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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Extended leaf senescence promotes carbon gain and nutrient resorption: importance of maintaining winter photosynthesis in subtropical forests

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Abstract The relative advantages of being deciduous or evergreen in subtropical forests and the relationship between leaf phenology and nutrient resorption efficiency are not well understood. The most successful deciduous species (*Lyonia ovalifolia*) in an evergreen-dominated subtropical montane cloud forest in southwest (SW) China maintains red senescing leaves throughout much of the winter. The aim of this study was to investigate whether red senescing leaves of this species were able to assimilate carbon in winter, to infer the importance of maintaining a positive winter carbon balance in subtropical forests, and to test whether an extended leaf life span is associated with enhanced nutrient resorption and yearly carbon gain. The red senescing leaves of *L. ovalifolia* assimilated considerable carbon during part of the winter, resulting in a higher yearly carbon gain than co-occurring deciduous species. Its leaf N and P resorption efficiency was higher than for co-occurring non-anthocyanic deciduous species that dropped leaves in autumn, supporting the hypothesis that anthocyanin accumulation and/or extended leaf senescence help in nutrient resorption. Substantial winter carbon gain and efficient nutrient resorption may partially explain the success of *L. ovalifolia* versus that of the other deciduous species in this subtropical forest. The importance of maintaining a positive carbon balance for ecological success in this forest also provides indirect evidence for the dominance of evergreen species in the subtropical forests of SW China.

Keywords Deciduousness · Leaf phenology · Carbon balance · Anthocyanin · Cloud forest

Introduction

Deciduous trees are defined as trees that remain completely leafless for a certain period of time during the year (for a

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review, see Kikuzawa and Lechowicz 2011). The habit of deciduousness originated in the Cretaceous period when angiosperms spread from low-latitude to mid-latitude regions (Axelrod 1966; Wolfe 1987). Deciduousness is thought to be an adaptation to the seasonal low temperatures in the mid-latitude region of the northern hemisphere, or to the seasonal drought in some tropical or subtropical regions (Givnish 2002). Being deciduous avoids the investment in low-temperature (or drought) resistance (e.g., high nitrogen partitioning to structural components; increasing osmo-regulation and membrane phospholipids in acclimation), at a cost of a relatively short period of carbon assimilation (Givnish 2002). Therefore, whether evergreen trees are able to assimilate considerable carbon during the unfavorable season is an important factor in determining the relative dominance of evergreen or deciduous trees in a forest ecosystem. The influence of an unfavorable season on tree carbon assimilation has been investigated in tropical, temperate, boreal, and Mediterranean forests/shrublands (Malhi et al. 1999; Oliveira and Penuelas 2004; Taneda and Tateno 2005), but not in subtropical forests. In the Asian subtropics, tree photosynthesis might be negatively affected by low temperatures in winter and high cloud cover (low incoming solar radiation) in summer. The advantages of being deciduous or evergreen in subtropical forests are poorly understood.

China harbors the largest area of subtropical broadleaf forests in the world (Wu 1980; Song et al. 2005). Evergreen broadleaf trees dominate the forests from 1,000 to 2,600 m in the subtropical area of southwest (SW) China, while subtropical forests from southeast (SE) China at similar elevations are dominated by deciduous trees (Wu 1980). The subtropical area in SW China has milder winters than SE China because the Tibetan Plateau and Yunnan–Guizhou Plateau prevent cold fronts from moving south in western China. If the medium and high elevation subtropical forests in SW China are dominated by evergreen broadleaf trees owing to a mild winter, it is reasonable to hypothesize that some coexisting deciduous species are subjected to selective pressures to extend their leaf life spans to partially utilize autumn and winter solar radiation.

Many deciduous species accumulate anthocyanins during leaf senescence (e.g., Matile 2000; Feild et al. 2001; Lee et al. 2003). There is some evidence that anthocyanins in leaves can act as a light-attenuation screen (Feild et al. 2001; Gould et al. 2002; Gould 2004; Hughes et al. 2005) or antioxidants (Hoch et al. 2001; Neill et al. 2002; Neill and Gould 2003; Kytridis and Manetas 2006) to reduce photo-inhibition and photo-oxidation. Since the leaves will senesce anyway, anthocyanin synthesis for photo-protection would be costly and without an apparent adaptive value. One hypothesis explaining the adaptive significance of anthocyanin synthesis in senescing leaves is that anthocyanins reduce photo-damage to protect the physiological processes of nutrient resorption (Feild et al. 2001; Lee et al. 2003; Hoch et al. 2003). That contention is based on the reasoning that free radicals induced by photo-oxidation oxidize the products of chlorophyll degradation (Matile et al. 1999), thereby interrupting the resorption process of nutrients. Three anthocyanic deciduous species recycled more N than acyanic mutants (Hoch et al. 2003), and leaf anthocyanin content was negatively correlated with leaf N concentration in senescent leaves of deciduous species in New England, USA (Lee et al. 2003), supporting this hypothesis. Photo-protection by anthocyanins will allow senescing leaves to maintain some degree of carbon assimilation, at least during the early stage of leaf senescence. The carbon assimilation of senescing leaves has not been investigated, probably because the senescence process is usually short. Carbon assimilation by senescing leaves, however, could be of importance to some deciduous species with prolonged senescence.

