

Effects of sample size on sap flux-based stand-scale transpiration estimates

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Summary In this study, we aimed to assess how sample sizes affect confidence of stand-scale transpiration (E) estimates calculated from sap flux (F_d) and sapwood area (A_{S_tree}) measurements of individual trees. In a Japanese cypress plantation, we measured F_d and A_{S_tree} in all trees ($n = 58$) within a 20×20 m study plot, which was divided into four 10×10 m subplots. We calculated E from stand A_{S_tree} (A_{S_stand}) and mean stand F_d (J_S) values. Using Monte Carlo analyses, we examined the potential errors associated with sample sizes in E , A_{S_stand} and J_S using the original A_{S_tree} and F_d data sets. Consequently, we defined the optimal sample sizes of 10 and 15 for A_{S_stand} and J_S estimates, respectively, in the 20×20 m plot. Sample sizes larger than the optimal sample sizes did not decrease potential errors. The optimal sample sizes for J_S changed according to plot size (e.g., 10×10 and 10×20 m), whereas the optimal sample sizes for A_{S_stand} did not. As well, the optimal sample sizes for J_S did not change in different vapor pressure deficit conditions. In terms of E estimates, these results suggest that the tree-to-tree variations in F_d vary among different plots, and that plot size to capture tree-to-tree variations in F_d is an important factor. The sample sizes determined in this study will be helpful for planning the balanced sampling designs to extrapolate stand-scale estimates to catchment-scale estimates.

Keywords: granier-type sensor, Monte Carlo sampling, sap flow, sapwood area, scaling procedures.

Introduction

To quantify water use in forests, several methods are available for assessing spatial and temporal variations in water use. The sap flux measurement technique is the most promising, especially in a mountainous country, because this

technique is not limited by complex terrain and spatial heterogeneity (e.g., Wilson et al. 2001). Previously, three spatial levels of scaling had been used to obtain bottom-up transpiration estimates based on the sap flux technique: from within-tree to tree (Phillips et al. 1996, Vertessy et al. 1997, Lu et al. 2000, Delzon et al. 2004, Tateishi et al. 2008), from tree to stand (Hatton et al. 1995, Schäfer et al. 2002, Delzon and Loustau 2005) and from stand to catchment or landscape (e.g., Wilson et al. 2001, Ford et al. 2007, Kumagai et al. 2007). Although there is a considerable variation that must be taken into account at each scaling step, variations in transpiration among different stands could be a major source of error for catchment- or landscape-scale transpiration estimates in terrestrial ecosystems (Mackay et al. 2002, Williams et al. 2004, Ford et al. 2007). This suggests that sap flux measurements in several stands within a catchment or landscape are required for catchment-scale or landscape-scale estimates. Thus, efficient procedures for stand-scale transpiration estimates are important to assess water use within forested catchments or landscapes.

To extrapolate individual tree-scale sap flux measurements to stand-scale estimates, the following scaling procedures are required: (1) estimation of the total sapwood area of the stand (A_{S_stand}); (2) estimation of mean stand sap flux (J_S); and (3) calculation of stand transpiration as the products of A_{S_stand} and J_S (e.g., Granier et al. 1996, Pataki and Oren 2003). Thus, scaling procedures need to account for two sources of errors, that is, determination of A_{S_stand} and J_S . Previously, Oren et al. (1998a) showed that different sample sizes were required to estimate J_S in different forest types. This suggests that optimal sample sizes vary with stand conditions and types. Furthermore, recent studies reported the spatial variations in tree transpiration with changing atmospheric conditions (Adelman et al. 2008, Loranty et al. 2008), suggesting that the sample size required to estimate J_S could change with environmental conditions.

Table 1. List of abbreviations and definitions.

