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Leaf Senescence can be Induced by Inhibition of Root Respiration

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Abstract

Constituting the last stage of leaf development, leaf senescence is a complicated process that involves many senescence-associated genes, and numerous factors can induce leaf senescence. To elucidate the relationship between root respiration and leaf senescence, we treated the roots of *Alhagi sparsifolia* with nitrogen (N_2) with the purpose of inhibiting root respiration (denoted as the N2 group). The results showed that compared with the control group, N_2 treatment decreased the root respiration rate, chlorophyll (Chl) *a*, Chl *b* and carotenoid (Car) contents, the Chl/Car ratio, stomatal conductance (G_s), photosynthesis rate (P_n), maximum photochemical efficiency (φ_{Po}), and performance index on absorption basis (PI_{abs}). In contrast, it increased leaf proline (Pro), malonaldehyde (MDA), and abscisic acid (ABA) contents. Moreover, no significant decline of Chl *a*/*b* was found in the N2 group. The results of the present work implied that leaf senescence can be induced by root respiration inhibition. Root respiration inhibition may result in ABA accumulation in leaves and thus induce leaf senescence. Another mechanism may be that root respiration inhibition resulted in the decrease of root water uptake, which subsequently led to water stress-induced leaf senescence. Root respiration inhibition-induced leaf senescence is a highly regulated process that is similar to natural senescence. In this process, no significant decline of Chl *a*/*b* was found. Root respiration inhibition-induced senescence is a "mild" process, in which most of the function of the photosynthetic apparatus performed well. Carotenoids play a key photoprotective role in leaf senescence. Overall, root respiration inhibition accelerated leaf senescence. In both types of senescence (root respiration induced senescence and natural senescence), the photosynthetic apparatus maintains a good performance until the last stage of senescence.

Keywords *Alhagi sparsifolia* · Chlorophyll · Leaf senescence · Nitrogen · Root respiration

Introduction

Constituting the last stage of leaf development in plants (Fukao et al. 2012; Sakuraba et al. 2012; Tang et al. 2015), leaf senescence involves the degeneration of cells and recycling of released nutrients to seeds, storage organs, or actively growing tissues (Besseau et al. 2012; Gregersen et al. 2013; Kotakis et al. 2014; Zhang et al. 2012) from vegetative plant parts that will eventually die off (Gregersen et al. 2013). Senescence in plants often appears as leaf yellowing, which results from chlorophyll loss (Mutui et al. 2012). Although such changes are not visibly obvious at the early stages of leaf senescence (Kusaba et al. 2013), they are soon easily recognizable due to the breakdown of photosynthetic pigments (Buchanan-Wollaston et al. 2003; Kusaba et al. 2013). However, the leaf senescence process is far more complicated than the degradation of chlorophyll and protein; declines in photosynthetic rate (Wingler et al. 2006, 1998; Tang et al. 2015b) and change of resources distributed

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to plant vegetative organs are also obvious characteristics of leaf senescence (Buchanan-Wollaston 1997; Gan and Amasino 1997; Miller et al. 2000; Tang et al. 2016a). Although leaf senescence is an age-dependent process, the initiation of senescence, and its acceleration and delay, can be triggered by various internal and external factors (Zhang et al. 2012). Previous studies showed that darkness (Buchanan-Wollaston et al. 2005; Gregersen et al. 2013), high light intensity (Fu et al. 2012), high light dosage (Gregersen et al. 2013; Noodén et al. 1997), heat (Gregersen et al. 2013; Lobell et al. 2012), water stress (Yang et al. 2003), nitrogen starvation (Koeslin-Findeklee et al. 2014), salicylic acid (SA) (Vogelmann et al. 2012), abscisic acid (ABA) (Gregersen et al. 2013; Setter et al. 1980), jasmonic acid (JA) (Jiang et al. 2014), carbon famine (Brouquisse et al. 2001), carbon feast (Moore et al. 2003; Parrott et al. 2005), and disease (Gregersen et al. 2013; Robert-Seilaniantz et al. 2011) accelerate senescence, whereas cytokinins delay leaf senescence (Zwack et al. 2013). Although leaf senescence is a degenerative process, it is important to whole plants because of the nutrient cycling process that occurs during senescence, especially for the reactivation of nutrient elements (Himelblau and Amasino 2001; Wingler et al. 2006). During early senescence, cellular membranes remain intact so that the plants can recycle nitrogen required by the photosynthetic apparatus (Hörtensteiner and Feller 2002; Wingler et al. 2006). In addition, in contrast to programmed cell death, the nucleus and mitochondria remain active for a long period of time during senescence (Roberts et al. 2012; Wingler et al. 2006).

Respiration is a controlled and multiple-stepped process, using organic compounds (carbohydrates, lipids, proteins, etc.) to produce energy which is used for various cellular and plant metabolic activities, such as cell division, plant growth, mineral element absorption, water transport, etc. (Huang et al. 2005). Plant respiration is related to temperature (Brestic et al. 1995; Huntingford et al. 2017; Lombardozzi et al. 2015; O'Leary et al. 2017; Slot and Kitajima 2015). For roots, respiratory activity should be related to the energy required to produce new roots, take up and transport nutrients, and repair tissues (Jarvi and Burton 2013) for root growth and maintenance, as well as for symbiotic processes and defense (Huang et al. 2005; Martinez et al. 2002). Previous studies revealed that photosynthetic product constituted the main source of C for root respiration (Högberg et al. 2001; Lynch et al. 2013; Trueman and Gonzalez-Meler 2005). In addition, removal of roots causes a decrease in protein content and enhances senescence in leaves (Sitton et al. 1967). This may be because cytokinins formed in the roots are transported to the shoot and leaves, which subsequently influences leaf senescence (Sitton et al. 1967). It was also found that Fe-deficiency in roots induced expression of many senescence-associated genes which may affect the leaf senescence process (Sperotto et al. 2008). Waterlogging displaces oxygen and other gases (Stieger and Feller 1994a), which causes inhibition of root respiration. Moreover, it was reported that waterlogging accelerates senescence in the oldest leaves of young wheat plants (Stieger and Feller 1994; Trought and Drew 1980). Therefore, root respiration may have a strong relationship with leaf senescence.

Despite the great importance of leaf senescence and root respiration to the whole plant, little attention has been paid to the relationship between leaf senescence and root respiration. However, root respiration will influence many physiological processes, such as synthesis and transport of cytokinins and ABA, which may affect the leaf senescence process. Thus, we conducted a study using N_2 to inhibit the root respiration of *Alhagi sparsifolia* and then analyzed the leaf senescence process. The objective of this paper was to elucidate whether there is a relationship between leaf senescence and root respiration and, if so, what is the possible mechanism through which root respiration affects leaf senescence.

