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Impact of aerosols and atmospheric particles on plant leaf proteins

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HIGHLIGHTS

- We obtain atmospheric particles and aerosols data from 2007 to 2012.
- Strong and weak diffuse solar radiation regions are classified.
- Aerosols and atmospheric particles stimulate plant photosynthesis.

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ABSTRACT

Aerosols and atmospheric particles can diffuse and absorb solar radiation, and directly affect plant photosynthesis and related protein expression. In this study, for the first time, we performed an extensive investigation of the effects of aerosols and atmospheric particles on plant leaf proteins by combining Geographic Information System and proteomic approaches. Data on particles with diameters of 0.1–1.0 μm (PM_1) from different locations across the city of Beijing and the aerosol optical depth (AOD) over the past 6 years (2007–2012) were collected. In order to make the study more reliable, we segregated the influence of soil pollution by measuring the heavy metal content. On the basis of AOD and PM_1, two regions corresponding to strong and weak diffuse solar radiations were selected for analyzing the changes in the expression of plant proteins. Our results demonstrated that in areas with strong diffuse solar radiations, plant ribulose bisphosphate carboxylase was expressed at higher levels, but oxygen evolved in enhancer protein and light-harvesting complex II protein were expressed at lower levels. The expression of ATP synthase subunit beta and chlorophyll a–b binding protein were similar in both regions. By analyzing the changes in the expression of these leaf proteins and their functions, we conclude that aerosols and atmospheric particles stimulate plant photosynthesis facilitated by diffuse solar radiations.

1. Introduction

Aerosols and atmospheric particles are considered to have a significant influence on solar radiance incidents on the Earth's surface, as they can reduce total irradiance and diffuse solar radiations (Cohan et al., 2002). In ecosystems, plants intercept sunlight and utilize solar energy, which is the basis for photosynthesis. The efficiency of photosynthesis has direct implications for plant health, generally by inducing changes in protein expression. Previous reports have indicated that photosynthesis conditions can impact protein expression in plants (Jiang et al., 2001).

Several investigations on photosynthetically active radiation (PAR) have demonstrated that diffuse solar radiation facilitated by aerosols and atmospheric particles is more advantageous for plant photosynthesis (Hollinger, 1998; Gu et al., 2002; Rocha et al., 2004). The scattering effect of aerosols on light would reduce downwelling solar radiation, but simultaneously increase plant photosynthesis (Matsui et al., 2008). Since aerosols enhance solar radiation scattering, the plant productivity can increase ultimately (Roderick et al., 2001). In addition, aerosol optical depths (AOD) also tend to increase the daytime carbon sink that benefit plant photosynthesis (Niyogi, 2004). In contrast, some studies have reported a negative

Abbreviations: AOD, aerosol optical depth; ATP-SSB, ATP synthase subunit beta; Chl-ab, chlorophyll a-b binding protein; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; LHC II, light-harvesting complex II protein; OEEP, oxygen evolved in enhancer protein; PAR, photosynthetically active radiation; PM, particulate matters; Rubisco, ribulose bisphosphate carboxylase; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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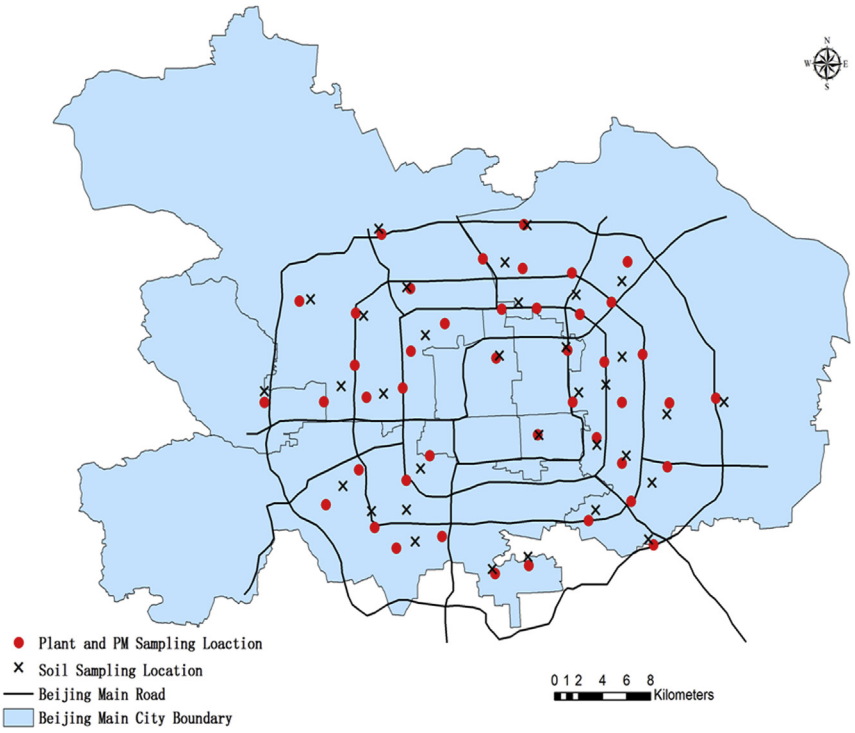

Fig. 1. Distributions of sampling locations in Beijing.

effect of aerosols and atmospheric particles on plant growth. Chameides (1999) found that aerosols in China could decrease crop productivity because they were always accompanied with other pollutants. Zhai et al. (2006) reported that heavy metals were widely found in atmospheric particles, and in response to heavy metal exposures plants usually decreased the synthesis of soluble proteins and chlorophyll. Kanniah et al. (2006) reported that high aerosol loadings can depress the carbon sink and is disadvantageous for plant photosynthesis.