Abbreviation	Definition
A_G	Ground area
A_{S_stand}	Stand sapwood area
A_{S_tree}	Individual sapwood area
CV	Coefficient of variation
dCV/dn	Changes in CV in accordance with n
DBH	Diameter of breast height
dT_{max}	Maximum temperature difference between the two proves
E	Stand-scale transpiration
$\Delta E/E$	Potential errors in E estimate due to sample sizes
F_d	Sap flux
F_{d_ave}	Tree-specific sap flux average over sapwood area (= Q/A_{S_tree})
J_S	Mean stand sap flux
LAI	Leaf area index
n	Sample size
PDF	Probability density function
Q	Whole-tree sap flux
R_s	Solar radiation
SD	Standard deviation
VPD	Vapor pressure deficit

In some previous studies, A_{S_stand} values have been estimated from sapwood area (A_{S_tree}) measurements done on a large number of sample trees (> 60 trees) (Wullschleger and King 2000, Roberts et al. 2001). A_{S_stand} values are usually determined in about 5–20 individuals (Vertessy et al. 1995, Cienciala et al. 2000, Wilson et al. 2001). In addition, J_S values have been estimated from sap flux measurements of < 15 individual trees (Cienciala et al. 2000, Granier et al. 2000, Vertessy et al. 2001, Kume et al. 2007, Kume et al. 2008a). Assessing how sample size impacts stand-scale estimation will improve our understanding of errors and uncertainties in estimations and will enable the design of effective sampling methods for sap flux-based transpiration estimates. Some potential errors in stand-scale transpiration estimates that may result from sample size have been described previously (Čermák et al. 1995, Hatton et al. 1995, Oren et al. 1998a, 1998b, Kumagai et al. 2005a, 2005b). However, these studies did not examine the sample size required to estimate J_S and A_{S_stand} at the same site simultaneously. Furthermore, there is little information on how stand properties and atmospheric conditions affect the potential errors resulting from sample sizes.

In this study, we aimed to define an optimal and effective sampling design for stand-scale transpiration estimates calculated from sap flux measurements. To do this, we examined how sample sizes for A_{S_stand} and J_S impact on stand-scale transpiration estimates. Also, we examined whether the estimation errors due to sample sizes change with the variations in stand conditions and atmospheric conditions. In this study, we conducted sap flux and sapwood area measurements for all trees ($n = 58$) in a

20 × 20 m study plot in a plantation of Japanese cypress, which is one of the most dominant plantation species in Japan. Based on the assumption that stand-scale transpiration was accurately determined from the tree measurements, we evaluated the impacts of tree-to-tree variations in A_{S_tree} and sap flux (F_d) on stand-scale transpiration estimates using Monte Carlo analyses of the original data sets. This analysis predicted how many samples are required to account for tree-to-tree variations.

Materials and methods

Site

This study was conducted in the Ochozu Experimental Watershed (OEW) in the Kasuya Research Forest, Kyushu University. This is a mountainous watershed located about 15 km east of Fukuoka City in western Japan (33°38' N and 130°32' E; Figure 1). A mountainous stream at this site flows into an enclosed sea area, Hakata Bay, via the Tataru River. The watershed area is 9.5 ha, the main stream is 265 m long and the main catchment is 358 m wide. The mean stream gradient (tangent) is 0.22 and the mean slope gradient (tangent) is 0.37. The mean air temperature recorded between 1995 and 2005 at the nearest Meteorological observatory in the city of Fukuoka, 15 km from the study site, was about 16 °C and the mean annual precipitation was 1790 mm.

About 46% of the OEW is covered in a Japanese cypress (*Chamaecyparis obtusa*) plantation. This forest was established in 1957 along a stream channel. The continuous canopy layer in this forest is at about 15 m. The tree density is about 1900 trees ha⁻¹. The plantation is not managed, i.e., the trees have not been pruned or thinned since 1993. Thus, the canopy of the plantation forest is closed with a mean canopy openness of 6–8% (Miyazawa et al. 2008), and the forest floor is considerably exposed as there is scarce

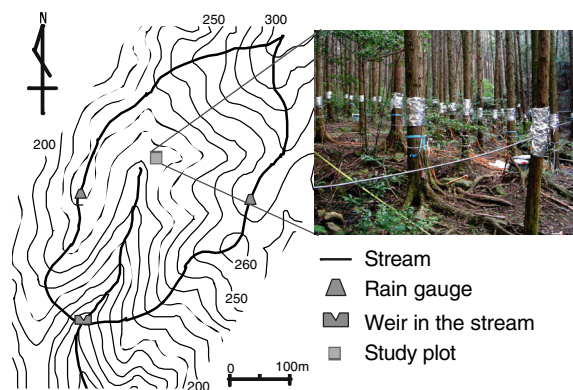


Figure 1. Location of the OEW, Kyushu, Japan. This figure appears in color in the online version of *Tree Physiology*.

vegetation cover in the riparian area (Ide et al. 2008). The forest soil is yellow-brown soil with medium porosity (40–50%) originated from serpentinite and chlorite schist, and the soil texture is clay loam (Kume et al. 2008b).

