A peer-reviewed version of this preprint was published in PeerJ on 13 January 2020.

View the peer-reviewed version (peerj.com/articles/8354), which is the preferred citable publication unless you specifically need to cite this preprint.

Wang Y, Gao S, He X, Li Y, Zhang Y, Chen W. 2020. Response of total phenols, flavonoids, minerals, and amino acids of four edible fern species to four shading treatments. PeerJ 8:e8354
https://doi.org/10.7717/peerj.8354

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

Yanlin Wang [Equal first author, 1], **Shanshan Gao** [Equal first author, 1], **Xingyuan He** [1], **Yan Li** [1], **Yue Zhang** [1], **Wei Chen** [Corresp. 1]

[1] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, Liaoning, China

Corresponding Author: Wei Chen
Email address: chenwei@iae.ac.cn

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 flavonoid, 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.

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

Yanlin Wang [1,2,†], Shanshan Gao [1,2,†], Xingyuan He [1,2,3], Yan Li[1], Yue Zhang [1], Wei Chen [1,2,3,*]

[1] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang, China;
[2] University of Chinese Academy of Sciences, Beijing, China
[3] Key Laboratory of Forest Ecology and Management, Chinese Academy of Sciences, Shenyang, China

Corresponding Author:
Wei Chen
72 Wenhua Road, Shenyang 110016, China
Email address: chenwei@iae.ac.cn

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, *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 acidsand flavonoids content, and *Pteridium aquilinum* L. Kuhn var *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.

Keywords

edible fern species; shading; total phenols; flavonoids; minerals; amino acids

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 *Matteuccia struthiopteris* (L.) Todaro (MS), *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 defensing 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 multidentatum* (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℃ for 30 min, then dried at 60℃ 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 0.1g 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℃ for 30 min. The mixture was centrifuged at 12000 rpm, 25℃ 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 0.1g 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℃ for 30 min. The mixture was centrifuged at 12000 rpm, 25℃ 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 power 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℃ 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℃ 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 $P \leqslant 0.05$.

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 flavonoidsr 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 in13% 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 α-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 H_2O_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 H_2O_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. Lettue (*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.

Acknowledgements

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.

References

Agati G, and Tattini M. 2010. Multiple functional roles of flavonoids in photoprotection. New

Phytologist 186:786-793. https://doi.org/10.1111/j.1469-8137.2010.03269.x

Andrae-Marobela K, Ghislain FW, Okatch H, and Majinda RRT. 2013. Polyphenols: A Diverse Class of Multi-Target Anti-HIV-1 Agents. Current Drug Metabolism 14:392-413. https://doi.org/10.2174/13892002113149990095

Bystriakova N, Bader M, and Coomes DA. 2011. Long-term tree fern dynamics linked to disturbance and shade tolerance. Journal of Vegetation Science 22:72-84. https://doi.org/10.1111/j.1654-1103.2010.01227.x

Chen CL, Luo XH, Jin GR, Cheng Z, Pan XY, Zhu GL, Li S, Zhu YG, and Tang NN. 2017. Shading effect on survival, growth, and contents of secondary metabolites in micropropagated Anoectochilus plantlets. Brazilian Journal of Botany 40:599-607. https://doi.org/10.1007/s40415-017-0365-4

Chen L, Teng H, Xie ZL, Cao H, Cheang WS, Skalicka-Woniak K, Georgiev MI, and Xiao JB. 2018. Modifications of dietary flavonoids towards improved bioactivity: An update on structure-activity relationship. Critical reviews in food science and nutrition 58:513-527. https://doi.org/10.1080/10408398.2016.1196334

Colonna E, Rouphael Y, Barbieri G, and De Pascale S. 2016. Nutritional quality of ten leafy vegetables harvested at two light intensities. Food Chemistry 199:702-710. https://doi.org/10.1016/j.foodchem.2015.12.068

Dong R, Liu S, Wen LK, Zhao SC, and Fu L. 1993. Determination of nutrient elements and health value of dried *Pteridium aquilinum* and *Athyrium multidentatum*. Chinese Wild Plant Resources 24(1):10-12.

Fiutak G, Michalczyk M, Filipczak-Fiutak M, Fiedor L, and Surowka K. 2019. The impact of LED lighting on the yield, morphological structure and some bioactive components in alfalfa (*Medicago sativa* L.) sprouts. Food Chemistry 285:53-58. https://doi.org/10.1016/j.foodchem.2019.01.086

Franzoni G, Trivellini A, Bulgari R, Cocetta G, and Ferrante A. 2019. Chapter 10 - Bioactive Molecules as Regulatory Signals in Plant Responses to Abiotic Stresses. In: Khan MIR, Reddy PS, Ferrante A, and Khan NA, eds. Plant Signaling Molecules: Woodhead Publishing, 169-182.

