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Stem CO_2 efflux of *Abies fabri* in subalpine forests in the Gongga Mountains, Eastern Tibetan Plateau

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ABSTRACT

Aims

Despite the importance of stem CO_2 efflux (E_s) in ecosystem carbon cycling and energy balance, little is known about temporal variation in the temperature coefficient (Q_{10}) and sapwood nitrogen concentrations ([N]) and their intrinsic links with E_s. The objectives of this study were: (1) to examine the response of E_s to temperature in a subalpine region and (2) to explore the influence of stem diameter and [N] on E_s. Also, we will test the hypothesis that (1) E_s in trees has thermal acclimation and (2) E_s will be well correlated with DBH and [N].

Methods

Here, a horizontally oriented soil chamber technique was applied to measure E_s

of *Abies fabri* in two subalpine forest stands in Southwestern China from May to December 2014. We also examined the variability in E_s, Q_{10} and [N] in trees and monitored the relationship between temperature, [N], DBH and E_s.

Important findings

During the measurement period, E_s showed an apparent seasonal trend, following the change in air temperature, increasing from May and peaking in July, then continuously decreasing until December. The mean E_s for the growing and dormant seasons were 1.45 and 0.25 μmol·m^{-2}·s^{-1}, respectively, and E_s in the mature forest was significantly higher than in the immature forest. The area-based E_s was positively correlated with DBH and sapwood width (SW), while volume-based E_s showed negative relationship with DBH and SW. Across the five diameter classes, 69.8–89.0% of the variation in E_s could be explained by air temperature. The temperature sensitivity (Q_{10}) of E_s ranged from 2.98 to 5.61 during the measurement period, with a higher Q_{10} appearing in the growing season than in the dormant season. There was a significant linear relationship ($P < 0.01$) between [N] and E_s (expressed based on two different units). Additionally, exponential models of E_s against [N] and air temperature were applied to estimate E_s.

Keywords: Stem CO_2 efflux; Q_{10}; DBH; Sapwood nitrogen contents; Subalpine forests.

INTRODUCTION

Forest ecosystems play a crucial role in the global carbon cycle (Dixon *et al.*

1994; Xu *et al.* 2001). Woody tissue CO_2 flux is thought to contribute about 25~50% of aboveground plant tissue respiration (Lavigne and Ryan 1997; Edwards *et al.* 2002). The stem, which accounts for the largest portion of the forest biomass, continuously increases during forest stand development (Kim and Nakane 2005). Therefore, there is substantial interest in E_s, which has been commonly regarded as a significant component in the tree or ecosystem carbon balance (Ryan *et al*. 1996; Bowman *et al*. 2008; Acosta *et al*. 2011; Rodríguez-Calcerrada *et al*. 2014).

Temporal patterns of E_s have been investigated for a long time and it is widely accepted that ambient temperature is a critical determinant in affecting diurnal and seasonal changes in E_s (Amthor 1994; Atkin *et al*. 2005). Most studies utilise an exponential function, which has gained wide acceptance in modelling respiration responses to temperature change and estimating annual carbon flux at different scales (Tjoelker *et al.* 2001), to calculate E_s under any temperature with a constant-temperature coefficient near 2.0 (fractional change in E_s with a 10^{o}C increase in temperature) (Edward and Hanson 1996; Ceschia *et al.* 2002). However, there is increasing evidence that the response of plant respiration to temperature is highly variable (Atkin and Tjoelker 2003; Atkin *et al*. 2005; Rodríguez-Calcerrada *et al.* 2014). Short- and long-term acclimation to changing thermal environments has been observed in several studies for leaves and roots (Atkin and Tjoelker 2003; Atkin *et al.* 2005; Lee *et al.* 2005), and the response of respiration to temperature can also be influenced by abiotic factors such as ambient CO_2 concentration (Acosta *et al.* 2010). The mature tissue method (Amthor 1989) that divides total respiration into growth

and maintenance respiration has been widely used to evaluate respiration components and to estimate plant respiration. However, further study is needed to test the validity of the mature tissue method (Atkin and Tjoelker 2003), because the method fails to take into account the variability in the plant temperature coefficient (Maier *et al.* 2001, 2004; Ceschia *et al.* 2002) and will result in large over-estimates of respiration efflux from the forest to the atmosphere during global climate warming. Consequently, understanding variability in the Q_{10} and its biological mechanism in thermal acclimation can observably improve the accuracy of modelling the carbon cycle at different scales. Currently, little information exists on the dynamic responses of respiration to temperature and the differences in respiration change mechanism among stem, leaf and root, so more studies are needed on stem respiration, especially long-term field observations.

To date, many studies have observed a strong relationship between E_s and plant organic nitrogen ([N]; Amthor 1989; Ryan 1991; Ryan *et al.* 1994), and 90% of plant nitrogen in cellular tissues exists as protein (Ryan *et al.* 1996) which has a direct relationship with cell division and growth. Based on the assumption that [N] is related to growth rate, Ryan (1991) proposed a general model to estimate maintenance respiration from temperature and [N]. Maintenance respiration might support protein repair and replacement, and it is frequently linked with protein in plants (Bouma *et al.* 1994; Vose and Ryan 2002). Maier *et al.* (1998) developed a two-factor model to describe stem and branch respiration as a function of stem temperature and [N] using a two-stage process, in an attempt to accurately predict

stem maintenance respiration in the dormant season. Vose and Ryan (2002) hypothesised that differences in maintenance respiration might largely be caused by differences in the nitrogen content, and a N-based model would be useful for estimating maintenance respiration budgets for forest ecosystems. Despite the probable importance of nitrogen in predicting cellular activity and the variation in respiration, there are few reports concerning the seasonal variation in stem-tissue nitrogen and its intrinsic relevance to respiration.

