. asiatica, PAL = P. aquilinum L. Kuhn var. latiusculum, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS = 4% Full sunlight. Different lowercase letters mean significant difference among different treatments in the same fern at \(P \leq 0.05\). Different uppercase letters mean significant difference between four fern species at \(P \leq 0.05\) (Duncan’s test). Error bars are ±SD (\(n = 3\)).
Effect of shading on minerals content (mg g\(^{-1}\) DW) in four fern species.
Figure 2  Effect of shading on minerals content (mg g⁻¹ DW) in four fern species. (A) K content; (B) Ca content; (C) Mg content; (D) Fe content; (E) Mn content; (F) Cu content; (G) Zn content; (H) Na content; (I) Total mineral content. MS = M. struthiopteris, AM = A. multidentatum, OCA = O. cinnamomea (L.) var. asiatica, PAL = P. aquilinum L. Kuhn var. latiusculum, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS = 4% Full sunlight. Different lowercase letters mean significant difference among different treatments in the same fern at $P \leq 0.05$. Different uppercase letters mean significant difference between four fern species at $P \leq 0.05$ (Duncan’s test). Error bars are ±SD (n = 3).
Figure 3 (on next page)

Changes of mineral element content (mg g\(^{-1}\) DW) under four shading treatments (percentage relative to 35% FS) (A) and among four fern species (percentage relative to MS) (B).
Figure 3  Changes of mineral element content (mg g⁻¹ DW) under four shading treatments (percentage relative to 35% FS) (A) and among four fern species (percentage relative to MS) (B). MS = M. struthiopteris, AM = A. multidentatum, OCA = O. cinnamomea (L.) var. asiatica, PAL = P. aquilinum L. Kuhn var. latusculum, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS = 4% Full sunlight.
Changes of 16 amino acids content of four fern species under four shading treatments (percentage relative to Threonine), and effect of shading on essential amino acid content, nonessential amino acid content, and total amino acids content (mg 100g⁻¹)
Figure 4  Changes of 16 amino acids content of four fern species under four shading treatments (percentage relative to Threonine) (A), and effect of shading on essential amino acid content, non-essential amino acid content, and total amino acids content (mg 100g\(^{-1}\) DW) in four fern species. (B) Essential amino acid content; (C) Non-essential amino acid content; (D) Total amino acid content. MS = M. struthiopteris, AM = A. multidentatum, OCA = O. cinnamomea (L.) var. asiatica, PAL = P. aquilinum L. Kuhn var. latiusculum, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS= 4% Full sunlight. Different lowercase letters mean significant difference among different treatments in the same fern at \(P \leq 0.05\). Different uppercase letters mean significant difference between four fern species at \(P \leq 0.05\) (Duncan’s test). Error bars are ±SD (n = 3).
Figure 5 (on next page)

Changes of 7 essential amino acid, 10 non-essential amino acid, total amino acids, total essential amino acids and total non-essential amino acids content (mg 100g$^{-1}$ DW) under four shading treatments.
Figure 5  Changes of 7 essential amino acid, 10 non-essential amino acid, total amino acids, total essential amino acids and total non-essential amino acids content (mg 100g\(^{-1}\) DW) under four shading treatments. (A, B, C) Percentage relative to MS. (C, D, E) Percentage relative to 35% FS. MS = *M. struthiopteris*, AM = *A. multidentatum*, OCA = *O. cinnamomea* (L.) var. asiatica, PAL = *P. aquilinum* L. Kuhn var. *latiusculum*, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS = 4% Full sunlight.