Frede K, Schreiner M, and Baldermann S. 2019. Light quality-induced changes of carotenoid composition in pak choi Brassica rapa ssp. chinensis. Journal of Photochemistry and

346 Photobiology B-Biology 193:18-30. https://doi.org/10.1016/j.jphotobiol.2019.02.001
347 Fukuda N. 2019. Chapter 2.2 - Plant Growth and Physiological Responses to Light Conditions.
348 In: Anpo M, Fukuda H, and Wada T, eds. Plant Factory Using Artificial Light: Elsevier,
349 71-77.
350 Galieni A, Di Mattia C, De Gregorio M, Speca S, Mastrocola D, Pisante M, and Stagnari F.
351 2015. Effects of nutrient deficiency and abiotic environmental stresses on yield, phenolic
352 compounds and antiradical activity in lettuce (Lactuca sativa L.). Scientia Horticulturae
353 187:93-101. https://doi.org/10.1016/j.scienta.2015.02.036
354 Georgieva K, Dagnon S, Gesheva E, Bojilov D, Mihailova G, and Doncheva S. 2017.
355 Antioxidant defense during desiccation of the resurrection plant Haberlea rhodopensis.
356 Plant Physiology and Biochemistry 114:51-59.
357 https://doi.org/10.1016/j.plaphy.2017.02.021
358 Graham TL. 1998. Flavonoid and flavonol glycoside metabolism in Arabidopsis. Plant
359 Physiology and Biochemistry 36:135-144. https://doi.org/10.1016/S0981-
360 9428(98)80098-3
361 Han XZ, Ma R, Chen Q, Jin X, Jin YZ, An RB, Piao XM, Lian ML, Quan LH, and Jiang J. 2018.
362 Anti-inflammatory action of Athyrium multidentatum extract suppresses the LPS-induced
363 TLR4 signaling pathway. Journal of Ethnopharmacology 217:220-227.
364 https://doi.org/10.1016/j.jep.2018.02.031
365 Hänsch R, and Mendel RR. 2009. Physiological functions of mineral micronutrients (Cu, Zn,
366 Mn, Fe, Ni, Mo, B, Cl). Current Opinion in Plant Biology 12:259-266.
367 https://doi.org/10.1016/j.pbi.2009.05.006
368 Häusler RE, Ludewig F, and Krueger S. 2014. Amino acids – A life between metabolism and
369 signaling. Plant Science 229:225-237. https://doi.org/10.1016/j.plantsci.2014.09.011
370 Heim KE, Tagliaferro AR, and Bobilya DJ. 2002. Flavonoid antioxidants: chemistry, metabolism
371 and structure-activity relationships. The Journal of Nutritional Biochemistry 13:572-584.
372 https://doi.org/10.1016/S0955-2863(02)00208-5
373 Hildebrandt Tatjana M, Nunes Nesi A, Araújo Wagner L, and Braun H-P. 2015. Amino Acid
374 Catabolism in Plants. Molecular Plant 8:1563-1579.
375 https://doi.org/10.1016/j.molp.2015.09.005
376 Ilić ZS, and Fallik E. 2017. Light quality manipulation improves vegetable quality at harvest and

postharvest: A review. Environmental and Experimental Botany 139:79-90. https://doi.org/10.1016/j.envexpbot.2017.04.006

Jones C, and Hartley S. 1999. A protein competition model of phenolic allocation. Oikos 86:27-44.

Kaiser E, Weerheim K, Schipper R, and Dieleman JA. 2019. Partial replacement of red and blue by green light increases biomass and yield in tomato. Scientia Horticulturae 249:271-279. https://doi.org/10.1016/j.scienta.2019.02.005

Kataria S, Baghel L, Jain M, and Guruprasad KN. 2019. Magnetopriming regulates antioxidant defense system in soybean against salt stress. Biocatalysis and Agricultural Biotechnology 18:101090. https://doi.org/10.1016/j.bcab.2019.101090

Kumari R, Singh S, and Agrawal SB. 2009. Combined effects of Psoralens and ultraviolet-B on growth, pigmentation and biochemical parameters of Abelmoschus esculentus L. Ecotoxicology and Environmental Safety 72:1129-1136. https://doi.org/10.1016/j.ecoenv.2008.12.009

Kyriacou MC, Rouphael Y, Di Gioia F, Kyratzis A, Serio F, Renna M, De Pascale S, and Santamaria P. 2016. Micro-scale vegetable production and the rise of microgreens. Trends in Food Science & Technology 57:103-115. https://doi.org/10.1016/j.tifs.2016.09.005

Less H, and Galili G. 2008. Principal transcriptional programs regulating plant amino acid metabolism in response to abiotic stresses. Plant Physiology 147:316-330. https://doi.org/10.1104/pp.108.115733

Liao X, Greenspan P, and Pegg RB. 2019. Characterizing the phenolic constituents and antioxidant capacity of Georgia peaches. Food Chemistry 271:345-353. https://doi.org/10.1016/j.foodchem.2018.07.163

Liu B, and Li X. 1995. Resources of economic plant Pteridophyte in Northeast China. Chinese Wild Plant Resources (04):36-38+35.

Liu HX, Yu P, and Wang HZ. 2011. Determination of amino acid content in five species of fern by HPLC with Precolumn Derivation. Amino Acids & Biotic Resources 33:30-33. https://doi.org/ 10.14188/j.ajsh.2011.03.018.

Liu Y, Fang S, Yang W, Shang X, and Fu X. 2018. Light quality affects flavonoid production and related gene expression in Cyclocarya paliurus. Journal of Photochemistry and

Photobiology B: Biology 179:66-73. https://doi.org/10.1016/j.jphotobiol.2018.01.002

Liu Y, and Wang D. 2018. Investigation on survival environment of Osmunda cinnamomea in high cold forest area. Forestry Science & Technology 43(06):24-25. https://doi.org/CNKI:SUN:LYKJ.0.2018-06-008.

Maathuis FJM. 2009. Physiological functions of mineral macronutrients. Current Opinion in Plant Biology 12:250-258. https://doi.org/10.1016/j.pbi.2009.04.003

Manoj GS, and Murugan K. 2012. Phenolic profiles, antimicrobial and antioxidant potentiality of methanolic extract of a liverwort, Plagiochila beddomei Steph.