Meteorological stations were installed on the western and eastern ridges (Figure 1). At each site, solar radiation (R_s) was measured using a solar radiometer (LI-200, Li-Cor Inc., Lincoln, NE), and the air temperature and humidity were measured using a thermohygrograph (HMP45A, Vaisala, Helsinki, Finland). Samples were taken every 10 s and stored in data loggers (CR10X, Campbell Scientific Inc., Logan) at 10-min intervals.

We established a 20 × 20 m plot consisting of four 10 × 10 m subplots in the center of the OEW (Figure 1). The plot has a relatively gentle slope (< 10°) and is in a riparian area in which the groundwater table is located at a soil depth of 20–50 cm throughout the year (Higashi et al. 2005). The study plot contained 60 Japanese cypress trees, but two individuals were seriously damaged during a typhoon that occurred within the study period (September and October, 2006). The leaf area index (LAI) was $3.24 \pm 0.56 \text{ m}^2 \text{ m}^{-2}$ with small seasonal variations (Miyazawa et al. 2008). The stand conditions in the study plots are shown in Table 2. Our detailed sap flux measurements were done during the growing season between September and October, 2006. Here, the growing season is defined as the period that begins when the temperature exceeds 5 °C on five consecutive days and ends after temperatures drop below 5 °C on five consecutive days. At this site, the growing period is typically from March to November. In the study period, the mean temperature, vapor pressure deficit (VPD) and R_s were 21.0 °C, 6.6 hPa and 13.7 MJ d⁻¹, respectively. These values were similar to the mean values of the 2003–2006 growing seasons (temperature, 22 °C; VPD, 8 hPa; and R_s , 15 MJ d⁻¹; Shinohara et al. 2008).

Sapwood analysis

Sapwood thickness was measured in 58 individuals using a ruler on a core extracted with a 5-mm increment borer at 1.3 m above the ground and assessed as the mean of two orthogonal measurements. Distinct color differences were used to identify the boundary between sapwood and heartwood. The individual sapwood area (A_{S_tree}) was obtained from the difference between the heartwood area and the stem cross-sectional area beneath the bark, and we assumed that the stem cross-sections were circular.

Sap flow measurements

The sap flux measurements were conducted with the thermal dissipation method using Granier-type sensors (e.g., Granier 1987). Each sensor consisted of a pair of probes 20 mm in length and 2 mm in diameter, which were inserted into the sapwood about 150 mm apart. The upper probe supplies power at 0.2 W and heats the sapwood, whereas the lower probe represents the sapwood temperature. The heat dissipates into the sapwood and gives an estimate of vertical sap flux surrounding the probe. All signal cables were connected to double-shielded cable wires, and each of them was differentially connected to a data logger with peripheral multiplexers (CR10X and AM16/32, Campbell Scientific Inc.). Measurements were done every 30 s, and 30-min averages were recorded. The recorded temperature difference between the two probes was converted into sap flux (F_d) as described by Granier (1987). To account for potential nocturnal transpiration, we used the maximum temperature difference (dT_{max}) measured at night at VPD lower than 0.2 kPa (e.g., Dawson et al. 2007, Oishi et al. 2008).

F_d was measured in all 58 individuals in the study plot. One to two sensors were inserted in each individual at

Table 2. Stand conditions in the 20 × 20 m plot consisting of 10 × 10 m study plots and optimal sample sizes for estimating A_{S_stand} and J_S . Potential errors were determined from Monte Carlo analyses (see Figure 5).