Lyonia ovalifolia Drude var. *ovalifolia* (Ericaceae), the most common deciduous tree species in the subtropical evergreen broadleaf forest of Ailao Mountain in SW China (Qiu and Xie 1998), maintains red leaves on trees for a long period in winter (more than 2 months) when other deciduous trees are leafless. The objectives of this study were: (1) to determine the winter photosynthetic performance of *Lyonia ovalifolia* red senescing leaves; (2) to infer the relative contribution of winter carbon gain to the yearly carbon balance of *L. ovalifolia* and the importance of maintaining positive winter carbon balances for trees in subtropical forests of SW China; and (3) to test whether *L. ovalifolia* with extended leaf senescence and anthocyanin accumulation has higher nutrient resorption efficiency than co-occurring deciduous species without extended leaf senescence and anthocyanin accumulation.

Materials and methods

Study site

This study was performed in an evergreen broadleaf forest at the Ailaoshan Station for Subtropical Evergreen Broadleaf Forest Ecosystem Studies (24°32′N, 101°01′E, elevation 2,460 m), located at Ailao Mountain, Jingdong County, Yunnan Province, SW China. The station is a member of the Chinese Ecosystem Research Network (CERN), and the Chinese National Ecosystem Observation and Research Network (CEORN). Annual average temperature at the study site is 11.3 °C, and annual average precipitation is 1,931 mm (5-year average; Qiu and Xie 1998). The weather in 2009, when we conducted the present study, is shown in Fig. 1. Sunshine duration in the

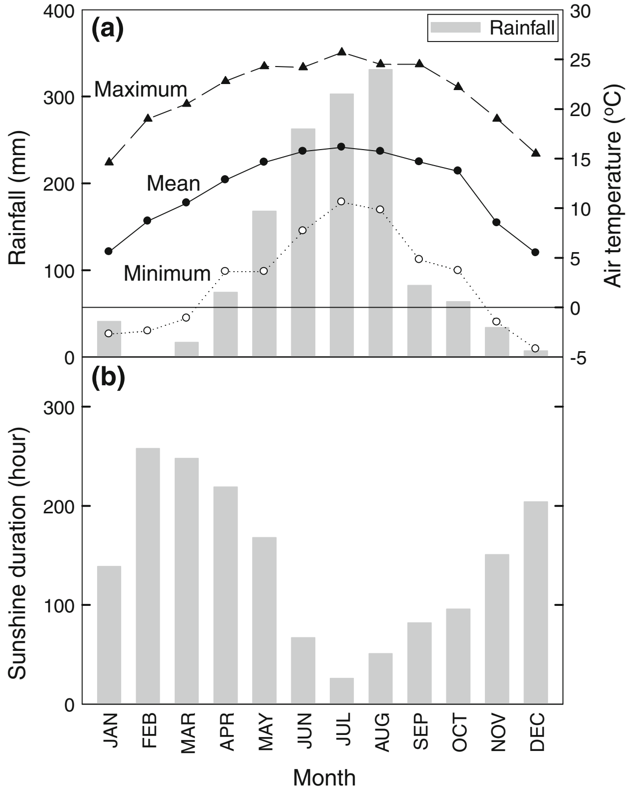

Fig. 1 **a** Mean monthly rainfall (*bars*), absolute maximum and minimum temperatures, average mean temperatures, and **b** sunshine duration in Ailao Mountain for 2009. (Data from Ailaoshan Station for Subtropical Forest Ecosystem Studies; elevation 2,460 m)

summer/wet season is much lower than that in the winter season. Because of abundant moisture and persistent cloud cover, this forest is known as a subtropical cloud forest. The forest canopy is approximately 20–25 m in height (Qiu and Xie 1998). The soils of the study site are loamy yellow–brown soils. The surface soil layer (0–15 cm) contains 12.15 % organic matter, 0.42 % total N, and 0.16 % total P (Qiu and Xie 1998). This forest is dominated by evergreen broadleaf trees, with some sparsely scattered deciduous species.

The species examined in the present study, *Lyonia ovalifolia* Drude var. *ovalifolia* (Ericaceae), is the most successful deciduous canopy species in terms of relative abundance and importance values (Qiu and Xie 1998). The leaf life span can be as long as 11 months but, during the last 2 months, before the trees are completely leafless, only a few leaves remain on the trees. The leaves of *L. ovalifolia* turn red at the end of October or the beginning of November. The red leaves are maintained on *L. ovalifolia* trees in November and December, and a small portion of the red leaves last until February on some trees, about a month before the new leaves flush in March. Nutrient resorption efficiencies of four co-occurring deciduous tree species (*Alnus nepalensis* D. Don, *Betula alnoides* Hamilt, *Populus yunnanensis* Dode, *Clethra brammeriana* Hand.-Mazz.) were also studied for comparison. Leaves of these four deciduous species turn yellow and drop in October or early November. Sun-exposed trees (2–3 m high) on the forest edges were chosen for the physiological measurements and to minimize shading effects. We performed the physiological measurements from April 2009 to February 2010.