Materials and Methods

Plant Material

Alhagi sparsifolia Shap. was used for this study. This plant is a spiny, clonal, perennial leguminous herb growing in arid and semi-arid regions with few leaves, and is approximately 1 m tall (Tang et al. 2015b). We chose *A. sparsifolia* as our plant material because it is a deep-rooted plant. *A. sparsifolia* also has a large root/shoot ratio, and the roots account for a large part of the whole plant. In general, the root mass in a 3-year-old *A. sparsifolia* accounts for approximately 90% of the whole plant mass (Liu et al. 2013). So, the inhibition of root respiration in *A. sparsifolia* may affect leaf status more obviously.

Study Site

The study was performed at the Fu-Kang Station of Desert Ecology, Chinese Academy of Sciences, in the hinterland of the Eurasian continent (44°17′ N, 87°56′E and 475 m a.s.l.). The station is 8 km from the southern edge of the Gurbantonggut Desert and 72 km south of the highest peak of the eastern Tianshan Mountains (5445 m a.s.l.). The study site exhibits a typically continental arid temperate climate with dry, hot summers and cold winters. The annual mean temperature is 6.6 °C, annual mean precipitation is 163 mm, and approximately 60% distributes during the growing season (from May to September); pan evaporation (*E*) is nearly 2000 mm.

Experimental Design

In our study, we employed an original method to inhibit the root respiration of *A. sparsifolia*, and then observed the change of plant status in the later time point. Especially, we aimed to elucidate the senescence process after root respiration inhibition. One-year-old potted *A. sparsifolia* plants were cultured in a greenhouse from May 1, 2014 to July 31, 2014, and 18 plants of comparable size were chosen for our treatment on August 1, 2014. Plants were divided into two groups, nine for the control group and nine for the N2 group. Transparent plastic film was used to cover the pots. In the N2 group, the soil of the potted *A. sparsifolia* was infiltrated with N_2 gas, and the width of the vent hole for N_2 was 1 cm. We used a nitrogen pump to fill in N_2 for 30 min, and the flux of N_2 was 10 mL s^{-1}. We then used silica gel to paste plastic film around the stem of *A. sparsifolia* to prevent N_2 from escaping from the soil. In the control group, we utilized only a transparent plastic film to cover the pot, so that the *A. sparsifolia* were exposed to normal air in the pot. The soil that we chose was sandy soil, and the porosity of the soil was approximately 42%. An outline of the treatment is presented in Fig. 1. Four, eight, and twelve days after treatment, photosynthetic pigment content, photosynthesis rate (P_n), chlorophyll (Chl) fluorescence parameters, and malonaldehyde (MDA) content were measured to evaluate the extent of leaf senescence. In addition, root respiration rate, stomatal conductance (G_s), proline (Pro) content, abscisic acid (ABA) content, soil water content, and water potential (Ψ) were measured at the same time points to track the mechanism of root respiration induced leaf senescence . As the measurement of root respiration is a destructive process and all assays were performed three times (on the 4th, 8th, and 12th day, respectively), for every time point we selected three *A. sparsifolia* from each group (control and N2) for the measurements. Utilized plants were removed from the experiment (i.e., excluded from the assays performed at the later dates).

Soil Condition and Plant Water Potential

Before we started our experiment, soils were fully watered. In addition, the physical and chemical characteristics of the soil were evaluated, and no obvious differences of soil status were found between the control group and N2 treatment (Table 1).

On the 4th, 8th, and 12th days, soil water content was measured using a traditional method. Specifically, soils were collected at 10 cm depth and placed in an aluminum box. Then, fresh mass ($W1$) was weighed using a balance with a precision of 1,000. After drying in an oven for 72 h at 120 °C to determine the constant dry mass ($W2$), soils were poured out of the aluminum box, and then the aluminum box was weighted ($W3$). Three replicate measurements (biological replicate) were performed for each treatment.

$$\text{Soil water content } (\%) = [(W1 - W2)/(W1 - W3)] \times 100$$

Ψ_{leaf} reading$_s$ were conducted according to our previous studies (Tang et al. 2017). Predawn water potential (Ψ_P)

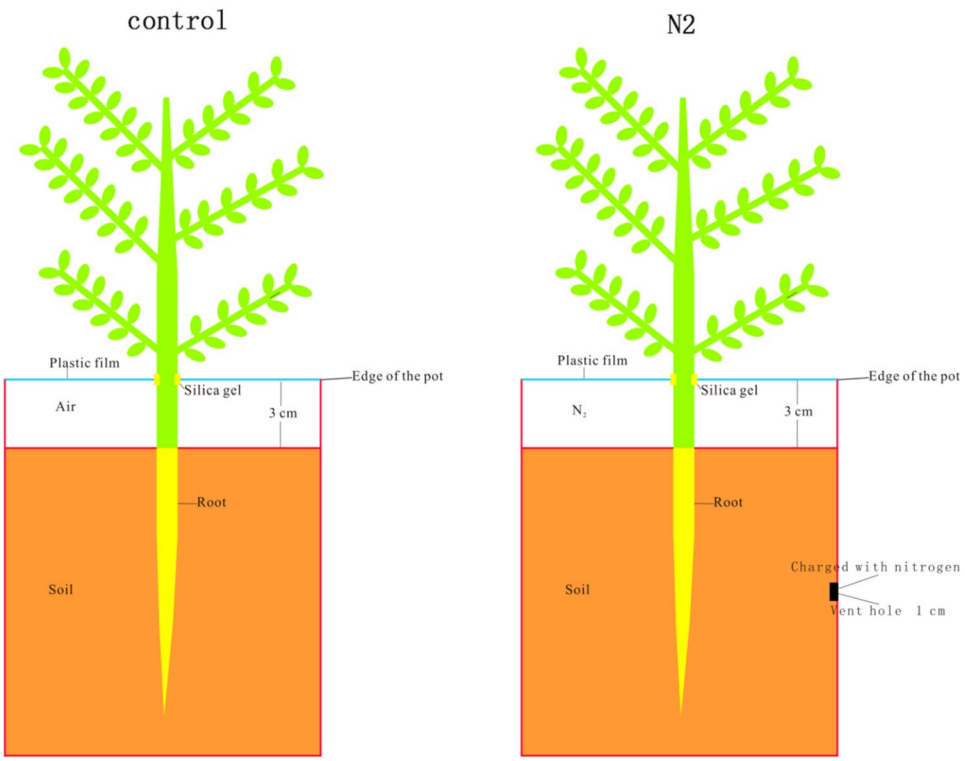

Fig. 1 Sketch of treatment of N_2 on leaf senescence of *Alhagi sparsifolia*. Control: pot filled with air; N2: treated with N_2 (pot filled with N_2). The orange part in pot was soil, the yellow part in the middle of soil was root, stems and leaves show green color in the sketch. The blue line means plastic film. At the border of plastic film and stem there was a silica gel (show yellow in the sketch) to prevent N_2 escaping from the pot. The black part in the middle of the right border indicates the vent hole for N_2, its width was 1 cm. The distance from the soil to plastic film was 3 cm. In the control group, the pot was filled with air and in the N2 group, the pot was filled with N_2. The volume of the pot was 18 l, and the flux of N_2 was 10 ml s^{-1}. Sandy soil was used to facilitate N_2 access to the root system. (Color figure online)