	Stem density (ha ⁻¹)	Mean DBH (cm ²)	Range DBH (cm ²)	Mean height (m)	Mean A_{S_tree} (cm ²)	A_{S_stand}		J_S		$\Delta E/E$ (%)
						n	CV(%)	n	CV	
<i>10 × 10 m plot</i>										
Plot 1	1700	21.2	11.9–28.9	15.7	128.9	10	4.1	15	10.4	14.5
Plot 2	1600	21.9	13.5–32.6	15.8	150.8	10	5.5	15	10.2	15.7
Plot 3	1400	19.9	11.8–29.3	15.1	134.3	10	4.8	20	15.7	20.5
Plot 4	1100	20.6	8.7–27.2	15.5	154.0	10	5.3	10	11.3	16.6
<i>10 × 20 m plot</i>										
Plot 1 + 2	1650	21.5	11.9–32.6	15.7	139.5	10	5.0	15	10.3	15.3
Plot 3 + 4	1300	20.2	8.7–29.3	15.4	143.0	10	5.4	15	14.0	19.4
Plot 1 + 4	1400	21.0	8.7–28.9	15.7	138.7	10	6.0	10	12.0	18.0
Plot 2 + 3	1500	21.0	11.8–29.3	15.5	143.1	10	5.2	15	13.8	19.0
20 × 20 m plot	1450	21.0	8.7–32.6	15.6	141.0	10	5.8	15	12.0	17.8
VPD < 10 hPa	–	–	–	–	–	–	–	15	15.4	21.2
VPD > 10 hPa	–	–	–	–	–	–	–	15	10.4	16.2

depths of 0–20 and 20–40 mm, to cover all the sapwood. These sensors were inserted 150 mm apart around the circumference of the tree, at about 1.3 m height. Sensors were always placed on the north-facing side of the trunk to avoid the sun-exposed side, and the sites where probes were inserted were fully insulated to prevent any direct radiation. There were few natural thermal gradients along the trunk measured (< 0.4 °C) and therefore the data need not have to be corrected.

Tree-specific F_d averaged over sapwood area ($F_{d,ave}$) was calculated from the whole-tree sap flux (Q) divided by A_{S_tree} in each tree. In this study, Q was calculated as the sum of the product of the F_d measured at 0–20-mm depth in individuals with thinner sapwood, and at 0–20 and 20–40-mm depths in individuals with thicker sapwood. In some sample trees, the sapwood thickness was less than the probe length. In such cases, we used the equation of Clearwater et al. (1999) to correct for the underestimation of the sap flux values. We did not measure F_d at multiple orientations within each tree trunk. Oren et al. (1999) reported the significant variations in F_d around the circumference of conifer trees with very regular circular stem and sapwood tissues. However, we assumed that the tree-to-tree variation in F_d was less significant than the circumference variations in F_d in coniferous stands, and that the circumference variation in F_d was included in the tree-to-tree variation in F_d (Kumagai et al. 2005b, 2007). Furthermore, within-tree variations in F_d are a minor source of error for sap flux-based transpiration estimates at the catchment scale (Ford et al. 2007).

Method of analysis

Individual tree-scale measurements can be scaled up to stand scale using the following equation (Granier et al. 1996, Wilson et al. 2001, Wullschlegler et al. 2001, Pataki and Oren 2003):

$$E = J_S \cdot \frac{A_{S_stand}}{A_G}, \quad (1)$$

in which

$$A_{S_stand} = \sum_{i=1}^{58} A_{S_tree_i} = \sum_{i=1}^{58} (b \cdot DBH_i + a), \quad (2)$$

$$J_S = \frac{\sum_{i=1}^n F_{d,ave_i} \cdot A_{S_tree_i}}{\sum_{i=1}^n A_{S_tree_i}}, \quad (3)$$

where E is the stand-scale transpiration, J_S is the mean stand sap flux, A_{S_stand} is the stand sapwood area, A_G is the ground area, A_{S_tree} is the individual sapwood area, DBH is the diameter at breast height, a and b are the fitted parameters and n is the number of samples. Note that both A_{S_stand} and J_S esti-

mates generate errors in E estimates, and that the fitted parameters (a and b) for A_{S_stand} and the J_S are usually derived from a limited number of samples. Furthermore, DBH can be measured in all individuals in a stand.