Gas exchange measurements

Six sun-exposed leaves from different *L. ovalifolia* individuals were chosen for gas exchange measurements. Light-saturated net photosynthetic rates (A_{sat}) were measured by a LI-6400 portable photosynthetic measurement system (LI-COR, NE, USA) under a saturation photosynthetic photon flux density (1,200 μmol m^{-2} s^{-1}) generated by a red–blue LED light source (LI-COR, NE, USA), ambient temperatures, and ambient CO_2 concentrations. Gas exchange measurements were repeated monthly from April to December. In January and February, measurements were not performed because only few leaves remained on some of the trees. Gas exchange was measured at 0900–1100 hours on typical sunny days from September to December, and occasionally on cloudy days from April to August, when sunny days were rare.

The leaf photosynthetic light response curves were determined in August and November 2009. Each leaf was exposed to a gradient of PPFD ranging from 0 to 2,000 μmol m^{-2} s^{-1}, starting from the high light. The light was generated by a red–blue LED light source (LI-COR, NE, USA) attached to the photosynthetic measurement system. When a steady state of net photosynthesis (maintained almost constant during 1 min) was obtained after exposing the leaf surface to a specific light intensity for about 3–5 min, the net photosynthetic rate was recorded. The relationship between *A* and PPFD was fitted with an exponential equation described by Bassman and Zwier (1991). Then, the light-saturated net photosynthetic rate (A_{sat}), dark respiration rate (R_d), apparent quantum yield (AQY), light saturation point (LSP), and light compensation point (LCP) were calculated according to Bassman and Zwier (1991). AQY is the light utilization efficiency by photosynthesis (Evans 1989). Leaf daily CO_2 assimilation was estimated by the light response curve and PPFD data. PPFD was measured by a Li-190SB PPFD quantum sensor (LI-COR) every second, and 1 h averages were recorded by a CR3000 datalogger (Campbell Science, Logan, UT, USA).

Leaf mass per area and leaf pigment concentration

Six sun-exposed leaves of *Lyonia ovalifolia* from different individuals were collected in August (green leaf stage) and

November (red leaf stage) to measure leaf mass per area (LMA), chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations. For LMA determinations, leaf areas were measured by a LI-3000A area meter (LI-COR), then the leaves were oven-dried at 70 °C to constant weight, and weighed. Leaf pigment concentrations were determined using the wet chemical method. After pigment extraction using 99 % ethanol, spectrophotometric determination was conducted using a UV 2501 spectrophotometer (Shimadzu, Japan). Chlorophyll (Chl *a*, Chl *b*) and carotenoid (Car) concentrations were calculated according to Lichtenthaler and Wellburn (1983):

$$\text{Chl } a \text{ concentration} = (13.95A_{665}) - (6.88A_{649}) \tag{1}$$

$$\text{Chl } b \text{ concentration} = (24.96A_{649}) - (7.32A_{665}) \tag{2}$$

$$\text{Car} = (1000A_{470} - 2.05\text{Chl } a - 114.8\text{Chl } b)/245 \tag{3}$$

In the equations, *A* refers to absorbance, and the subscripts refer to specific wavelengths.

Leaf reflectance and transmission spectrum

A USB-4000 spectrometer (Ocean Optics, USA) attached to an integrating sphere by fiber optics was used to study the leaf spectral reflectance and transmittance at wavelengths from 400 to 740 nm, setting integration time at 4 ms and light source at 100 %. Dark and reference scans were conducted before sample measurement. During measurement, dark reflectance was autocorrected for each measurement. A modified normalized difference vegetation index (NDVI), which is strongly correlated with leaf chlorophyll content (Gamon et al. 1997; Gamon and Surfus 1999) was calculated. Leaf anthocyanin concentration was estimated by an anthocyanin reflectance index (ARI), which has been shown to be well correlated with leaf anthocyanin concentration (Gitelson et al. 2001). The calculations were:

$$\text{NDVI (chlorophyll)} = (R_{750} - R_{705})/(R_{750} + R_{705}) \tag{4}$$

$$\text{ARI} = (R_{550})^{-1} - (R_{700})^{-1} \tag{5}$$

In the equations, *R* refers to reflectance, and the subscripts refer to specific wavelengths.