High-elevation and subalpine regions represent sites of great climatic instability at most scales (Rangwala *et al.* 2009), and will experience substantially alterations in ecological boundaries and transition zones due to future climate change (Kullman 1998). Although distributed over a small spatial area, subalpine forests will experience more rapid and larger climate changes than forests at low elevations because of snow-albedo feedback (Kueppers and Harte 2005). Consequently, subalpine forest ecosystems are perceived to be an optimal site to examine forest respiration or carbon cycle in response to global climate change. In this study, an experiment on E_s in *A. fabri* was conducted in subalpine forests. The objectives were: (1) to examine the response of E_s to temperature in a subalpine region and (2) to explore the influence of stem diameter (DBH) and [N] on E_s. We hypothesized that: (1) E_s in trees has thermal acclimation and (2) E_s will be well correlated with DBH and [N].

MATERIALS AND METHODS

Study site

The Gongga Mountains (29°20′–30°20′N, 101°30′–102°15′N, 7,556 m a.s.l), located in the southeastern fringe of the Tibetan Plateau represent the southern section and peak of the Daxue Mountain Range, belong to the Hengduan Mountain System in the transitional zone between the Qinghai-Tibetan Plateau and the Sichuan Basin. The climate is dominated by the southeastern Pacific monsoon. Reaching the Dadu River valley at 1,100 m a.s.l over 30 km, the eastern slope of Mt. Gongga has an intact and continuous vertical vegetation spectrum, consisting of subtropical evergreen broad-leaved vegetation (1,100−2,200 m a.s.l), temperate coniferous and broad-leaved mixed forests (2,200−2,800 m a.s.l), frigid dark coniferous forests (2,800−3,600 m a.s.l), alpine sub-frigid shrub and meadow vegetation (3,600−4,200 m a.s.l), alpine frigid meadow vegetation (4,200−4,600 m a.s.l), alpine frigid sparse grass and desert zone (4,600−4,800 m a.s.l), and a higher-altitude alpine ice-and-snow zone (>4,800 m a.s.l).

The study was carried out at the Alpine Ecosystem Observation and Experiment Station of the Gongga Mountain (3,000 m a.s.l), located on the eastern slope of Gongga mountain. The climate of the experimental site is characterised by an annual mean temperature of 3.8^{o}C (-4.3^{o}C in January and 11.9^{o}C in July), an annual mean precipitation of 1,940 mm (most of which occurs from June to September), transpiration of 1,579 mm, and an mean annual air relative humidity of 90% (data collected from automatic meteorological station in the Alpine Ecosystem Observation

and Experiment Station of the Gongga Mountain). The soil of the study site is classified as typical mountain dark-brown earth with a high sand content and strong permeability, and the vegetation mainly consists of dark coniferous forest dominated by *A. fabri*. Other non-dominant ground vegetation contains *Aacer maximowiczii*, *Sorbus multijuga* and *PopulussimoniiCarr*. Coarse woody debris and deciduous leave are scattered on the forest floor.

The two 20 × 20 m plots were set up in immature and mature *A. fabri* forest stands, respectively. The topography of two plots (30 m apart) is nearly flat and the mean height, diameter at breast height (DBH) and stem density of trees with a DHB >10 cm were measured in immature and mature stands, respectively. Fifteen trees were selected for measurements, which were then pooled into five diameter classes. Sample trees encompassed the range of diameters at the site. The three smallest diameter classes were selected from the immature forest, whereas the other two belonged to the mature forest. All classes contained three trees that were similar in diameter (<3 cm) for repetition (15 trees in total). Detailed characteristics of the sampled tree are shown in Table 1.

Measurements

In this study, a technique called horizontally oriented soil chamber (HOSC) (Xu *et al*. 2000) was applied to measure the CO_2 released by stems. An opaque PVC collar, with an inner diameter of 10.7 cm and a height of 5 cm, was cut to match the approximate curvature of the stem and the other end was cut flat. Then, the custom-built PVC collar was fastened to the south of the stem at a height of 1.3 m,

using 100% silicone sealant 24 h before measurement. Loose bark and moss were carefully removed from the stem surface curved by the PVC collar using a hairbrush, without damaging the underlying cambium before installing the PVC collars. The measuring of volume of the stem-attached collar has been described in details by Xu *et al*. (2000). A cardboard was attached to the stem to measure the stem surface area enclosed by the collar after the experiment.

The E_s was measured for 15 trees every month from May to December 2014, using a portable infrared gas analyzer (LI-6400, Li-Cor Inc., Lincoln, NE, USA) coupled to a soil CO_2 efflux chamber (LI-6400-09). Measurements were conducted for three cycles at each sampling point, every 2 h from 8:00 to 18:00 in a day. Air temperature was obtained from an automatic weather station in the Alpine Ecosystem Observation and Experiment Station of Gongga Mountain.

Sampling

Increment cores of 15 trees were extracted from below the stem-attached collar on 27 September 2014 and two cores from south and north were collected from each tree (30 cores in total). The cores were mounted onto the custom-made board after being air-dried in the laboratory and then sanded with successively finer grades of sandpaper until the annual rings could be clearly distinguished. The tree-ring widths of cores were measured using the image analysis system WinDENDRO (Regent Instruments Inc.) and tree ages were ascertained by tree rings. Furthermore, sapwood width was calculated according to the different colours and their radii.

Sapwood tissue was collected monthly (June to December) by sampling the outer

2 cm of the stem at 1.2 cm from east and west using an increment hammer and the bark was removed. All samples were dried at 60°C for three days and then ground through a 0.2-mm mesh in a Wiley Mill. The tissue nitrogen concentration was measured using an automatic kjeldahl apparatus (Zddn-ii, Top Instrument, CN).

Data analysis

As mentioned in measurement section, customised collars but original ones were used. For convenience, default parameters were applied during measurement. To calculate the actual stem CO_2 efflux rate, we used the following equation (Shi *et al*. 2010):

$$Es = E \times \frac{V_0 + V - \pi \times (\frac{D}{2})^2 \times H}{V_0} \times \frac{A_0}{A} \quad (1)$$

where E_s is actual stem CO_2 efflux rate (μmol m^{-2} s^{-1}), E is measured stem CO_2 efflux rate (μmol m^{-2} s^{-1}), V_0 is the system default volume of the chamber (991 cm^3), V is the volume (cm^3) of the stem-attached collar (cm), H is the length of the stem attached collar (5 cm), D is the diameter of the collar (10.7 cm), A_0 is the system default area of the chamber and A is the stem surface area enclosed by the collar (cm^2).Among the parameters, V and A may vary between each collar assembly.