To date, aerosols and atmospheric particles have been shown to have important effects on plant growth and health. Although McCree (1981) reported that particles with diameters of 0.1–1.0 μm had more efficient light-scattering ability and influenced plant photosynthesis, the mechanism by which they affect plant protein expression is still unknown. It is highly important to understand the expression profile of plant leaf proteins related to plant photosynthesis, productivity, and health status under different aerosol and atmospheric particle pollution conditions over a long-term period. In this work, we attempt to combine Geographic Information System (GIS) and proteomic approaches to investigate specific protein changes in plant leaves that are facilitated by aerosols and atmospheric particles. We tracked and continuously measured the atmosphere particles with diameters of 0.1–1.0 μm (PM_1) during the last 6 years (2007–2012). Meanwhile, AOD data were obtained by means of MODIS AOD products and remote sensing inversion. On the basis of these data, we selected two regions that have strong or weak diffuse solar radiation for further analyzing the expression patterns and changes in plant leaf proteins. Our results provide new insights into the biochemical mechanisms by which aerosols and atmospheric particles influence plant growth and health.

Table 1
Sampling time.

Years	PM_1
2007	May–June
2008	July–August
	December–January
2009	June–July
	December–January
2010	May–June
	December
2011	June–July
	December–January
2012	June 20–June 27
	June–July

2. Materials and methods

2.1. Study area and plant materials

This study was conducted in the city of Beijing, the capital city of China, where atmospheric particles present a significant problem (Wang and Xie, 2009). Beijing lies at east longitude 39.92°, north latitude 116.46° and covers an area of 16507.5 km^2. It is located at the northern edge of the North China Plain at the junction of Inner Mongolia Plateau, Loess Plateau, and North China Plain.

Euonymus japonica is native to China, Korea, and Japan. It is an evergreen shrub or small tree, which grows to 2–8 m tall, with opposite, oval leaves that are 3–7-cm long with finely serrated margins. The flowers are inconspicuous, greenish-white, and 5 mm in diameter. It is a popular ornamental plant for parks and gardens, both in its native area and also in North America and Europe. *E. japonica* is one of the main plant species planted near roads Beijing City Council, and was used as the experimental plant.

Mature leaves from 1-m high *E. japonica* were collected from different locations to ensure that the collected leaves had consistent growth time and received solar radiation under similar conditions. These leaves were stored in zip-lock bags and placed in an ice box

Table 2
Statistical results for soil heavy metals.

Elements	Cd/mg kg^{-1}	Cu/mg kg^{-1}	Zn/mg kg^{-1}	Pb/mg kg^{-1}	V/mg kg^{-1}	Ti/μg kg^{-1}	Mn/mg kg^{-1}	Al/μg kg^{-1}	Fe/μg kg^{-1}
Average	1.28	26.45	79.70	32.86	79.11	3.76	531.4	56.59	21.78
95% CI	1.1–1.5	20.8–30.1	59.5–99.9	26.8–38.9	75.5–82.7	3.5–3.9	499–562	55.3–57.8	20.8–22.7
Stdev	0.5	16.1	57.8	17.5	10.2	0.5	90.2	3.6	2.7
CV	0.42	0.61	0.73	0.53	0.13	0.15	0.17	0.06	0.13
Min	0.1	11.6	14.7	15.9	56.3	2.3	334	48.9	14.9
Max	2.3	90.9	261.1	88.0	113.7	5.2	714	64.8	27.6
Skewness	−0.2	2.6	1.7	2.1	0.9	0.2	−0.1	0.1	−0.2
Kurtosis	−0.1	7.8	3.1	4.4	2.8	0.6	−0.3	0.5	−0.1

prior to analysis. Experimental leaf samples were collected from 2007 to 2012 at 6 sampling locations and in 2012 at 44 sampling locations, and their geographical distributions are shown in Fig. 1. Samples of three biological replicates from each location were collected.

2.2. Collection of particulate matters (PM)

Data on the PM were collected using a miniature laser aerosol particle counter (KANOMAX, 3886GEOX), which can record PM (count/m^3) with sizes of 1 μm. Sampling was performed during summer (May–September) and winter (November–January) in the years of 2007–2012 (Table 1).