Leaf freezing resistance

To test whether *L. ovalifolia* leaves undergo cold acclimation, leaf freezing resistance was determined for green (August) and red (January) leaves using chlorophyll fluorescence emission (Boorse et al. 1998). Sun-exposed leaves from different individuals ($n = 6$) were collected in the late afternoon, and then kept in black plastic bags with slightly moist paper towels for 12 h for dark adaptation. Maximum quantum yield of PSII (F_v/F_m) of the leaves were then determined with a Dual PAM-100 (Walz, Germany). Leaf F_v/F_m was determined by illuminating the leaf with a white saturating light pulse with a photon flux density of 10,000 µmol m^{-2} s^{-1} for 600 ms. After measuring the control value of F_v/F_m (before low temperature treatment), leaves were treated with different temperatures generated by a freezer (10, 5, 2.5, 0, −2.5, −5, −7.5, −10, −12.5, −15, −17.5, −20, −22.5, and −25 °C) for 30 min. The leaf temperature was monitored with copper–constantan thermocouples and a CR1000 datalogger (Campbell Science) connected to a computer. After the different low temperature treatments, leaf samples were removed from the freezer, and the leaves were allowed to thaw in the dark at room temperature (15 °C) for 12 h. Leaves were kept in plastic bags with wet paper towels to avoid water loss. Then, the leaf F_v/F_m was re-measured with the Dual PAM-100. Relative F_v/F_m was calculated as the percentage of the control values before low temperature treatment. The relationship between relative F_v/F_m and treatment temperature was fitted with a sigmoid function, and the temperature at 50 % loss of F_v/F_m was interpolated. The chlorophyll fluorescence method is widely used to assess the sensitivity of leaves to low temperatures (e.g., Boorse et al. 1998; Sierra-Almeida and Cavieres 2010), and the temperature at 50 % loss of F_v/F_m was defined as the leaf lethal temperature (LT_{50}).

Leaf nutrient concentration and nutrient resorption efficiency

Sun-exposed mature leaves of *L. ovalifolia* and the four co-occurring deciduous species (*A. nepalensis*, *B. alnoides*, *P. yunnanensis*, *C. brammeriana*) were collected in the field in August to determine foliar nutrient concentrations ($n = 6$). Leaves of the deciduous species in the cloud forest reached peak photosynthetic rates in June or July, so we collected leaves in August for nutrient concentration determination to make sure the leaves were fully mature. Leaves of *L. ovalifolia* were also collected in September, November, and January. Recently senesced leaves of *L. ovalifolia* were collected in January, and those of the other deciduous species were collected in October or early November. We collected senesced leaves when leaves dropped on windy days so that we could distinguish them among different individuals. Leaves were oven-dried at 70 °C for 48 h and analyzed for total N and P. Leaf total N concentration was determined using a Vario MAX CN auto element analyzer (Elementar Analysensysteme, Hanau, Germany). Leaf P concentration was measured using an inductively coupled plasma atomic-emission spectrometer (IRIS Advantage-ER; Thermo Jarrell Ash, MA, USA) after the samples were digested with concentrated HNO_3–

$HClO_4$. Leaf N or P resorption efficiency was calculated as the percentage of total nutrient recycled after senescence by using leaf N or P concentration of mature green leaves as 100 % (Killingbeck 1996).

Data analysis

The differences in mean A_{sat}, R_d, LSP, LCP between August green leaves and November red leaves were tested by one-way ANOVA. The differences in mean Chl $a + b$, Chl *a*, Chl *b*, Car, and Chl *a*/*b* between August green leaves and November red leaves were tested by the Mann–Whitney *U* test, because variances were not homogenous.

Results

The leaves of *Lyonia ovalifolia* reached the yearly highest light-saturated net photosynthetic rate (A_{sat}; 16.3 μmol CO_2 m^{-2} s^{-1}) in June, and then started to decrease (Fig. 2a). The A_{sat} of *L. ovalifolia* leaves decreased sharply to 5.9 μmol CO_2 m^{-2} s^{-1} when the leaves turned red in November, and this A_{sat} was maintained in December (Fig. 2a). In January and February, only a few leaves remained on some trees of this species. The A_{sat}, R_d, AQY, LCP, and LSP of the winter red leaves (November) were significantly lower than those of the August green leaves (Fig. 2b; Table 1). Daily net CO_2 assimilation (estimated from light response curves and daily PPFD) in red leaves was lower than that of the green leaves (Fig. 2c). However, daily net CO_2 assimilation of red leaves fluctuated less compared to green leaves (Fig. 2c), probably owing to fewer rainfall events and less cloud cover in winter. Estimated monthly CO_2 assimilation in red leaves was close to half of that in green leaves (Fig. 2c).

Anthocyanin reflectance index (ARI) in *L. ovalifolia* leaves was zero in August, September, and October, while it reached a value of 0.09 in November, and tended to increase slightly in December, January, and February. The ARI of *L. ovalifolia* leaves in February was 0.11 (Fig. 3a). In contrast, the chlorophyll reflectance index (NDVI) of *L. ovalifolia* leaves was around 0.46 in August, September and October, and it decreased to 0.26 in November, and maintained this value in December, January, and February (Fig. 3b). The total chlorophyll concentration (Chl $a + b$), Chl *a* concentration, Chl *a*/*b* ratio and carotenoid concentration of *L. ovalifolia* red leaves (November) were significantly lower than those of the green leaves (Table 1). The total chlorophyll concentration and carotenoid concentrations of the red leaves were half of those of the green leaves. However, no significant difference was found in Chl *b* concentration between green and red leaves (Table 1). Red and green leaves did not differ significantly in LMA (Table 1).