Table 1 Physical and chemical characteristics of the soil under control and N_2 conditions

Factors	Control	N_2
Bulk density (g cm^{-3})	1.29 ± 0.03	1.31 ± 0.08
Moisture content (%)	25.32 ± 1.28	25.76 ± 1.71
pH	7.53 ± 0.31	7.32 ± 0.27
Total nitrogen content (g kg^{-1})	0.87 ± 0.03	0.82 ± 0.06
Total phosphorus content (g kg^{-1})	0.51 ± 0.02	0.56 ± 0.07

Control, pot filled with air; N2, treated with N_2. The data represent means of nine biological replicates ± SE

measurements began at approximately 4:30 am (GMT + 6) and were completed before sunrise using a pressure chamber (PMS Instruments Co., Corvallis, OR, U.S.A.). Midday water potential (Ψ_M) measurements were performed between 11:30 am and 12:30 pm (GMT + 6). Leaf blades used for the determination of Ψ_{leaf} were covered with a plastic bag and quickly sealed. Petioles were then cut within 1–2 s, and the time between leaf excision and chamber pressurization was generally less than 10–15 s. Leaves chosen for Ψ_M determination were exposed to direct solar radiation.

Measurement of Photosynthetic Pigment Content, ABA

Leaves of similar size were collected from the second to fifth leaves (counting from the tip of each branch) on each of the branches. We collected ten leaves from each *A. sparsifolia*, so each treatment (control, N2) had 30 leaves (3 × 10). Measurement of photosynthetic pigment content was performed as described by Lichtenthaler (1987). Specifically, for every ten leaves (a total of three groups and thirty leaves), Chl was extracted with a mortar and pestle in 10 mL 80% chilled acetone plus 1 g $MgCO_3$ and purified with sea sand. After centrifugation at 10,000×*g* for 2 min, the resultant solution of Chl *a*, Chl *b*, and carotenoids (Car) was analyzed spectrophotometrically at 663 nm (Chl *a*), 647 nm (Chl *b*), and 470 nm (Car) (Jenway 6400, Krackeler Scientific, London, UK). The concentration was calculated as previously described by Lichtenthaler (1987). Three replicates were performed at each treatment.

ABA analysis was performed using the method described by Veselov et al. (2008). All samples (the leaves obtained as described above) were harvested in the morning at approximately 10:00 am, weighed, and then frozen in liquid nitrogen. Frozen samples were ground to fine powder in liquid nitrogen. ABA was extracted in 80% ethanol and incubated overnight at 4 °C. Distilled water was added to dilute the aqueous residue, acidified with HCl (1 mol/l) to pH 2.5, and then partitioned twice with peroxide-free diethyl ether (ratio of organic to aqueous phases was 1:3). The organic phase was transferred into 1% sodium hydrocarbonate (pH 7–8, ratio of organic to aqueous phases was 3:1). The solution was re-extracted with diethyl ether, methylated with diazomethane, and immunoassayed using antibodies to ABA (Vysotskaya et al. 2004). For additional details regarding the method and calculations, please see Veselov et al. (2008).

Measurement of G_s and P_n

P_n and G_s were measured using the Portable Photosynthesis System (LI-COR 6400 Inc., Lincoln, NE, U.S.A.), according to previous work (Mittler et al. 2001; Tang et al. 2015). The parameters were measured on the 4th, 8th, and 12th days after treatment. All days were cloudless when G_s and P_n were measured. We conducted measurements at 10:00 am (GMT + 6), when the P_n of plants was the greatest during the day.

As the measured leaves did not reach the standard size of the leaf chamber (2 cm × 3 cm), a scanner was employed to scan the measured leaves. In addition, Image Pro Plus 6.0 software (Media Cybernetics, Silver Springs, MD, U.S.A.) read the surface area of the measured leaves, and was later utilized to calculate the actual values of P_n and G_s (the value read from the apparatus divided by the area of leaf that we measured and then multiplied by 6 cm^2 are the actual values of P_n and G_s when the leaf fills the leaf chamber). Measurements were done in five replicates. We selected the third leaf (counted from top to base) on the branch as our measured leaf.

Measurement of Pro, MDA Content, and Root Respiration

The measurement of Pro content was conducted based on the method described by Demiral and Türkan (2005). A sample (0.5 g) of fresh leaves was ground and mixed with 5 ml 3% (w/v) sulfosalicylic acid. The sample was then filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, the resulting mixture was heated at 100 ◦C for 1 h in a water bath. The mixture was then placed in an ice bath for approximately 30 min. The mixture was extracted with 25 ml toluene, which was taken as a control. The extract was then placed in a cuvette, and its absorbance was measured at 520 nm. Pro content was calculated using a calibration curve and expressed as molproline g^{-1} FW (fresh weight).

The measurement of MDA content was performed based on the method described by Rahman et al. (2012). Thiobarbituric acid (TBA) reactive substances, representing lipid peroxidation products, were extracted through the homogenization of a 0.2 g leaf sample in 5 ml of solution containing 20% trichloroacetic acid and 0.5% 2-TBA. The mixture was heated at 95 °C for 30 min, and the reaction was

arrested by a sudden ice bath. The cooled mixture was centrifuged at 5000×*g* for 10 min at 25 °C, and the absorbance of the supernatant at 532 and 600 nm was recorded. After subtracting the nonspecific turbidity at 600 nm, the MDA content was determined by its molar extinction coefficient, 155 mol^{-1} cm^{-1} (Rahman et al. 2012).