2.3. AOD data

In this study, the AOD data obtained by two means—MODIS AOD products from NASA and inversion method by using TM/ETM + images. MODIS 1000 m resolution 550 nm AOD products—were collected from 2007 to 2012 in Beijing City areas. At present, the MODIS remote sensing AOD products are widely used for routine monitoring, as it can detect the average aerosols on large scales. However, its AOD spatial resolution is low (Deng et al., 2003). Therefore, in addition to MODIS AOD products, we used 30-m spatial resolution with TM/ETM + images to obtain the AOD. Inversion method was based on Song and Guan (2008) and the TM/ETM + images were acquired during the summer from 2007 to 2012.

2.4. Protein extraction and SDS-PAGE

Leaf total proteins were extracted and quantified according to Gao et al. (2011) with minor modifications. Approximately 500 mg of fresh leaves from each sample were ground into a fine power in liquid nitrogen, and the ground tissue was suspended in 5 mL ice-cold 10% w/v trichloroacetic acid (TCA)/acetone solution containing 0.07% w/v dithiothreitol (DTT), 1 mM phenylmethanesulfonyl fluoride (PMSF), and incubated at −20 °C for protein precipitation. After centrifugation at 14,000 × *g* for 30 min at 4 °C, the supernatant was carefully discarded. The pellet was rinsed three times with ice-cold acetone containing 0.07% w/v DTT for 15 min at −20 °C, and then vacuum-dried and stored at −80 °C. Dried protein pellets of 10 mg were dissolved in 300 μL lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, and 65 mM DTT) over 3 h at room temperature. The proteins were harvested by centrifugation at 14,000 × *g* for 15 min at 4 °C to remove insoluble materials. The protein concentration was determined using a 2-D Quant Kit (Amersham Bioscience) using BSA (2 mg/mL) as a standard. The final concentration of the protein sample was 2 μg/μL. A 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed with the leaf soluble proteins using a Multiphor II apparatus (Amersham Pharmacia Biotech) following Laemmli's method (1970) with a 50-μg protein load for each sample. SDS-PAGE was carried out at a constant current of 10 mA per gel for 3 h, followed by 14 mA per gel until the tracking dye (bromophenol blue) eluted from the bottom of the gel. After electrophoresis, the gels were stained with colloidal Coomassie Brilliant blue G-250 (Sigma, St. Louis, MO, USA).

2.5. Protein identification and quantification by nano-LC–MS/MS

In order to study the impact of aerosols and atmospheric particles on plant proteins, specific protein bands of interest, which were detected in most samples but at different concentrations, were selected for further tandem mass spectrometry identification and quantification. Selected protein bands were excised from the SDS-PAGE gels and destained with 100 μL of 50% acetonitrile (ACN) in 50 mM ammonium hydrogen carbonate for approximately 1 h at room temperature. This step was repeated until the gel was colorless. After evaporation of the solvent by vacuum centrifugation, each of the gel plugs was rehydrated with 20 μL of 0.01 mg/mL sequencing-grade modified trypsin (Promega, Madison, WI, USA), and the mixture was agitated at 37 °C overnight (16 h). The supernatants were collected, and the gel pieces were rinsed once with 5% trifluoroacetic acid (TFA) in 50% ACN and then twice with 2.5% TFA in 50% ACN. The supernatants were combined and lyophilized. The lyophilized peptides were dissolved in 5 mg/mL CHCA (a-cynao-4-hydroxycinnamic-acid, Sigma, Germany) in 50% ACN and 0.1% TFA. All of the MS/MS experiments for peptide identification were performed using a nano-LC–MS/MS system, consisting of an ultimate HPLC system and a Q-TOF mass spectrometer (Waters, Milford, MA) equipped with a nano-ESI source. The peptides were subsequently eluted onto an analytical Atlantis C18 column (Waters Corporation) and separated at 1 μL/min with an increasing ACN gradient from 4% to 95% over 50 min. Mobile phase A consisted of 0.1% formic acid in water, and mobile phase B consisted of 0.1% formic acid in ACN. The mass spectrometer was operated in a positive ion mode with a source temperature of 80 °C and a cone gas flow of 10 L/h. The MS/MS data were processed using MassLynx version 4.0 software (Waters Corporation) to produce a PKL file and was analyzed using the NCBInr green plant protein sequence database using the Mascot search engine. The following search parameters were used in all of the Mascot searches: tolerance of one missed cleavage; and carbamidomethylation (Cys) and oxidation (Met) as the fixed and variable modifications, respectively. A maximum error tolerance of 100 ppm and a 0.3-Da fragment tolerance were allowed. Finally, the protein concentrations in every lane on the 1-D electrophoresis gels were calculated using Quantity One software.