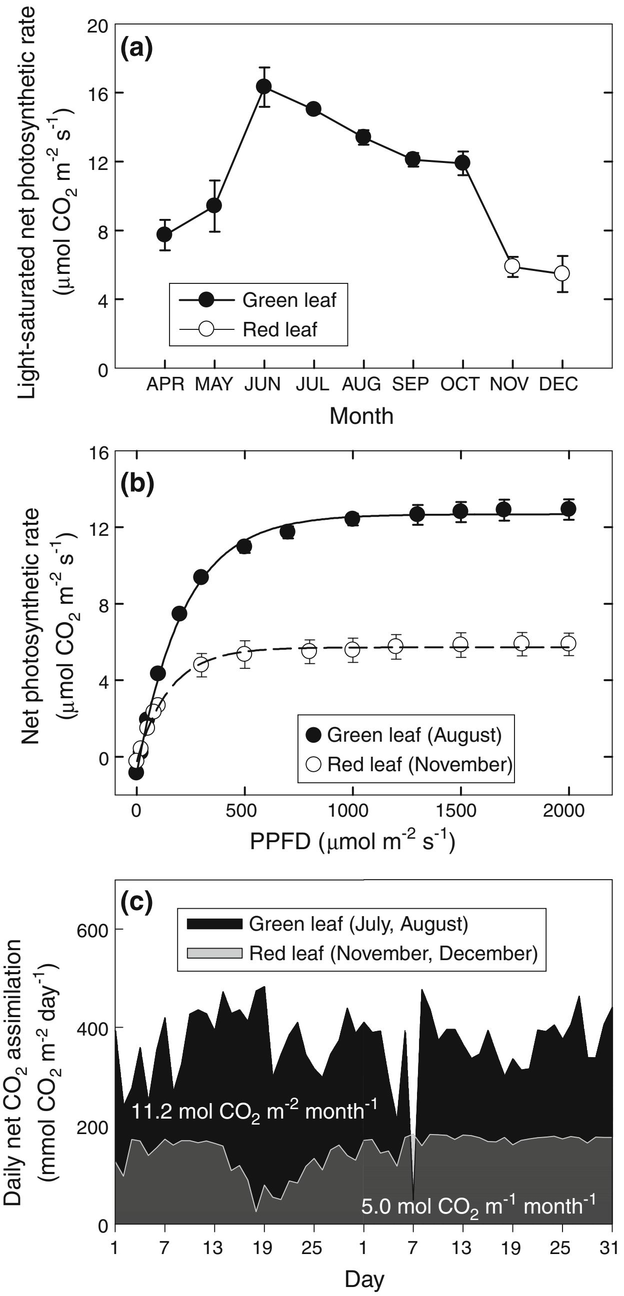

Fig. 2 **a** Seasonal dynamics in light-saturated net photosynthetic rate (A_{sat}) of *Lyonia ovalifolia*, and **b** light response curves of its green (August) and red (November) leaves, and **c** daily net carbon assimilation of *Lyonia ovalifolia* green (July and August) and red (November and December) leaves. *Closed symbols* green leaves, and *open symbols* red leaves in (**a**, **b**). Data in (**a**, **b**) are means ± SE ($n = 6$)

Winter red leaves resisted lower subzero temperatures compared to the summer leaves (Fig. 4). Relative PSII quantum efficiency (F_v/F_m), which reflects the status of the photosynthetic apparatus, started to decrease at −2.5 °C in green leaves, while it started to decrease at −10 °C in red leaves. The decrease in relative F_v/F_m of the green leaves

Table 1 Light-saturated net photosynthetic rate (A_{sat}), dark respiration rate (R_d), apparent quantum yield (*AQY*), light compensation point (*LCP*), light saturation point (*LSP*), leaf mass per area (*LMA*), total leaf chlorophyll concentration (*Chl a + b*), chlorophyll *a* concentration (*Chl a*), chlorophyll *b* concentration (*Chl b*), chlorophyll *a*/*b* ratio (*Chl a/b*), and carotenoid concentration (*Car*) of green (August) and red (November) leaves

Unit	Green leaf	Red leaf
A_{sat} (μmol m^{-2} s^{-1})	12.6 ± 0.5 a	5.7 ± 0.6 b
R_d (μmol m^{-2} s^{-1})	0.84 ± 0.14 a	0.22 ± 0.06 b
AQY	0.052 ± 0.001 a	0.032 ± 0.002 b
LCP (μmol m^{-2} s^{-1})	16.1 ± 2.6 a	7.1 ± 2.1 b
LSP (μmol m^{-2} s^{-1})	1,011 ± 60 a	718 ± 64 b
LMA (g m^{-2})	94.6 ± 3.9 a	92.9 ± 5.1 a
Chl *a* + *b* (mg g^{-1})	3.62 ± 0.46 a	1.88 ± 0.18 b
Chl *a* (mg g^{-1})	2.71 ± 0.36 a	0.98 ± 0.12 b
Chl *b* (mg g^{-1})	0.91 ± 0.11 a	0.90 ± 0.09 a
Chl *a*/*b*	2.96 ± 0.17 a	1.10 ± 0.11 b
Car (mg g^{-1})	0.58 ± 0.07 a	0.29 ± 0.03 b

Values are means ± SE ($n = 6$). Values followed by the same letter do not differ significantly between red and green leaves

was very sharp, and 100 % loss of relative F_v/F_m occurred at −7.5 °C. The decrease in relative F_v/F_m of the red leaves was more gradual, and 100 % loss of relative F_v/F_m occurred at −22.5 °C. The 50 % loss of relative F_v/F_m occurred at −5.4 °C in green leaves, and at −13.8 °C in red leaves (Fig. 4).