Meini M-R, Cabezudo I, Boschetti CE, and Romanini D. 2019. Recovery of phenolic antioxidants from Syrah grape pomace through the optimization of an enzymatic extraction process. Food Chemistry 283:257-264. https://doi.org/10.1016/j.foodchem.2019.01.037

Mir-Marqués A, Cervera ML, and de la Guardia M. 2016. Mineral analysis of human diets by spectrometry methods. TrAC Trends in Analytical Chemistry 82:457-467. https://doi.org/10.1016/j.trac.2016.07.007

Mullineaux PM, Exposito-Rodriguez M, Laissue PP, and Smirnoff N. 2018. ROS-dependent signalling pathways in plants and algae exposed to high light: Comparisons with other eukaryotes. Free Radical Biology and Medicine 122:52-64. https://doi.org/10.1016/j.freeradbiomed.2018.01.033

Naikoo MI, Dar MI, Raghib F, Jaleel H, Ahmad B, Raina A, Khan FA, and Naushin F. 2019. Chapter 9 - Role and Regulation of Plants Phenolics in Abiotic Stress Tolerance: An Overview. In: Khan MIR, Reddy PS, Ferrante A, and Khan NA, eds. Plant Signaling Molecules: Woodhead Publishing, 157-168.

Otálora G, Piñero MC, López-Marín J, Varó P, and del Amor FM. 2018. Effects of foliar nitrogen fertilization on the phenolic, mineral, and amino acid composition of escarole (Cichorium endivia L. var. latifolium). Scientia Horticulturae 239:87-92. https://doi.org/10.1016/j.scienta.2018.05.031

Paiva CL, Queiroz VAV, Simeone MLF, Schaffert RE, de Oliveira AC, and da Silva CS. 2017a. Mineral content of sorghum genotypes and the influence of water stress. Food Chemistry 214:400-405. https://doi.org/10.1016/j.foodchem.2016.07.067

Paiva CL, Vieira Queiroz VA, Ferreira Simeone ML, Schaffert RE, de Oliveira AC, and da Silva

CS. 2017b. Mineral content of sorghum genotypes and the influence of water stress. Food Chemistry 214:400-405. https://doi.org/10.1016/j.foodchem.2016.07.067

Perez-Lopez U, Sgherri C, Miranda-Apodaca J, Micaelli F, Lacuesta M, Mena-Petite A, Quartacci MF, and Munoz-Rueda A. 2018. Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO2. Plant Physiology and Biochemistry 123:233-241. https://doi.org/10.1016/j.plaphy.2017.12.010

Pinto-Marijuan M, and Munne-Bosch S. 2014. Photo-oxidative stress markers as a measure of abiotic stress-induced leaf senescence: advantages and limitations. Journal of Experimental Botany 65:3845-3857. https://doi.org/10.1093/jxb/eru086

Pourcel L, Routaboul J-M, Cheynier V, Lepiniec L, and Debeaujon I. 2007. Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends in Plant Science 12:29-36. https://doi.org/10.1016/j.tplants.2006.11.006

Qi GY, Yang LQ, Xiao CX, Shi J, Mi YS, and Liu XB. 2015. Nutrient values and bioactivities of the extracts from three fern species in China: a comparative assessment. Food & Function 6:2918-2929. https://doi.org/10.1039/c5fo00510h

Riachi LG, Simas DLR, Coelho GC, Marcellini PS, Ribeiro da Silva AJ, and Bastos de Maria CA. 2018. Effect of light intensity and processing conditions on bioactive compounds in maté extracted from yerba mate (Ilex paraguariensis A. St.-Hil.). Food Chemistry 266:317-322. https://doi.org/10.1016/j.foodchem.2018.06.028

Riga P, Benedicto L, Gil-Izquierdo A, Collado-Gonzalez J, Ferreres F, and Medina S. 2019. Diffuse light affects the contents of vitamin C, phenolic compounds and free amino acids in lettuce plants. Food Chemistry 272:227-234. https://doi.org/10.1016/j.foodchem.2018.08.051

Rouphael Y, Kyriacou MC, Petropoulos SA, De Pascale S, and Colla G. 2018. Improving vegetable quality in controlled environments. Scientia Horticulturae 234:275-289. https://doi.org/10.1016/j.scienta.2018.02.033

Ruangrak E, and Khummueng W. 2019. Effects of artificial light sources on accumulation of phytochemical contents in hydroponic lettuce. Journal of Horticultural Science & Biotechnology 94:378-388. https://doi.org/10.1080/14620316.2018.1504630

Saito K, Yonekura-Sakakibara K, Nakabayashi R, Higashi Y, Yamazaki M, Tohge T, and Fernie AR. 2013. The flavonoid biosynthetic pathway in Arabidopsis: Structural and genetic

diversity. Plant Physiology and Biochemistry 72:21-34. https://doi.org/10.1016/j.plaphy.2013.02.001

Sarker U, and Oba S. 2018. Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. Food Chemistry 252:72-83. https://doi.org/10.1016/j.foodchem.2018.01.097

Schenke D, Utami HP, Zhou Z, Gallegos M-T, and Cai D. 2019. Suppression of UV-B stress induced flavonoids by biotic stress: Is there reciprocal crosstalk? Plant Physiology and Biochemistry 134:53-63. https://doi.org/10.1016/j.plaphy.2018.06.026