To determine how many trees are needed to calculate the fitted parameters (a and b) for A_{S_stand} and J_S estimates, respectively, we used the unstratified Monte Carlo sampling technique. We calculated A_{S_stand} and J_S estimation errors caused by tree-to-tree variations using various sample sizes ($n = 3–58$) as follows: First, samples ($n = 3–58$) were extracted from the original data set of individuals (i.e., DBH and A_{S_tree} in A_{S_stand} or $F_{d,ave}$ in J_S) using the Monte Carlo sampling with replacement as described by Kumagai et al. (2005a, 2005b). The A_{S_stand} and J_S of the extracted samples were calculated using Eqs. (2) and (3), respectively. Applying Eq. (2), the linear regression equation for predicting A_{S_tree} from DBH (i.e., a and b) was determined for each sample size, and A_{S_stand} was then estimated for the given sampling run using the original DBH data set. The linear congruential method was used to generate random numbers. We repeated the above-mentioned step 1000 times, that is, we generated 1000 values of estimated A_{S_stand} and J_S , and then determined the probability density function (PDF) of A_{S_stand} and J_S . Finally, the coefficient of variance (CV) was calculated from the average and the standard deviation (SD) of the PDF. In this study, CV represents the potential estimation errors caused at a given sample size.

Additionally, this study examined the errors in E caused by the potential estimation errors in J_S and A_{S_stand} associated with the sample size. For the moment, we assume that the variance around E estimates associated with sample sizes was given by the combined variance of two categories such as A_{S_stand} and J_S estimates associated with sample sizes, and the total derivative of Eq. (1) is

$$dE = \frac{\partial E}{\partial J_S} dJ_S + \frac{\partial E}{\partial A_{S_stand}} dA_{S_stand}. \quad (4)$$

An approximate form of Eq. (4) can be transformed to the following equation:

$$\frac{\Delta E}{E} = \frac{\Delta J_S}{J_S} + \frac{\Delta A_{S_stand}}{A_{S_stand}}. \quad (5)$$

Equation (5) represents that the errors in E estimates are the multiple of the potential errors in J_S and A_{S_stand} associated with sample size. These analyses were performed using data sets collected on ideal days without rain in the growing season (see Figure 3).

To examine whether the potential errors due to sample sizes change in different stand conditions, we performed Monte Carlo analyses for subplots with different stand densities (i.e., four 10 × 10 m subplots and four 20 × 10 m subplots) (Table 2). To examine whether the potential errors due to sample sizes change with environmental conditions, we performed the Monte Carlo analyses of data collected

from individuals in lower and higher VPD conditions. The threshold was set at a daily mean VPD of 10 hPa and then diurnal variations in $F_{d,ave}$ were averaged in each individual measured at VPD < 10 hPa and VPD > 10 hPa. At this site, long-term measurements based on a limited number of samples ($n = 14$) showed that the daily mean J_S increased linearly with VPD from 0 to 10 hPa, whereas it remained constant at VPD > 10 hPa. This suggests that stomata controlled transpiration at VPD > 10 hPa (Hogg and Hurdle 1997).

Results

Sapwood area

Sapwood thickness was related to DBH ($R^2 = 0.43$, $P < 0.001$) and ranged from 11 to 37 mm, with a mean of 23 mm among 58 individuals. Comparisons between the two different orientation measurements resulted in < 5 mm differences in sapwood thickness in most cases. The mean difference between the two different orientations was 4 mm, which corresponded to 17% of the population mean. $A_{S,tree}$ had a strong linear relationship with DBH (Figure 2; $R^2 = 0.85$, $P < 0.0001$) and ranged from 43.7 to 273.0 cm² (mean: 140.3 cm²).