2.6. Measurement of heavy metals in the soil

Soil sampling locations are shown in Fig. 1. The sampling area in Beijing City has cinnamon soil and similar soil nutrient levels of N, P, K, etc., as well as the same annual rainfall (340–380 mm) and

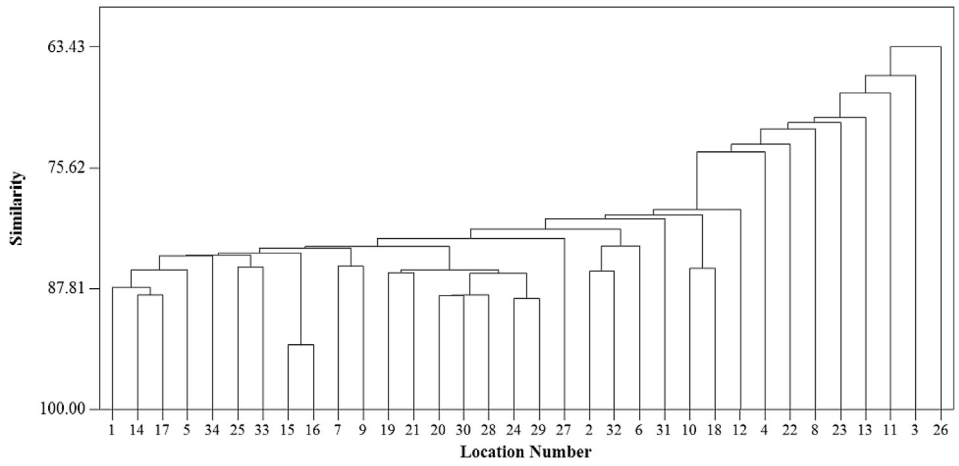

Fig. 2. Cluster analysis of heavy metals.

temperature (12–12.6 °C) as indicated in the government data. Chen et al. (2012) indicated that the most important factor that affects Beijing City soil is their heavy metal content. Zhao et al. (2001) also reported that the nutrients of Beijing soil near roads are significantly influenced by heavy metal content. According, on the basis of these studies, we used the heavy metal content to classify the soil. Some of the soil samples corresponded to two adjacent plant samples while most corresponded to specific plant samples. During the experiment, the samples (0.1 g) were held in Teflon inner tubes with 2 mL HF and 1 mL HNO_3, and then the inner tubes surrounded by steel cans were placed in an oven at 190 °C and insulated for 30 min. After the samples were cooled down, the tubes were placed on a hot plate for acid cleanup and the heating steps were repeated by adding 0.5 mL HNO_3. Finally, 5 mL HNO_3 was added and the tubes were heated for 3 h and 50 mL ultrapure water was added. The heavy metal concentration of the digested samples was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) based on the method employed by Eberhardt and Pan (2013).

3. Results and discussion

3.1. Exclusion of soil influence

Table 2 presents the descriptive statistical analysis of soil heavy metals. The coefficient of variation (CV) of soil heavy metals V, Ti, Mn, Al, and Fe was 0.13, 0.15, 0.17, 0.06, and 0.13, respectively, indicating that their contents are relatively stable and homogeneous in the study region. In contrast, the CV of Cd, Cu, Zn, and Pb was 0.42, 0.61, 0.73, and 0.53, respectively, suggesting that their content differ in their distribution in the study region and are influenced by external factors. In order to select samples with similar soil properties, cluster analysis was used for classification of the samples used in this study, and the results are shown in Fig. 2.

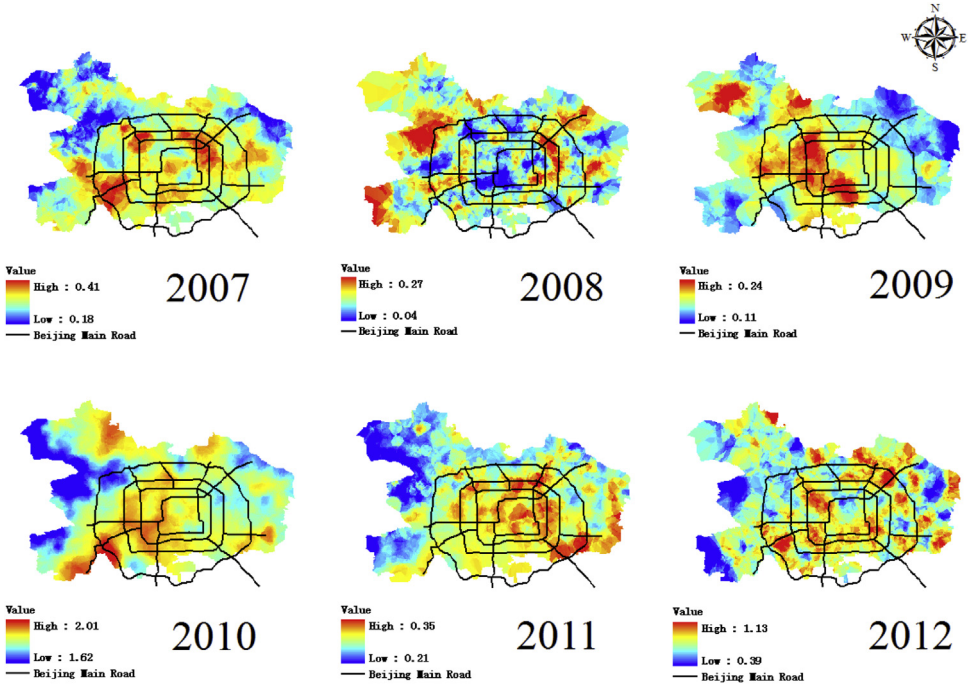

Fig. 3. AOD inversion image on a certain day in 2007–2012.