Shao QS, Wang HZ, Guo HP, Zhou AC, Huang YQ, Sun YL, and Li MY. 2014. Effects of Shade Treatments on Photosynthetic Characteristics, Chloroplast Ultrastructure, and Physiology of Anoectochilus roxburghii. Plos One 9. https://doi.org/10.1371/journal.pone.0085996

Shibuya T, Kishigami S, Endo R, and Matsuda R. 2019. Interaction between red to far-red ratio of light and vapor-pressure deficit on extension growth of cucumber seedlings. Scientia Horticulturae 248:98-104. https://doi.org/10.1016/j.scienta.2018.12.049

Siracusa L, and Ruberto G. 2014. Chapter 2 - Plant Polyphenol Profiles as a Tool for Traceability and Valuable Support to Biodiversity. In: Watson RR, ed. Polyphenols in Plants. San Diego: Academic Press, 15-33.

Soares C, Carvalho MEA, Azevedo RA, and Fidalgo F. 2018. Plants facing oxidative challenges—A little help from the antioxidant networks. Environmental and Experimental Botany. https://doi.org/10.1016/j.envexpbot.2018.12.009

Sonawala U, Dinkeloo K, Danna CH, McDowell JM, and Pilot G. 2018. Review: Functional linkages between amino acid transporters and plant responses to pathogens. Plant Science 277:79-88. https://doi.org/10.1016/j.plantsci.2018.09.009

Song YX. 2009. Effect of shading and restoration light intensity on photosynthetic characteristics, nitrogen metabolism, yield and quality in various relay cropping soybeanMaster. Sichuan Agricultural university.

Stagnari F, Galieni A, Cafiero G, and Pisante M. 2014. Application of photo-selective films to manipulate wavelength of transmitted radiation and photosynthate composition in red beet (Beta vulgaris var. conditiva Alef.). Journal of the Science of Food and Agriculture

94:713-720. https://doi.org/10.1002/jsfa.6312

Stagnari F, Galieni A, and Pisante M. 2015. Shading and nitrogen management affect quality, safety and yield of greenhouse-grown leaf lettuce. Scientia Horticulturae 192:70-79. https://doi.org/10.1016/j.scienta.2015.05.003

Szymańska R, Ślesak I, Orzechowska A, and Kruk J. 2017. Physiological and biochemical responses to high light and temperature stress in plants. Environmental and Experimental Botany 139:165-177. https://doi.org/10.1016/j.envexpbot.2017.05.002

Tounekti T, and Khemira H. 2015. NaCl stress-induced changes in the essential oil quality and abietane diterpene yield and composition in common sage. Journal of intercultural ethnopharmacology 4:208-216. https://doi.org/10.5455/jice.20150405064135

Tounekti T, Munne-Bosch S, Vadel AM, Chtara C, and Khemira H. 2010. Influence of ionic interactions on essential oil and phenolic diterpene composition of Dalmatian sage (Salvia officinalis L.). Plant Physiology and Biochemistry 48:813-821. https://doi.org/10.1016/j.plaphy.2010.08.007

Treutter D. 2006. Significance of flavonoids in plant resistance: a review. Environmental Chemistry Letters 4:147. https://doi.org/10.1007/s10311-006-0068-8

Vaknin H, Bar-Akiva A, Ovadia R, Nissim-Levi A, Forer I, Weiss D, and Oren-Shamir M. 2005. Active anthocyanin degradation in Brunfelsia calycina (yesterday–today–tomorrow) flowers. Planta 222:19-26. https://doi.org/10.1007/s00425-005-1509-5

Wahle KWJ, Brown I, Rotondo D, and Heys SD. 2010. Plant Phenolics in the Prevention and Treatment of Cancer. In: Giardi MT, Rea G, and Berra B, eds. Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. Boston, MA: Springer US, 36-51.

Wang H, and Wu S. 2013. Preparation and antioxidant activity of Pteridium aquilinum-derived oligosaccharide. International Journal of Biological Macromolecules 61:33-35. https://doi.org/10.1016/j.ijbiomac.2013.06.053

Williams RJ, Spencer JPE, and Rice-Evans C. 2004. Flavonoids: antioxidants or signalling molecules? Free Radical Biology and Medicine 36:838-849. https://doi.org/10.1016/j.freeradbiomed.2004.01.001

Xiang J, Apea-Bah FB, Ndolo VU, Katundu MC, and Beta T. 2019. Profile of phenolic compounds and antioxidant activity of finger millet varieties. Food Chemistry 275:361-

368. https://doi.org/10.1016/j.foodchem.2018.09.120

Xiao J. 2017. Dietary flavonoid aglycones and their glycosides: Which show better biological significance? Critical reviews in food science and nutrition 57:1874-1905. https://doi.org/10.1080/10408398.2015.1032400

Yang JH, Li ZY, Wang L, and Zhang L. 2002. Compositions of amino acids in two kinds of lobelia plant from Yunnan. Journal of Yunnan Minzu University (Natural Sciences Edition) 11(2):94-95. https://doi.org/10.3969/j.issn.1672-8513.2002.02.011

Yao YX. 2003. Analysis of nutritive components of Kochia scopania (L.) and Pteridium aquilinum (L.). Amino Acids &Biotic Resources 25(1):1-2. https://doi.org/10.3969/j.issn.1006-8376.2003.01.001

Yao YX, Cai JP, Li Z, M., and Wang Y, H. 2003. Analysis of the nutritional components of four edible wild vegetables. Acta Nutrimenta Sinica 25(4):441-442. https://doi.org/10.3321/j.issn:0512-7955.2003.04.027

Zhang M, Cao J, Dai X, Chen X, and Wang Q. 2012. Flavonoid Contents and Free Radical Scavenging Activity of Extracts from Leaves, Stems, Rachis and Roots of Dryopteris erythrosora. Iranian Journal of Pharmaceutical Research 11:991-997.