As the measurement of root respiration is a destructive process and as it was conducted three times (on the 4th, 8th, and 12th days, respectively), for every time point we selected three *A. sparsifolia* plants from each group (control and N2) for the measurement. These plants were then removed from the experiment (that is, not used for the later assay dates). Fresh fine roots (diameter < 2 mm) were taken from each plant and mixed well. The sample was used to measure the root respiration rate utilizing the Liquid-Phase Oxygen Measurement System (Hansatech Ltd., King's Lynn, Northfolk, UK) at a temperature of 25 °C. Measurements were done in three replicates.

Measurement of Chl Fluorescence

The measurement of Chl fluorescence was carried out according to a previous study (Lei et al. 2014). Chl *a* fluorescence was induced by a saturating photon flux density at 3.5×10^6 μmol photons m^{-2} s^{-1} provided by an array of three light-emitting diodes (peak 650 nm) to generate fluorescence curves expanding from F_o (minimal recorded fluorescence intensity) to F_m (maximal recorded fluorescence intensity) for the two treatments (control and N2). Data were initially sampled at 10 μm intervals for the first 300 μs to achieve an excellent time resolution of dark-adapted F_o, as well as the initial rise kinetics. The time resolution of digitization was then switched to slower acquisition rates.

Data Analysis

The significance of the differences of data between control and N_2-treated plants at 4, 8, and 12 d was analyzed by the t test using PASW Statistics 18.0 software (Macintosh, SPSS Inc., Chicago, IL, U.S.A.) for each parameter. A value of $p < 0.05$ was used to identify statistically significant differences. The results shown in the graphs are presented as the mean value ± standard error.

Results

Changes of Root Respiration After N_2 Treatment

In our study, we used N_2 to inhibit the root respiration of *A. sparsifolia*. As expected, N_2 treatment appeared to inhibit the root respiration of *A. sparsifolia*. Four days after treatment, root respiration in the N2 group decreased approximately 6.9% compared with control, and the decline of root respiration became more obvious as time passed (Fig. 2). On the 12th day after treatment, the inhibition of root respiration reached 45.1% (Fig. 2).

Effect of N_2 on Photosynthetic Pigment Contents in *A. sparsifolia* Leaves

As anticipated, N_2 treatment resulted in the degradation of photosynthetic pigments, as Chl *a*, Chl *b*, Chl (*a* + *b*), and carotenoid (Car) contents exhibited a significant decline in the N2 group compared with the control (Fig. 3a–d). It seems that the photosynthetic pigment (Chl *a*, Chl *b*, Chl, and Car) contents in both control and N2 plants showed a decline during our study; however, the N2 group showed a much more obvious decline compared with the control. On the 12th day, Chl *a*, Chl *b*, Chl (*a* + *b*), and Car contents in the N2 group were reduced by 33.7%, 30.9%, 33.0%, and 24.9%, respectively (Fig. 3a–d). Chl/Car in the control group showed a slight decline during our 12-day study; whereas, in the N2 group, the value of Chl/Car exhibited a significant decline. On the 12th day, Chl/Car in the N2 group decreased 10.7% compared with the control (Fig. 3f). Although it seems that both the control and N2 group showed a decline of Chl *a*/*b* during our 12-day study, the difference between the control and N2 was not significant (Fig. 3e).

Effect of N_2 on G_s and P_n in *A. sparsifolia* Leaf

As shown in Fig. 4a, G_s in the N2 group decreased substantially during the study, and the difference between the control and N2 group was significant at all three time points.

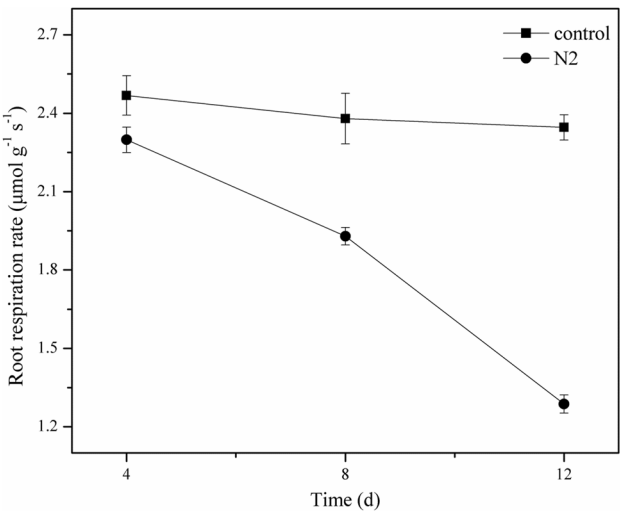

Fig. 2 Effect of N_2 on root respiration rate in *A. sparsifolia*. N2, treated with N_2. The data represent means of three biological replicates ± SE. *Significant difference at the 0.05 probability level