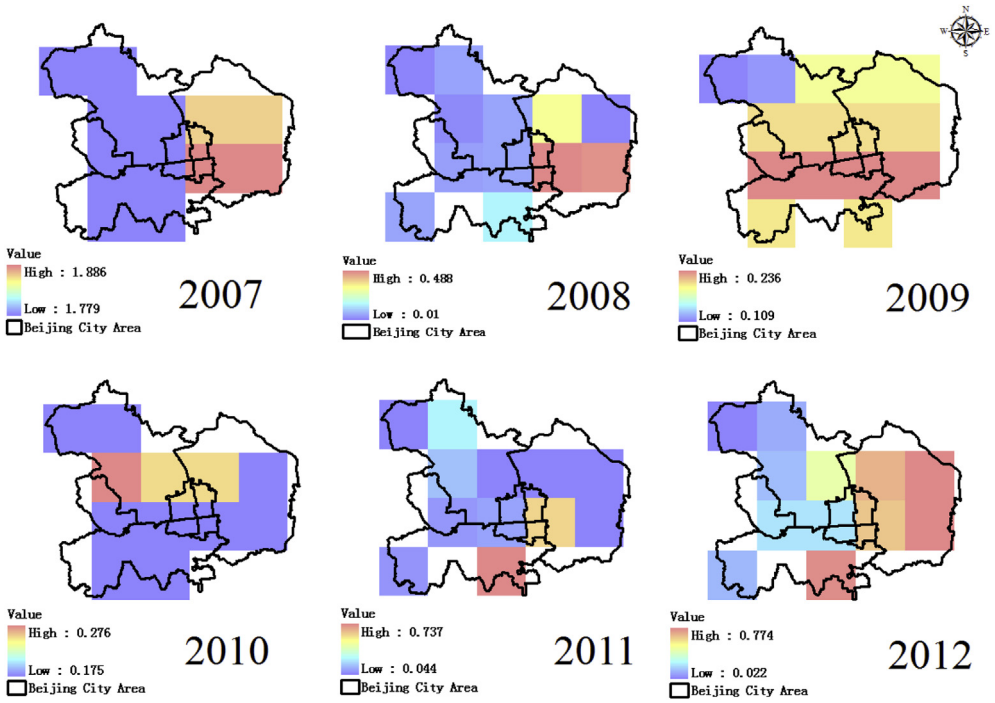

Fig. 4. MODIS AOD on a certain day in 2007–2012.

We excluded samples with lower than 75% similarity (Location No. 4, 22, 8, 23, 11, 3, and 26, these location numbers corresponded to 12 plant samples). Four plant samples were collected from Kan Dan Qiao, Fu Feng Qiao, Zhong Guan Cun, and Cao Qiao with low reliability because they are close to an engineering construction for the 2008 Olympics. Therefore, these samples were excluded and finally 28 plant samples with similar soil conditions were used for further analysis.

3.2. Classification of two regions by AOD and PM_1

We obtained approximately 680 MOIDS AOD products and 36 inversion AOD images from 2007 to 2012. Figs. 3 and 4 show the AOD spatial distribution in a certain day from inversion results and MODIS products. Table 3 presents the descriptive statistical information for AOD in each year. We found that AOD had a minimal mean value of 0.29 in 2008, which may because of the 2008 Olympic Games in Beijing, and that the government strengthened comprehensive administration of the environment during this year. The coefficient of variation in 2010 had a greater value of 100.22 relative to other years, which indicates that the AOD in this year was not stable and had much more variability relative to its mean value.

Figs. 5 and 6 show the mean values of AOD and PM_1 from 2007 to 2012, respectively, and the first and third quartiles are also presented. According to the results, PM_1 in the slight and severe zones ranged from 13,00,000 to 23,00,000 count/m^3 and from 35,00,000 to 43,00,000 count/m^3, respectively, whereas the AOD of the first and third quartile was 0.475 and 0.551, respectively. AOD is notably relatively lower in Xiang Shan Park, Ba Da Chu, and Jiu Gong Zhen, but comparatively higher in Song Jia Zhuang, Ma Lian Dao, and Beijing South Railway Station. Ba Da Chu displayed the lowest value, whereas Song Jia Zhuang had the highest value. PM_1's first and third quartiles were 21,72,591 and 35,08,284 count/m^3, respectively, which were relatively smaller in Shi Jin Shan, Tian Tan Park, and Shang Qing Qiao and the lowest value was 13,42,752 count/m^3 in Tian Tan Park. Relatively larger values were detected in Beijing South Railway Station, Chao Yang Park, and the Capital University of Economy, of which the largest value was detected in Chao Yang Park. Weak diffuse solar areas were determined if the mean values of AOD and PM_1 were near to or below the first quartile. Similarly, strong diffuse solar areas were determined if the mean values of AOD and PM_1 were near to or exceeded the third quartile. On the basis of these criteria, five locations (Xiang Shan Park, Shi Jin Shan, Ba Da Chu, Tian Tan Park, and Yuan Ming Yuan Villa) were classified as weak diffuse solar areas, whereas four locations (Song Jia Zhuang, Beijing South Railway Station, Dong Zhi Men Bridge, and the Capital University of Economy) were classified as strong diffuse solar areas.