We fitted the relationship between E_s and air temperature using an exponential equation:

$$Es = \alpha e^{\beta T} \quad (2)$$

where T is air temperature, and α, β are constants.

Q_{10} is the change in rate with a 10℃ change in temperature, which is calculated

as follows:

$$Q_{10} = e^{10\beta} \quad (3)$$

where β is the regression coefficient obtained from the Equation 3.

E_s was also modelled as a function including temperature and N content, as described by Maier *et al*. (1998):

$$Es = (a + bN)e^{cT} \quad (4)$$

where N is the sapwood nitrogen content ($g \cdot kg^{-1}$), and a, b and c are parameters.

A two-way analysis of variance (ANOVA) was used to test differences in E_s followed by Duncan's multiple comparison. All statistical analyses were carried out using SPSS version 19.0 (SPSS IBM, New York, USA).

RESULT

Seasonal variation in E_s

The seasonal change in E_s formed a single peak pattern (Fig. 2a and b). The mean E_s for the growing season (May to October) and the dormant season (November and December) was 1.45 and 0.25 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. The E_s for immature and mature forests showed a similar seasonal trend, but the value for the mature forest was significantly higher than that for the immature forest. The E_s increased from May, and peaked in July, with a value of 1.69 and 2.76 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for immature and mature forests, respectively and then E_s declined dramatically, with the lowest value in December. Consistent with diurnal changes, the seasonal E_s trend was correlated with the mean monthly temperature, with both maxima and minima occurring in July

and December.

Relationship of E_s, Q_{10} to temperature and DBH

The E_s was exponentially dependent on T_a in five diameter classes, and T_a explained 69.8–89.0% of the variation in E_s (Table 3). Relatively, E_s and T_a in immature forest showed stronger correlation than in mature forest. According to the ANCOVA in Table 4, the month and diameter class significantly affected the E_s of *A. fabri*. Figure 4 illustrates the relationship between E_s (per surface area and per sapwood volume) and DBH and sapwood width (SW). Area-based E_s showed positive correlation to DBH and SW with R^2 0.404 and 0.478, respectively. Volume-based E_s showed negative correlation to DBH and SW with R^2 0.521 and 0.347, respectively. In addition, the SW was linearly dependent on T_a (Fig. 5).

The stem Q_{10} for E_s in the five diameter classes during the growing and dormant seasons was calculated and is shown in Figure 3. The Q_{10} ranged from 1.99 to 4.27 in the growing season, and from 3.05 to 5.02 in the dormant season. Except for D_3, the Q_{10} was generally higher in the dormant season than in the growing season. The mean Q_{10} of immature (D_1 to D_3) and mature (D_4 and D_5) forests during the growing season was 3.36 and 2.31, respectively. But for the dormant season, the mean Q_{10} in the mature forest (D_4 and D_5) reached a higher Q_{10} with 5.02 than in the immature forest (D_1 to D_3) with 3.91.

Relationship between [N] and E_s

There were clear seasonal changes in the [N] of *A. fabri* (Fig. 6). Except for D_2,

the [N] in the stems of other diameter classes showed a similar trend, with the concentration increasing from June and peaking in July for D_3 to D_5 and in August for D_1. The concentration then declined and reached the lowest value in December. In addition, the [N] in D_2 decreased continuously from June to December. The [N] in five diameter classes varied from 1.10 to 1.77 $g \cdot kg^{-1}$ during the growing and dormant seasons.

For the forests, E_s based on surface area or sapwood volume was significantly correlated with [N] (Fig. 7a and b). Regardless of the expressing unit, R^2 between E_s and [N] was invariably higher in the mature forest than in the immature forest. In the immature forest, R^2 improved when E_s was expressed per unit sapwood volume instead of based on surface area and decreased from 0.405 to 0.389; for the mature forest, the opposite result was obtained.

The temperature-nitrogen model was used to predict E_s (Table 4). In D_2 and D_4, adding nitrogen to the exponential model improved the R^2 compared to using temperature alone for both expression units of E_s. However, the predictions from the single-factor model containing air temperature showed a higher correlation with the temperature-nitrogen model for D_1, D_3 and D_5.

DISCUSSION

Seasonal variation in E_s

Seasonal variation in E_s has been reported in many studies (Ceschia *et al.* 2002; Vose and Ryan 2002; Acosta *et al.* 2008; Stahl *et al.* 2011), reflecting the physiological

adaptation of trees to seasonal changes in temperature and metabolic activity (Zha *et al.* 2004). In this study, typical seasonal variations in E_s were found in both forests (Fig. 2a and b). The E_s declined dramatically from August to December after peaking in July and was strongly associated with fluctuations in T_a. This phenomenon indicates that variations in E_s are strongly influenced by seasonal changes in T_a. However, seasonal variations in plant E_s could not solely be explained by temperature (Damesin *et al.* 2002; Zha *et al.* 2004; Yang *et al.* 2014). According to the functional model (Amthor 1989), total respiration is partitioned into maintenance and growth components, and maintenance respiration varies primarily with temperature fluctuation (Lavigne and Ryan 1997). Nonetheless, growth respiration is mainly controlled by plant growth, implying that plant physiological activity (such as leave photosynthesis and vascular cambium activity) can also affect seasonal dynamic changes in E_s (Edwards *et al.* 2002; Maseyk *et al.* 2008). During the study period, the E_s values decreased 32.2 %~70.9 % from August to September, while the T_a dropped little (Fig. 2a and b). This may attribute to the tree seasonal physiological activity. Because of the changes in phenology that leaves turned color in September (Table 2), *A. fabri* significantly decreased the E_s to adapt itself to the physiological activity before dormant season.