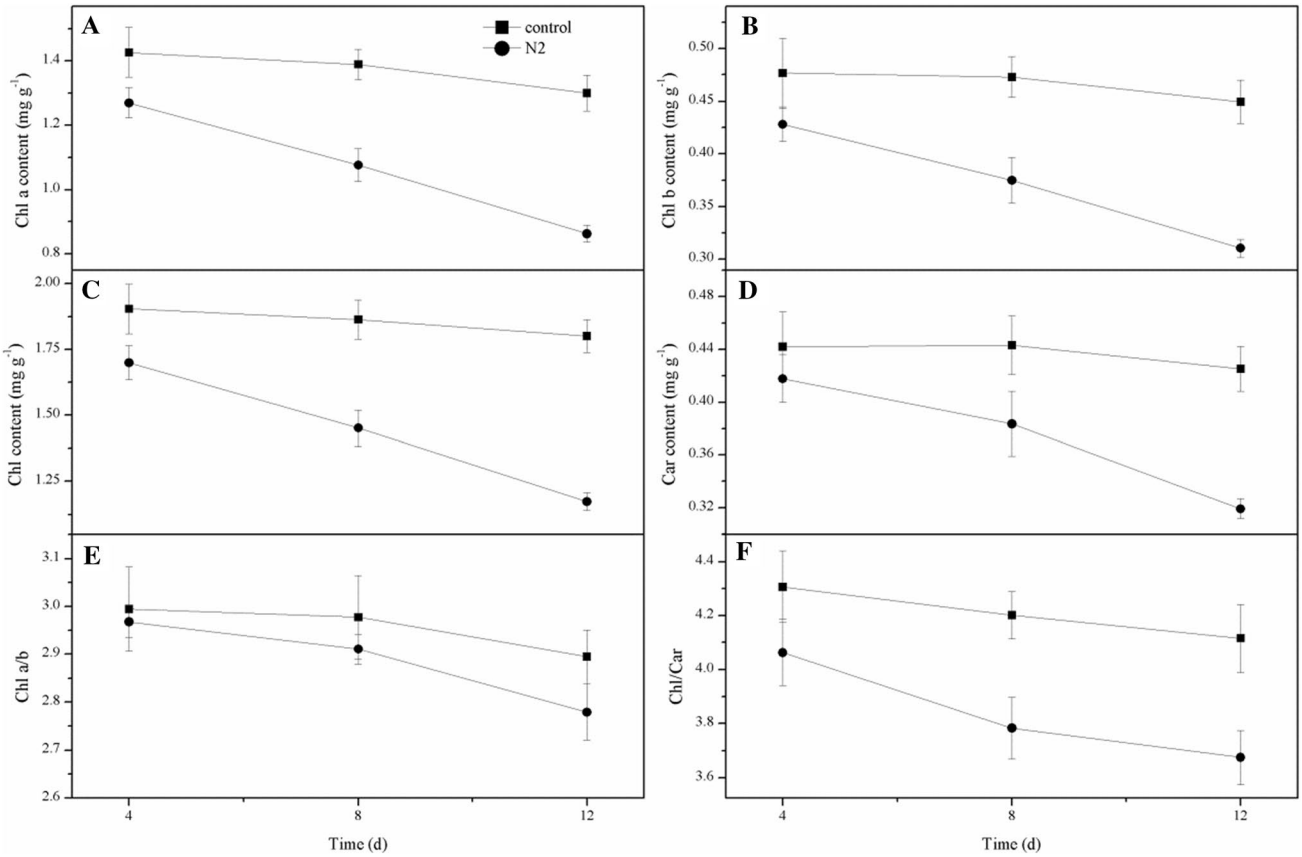

Fig. 3 Effect of root N_2 treatment on leaf Chl *a* content, Chl *b* content, Chl content, Car content, Chl *a*/*b*, and Chl/Car in *A. sparsifolia*. **a** Chl *a* content; **b** Chl *b* content; **c** Chl (*a* + *b*) content; **d** Car content; **e** Chl *a*/*b*; **f** Chl/Car. *Chl* chlorophyll, *Car* carotenoids, *N2* treated with N_2. The data represent means of three biological replicates ± SE. *Significant difference at the 0.05 probability level

On the 4th day, G_s in the N2 group was reduced by 13.7% as compared to control; on the 8th day, the decline of G_s in N2 was 23.7%; and on the 12th day, it reached 28.0% (Fig. 4a). P_n showed a similar trend during the 12-day study; however, the decline of P_n in the N2 group was more obvious and rapid than G_s. On the 4th, 8th, and 12th day, compared with the control, P_n under N_2 treatment was reduced by 15.1%, 18.6%, and 40.1%, respectively (Fig. 4b).

Change of Leaf Pro, MDA, and ABA Contents After Treatment of *A. sparsifolia* Roots with N_2

In contrast to the parameters described above, Pro, MDA, and ABA contents increased during the 12-day study, and even the control treatment showed a slight increase, while Pro, MDA, and ABA content in the N2 group increased quickly. Among these three indicators, ABA seems more sensitive to N_2 treatment, as ABA increased more in the N2 group compared with the control relative to Pro and MDA (Fig. 5a–c). On the 4th, 8th, and 12th day, compared with the control, Pro in the N2 group increased by 10.6%, 16.8%, and 28.2%, respectively (Fig. 5a); MDA in the N2 group increased by 8.0%, 19.7%, and 40.9%, respectively (Fig. 5b); and ABA in the N2 group increased by 14.3%, 31.0%, and 63.2%, respectively (Fig. 5c).

Chlorophyll Fluorescence Characteristics After Treatment of *A. sparsifolia* Roots with N_2

As expected, N_2 appeared to change the Chl fluorescence characteristics in photosystem II (PSII). The maximum quantum yield for primary photochemistry (φ_{Po}) declined with time in control and N_2-treated plants, and the N2 group exhibited a more significant decrease. For N_2 treatment, φ_{Po} decreased by 5.4% on the 4th day compared with control; whereas, on the 12th day, the decline of φ_{Po} was 21.0% (Fig. 6a). Relative to φ_{Po}, PI_{abs} changed more quickly during our 12-day study, although the trend was also declining and similar to φ_{Po}. On the 4th, 8th, and 12th day, compared with the control, PI_{abs} in the N2 group was reduced by 4.3%, 29.3%, and 54.9%, respectively (Fig. 6b). The electron transport rate per RC (ET_o/RC) and exciton dissipation rate per RC (DI_o/RC) were very sensitive to leaf senescence. For N_2 treatment, on the 4th day ET_o/RC decreased by 23.1%

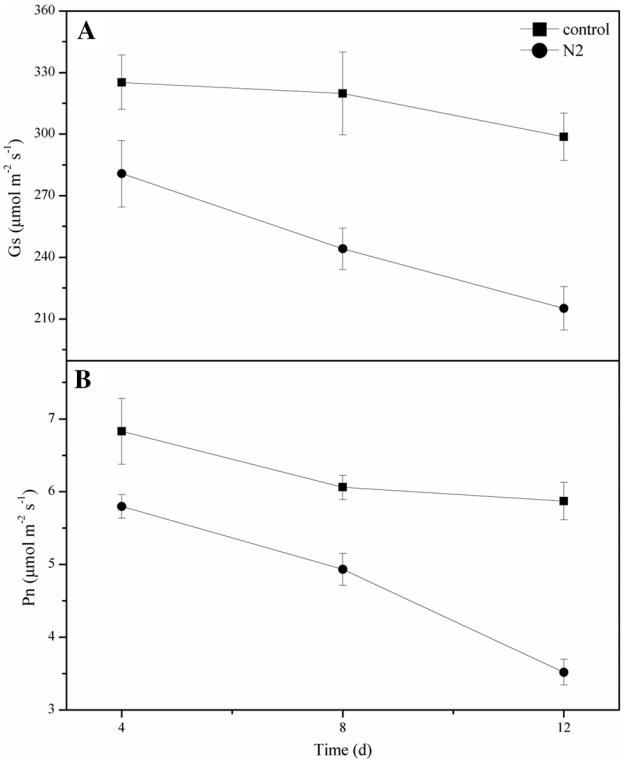

Fig. 4 Effect of root N_2 treatment on leaf G_s and P_n in *A. sparsifolia*. **a** Stomatal conductance; **b** net photosynthesis rate; N2, treated with N_2. The data represent means of five biological replicates ± SE. *Significant difference at the 0.05 probability level