Table 3
Descriptive statistical information of AOD from 2007 to 2012.

	N	Mean	StDev	Variance	CoefVar
2007	115	0.54	0.47	0.22	88.12
2008	103	0.29	0.18	0.10	64.16
2009	141	0.40	0.39	0.15	97.67
2010	120	0.42	0.42	0.18	100.22
2011	124	0.48	0.43	0.19	90.08
2012	128	0.58	0.44	0.19	76.43

StDev indicates standard deviation, CoefVar indicates coefficient of variation.

3.3. Comparison of leaf protein changes between two specific regions

The leaf soluble proteins were extracted and separated by SDS-PAGE (Fig. 7). On the basis of the changes in their expression, 5 representative protein bands, denoted as A, B, C, D, and E, were further identified by nano LC-MS/MS, and the results are shown in Table 4. Five proteins were identified: A: ATP synthase subunit beta (ATP-SSB), B: Ribulose bisphosphate carboxylase (Rubisco), C: Oxygen-evolving enhancer protein (OEEP), D: Light-harvesting complex II protein (LHC II), and E: Chlorophyll a-b binding

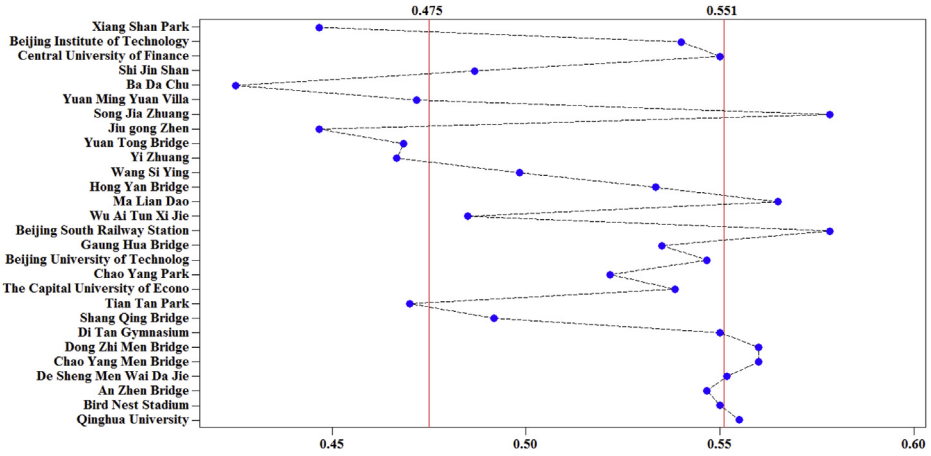

Fig. 5. AOD mean value in 2007–2012. The first quartile and the third quartile were 0.475 and 0.551, respectively.

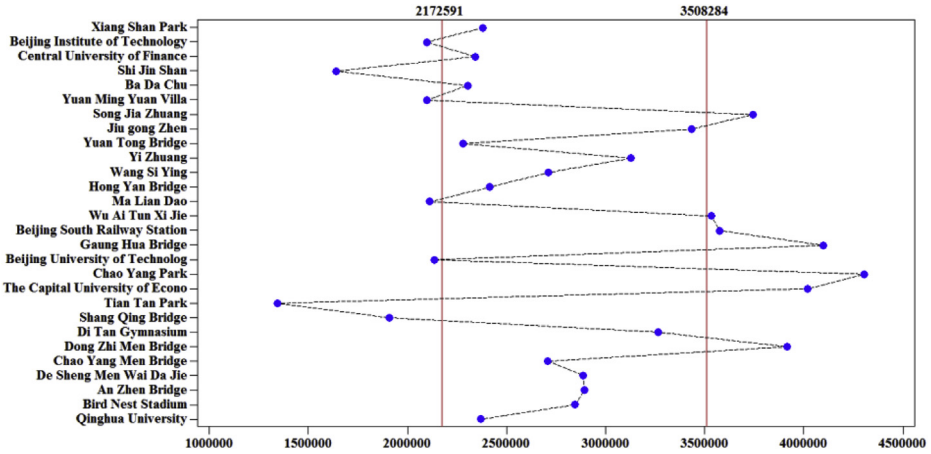

Fig. 6. PM_1 mean value in 2007–2012, 21, 72,591 was the first quartile, and 35, 08,284 was the third quartile (unit: count/m^3).

protein (Chl-ab). Their expression levels were determined using Quantity One Software.