During growing season, the magnitude of measured E_s values (0.58–1.69 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the immature forest; 0.96–2.76 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the mature forest) were significantly lower than the studies for *Pinus ponderosa* (3.5–7.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$; Xu *et al.* 2000) and for *Picea abies* (0.34–6.52 $\mu mol \cdot m^{-2} \cdot s^{-1}$; Acosta *et al.* 2008). One

possible explanation for this phenomenon is the low-temperature environment of the experimental site in the subalpine region. Zach *et al.* (2008) and Robertson *et al.* (2010) conducted experiments to measure E_s across elevation transects, and both observed significant decreases in E_s with increasing elevation. They assumed that adaptation in respiratory depression allows trees to meet energy requirement in low temperature environment. This study was conducted at the frigid dark coniferous forests (3,000 m a.s.l.), with a mean T_a of 10^{o}C for the growing season of *A. fabri*. Low temperature obviously limits the physiological activity and carbohydrate metabolism in *A. fabri*, keeping maintenance respiration at a low-level and limiting the E_s (Cavieres *et al.* 2000).

Response of E_s to temperature

Temperature plays a pivotal role in the regulation of E_s (Amthor 1994), because it affects enzymatic activity, the availability of substrates and adenylates (Atkin and Tjoelker 2003; Bowman *et al*. 2008), and the velocity of the xylem sap flow (Levy *et al*. 1999). Our results suggest that more than 69% of seasonal variation in E_s could be explained by T_a (Table 3). Similarly, Maier (1998) documented that cambium temperature accounted for 61% of the variation in E_s in the dormant season for young loblolly pine. Zha (2004) observed the annual E_s in Scots pine for three years and found that stem temperature could explain >70% of the variation in E_s. Additionally, higher correlation was found between E_s and T_a for immature forests than for mature forests. This may due to the inertia of thick bark and large body of bigger trees to air temperature fluctuation. That is, stems of small trees gain and lose heat faster than

larger trees (Damesin *et al.* 2002).

For *Abies* and *Pinus* species, Q_{10} values were observed close to a constant value near 2.0 (Damesin *et al.* 2002). However, in this study, our estimated Q_{10} values (2.98–5.61; Table 2) were substantially higher than previous studies (Lavigne *et al.* 1996; Stockfors and Linder 1998; Vose and Ryan 2002; Acosta *et al.* 2008). Presumably, the thermal acclimation of plant respiration to temperature may lead to the high value. According to the literature analysis by Tjoelker (2001), mean Q_{10} values appeared 2.14 for tropical, 2.26 for temperate, 2.20 for boreal, and 2.56 for arctic biomes. Apparently, the Q_{10} values of plant growing in cold regions are higher than those of warm-grown plants. As a consequence, cold environment of the subalpine region (with a mean annual temperature of only 3.8^{o}C) may lead to the high Q_{10} in *A. fabri*. In addition, higher Q_{10} values were observed during the cold season (except for D_3), which was in agreement with reports by Carey *et al.* (1997) and Lavigne and Ryan (1997) and Acosta *et al.* (2008). Atkin (2005) concluded that the two assumptions: 1) plant respiration increases exponentially with an increase in temperature under a constant Q_{10} value and 2) respiration shows no acclimation to long-term changes in temperature, might be incorrect. Also, some researchers (Carey *et al.* 1997; Atkin and Tjoelker 2003; King *et al.* 2006) suggested that the temperature sensitivity of plants can fluctuate seasonally and will increase as the temperature decreases. Based on theory above and observations that higher Q_{10} values were observed in the cold season and region, we conclude that the first hypothesize (E_s in trees has thermal acclimation) was supported by our results.

Given that Q_{10} is a critical parameter in estimating ecosystem and global carbon balance (Zha *et al.* 2004; Kim *et al.* 2007; Yang *et al.* 2014), there has been considerable attention devoted to accurate understanding the variability in the temperature response of plant respiration (King *et al*. 2006; Tjoelker *et al*. 2001, 2008). To date, the thermal acclimation of plant respiration to temperature has been observed for soil and root (Bryla *et al.* 1997; Atkin *et al*. 2000; Peng *et al.* 2009), leaf (Larigauderie and Körner, 1995; Tjoelker *et al.* 1999) and stem (Carey *et al.* 1997; Brito *et al.* 2010; Yang *et al.* 2012b). If so, still defining Q_{10} values as a constant value is likely to result in overestimating of plant respiration under global warming. Further study should focus on how to take into account the degree and speed of thermal acclimation of respiration, when modelling E_s at a range of scales from individual level to global carbon cycle (Atkin *et al.* 2005).

Response of E_s to DBH

It is generally acknowledged that respiring cells are the most likely local source of the CO_2 we measured (Teskey *et al.* 2008), and sapwood xylem parenchyma contributes the most respiring cells among stem tissues (Maier and Clinton 2006; Saveyn *et al.* 2008; Rodríguez-Calcerrada *et al.* 2015). Also, the proportion of live cells in the xylem was found to vary with tree size (Teskey *et al.* 2008). Because of this, researchers should pay much attention to stem diameters when focusing on the intraspecific variations of E_s. For tropical and temperate forests, correlations between E_s and DBH have been reported in some studies (Lavigne *et al.* 1996; Lavigne and Ryan 1997; Levy and Jarvis 1998; Cavaleri *et al.* 2006; Kim *et al.* 2007; Zach *et al.*

2008; Robertson *et al.* 2010; Yang *et al.* 2012a). However, much uncertainty exists in the relationship between E_s and DBH among these studies. Based on the measurement of *Fagus sylvatica* and *Pinus cembra*, Damesin *et al.* (2002) and Wieser and Bahn (2004) suggested that the inconsistency among studies was relevant to the expression unit of E_s. For example, the area-based E_s for *Pinus densiflora* showed no obvious fluctuation with increasing diameters, while volume-based efflux increased with DBH enlarging (Kim *et al.* 2007). This is confirmed by our results that there was a positive correlation between area-based E_s and DBH, but a negative correlation when E_s expressed on volume (Fig. 4a and c). Additionally, several studies indicated that most living cells appeared in the sapwood and close correlations existed between sapwood indices and DBH (Ryan 1990; McGuire *et al.* 2007; Moore *et al*. 2008). In accordance with these observations, our area-based E_s showed higher correlation with sapwood width (R^2=0.478; Fig. 4b) than DBH (R^2=0.407; Fig. 4c) and increase in sapwood width was well coincided with increases in DBH (Fig. 5). Combined with these findings, we suggest that the variability among trees is mainly affected by sapwood width but DBH and the proportion of sapwood tissue in stem may determine the correlation between E_s and DBH.