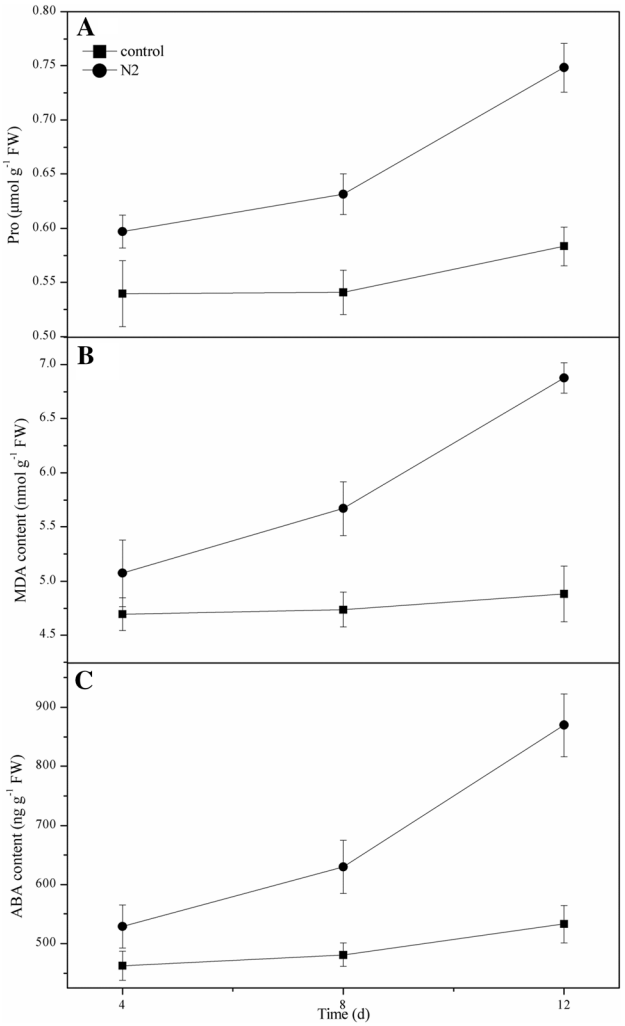

Fig. 5 Effect of root N_2 treatment on leaf Pro, MDA, and ABA contents in *A. sparsifolia*. **a** Pro content; **b** MDA content; **c** ABA content. *Pro* proline, *MDA* malonaldehyde, *ABA* abscisic acid, *FW* fresh weight, *N2* treated with N_2. The data represent means of three biological replicates ± SE. *Significant difference at the 0.05 probability level

(Fig. 7a) and DI_o/RC increased by 16.6% (Fig. 7b), respectively, compared with the control. On the 12th day, ET_o/RC decreased by 50.0% (Fig. 7a) and DI_o/RC increased by 102.3% (Fig. 7b), respectively, compared with the control.

Effect of N_2 on Water Potential in *A. sparsifolia* Leaves and Soil Water Content

As shown in Fig. 8b, c, $\Psi_{P\ and}$ Ψ_M did not exhibit any large difference during the study, whereas after N_2 treatment, both Ψ_P and Ψ_M decreased at all three time points. Especially on the 12th day, Ψ_P and Ψ_M in the N2 group was reduced by 30.0% and 20.6%, respectively, as compared to the control (Fig. 8b, c). Change of soil water content was much less after N_2 treatment until the 12th day, at which time the water content in the N2 group increased by 8.74% as compared to the control (Fig. 8a).

Discussion

Leaf senescence constitutes an age-dependent process that is highly regulated by genes. These genes have been identified to always be upregulated in senescing leaves, which are collectively termed senescence-associated genes (SAG_s), such as *SAG12* (Lim et al. 2003). However, leaf senescence can also be influenced by numerous biotic, abiotic, internal, and external factors (Gregersen et al. 2013; Koeslin-Findeklee et al. 2014; Zwack et al. 2013). Previous studies have preferentially considered the status of the leaf itself during leaf senescence (Tang et al. 2015), while few researchers have investigated the relationship between the root status and leaf senescence. Our aim was to determine whether leaf senescence can be induced by inhibition of root respiration and, if so, which mechanism(s) is/are responsible for leaf senescence. With this purpose, we inhibited the root respiration of *A. sparsifolia* by utilizing N_2. As expected, root respiration under N_2 treatment showed a significant decline

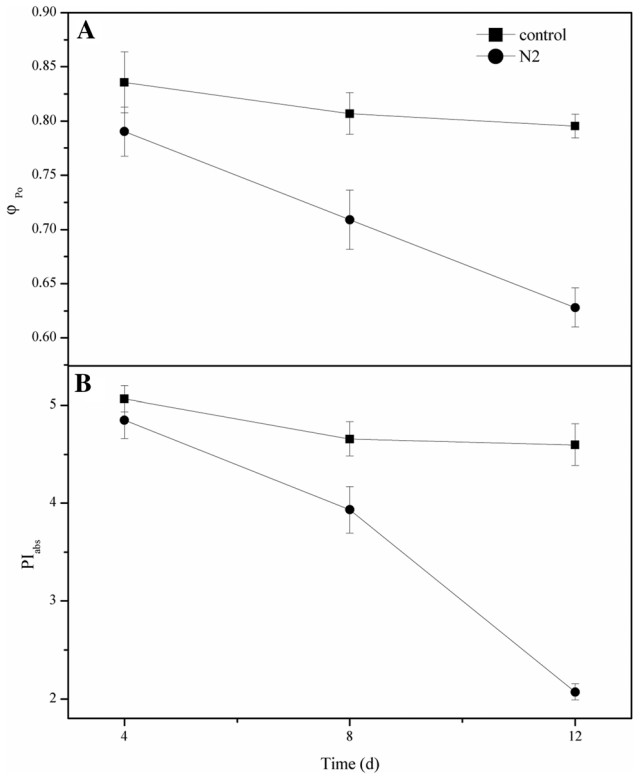

Fig. 6 Effect of root N_2 treatment on leaf φ_{po} and PI_{abs} in *A. sparsifolia*. **a** φ_{Po}; **b** PI_{abs}. φ_{Po}, maximum quantum yield for primary photochemistry (at $t=0$); PI_{abs}, performance index on absorption basis; N2, treated with N_2. The data represent means of five biological replicates ± SE. *Significant difference at the 0.05 probability level