Zhao ML, Liu MC, Zhong QW, He HJ, Ji YM, and Li L. 2018. 29 Jerusalem artichoke germplasm resources amino acid content and nutritional value evaluation. Seed 37:55-60. https://doi.org/10.16590/j.cnki.1001-4705.2018.03.055

Zhao SC, Fu L, and Liu ML. 1991. Analysis of amino acids and inorganic elements in Pteridium aquilinum. Food Science (05):31-33.

Zhen YD, Yu M, Xiao HD, Wang HZ, Xie HS, and Zeng XF. 2010. Influences of silicon on content of soluble sugars, amino acids in Turf-grasses under shading stress. Journal of Huazhong Agricultural University 29:317-320.

Zhu LJ, Yan F, Chen JP, Zhang N, Zhang X, and Yao XS. 2016. 8-O-4 ' Neolignan glycosides from the aerial parts of Matteuccia struthiopteris. Chinese Chemical Letters 27:63-65. https://doi.org/10.1016/j.cclet.2015.08.016

Zrig A, Tounekti T, AbdElgawad H, Hegab MM, Ali SO, and Khemira H. 2016. Essential oils, amino acids and polyphenols changes in salt-stressed Thymus vulgaris exposed to open-field and shade enclosure. Industrial Crops and Products 91:223-230. https://doi.org/10.1016/j.indcrop.2016.07.012

Figure 1(on next page)

Effect of shading on total phenols and flavonoids content (mg g^{-1} DW) in fourfern species.

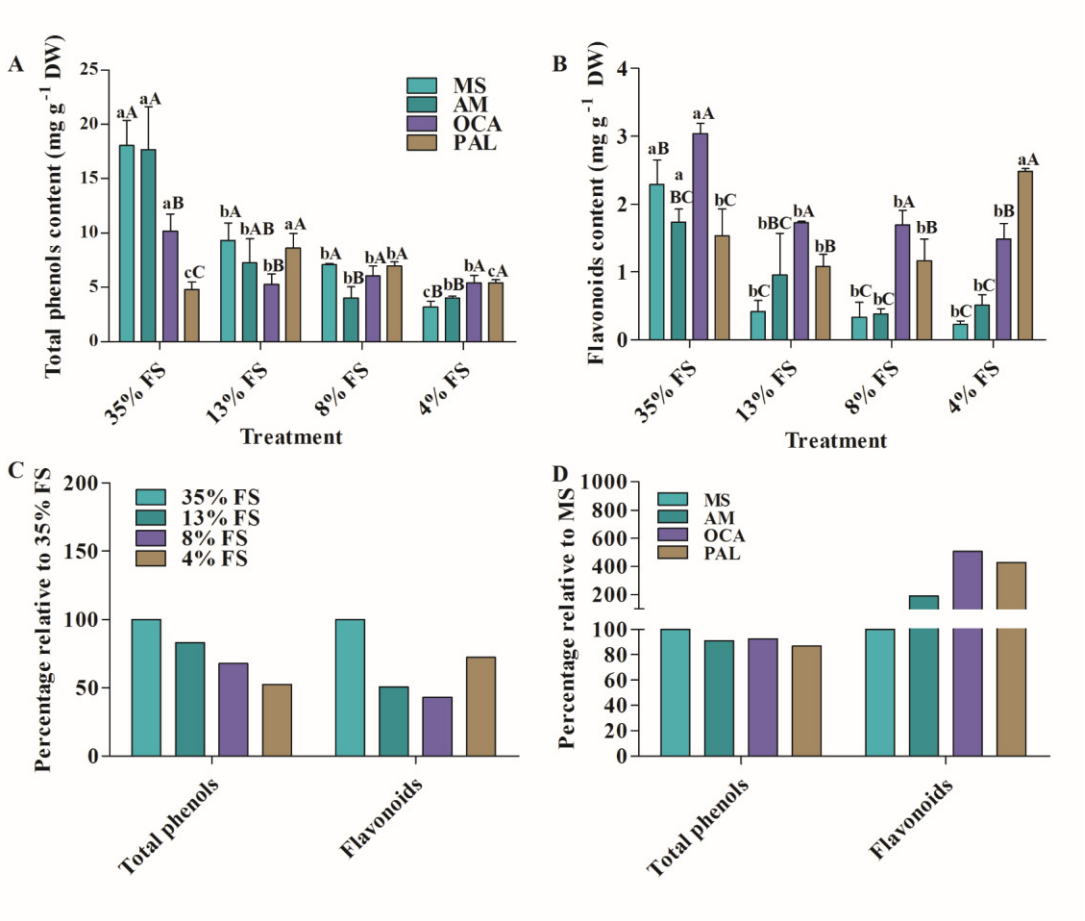

Figure 1 **Effect of shading on total phenols and flavonoids content (mg g^{-1} DW) in four fern species.** (**A**) Total phenols content; (**B**) Flavonoids content. Changes of total phenols content and flavonoids content (mg g^{-1} DW) under four shading treatments (percentage relative to 35% FS) (**C**) 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).

Figure 2(on next page)

Effect of shading on minerals content (mg g^{-1} DW) in four fern species.