Although the sapwood area can be estimated from a power function-based regression (Vertessy et al. 1995, Wullschlegel and King 2000), this regression did not significantly strengthen the relationship between $A_{S,tree}$ and DBH because there were no individuals with a DBH smaller than about 10 cm at this site. Using the linear relationship and DBH data from 58 individuals, we estimated $A_{S,stand}$ as 20.4 m² ha⁻¹, which was identical to the measured $A_{S,stand}$ (= 20.4 m² ha⁻¹). In

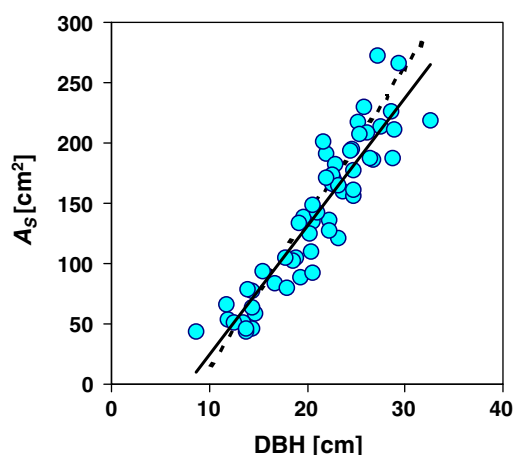


Figure 2. DBH versus sapwood area (A_S) for 58 Japanese cypress trees. Black line represents regression equation derived in this study ($y = 10.7x - 83.6$ ($R^2 = 0.85$)). Dashed line represents regression equation generated from the relationship between DBH and A_S measured in 1226 Japanese cypress by Kumagai et al. (2005a) ($y = 12.2x - 105.3$ ($R^2 = 0.79$)). This figure appears in color in the online version of *Tree Physiology*.

this study, there was no difference between the measured and estimated $A_{S,stand}$. A linear relationship found by Kumagai et al. (2005a) is also shown in Figure 2; this was generated from an allometric data set based on 1226 Japanese cypress trees in the Shiiba Research Forest, Kyushu University, located ca. 170 km south of this study site. The regression line found by Kumagai et al. (2005a, 2005b) was nearly identical to the regression line in this study, thus, the $A_{S,stand}$ estimation (= 21.8 m² ha⁻¹) based on the previous study's regression was close to the measured $A_{S,stand}$ value.

Variations in sap flux

Diurnal variations in the $F_{d,ave}$ of 58 individuals, R_s and VPD on given days without rain are shown in Figure 3. In the cross-correlation analysis, the diurnal patterns of $F_{d,ave}$ averaged over 58 individuals showed maximum correlation with VPD after a time lag of 1.5 h ($R = 0.93$) and with R_s after a time lag of 2.5 h ($R = 0.95$). The time lag of $F_{d,ave}$ with VPD was shorter than that of R_s . The peaks of the diurnal patterns of $F_{d,ave}$ appeared around 14:00–15:00, and the peaks of the $F_{d,ave}$ varied significantly among individuals, which ranged between 0 and 30 cm³ s⁻¹ m⁻² (Figure 3). The daytime mean $F_{d,ave}$ was not strongly correlated with DBH ($R^2 = 0.21$, $P < 0.01$; Figure 4), sapwood thickness ($R^2 = 0.10$, $P < 0.01$) or tree height ($R^2 = 0.16$, $P < 0.01$). These results indicate that the tree-to-tree variations in $F_{d,ave}$ were not related to tree-size parameters at this site.

Sample size

The relationship between sample size and CV in $A_{S,stand}$ in the 20 × 20 m plot is shown in Figure 5A. To determine the optimal sample size for estimating $A_{S,stand}$ and J_S in this study, we used -0.5 as the threshold of the changes in CV (dCV/dn). At the range of dCV/dn < -0.5, CV significantly decreased with increasing n , and the increasing n improved the precision of estimates. At dCV/dn > -0.5, CV slightly decreased with increasing n , and the increasing n did not improve the precision of estimates. We defined minimum n with dCV/dn > -0.5 as the optimal sample size in this study. The dCV/dn in $A_{S,stand}$ was smaller than -0.5 at $n < 10$, and dCV/dn was larger than -0.5 at $n > 10$ (Figure 5A). At the optimal sample size of $n = 10$, CV in $A_{S,stand}$ was 5.8%. On the other hand, the dCV/dn in J_S was smaller than -0.5 at $n < 15$, and the dCV/dn was larger than -0.5 at $n > 15$ (Figure 5B). At the optimal sample size of $n = 15$, CV in J_S was 12.0%. The CV at the optimal sample size for J_S was larger than that of $A_{S,stand}$ when we did not consider the changes in atmospheric conditions in the 20 × 20 m plot. Figure 6 shows the multiple errors in E estimates associated with the sample size of J_S and $A_{S,stand}$ using Eq. (5). The multiple errors were less than 20% when $n = 10$ for $A_{S,stand}$ and $n = 15$ for J_S . Furthermore, changes in the multiple errors were conservative when $n > 10$ for $A_{S,stand}$ and $n > 15$ for J_S , and the mul-