Fig. 8 shows the mean expression levels of 5 proteins from 2007 to 2012 in six simple sites (Xiang Shan Park, Shi Jin Shan, Beijing Nan Zhan, Song Jia Zhuang, Shang Qing Bridge and Yuan Ming Yuan Villa). It presents five protein contents varied slightly over six years, OEEP, LHC II, ATP-SSB, Rabisco and CHI-ab were 0.30, 0.27, 0.15, 0.15 and 0.13, respectively. Particularly, five proteins levels in 2012 were

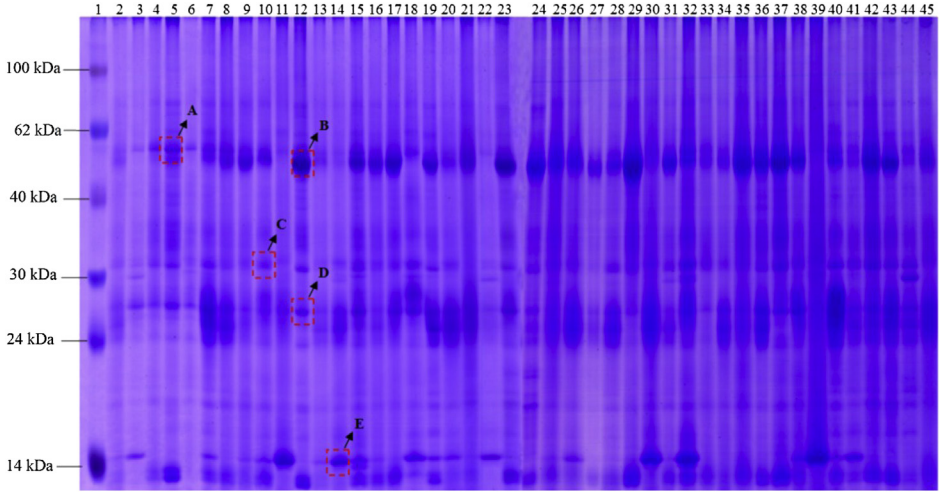

Fig. 7. SDS-PAGE analysis results. 1: Protein marker (14–100 kDa); A: ATP synthase subunit beta (ATP-SSB); B: Ribulose bisphosphate carboxylase (Rubisco); C: Oxygen evolving enhancer protein (OEEP); D: Light-harvesting complex II protein (LHC II); E: Chlorophyll a-b binding protein (Chl-ab).

Table 4
Identification of 5 leaf proteins in *Euonymus japonica* by nano-LC–MS/MS.

Bands on SDS-PAGE[a]	Pep count sequences	Identified proteins	Unique pep count[b]	Accession no.	Sequence coverage (%)	MW[c] (Da)	p*I*[d]
A	87	ATP-SSB	18	81175147	51.13	52357	5.34
B	160	Rubisco	30	132051	54.62	51587	6.00
C	16	OEEP	8	355513195	30.42	35022	6.48
D	41	LHC II	9	27542565	46.97	28053	5.29
E	6	Chl-ab	3	355519418	100.00	3801	8.24

[a] Protein bands as indicated in Fig. 7.
[b] Multiple matches to peptides with the same primary sequence count.
[c] Molecular mass of the predicted protein.
[d] Isoelectric point (p*I*) of the predicted protein.

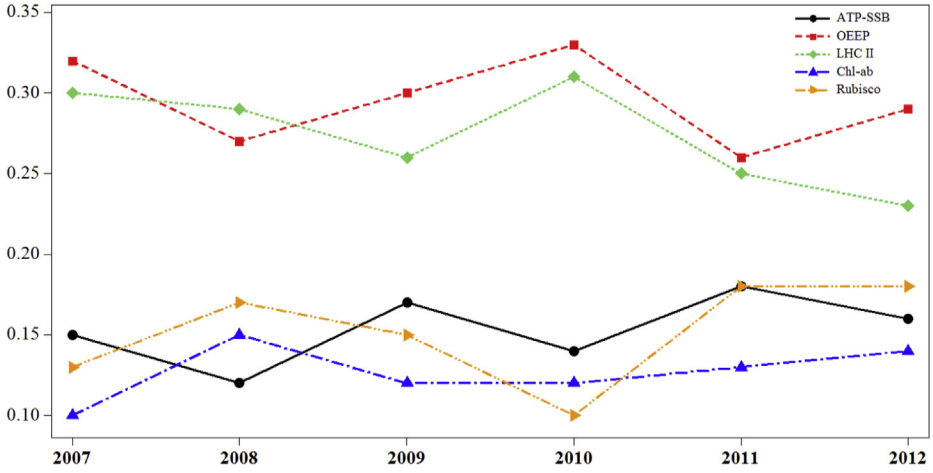

Fig. 8. Changes of five protein expression levels from six simple sites.

0.29, 0.23, 0.16, 0.18 and 0.14 which can enough represent the six years' protein expression levels. Thus, we focus on the protein expression differences in 2012 of 44 samples from two regions of distinctive solar diffuse area as classified by AOD and PM_1.