Total N concentration of *L. ovalifolia* leaves was 13.5 g kg^{-1} in August and September, and it decreased to 10.3 g kg^{-1} in November and to 7.4 g kg^{-1} in January (Fig. 5a). Total P concentration of *L. ovalifolia* leaves was 0.98 g kg^{-1} in August and September, and it decreased to 0.87 g kg^{-1} in November and to 0.51 g kg^{-1} in January (Fig. 5b). Nutrient resorption efficiency of *L. ovalifolia* was much higher than that of four co-occurring deciduous tree species (Table 2).

Discussion

Our results reveal that a deciduous species in a subtropical evergreen forest can use red senescing leaves to assimilate substantial amounts of carbon during 2 months of the winter season. Cold acclimation and photoprotection corresponding with anthocyanins helped winter CO_2 assimilation. We also observed continuing nutrient resorption in *L. ovalifolia* during the red leaf stage, and a higher nutrient resorption efficiency than for co-occurring deciduous species without extended leaf senescence, supporting the hypothesis that anthocyanin accumulation in senescing leaves protects the nutrient resorption process (Feild et al. 2001; Lee et al. 2003; Hoch et al. 2003).

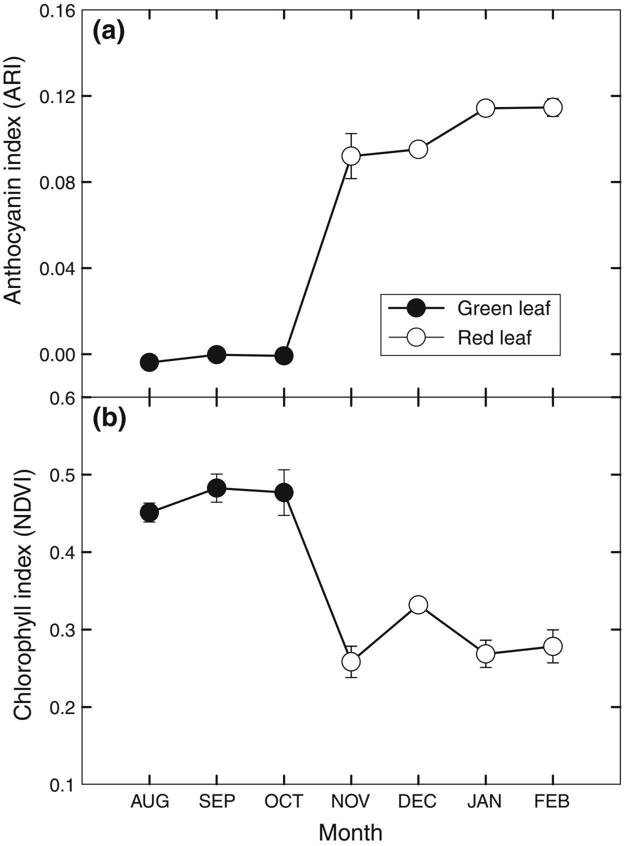

Fig. 3 Seasonal dynamics in **a** anthocyanin reflectance index (ARI) and **b** NDVI of *Lyonia ovalifolia* leaves. *Closed symbols* green leaves, and *open symbols* red leaves. Data are means ± SE ($n = 6$)

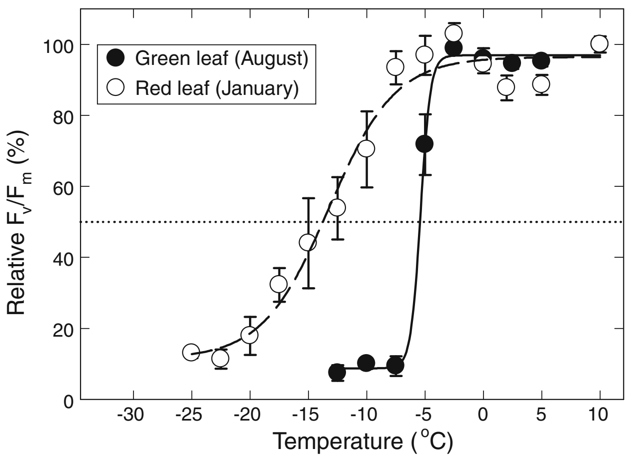

Fig. 4 Freezing resistance of green (August), and red (January) *Lyonia ovalifolia* leaves. *Closed symbols* green leaves, and *open symbols* red leaves. Data are means ± SE ($n = 6$)

Winter carbon assimilation

The photosynthetic rates of *L. ovalifolia* winter red senescing leaves were lower than that of the summer green leaves, but the red leaves still maintained relatively high