Although several expression units have been utilized to represent the E_s and scale estimates to the stand level, there is no generally acceptance of expressing basis for E_s (Bowman *et al.* 2005). This may attribute to large differences in the proportion of respiratory activity in woody tissues within trees and between stands (Bowman *et al.* 2008). For instance, Stockfors and Linder (1998) found surface area was the best

unit for expressing of *Picea abies* on account of the largest concentration of living cells in outer stem. While, for *Platanus occidentalis* L. (McGuire *et al.* 2007), volume was preferable over surface area when calculating E_s, because sapwood is observed proportional to the amount of living cells. In the current experiment, we conclude that volume-based E_s is best-suited for scaling E_s from sample measurements to the whole stem or stand level. A stronger relationship between DBH and volume-based E_s than area-based is found in the study (Fig. 4a and c), suggesting that the volume component is more important than surface area in the stem tissues. As a consequence, applying volume as a basis of *A.fabri* for extrapolating E_s to the stand-level can reduce the estimating errors on account of xylem anatomical characters and ontogeny (Wieser and Bahn 2004).

Relationship between E_s and [N]

The second hypothesis, that E_s will be well correlated with DBH and [N], was also confirmed by our study (the relationship between E_s and DBH was already discussed above). Similar results on relationship between E_s and [N] have been reported for *Pinus taeda* by Maier (1998; 2001) and for *Pinus strobes* by Vose and Ryan (2002). As the most biochemically relevant element in relation to plant respiration (Amthor, 1989), N is recognized to play an important role in respiratory processes of living tissues (Amthor 1989; Ryan 1991; Ryan *et al.* 1994). Maier (2001) suggested that seasonal variations in [N] may result in changes in maintenance respiration because protein metabolic activity contributes most maintenance respiration. In this study, the temporal variation in [N] coincided with seasonal

fluctuation in E_s (Fig. 6) and stem [N] explained 29.5 to 40.5 % of the seasonal variation in E_s. This phenomenon implies that trees can adjust the demand for nitrogen in stems in response to changes in the plant phenology and external environment (Tjoelker *et al.* 2008), and temporal variations in [N] may affect the seasonal pattern of E_s independently of temperature (Maier 2001). In addition, larger trees had more sapwood nitrogen (Fig. 6), suggesting that dominant trees tend to out compete smaller trees for nutrients (Vose and Ryan, 2002). However, in other studies (Lavigne and Ryan 1997; Bowman et al. 2005), no significant effect of nitrogen in living cells on E_s was found for aspen, black spruce, jack pine and *Dacrydium cupressinum* in dormant season. We speculate the inconsistency between studies is probably associated with the low [N] in these tress relative to other tree species.

Several researchers recommend [N] as a predictable variable in models for simulating and scaling up E_s (Ryan 1991; Ryan *et al.* 1996; Stockfors *et al.* 1998; Maier 2001; Reich *et al.* 2008), because nitrogen was an integral element in many plant physiological and a N-based model could establish direct contact with photosynthesis, carbon allocation, fine root turnover and decomposition (Ryan *et al*. 1995). Additionally, Lee *et al.* (2005) suggested that nitrogen concentrations in tissues may contain thermal effects, that is, temperature variation could induce changes in [N] to affect respiration rates indirectly. Some other studies showed that temperature acclimation may be associated with variation in tissue [N] (Tjoelker *et al.* 1999, 2008; Reich *et al.* 2008), and changes in [N] can help explain the acclimation of respiration to temperature. Thus, a mixed-effected model involving T_a and [N] is

essential in estimate of E_s. However, in this study, the pronounced double-factor model (involving T_a and [N] both) did not significantly improve R^2 in comparison with the exponential model involving T_a only (Table 4). Probably nitrogen concentration in other specific organs, such as leaf and root, can further contribute to the improvement in prediction because of closely links between E_s and leaf photosynthesis and root turnover. Nevertheless, the double-factor model represents a novel method that involving biological factor to model carbon cycling on different scales, especially when accounting for the adaptation and acclimation of respiration in response to temperature (Tjoelker *et al.* 2008).

CONCLUSION

This study demonstrated that E_s varied markedly with the season. This variation might be the result of seasonal change in environmental temperature and phenology. The E_s value in different stem diameter classes increased exponentially with an increase in air temperature and this relationship was stronger for trees with a small diameter. The temperature response was affected by seasonal variation and DBH. The Q_{10} was higher in the dormant-season and differed among different diameter classes when expressed per surface area and sapwood volume. Our results suggest that *A. fabri* might have evolved thermal acclimation to cooler environments in subalpine regions. The [N] in stems exhibited a similar seasonal trend as air temperature. The exponential model that considered air temperature only or including air temperature and [N] simultaneously as independent variables, can be preferentially used to predict E_s in the stem.

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Table 1: Basic characteristics of the sampled trees.

Diameter	Number	DBH (cm)	Sapwood width (cm)	Tree ages	Stand
D_1 (10~20 cm)	Tree 1	14.51	3.7	47	immature
	Tree 2	14.83	3.8	44	immature
	Tree 3	15.92	3.7	42	immature
D_2 (20~30 cm)	Tree 4	23.36	5.3	72	immature
	Tree 5	24.29	6.3	46	immature
	Tree 6	25.94	5.2	40	immature
D_3 (30~40 cm)	Tree 7	32.53	6.2	49	immature
	Tree 8	33.80	5.2	46	immature
	Tree 9	34.38	6.5	47	immature
D_4 (65~75 cm)	Tree 10	67.48	11.0	211	mature
	Tree 11	69.39	9.3	212	mature
	Tree 12	71.94	8.6	194	mature
D_5 (80~90 cm)	Tree 13	84.67	10.0	219	mature
	Tree 14	84.67	8.9	228	mature
	Tree 15	85.94	8.8	238	mature

Table 2: Phenological phase of *A. fabri* in the experimental field.

Phenological period	Bud burst	Leaf expansion	Flowering	Maturity period	First leaf colouring	Defoliating period
Beginning time	1 May 2014	7 May 2014	19 May 2014	2 Jun 2014	20 Sep 2014	10 Oct 2014

Table 3: Fitted equations for Es per surface area (μmol·m-2·s-1) and air temperature of A. fabri.