A boxplot of the expression amounts of the 5 proteins in the two specific regions is shown in Fig. 9. The OEEP (0.26) and LHC II (0.27) in the weak diffuse solar areas had a higher expression level than those in the strong diffuse solar areas, which was 0.11 and 0.10, respectively. Light protection is one of the most important functions of LHC II, such as dealing with excessive light energy. In the weak diffuse solar areas, the direct solar radiation to plants usually exceeds the plant photosynthesis capacity (Matsui et al., 2008). Thus, under this condition, a higher expression of LHC II could be induced to facilitate different biochemical reactions to consume excess energy. The main function of OEEP is to split water and release oxygen (Weng and Xu, 2003). Direct radiation increases plant temperature more than diffuse radiation, and at the same time the plant will consume more water as temperature is

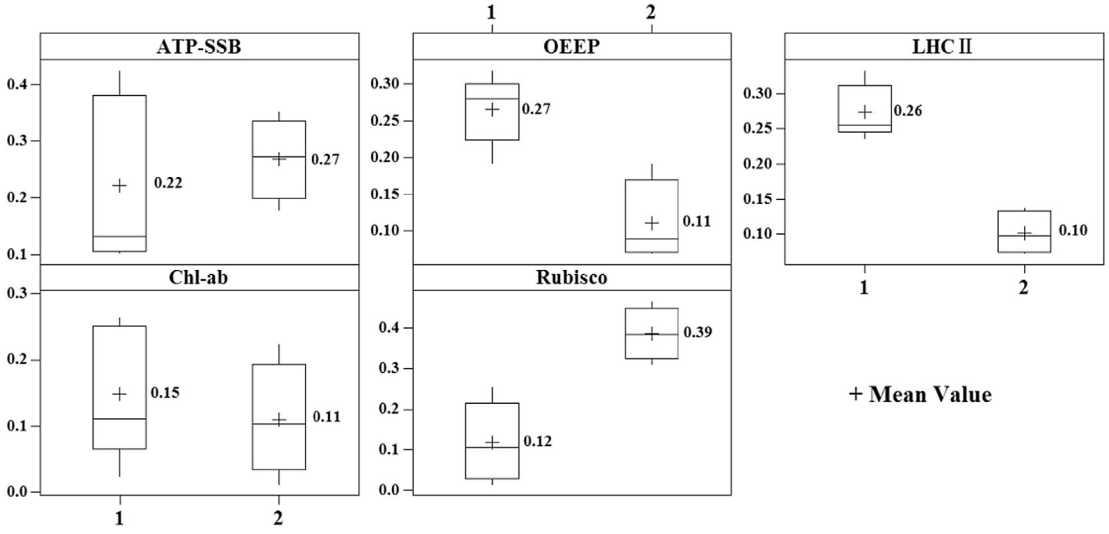

Fig. 9. Boxplot of the expression of five proteins (1 denotes weak diffuse solar area, 2 denotes strong diffuse solar area).

increased (Ananyev et al., 2001). For this reason, OEEP' expression in weak diffuse solar areas is higher than that in strong diffuse solar areas.

However, expression of Rubisco in the weak and strong diffuse solar areas was 0.12 and 0.39, respectively. Apparently, the former is much lower than the latter. Rubisco is a key enzyme in the plant photorespiration process, which catalyzes two reactions: the carboxylation of D-ribulose 1,5-bisphosphate, the primary event in carbon dioxide fixation, and also the oxidative fragmentation of the pentose substrate. Thus, its expression level can determine the net efficiency of plant photosynthesis (Lan et al., 1992). Rubisco expression in the high AOD and PM_1 areas was much higher than low in the AOD and PM_1 areas. This is due to the presence of high aerosol and atmospheric particle contents that can increase diffuse radiation and enhance plant photosynthesis. Diffuse light can enter a canopy from all directions and reach leaves more evenly than a direct solar beam; therefore, it may be more effective for plant photosynthesis (Sinclair et al., 1992; Cohan et al., 2002). Hollinger (1998) and Gu et al. (2002) also found that diffuse solar radiation would increase plant net primary productivity; simultaneously, this progress is accompanied by changes in the expression of Rubisco. Because of the increased photosynthesis, Rubisco expression would increase proportionately (Feller et al., 1998). The expression of ATP-SSB and Chl-ab proteins were similar in both specific regions (Fig. 9).

4. Conclusions

This study showed that aerosols and atmospheric particles can directly impact protein expression in plants and photosynthesis efficiency. This influence is particularly obvious for leaf Rubisco, OEEP, and LHC II proteins that are highly important for plant photosynthesis. Compared to weak diffuse radiation areas, the expression of Rubisco was higher, whereas OEEP and LHC II were lower, in the strong diffuse radiation areas. On the basis of these results, we consider that aerosols and atmospheric particles are more advantageous for plant photosynthesis by facilitating diffuse solar radiations. These findings are consistent with previous studies (Niyogi, 2004), which were further clarified at the plant protein levels in the current study. However, the impact of aerosols and atmospheric particles on plant growth and development appear to be complicated, as they may be accompanied by the influence of other pollutants. Hence, further studies are needed to comprehensively understand the impact of these factors on various physiological and biochemical processes during plant growth and development.

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