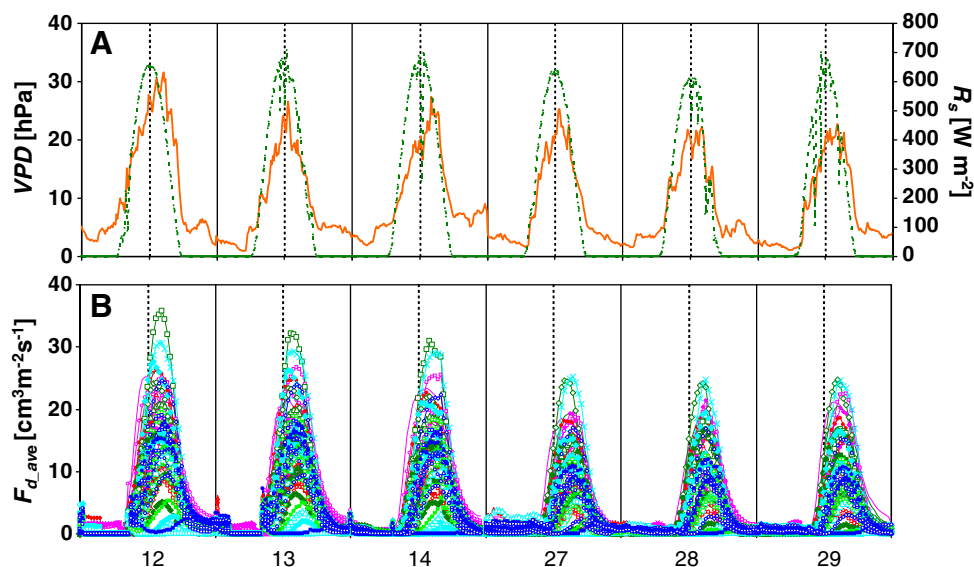


Figure 3. Diurnal variations in (A) solar radiation (R_s ; dashed lines), vapor pressure deficit (VPD; bold line) and (B) tree-specific sap flux averaged over sapwood area for each tree ($F_{d,ave}$) measured in 58 Japanese cypress trees, October of 2006. Each type of symbol represents a different individual. The vertical dotted lines represent midday (1200 h). This figure appears in color in the online version of *Tree Physiology*.

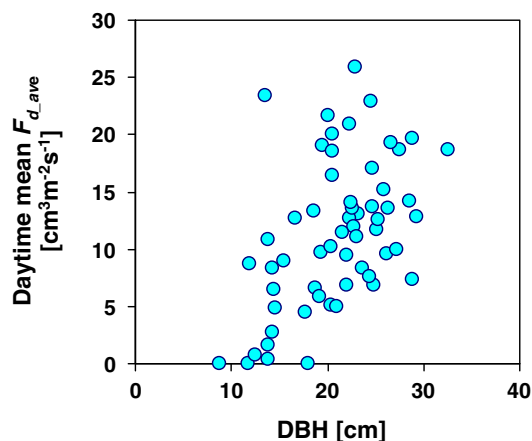


Figure 4. Tree-to-tree variations in the daytime mean $F_{d,ave}$ plotted as a function of DBH. This figure appears in color in the online version of *Tree Physiology*.

multiple errors were always $> 10\%$ (Figure 6). These results indicate that larger sample sizes greatly improved the E estimates when $n < 10$ for $A_{S,stand}$ and $n < 15$ for J_S , respectively, but if n values were > 10 and > 15 , respectively, greater sample sizes did not improve precision.

Monte Carlo analyses were also done on data from the four 10×10 m subplots and the four 20×10 m subplots (Table 2). All analyses for $A_{S,stand}$ showed optimal sample sizes of $n = 10$ with the CV of 4.1–6.0. On the other hand, different optimal sample sizes were indicated in J_S analyses for two 10×10 m subplots and one 20×10 m subplot. The optimal sample size

was $n = 20$ with a CV of 15.7 in plot 3 and $n = 10$ with CVs of 11.3 and 13.8 in plot 3 and plot 1 + 4, respectively.