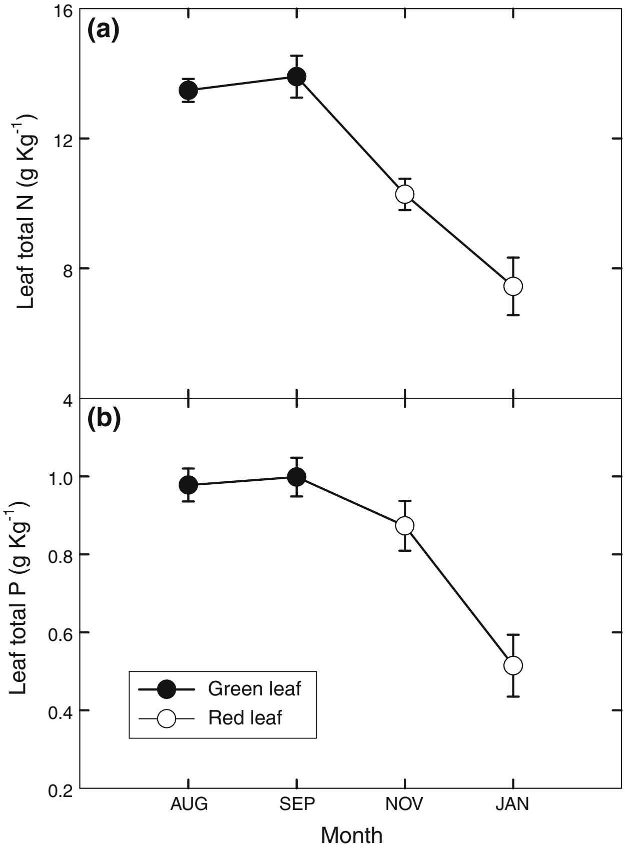

Fig. 5 Seasonal dynamics in **a** leaf total N and **b** P concentrations of *Lyonia ovalifolia*. *Closed symbols* green leaves, and *open symbols* red leaves. Data are means ± SE ($n = 6$)

winter CO_2 assimilation in November and December (Fig. 2). The substantial down-regulation in photosynthetic capacity in winter is likely the result of the significant decreases in Chl *a*, apparent quantum yield (Table 1), Rubisco and/or chlorophyll binding protein contents (suggested by reduced leaf N; Fig. 5), and enzyme activities (Holaday et al. 1992). On the other hand, carbon assimilation in red leaves was facilitated by higher solar radiation in winter than in the summer/wet season (Fig. 1b). A substantially lower leaf respiration rate in red leaves compared to the green leaves (Table 1; probably due to lower air temperatures in winter) also promoted the maintenance of positive carbon assimilation during the red leaf stage.

The considerable winter carbon assimilation of *L. ovalifolia* may provide advantages over the other deciduous trees, which dropped leaves before winter, in terms of their yearly carbon balances. The leaf level yearly net carbon gain of *L. ovalifolia* (88.3 mol CO_2 m^{-2} $year^{-1}$; estimated by the light response curves and PPFD data) was higher than that of other deciduous species (ranging from 72.3 in *Clethra brammeriana* to 79.4 mol m^{-2} $year^{-1}$ in *Betula alnoides*) despite similar summer photosynthetic capacities (Zhang, unpublished data). Therefore, substantial winter carbon gain could be one of the reasons why *L. ovalifolia* is the most successful deciduous species in this subtropical forest dominated by evergreen broadleaf trees (Qiu and Xie 1998). Net winter carbon gain in *L. ovalifolia* (10.2 mol m^{-2}; November and December), however, was lower than that of the co-occurring dominant evergreen broadleaf species (e.g., 31.1 and 28.2 mol m^{-2} for *Lithocarpus jingdongensis* and *Schima noronhae*, respectively; November to February), resulting in a lower yearly carbon gain in *L. ovalifolia* compared to that of the evergreen broadleaf species (e.g., 109.0 and 105.0 mol m^{-2} $year^{-1}$ for *L. jingdongensis* and *S. noronhae*, respectively; Y-J Zhang, unpublished data). The substantial contribution of winter carbon gain (10.2/88.3 = 12 %) to the yearly carbon balance of this deciduous species, and the importance of maintaining a positive winter carbon balance for achieving high yearly carbon gains provides indirect evidence for the dominance of evergreen species in the subtropical forests of SW China.

Cold acclimation and photoprotection in red leaves

Carbon assimilation in *L. ovalifolia* senescing leaves is facilitated by cold acclimation. Considerable cold acclimation was found in deciduous *L. ovalifolia*; winter red leaves of *L. ovalifolia* resisted lower subzero temperatures than summer green leaves (Fig. 4). Evergreen species are known to undergo structural and physiological changes to help in cold acclimation during the transition from warm to cold seasons, enhancing the resistance (either tolerance or avoidance) to subzero temperatures (for reviews, see Thomashow 1999; Xin and Browse 2000). Since freezing events occur frequently in this high elevation subtropical forest, development of freezing resistance in *L. ovalifolia*

Table 2 Leaf N and P resorption efficiency of *Lyonia ovalifolia* and four co-occurring deciduous tree species

Resorption efficiency	*Lyonia ovalifolia*	*Betula alnoides*	*Clethra brammeriana*	*Populus yunnanensis*	*Alnus nepalensis*
N (%)	46.5 ± 6.4	28.3 ± 4.2	10.2 ± 4.6	34.7 ± 3.3	17.7 ± 2.6
P (%)	48.5 ± 7.9	25.4 ± 7.9	20.1 ± 3.0	26.6 ± 4.5	41.7 ± 7.6

Values are means ± SE ($n = 6$)

allowed red leaves to remain metabolically active in winter. Anthocyanins could act as osmo-regulators (Chalker-Scott 1999, 2002; but see Archetti et al. 2009; Hughes et al. 2013), potentially enhancing leaf resistance to cell dehydration induced by extracellular ice growth. Also, anthocyanins are associated with higher sugar concentrations, which may add to osmo-regulation and freezing resistance (Goldstein and Nobel 1994; Lipp et al. 1994; Murakami et al. 2008).