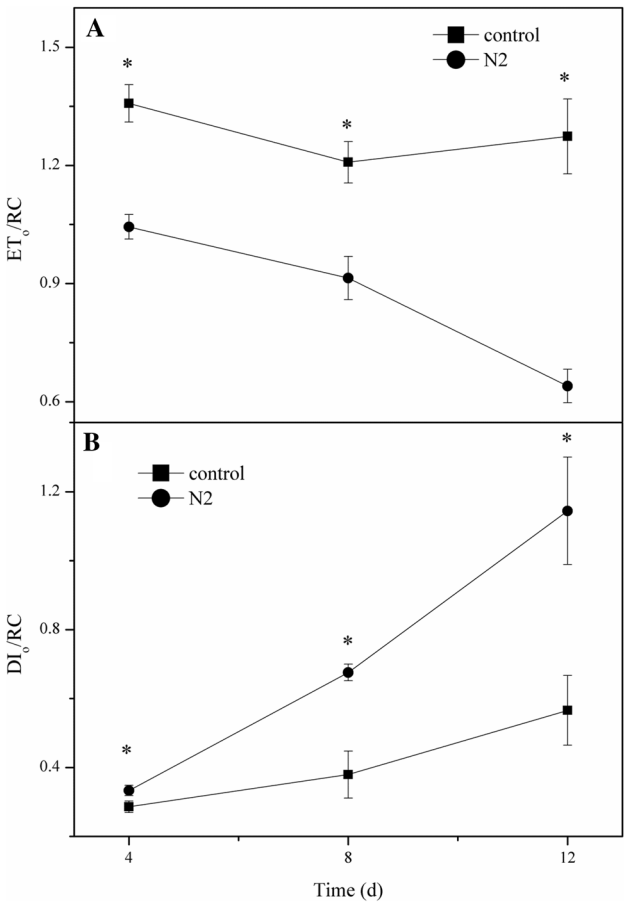

Fig. 7 Effect of root N_2 treatment on ET_o/RC, and DI_o/RC in *A. sparsifolia*. **a** ET_o/RC; **b** DI_o/RC. ET_o/RC, electron transport rate per RC; DI_o/RC, exciton dissipation rate per RC; N2, treated with N_2. The data represent means of five biological replicates ± SE. *Significant difference at the 0.05 probability level

(Fig. 2). Root respiration inhibition was the most direct response to N_2, and we attribute the change of other indicators in leaves to the effect of root respiration inhibition.

Traditionally, degradation of Chl and decline of P_n are early symptoms of leaf senescence (Bleecker and Patterson 1997; Hörtensteiner 2006; Smart 1994; Wingler et al. 2006), and in previous studies, decrease of Chl and P_n was considered as evidence of senescence (Dai and Dong 2011). To study the effect of root respiration inhibition on leaf senescence, we analyzed the Chl content and P_n after 4, 8, and 12 d of N_2 treatment. The present study showed that Chl *a*, Chl *b*, and Chl ($a+b$) decreased in the N2 group (Fig. 3a–c); thus, P_n in the N2 group also showed a significant decline compared with the control group (Fig. 4b). Malondialdehyde (MDA) is always considered as a stress maker. Moreover, in some studies, MDA together with Chl content and P_n have also been utilized as valid markers of leaf senescence (Dai and Dong 2011; Dong et al. 2008). In the present study, MDA increased in the N2 group (Fig. 5b), which further proved that root respiration inhibition can induce leaf senescence.

In addition to the indicators described above, the extant literature has also proven that visible leaf senescence is correlated with a decline of maximum photosynthetic efficiency (F_v/F_m), a fluorescence parameter that can be measured rapidly and non-destructively by employing chlorophyll fluorescence imaging (Pourtau et al. 2004, 2006; Wingler et al. 2004). In our research, we used a plant efficiency analyzer (PEA) to monitor the fluorescence parameters in photosystem II (PSII), including φ_{Po}. φ_{Po} reflects the maximum photochemical efficiency after dark adaptation, and has the same meaning as the parameter F_v/F_m determined by a modulated pulse fluorescence meter (Tang et al. 2015). Similar to the result described above, φ_{Po} also decreased in the senescent leaf in *A. sparsifolia*, as φ_{Po} declined during our 12-day study and was significantly lower in the N2 group compared with the control treatment (Fig. 6a). This result is consistent with previous studies that demonstrated that F_v/F_m declined with leaf senescence (Pourtau et al. 2004, 2006; Wingler et al. 2004). Furthermore, the decline of φ_{Po} in the N2 group further confirmed that root respiration inhibition can induce leaf senescence.