Each Es is the mean value of three measured cycles during the day.

Diameter class	Fitted equations	Coefficient of determination	Significance level	Temperature coefficient(Q_{10})	Sample size
D_1	$y = 0.162e^{0.1092x}$	0.869	$p < 0.01$	2.98	162
D_2	$y = 0.293e^{0.1535x}$	0.890	$p < 0.01$	4.64	150
D_3	$y = 0.236e^{0.1725x}$	0.865	$p < 0.01$	5.61	150
D_4	$y = 0.357e^{0.1362x}$	0.698	$p < 0.01$	3.90	144
D_5	$y = 0.364e^{0.1125x}$	0.768	$p < 0.01$	3.08	144

Table 4: ANCOVA test for the effect of month and diameter class on the Es of *A. fabri.*

Source	Degrees of freedom	Mean squares	Mean squares	*F value*	*P value*
Diameter class	4	18.65	4.66	27.58**	<0.01
Month	7	48.05	6.86	40.61**	<0.01
Error	108	18.26	0.17		
Total variation	119	84.95			

Note：** Significant difference at $p < 0.01$, *; Significant difference at $p < 0.05$.

Table 5: Nonlinear regression models of *Es* against [N] ($g \cdot kg^{-1}$) and air temperature (^{o}C) for Equation 4.

	Es expressed as surface area					*Es* expressed as sapwood volume				
	a	*b*	*c*	R^2	*n*	*a*	*b*	*c*	R^2	*n*
D_1	0.178	-0.001	0.101	0.846	21	6.828	-0.153	1.02	0.843	21
D_2	0.533	-0.063	0.128	0.898	21	14.298	-2.874	0.134	0.901	21
D_3	0.290	0.093	0.106	0.791	21	7.391	1.266	0.103	0.709	21
D_4	0.747	-0.127	0.114	0.830	21	9.267	-1.614	0.114	0.797	21
D_5	0.594	-0.153	0.124	0.707	21	7.325	-1.835	0.123	0.675	21

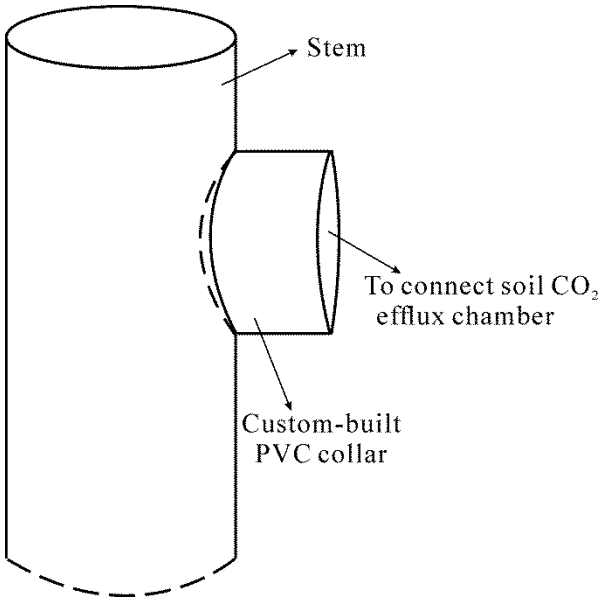

Figure 1: The schematic diagram of custom-built PVC collar attached on the stem for *Es* measurement