Anthocyanins may play a role in photoprotection in red senescing leaves of *L. ovalifolia*. Cold could induce photoinhibition and photooxidation even under normal or low light conditions because low temperatures affect light utilization more than light absorption (Germino and Smith 1999, 2000; Miyazawa et al. 2007). Decreased photosynthetic capacity and more destruction in energy-processing centers than in light harvesting apparatus (as suggested by decreased Chl *a*/*b*) in *L. ovalifolia* further increased the excess of photon flux for photosynthesis. Anthocyanins strongly absorb blue-green light (Harborne 1988; Smillie and Hetherington 1999; Barnes et al. 2000), which would potentially reduce the photons captured by chlorophylls and carotenoids (Smillie and Hetherington 1999; Feild et al. 2001; Gould et al. 2002; Oberbauer and Starr 2002; Gould 2004; Hughes et al. 2005; Hughes and Smith 2007). In addition, anthocyanins attenuate light that might oversaturate the xanthophyll cycle for thermal dissipation (Demmig-Adams and Adams 1992), because xanthophyll equilibrium is sensitive to the wavelength that anthocyanins absorb (Gamon et al. 1993, 1997). Anthocyanins can also attenuate light that photo-activates the high active chlorophyll intermediates during degradation such as red Chl catabolite (RCC), which has a peak absorbance in the wavelengths that anthocyanins absorb (Engel et al. 1991; Iturraspe et al. 1994; Mur et al. 2010).

Leaf senescence and nutrient resorption

The high nutrient resorption efficiency of *L. ovalifolia* with extended leaf senescence and anthocyanin accumulation agrees with the hypothesized protection effect of anthocyanin on leaf functioning including the nutrient resorption process. Association between anthocyanin accumulation and extended leaf senescence (greater leaf longevity and longer time for nutrient resorption) has also been reported in sugar maple (Schaberg et al. 2008). Nutrient resorption during leaf senescence is important for reproduction and leaf production during the following growing season (Aerts 1995; Killingbeck 1996; Matile et al. 1999; Matile 2000; Bogard et al. 2011), while anthocyanin synthesis in autumn leaves was hypothesized to protect the nutrient resorption process (Feild et al. 2001; Lee et al. 2003; Hoch et al. 2003). In the present study, leaf N and P resorption efficiency of anthocyanic *L. ovalifolia* leaves were 12–356 % higher than for four co-occurring non-anthocyanic deciduous tree species (Table 2), providing support for this hypothesis.

Extended leaf senescence may provide a longer time for nutrient resorption. Leaf N and P concentrations decrease continuously during leaf senescence (Fig. 5), probably because the protein degradation and/or nutrient resorption process is slow in *L. ovalifolia*. Notably, chlorophyll concentration (estimated indirectly by NDVI) remained constant during this time period, suggesting that the nutrients recycled did not result from continuous degradation of thylakoid membranes. Since 90 % of the nitrogen resorbed by plants during leaf senescence is from the degradation of stromal proteins and thylakoid membranes (Evans 1983), the nutrients recycled during this period were probably from stromal proteins, which would explain the reduced photosynthetic rates observed in red leaves. This protein degradation and nutrient resorption process possibly needs a relatively long time to achieve a high efficiency, and a positive correlation between leaf senescence duration and nutrient resorption efficiency has also been reported in winter wheat (Bogard et al. 2011).

However, the suggestion that anthocyanins are responsible for the extended leaf life span and enhanced nutrient resorption in *L. ovalifolia* is based on the correlations between the presence of anthocyanins, extended leaf life span, and enhanced nutrient resorption efficiency. Although correlation does not mean causation, results from this study and others (e.g., Feild et al. 2001; Lee et al. 2003; Schaberg et al. 2008) suggest potential causal links between anthocyanin accumulation, extended leaf life span, and enhanced nutrient resorption. Manipulative experiments could be done to connect these variables in a more mechanistic way.

In conclusion, the red senescing leaves of *L. ovalifolia* assimilated a substantial amount of carbon during part of the winter. To extend the carbon assimilation period to the winter months has the advantage that the incoming solar radiation is higher than during the summer. The substantial contribution of winter carbon gain to the yearly carbon budget of this deciduous species and its higher yearly carbon gain compared to co-occurring deciduous species without extended leaf senescence may partly explain its success in this subtropical forest. The importance of maintaining a positive winter carbon balance for achieving high yearly carbon gains and ecological success also provides indirect evidence for the dominance of evergreen species in the subtropical forests of SW China. In addition, our results support the hypothesis that photoprotection by anthocyanins and/or prolonged leaf senescence facilitates nutrient resorption.

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