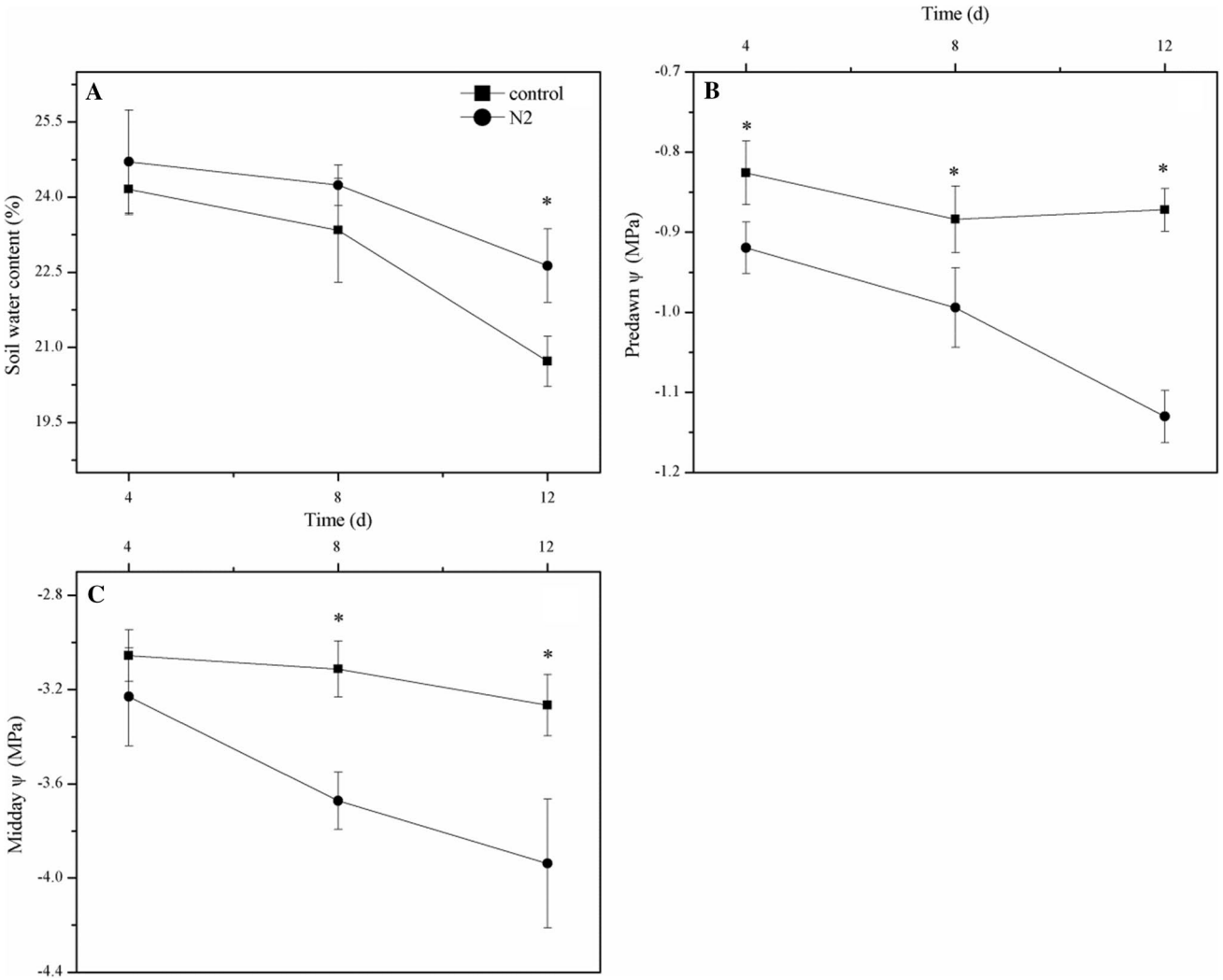

Fig. 8 Effect of root N_2 treatment on soil water content, predawn *y* and midday *y* in *A. sparsifolia*. **a** water content; **b** predawn *y*, (C) midday *y*. *y*, water potential; N2, treated with N_2. The data represent means of three biological replicates ± SE. *Significant difference at the 0.05 probability level

In the present study, PI_{abs} showed a similar trend to φ_{Po}, that is, PI_{abs} declined during our 12-day study. However, PI_{abs} in the N2 group reduced by 54.9% on the 12th day compared with the control (Fig. 6b); thus, the change of PI_{abs} in senescent leaves was more conspicuous than the change of φ_{Po}, as φ_{Po} only decreased by 21.0% on the 12th day in the N_2 treatment (Fig. 5a). This result is consistent with our previous conclusion that PI_{abs} is a superior parameter for measuring leaf senescence (Tang et al. 2015). Moreover, Fig. 2 clearly shows that N_2 significantly inhibited root respiration, and Figs. 2, 3, 4 and 5 strongly indicate that leaf senescence can be induced by root respiration inhibition. In the photosystem II (PSII) complex, the active reaction centres (RC) can absorb light energy, and these energies were used for electron transfer to downstream for the photochemical reaction or used for non-photochemical quenching (Tang et al. 2015). In the present study, root respiration inhibition can induce leaf senescence, which subsequently leads to the decrease of electron transport rate per RC (ET_o/RC) (Fig. 7a), and more energies absorbed by RC were used for exciton dissipation (DI_o/RC) (Fig. 7b). Our previous study also found that ET_o/RC decreased and DI_o/RC increased significantly during natural senescence and girdling induced senescence process (Tang et al. 2016).

Previous studies have demonstrated that ABA has a strong relationship with the leaf senescence process, and that ABA plays an important role in promoting leaf senescence (Kumar et al. 2014; Zhang et al. 2012). In our study, ABA content increased in the N2 group (Fig. 4c), which indicated that root respiration inhibition resulted in ABA accumulation in *A. sparsifolia* leaves. Previous investigations showed that when roots were exposed to salt stress or water stress,

ABA content increased in xylem vessels, shoots and leaves, which subsequently resulted in the closing of guard cells and a decline of G_s (Sauter et al. 2001; Wolf et al. 1990). So, these studies revealed that deterioration of root status will lead to ABA accumulation in leaves, which was similar to the findings in our present study. In our study, ABA is one of the factors which induced leaf senescence in the root respiration inhibited *A. sparsifolia*. The mechanism may be that inhibition of root respiration might stimulate ABA production in the roots, which is transported to the leaves, and the accumulation of ABA in leaves stimulates Pro (Fig. 5a) synthesis and induces leaf senescence. In addition, root respiration inhibition may influence water uptake in roots and thus lead to a deterioration of water status in leaves (Fig. 8b, c), which subsequently induces ABA accumulation and leaf senescence.

We discussed above that, during leaf senescence, non-photochemical quenching in senescent leaves was increased, and thus electrons from the electron transport chains leaked and reacted with O_2. This then resulted in the accumulation of reactive oxygen species (ROS) in leaves, which can be demonstrated by the increase of leaf MDA content (Fig. 5b). Our previous study demonstrated that, during leaf senescence, Car has a great degree of retention relative to Chl (shown as decline of Chl/Car) (Tang et al. 2015). This allows Car to absorb residual light energy and quench ROS, thus preventing membrane lipid peroxidation. Thus, Car plays a key photoprotective role in leaf senescence. This result is also proven in our present study because Chl/Car decreased obviously in the N2 group compared with the control (Fig. 3f), which indicated that in the root respiration inhibition induced senescence process, one of Car's most important roles is also photoprotective. Previous investigations have reported that there is an increase in content of proline and MDA in response to stress (Kamran et al. 2017; Kishor et al. 2014). We also found, in our study, that after N_2 treatment, leaf Ψ_P and Ψ_M decreased significantly (Fig. 8b, c), and thus the accumulation of proline and MDA were at least partially resultant from the deterioration of water status in plant leaves. Moreover, deterioration of water status in leaves may subsequently induce leaf senescence (Yang et al. 2002). It may also inhibit leaf transpiration, and thus retain more water in soil (Fig. 8a).

Our previous study also demonstrated that Chl *a*/*b* and the fast Chl fluorescence induction kinetic curve varies among different types of senescence (natural senescence vs. girdling induced senescence) (Tang et al. 2015). In the present research, although Chl *a*/*b* decreased during the 12-day study, the difference of Chl *a*/*b* between the N2 and control group was not significant (Fig. 3e). This result indicated that root respiration inhibition-induced senescence shows essentially the same symptoms as developmental senescence (natural senescence).

Overall, our study demonstrated that leaf senescence can be induced by root respiration inhibition. The symptoms of root respiration inhibition-induced senescence were similar to natural senescence. The reasons for leaf senescence in root respiration-induced leaf senescence may be that root respiration inhibition results in ABA accumulation in leaves, which subsequently induces leaf senescence. In addition, root respiration inhibition results in the deterioration of leaf water status (Fig. 8b, c), which subsequently induces leaf senescence.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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