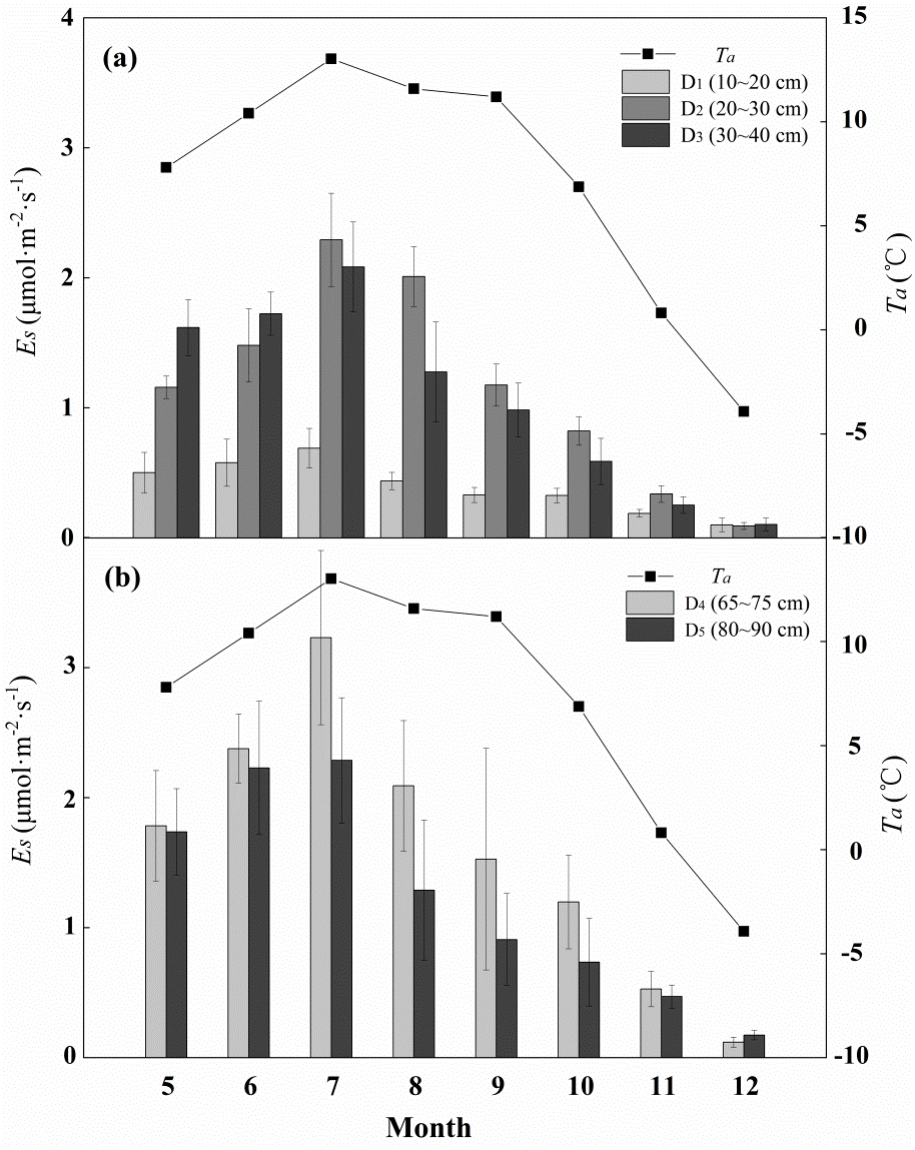

Figure 2: Seasonal changes in *Es* of (a) immature forest and (b) mature forest of *A. fabri*. Values of *Es* are means (± SE) from three trees in the same diameter classes and data for each month for individual tree is the mean of six measured values

at the same time of day (every 2 h once). The diamonds (T_a) represent the monthly mean air temperature.

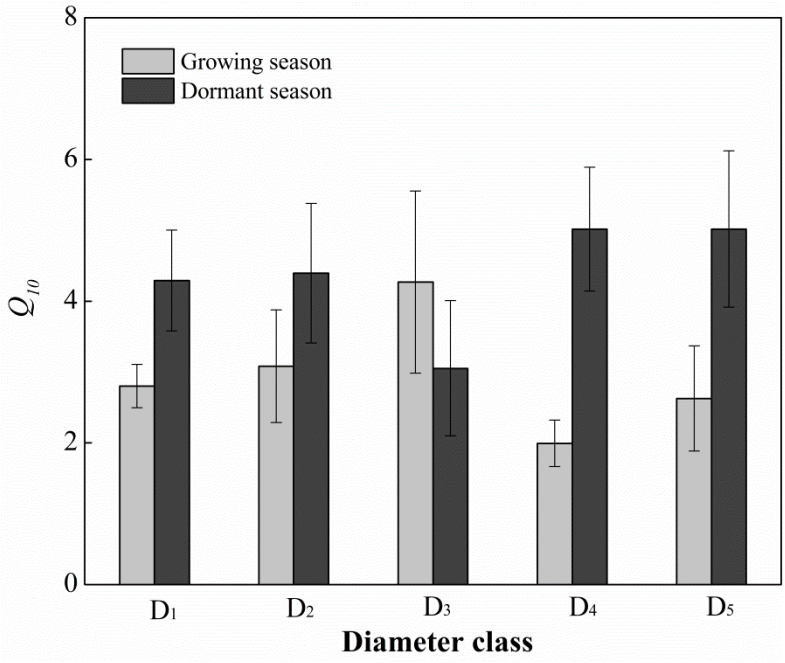

Figure 3: Mean Q_{10} in different diameter classes of *A. fabri* for the growing season and dormant season. Values are means (± SE) for three trees in the same diameter classes. The growing season was from May to September and the dormant season from November to December.

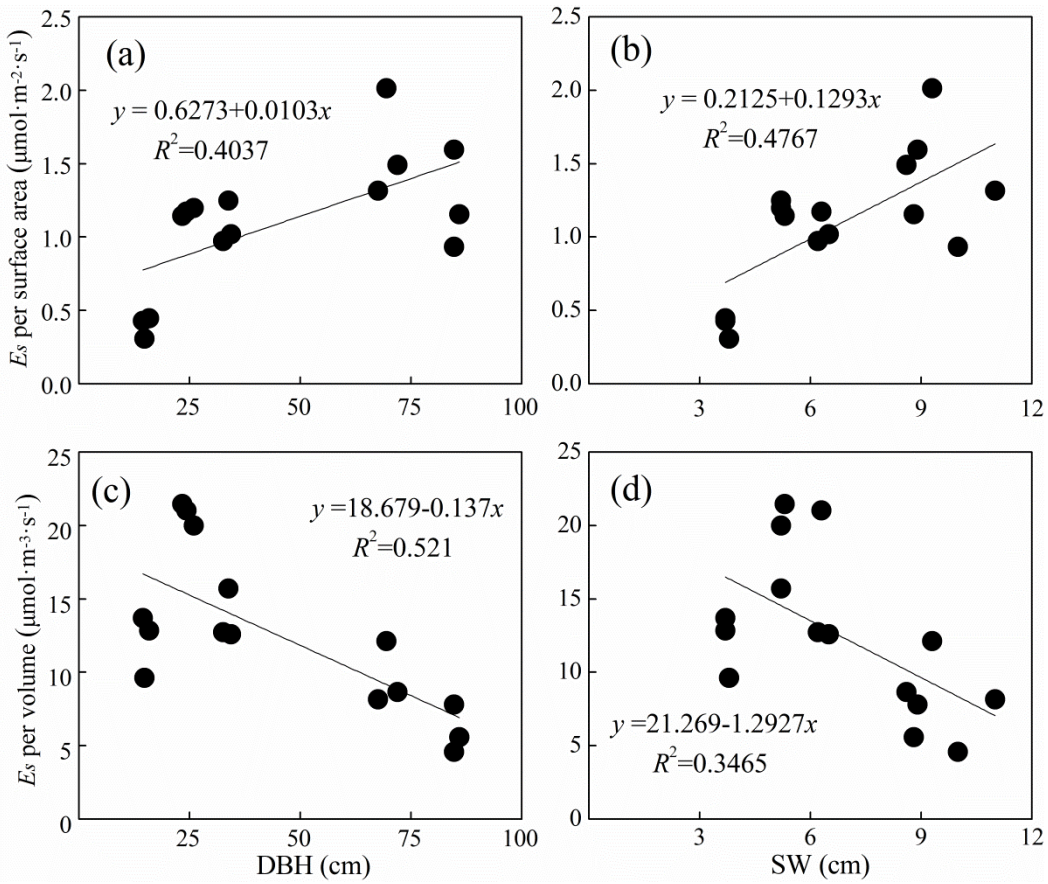

Figure 4: Relationship of E_S to diameter at breast height (DBH) and sapwood width (SW). (a) and (b) show the relationship

between area-based *Es* and DBH and SW, respectively. (c) and (d) show the relationship between volume-based *Es* and DBH and SW, respectively. Each point represents the mean *Es* of an individual tree over the study period