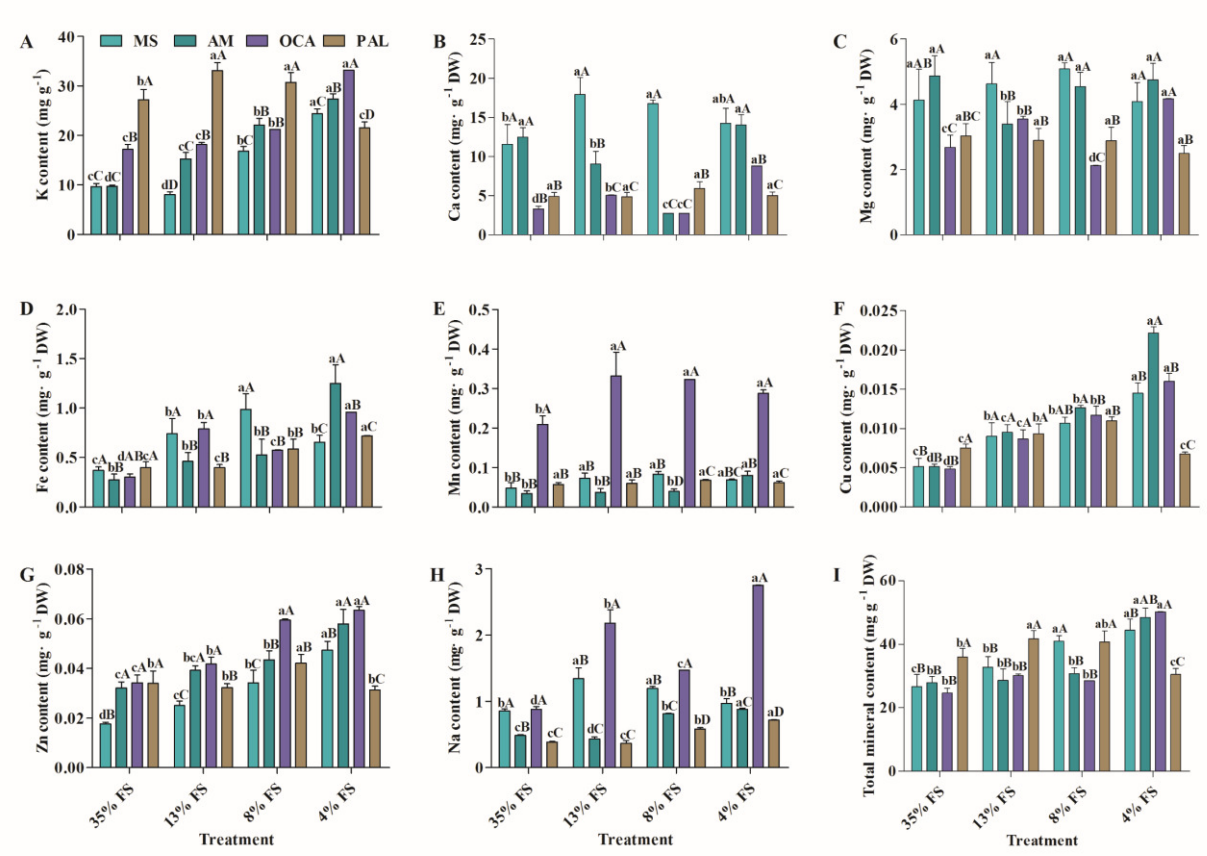

Figure 2 **Effect of shading on minerals content (mg g^{-1} DW) in four fern species.** (**A**) K content; (**B**) Ca content; (**C**) Mg content; (**D**) Fe content; (**E**) Mn content; (**F**) Cu content; (**G**) Zn content; (**H**) Na content; (**I**) Total mineral content. MS = *M. struthiopteris*, 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 (percentagerelative to 35% FS) (A) and among four fern species (percentage relative to MS) (B).