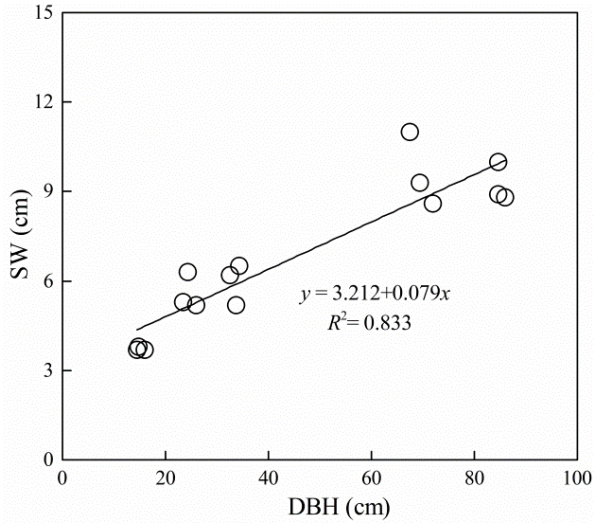

Figure 5: Relationship between sapwood width (SW) and diameter at breast height (DBH).

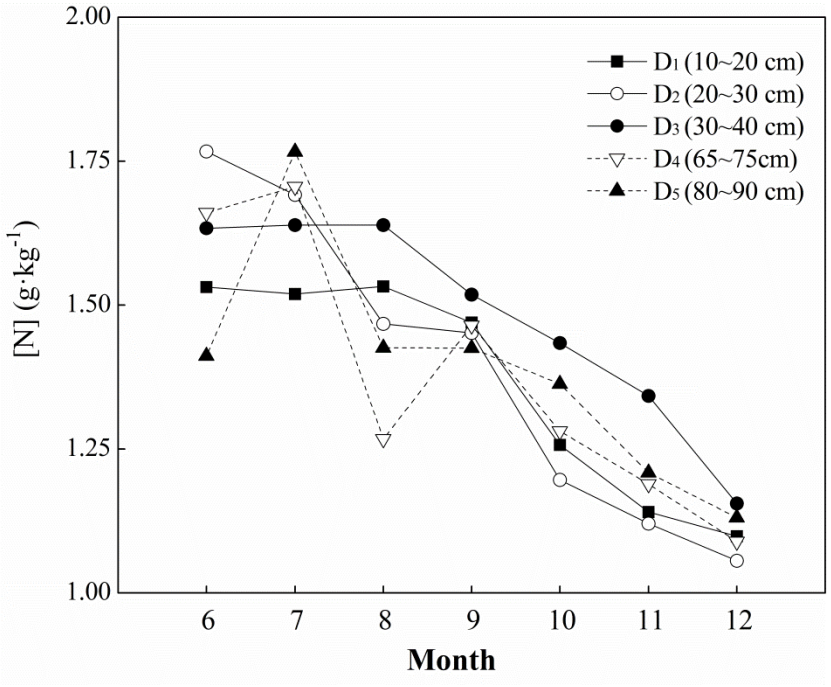

Figure 6: Seasonal variations in the sapwood nitrogen content of *A. fabri*. Values are means for three dates for each diameter class. Data in May are omitted due to a failure in sampling.

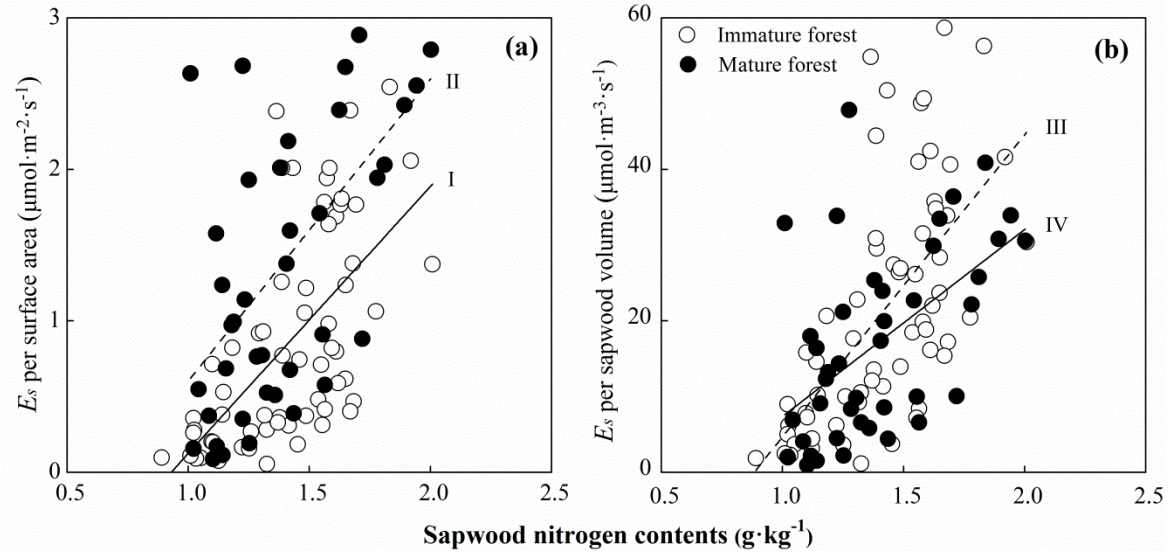

Figure 7: Relationship between [N] and (a) *Es* per stem surface; (b) *Es* per sapwood volume. Linear regression models significant at $p < 0.05$ are depicted for all pooled data: (I) $y = 1.764x\text{-}1.639$, $R^2 = 0.389$, $n = 64$ (Immature forest); (II) $y = 1.994x\text{-}1.393$, $R^2 = 0.303$, $n = 42$ (Mature forest); (III) $y = 24.68x\text{-}17.29$, $R^2 = 0.405$, $n = 64$ (Immature forest); (IV) $y = 39.918x\text{-}35.25$, $R^2 = 0.295$, $n = 42$ (Mature forest).