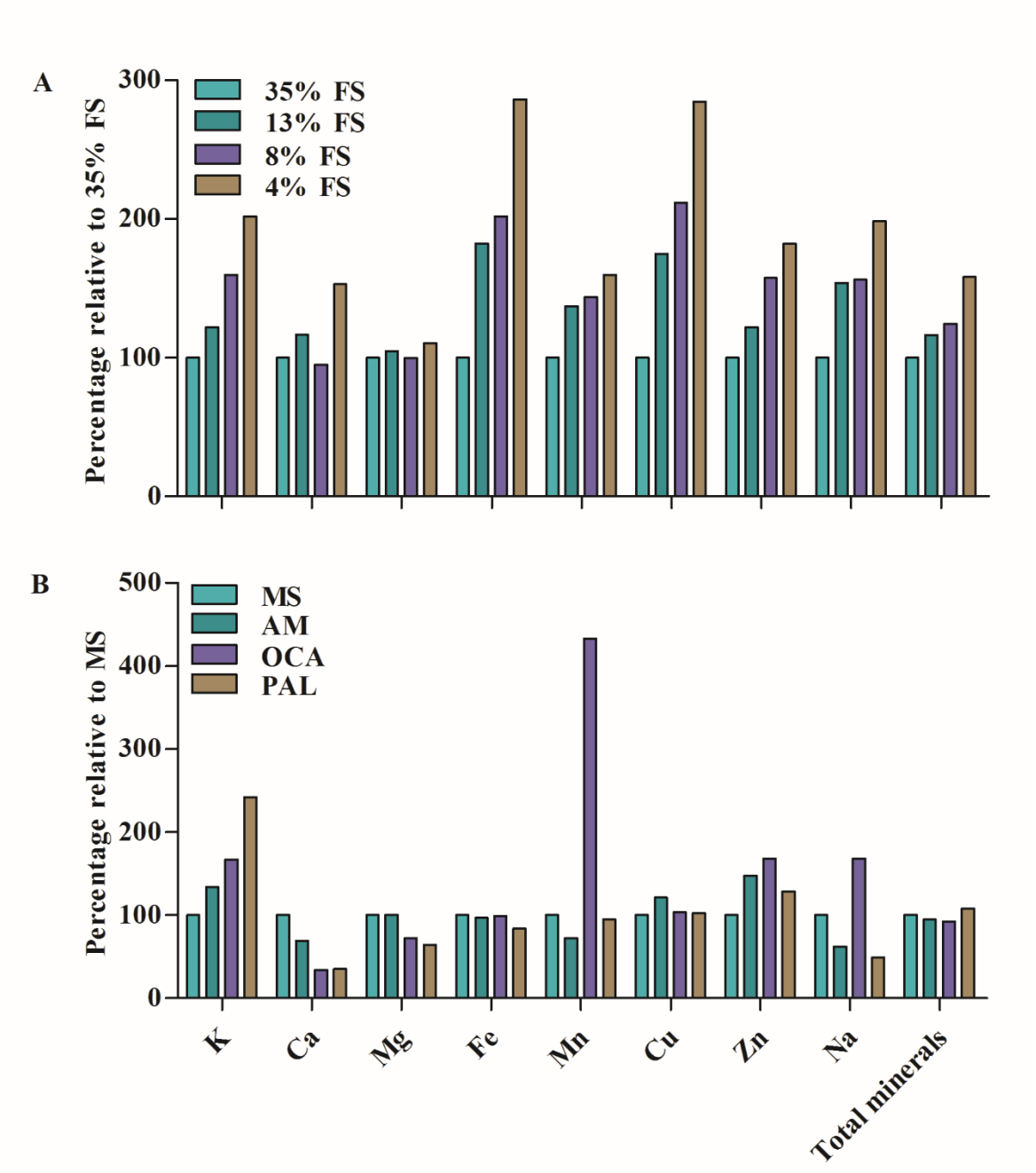

Figure 3 **Changes of mineral element content (mg g^{-1} DW) under four shading treatments (percentage relative to 35% FS) (A) and among four fern species (percentage relative to MS) (B).** MS = *M. struthiopteris*, AM = *A. multidentatum*, OCA = *O. cinnamomea* (L.) var. *asiatica*, PAL = *P. aquilinum* L. Kuhn var. *latiusculum*, 35% FS = 35% Full sunlight, 13% FS = 13% Full sunlight, 8% FS = 8% Full sunlight, 4% FS= 4% Full sunlight.

Figure 4(on next page)

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[sup]-1[s

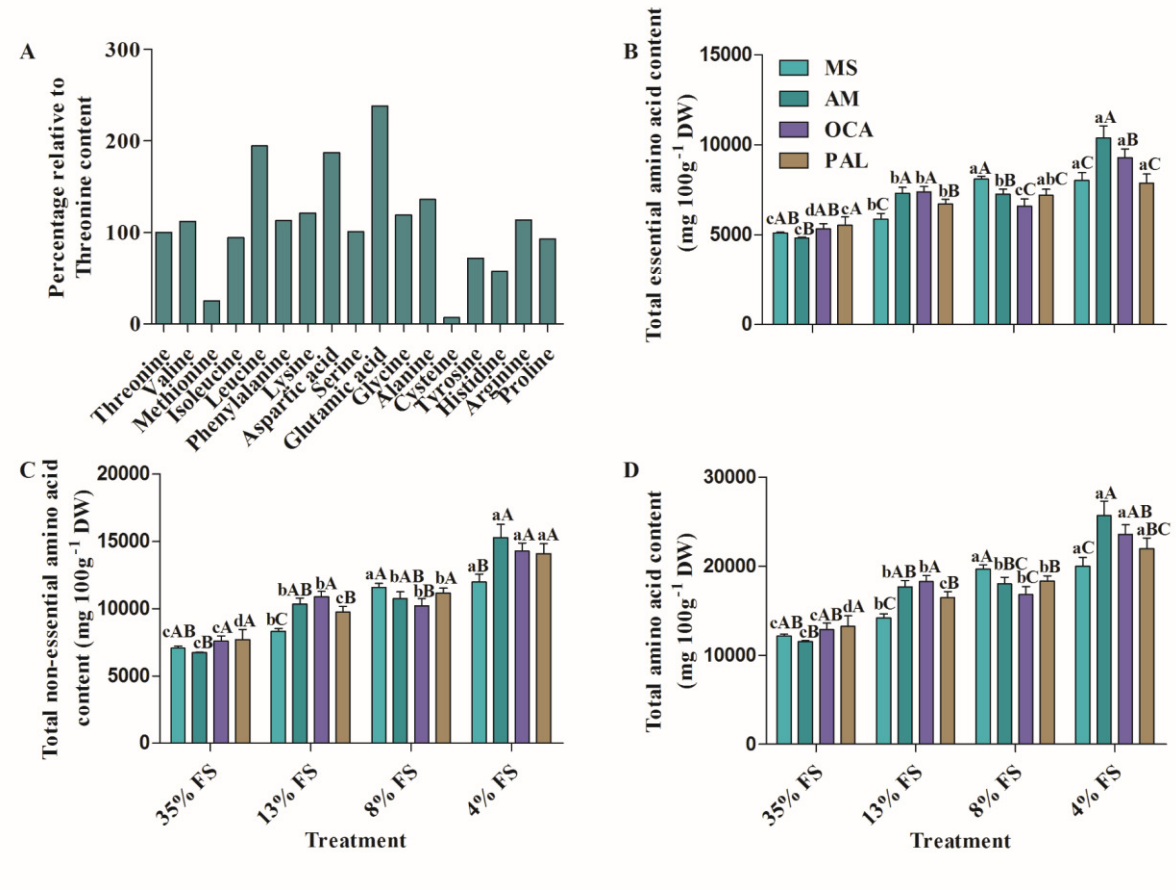

Figure 4 **Changes of 16 amino acids content of four fern species under four shading treatments (percentage relative to Threonine) (A), and effect of shading on essential amino acid content, non-essential amino acid content, and total amino acids content (mg 100g^{-1} DW) in four fern species.** (**B**) 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 acidscontent (mg $100g^{-1}$ DW) under four shading treatments.

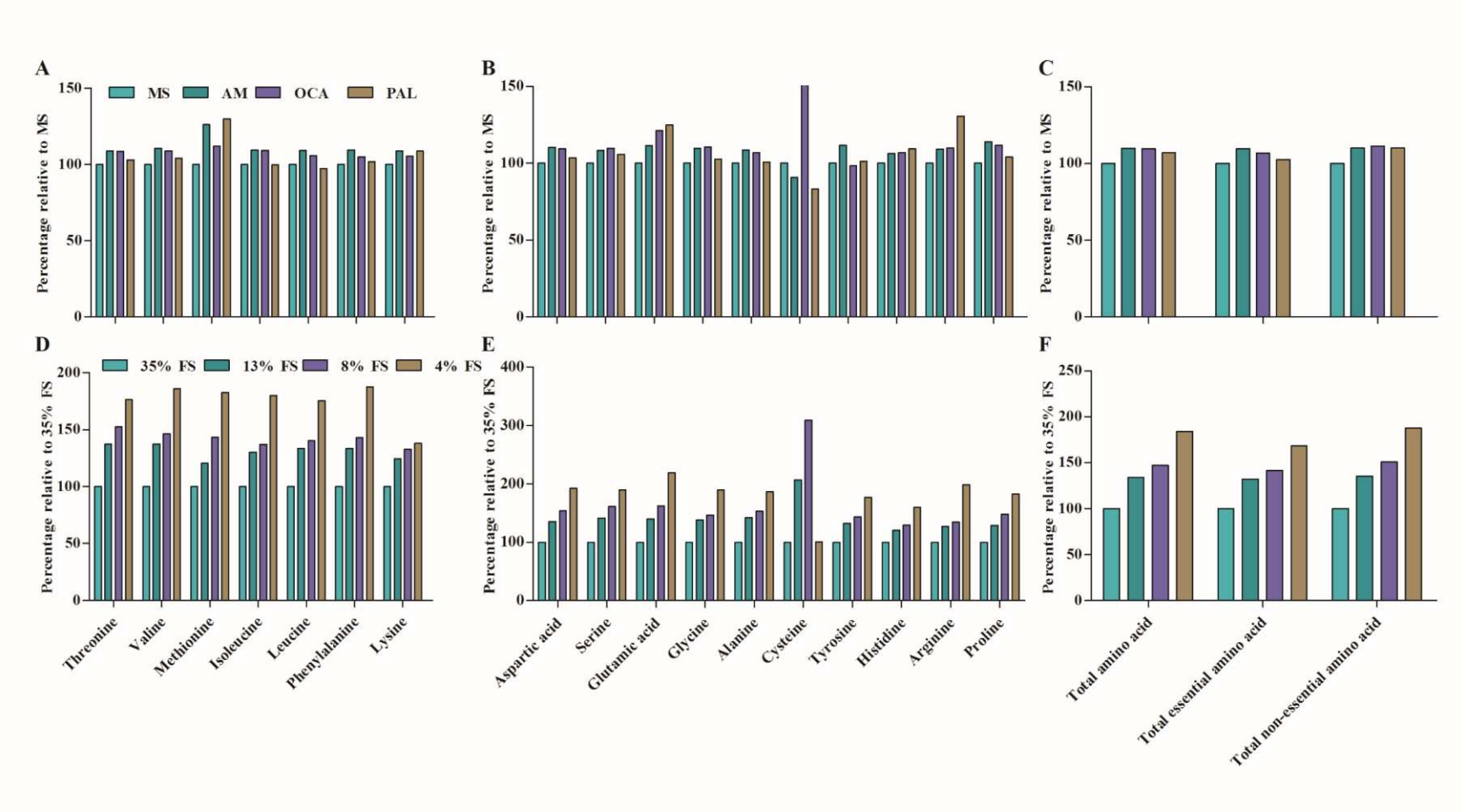

Figure 5 **Changes of 7 essential amino acid, 10 non-essential amino acid, total amino acids, total essential amino acids and total non-essential amino acids content (mg 100g^{-1} DW) under four shading treatments.** (**A, B, C**) Percentage relative to MS. (**C, D, E**) Percentage relative to 35% FS. 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.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.27782v1 | CC BY 4.0 Open Access | rec: 4 Jun 2019, publ: 4 Jun 2019