In both VPD conditions (< 10 and > 10 hPa), optimal sample sizes were $n = 15$ (Table 2). However, the CV for J_S in VPD conditions of < 10 hPa ($= 15.4$) was larger than that in VPD conditions of > 10 hPa ($= 10.4$).

Discussion

Sample size impacts on E estimates

In this study, we measured F_d and $A_{S,tree}$ in all trees (58 individuals) in a 20×20 m plot divided into four 10×10 m subplots in a Japanese cypress plantation. Using this data set, we first examined whether the optimal sample sizes and potential errors (i.e., CV) for $A_{S,stand}$ and J_S that result from sample sizes change with the variations in stand conditions. Our results showed that the optimal sample sizes for J_S differed among 10×10 m subplots, with the different potential errors ranging from 14.5% to 20.5% (Table 2). The ranges of the optimal sample sizes and the potential errors were smaller in the 10×20 m subplots and were more similar to those calculated for the 20×20 m plot (Table 2). This suggests that tree-to-tree variability differs among plots, and that measurements based on a small plot can introduce errors into E estimates based on sap flux measurements. In the $A_{S,stand}$ estimates, all analyses showed the same optimal sample sizes for $A_{S,stand}$ with a small range of potential errors (Table 2). Second, we examined whether the optimal sample sizes and the potential errors for J_S change in differ-

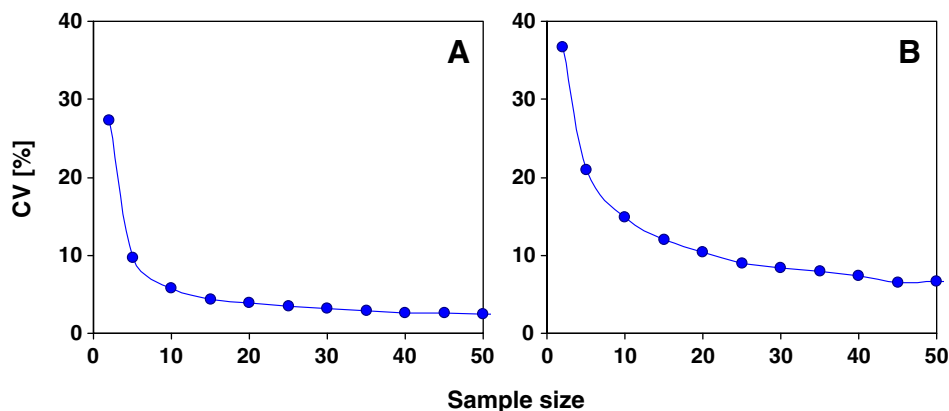


Figure 5. (A) Relationship between sample size and CV in A_{S_stand} (calculated using Eq. (2)). (B) Relationship between sample size and CV in daytime mean F_{d_ave} shown in Figure 3 (calculated using Eq. (3)). This figure appears in color in the online version of *Tree Physiology*.

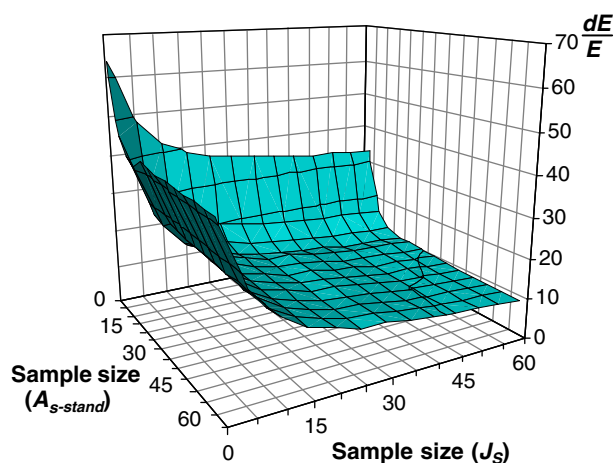


Figure 6. Relationship between errors in E estimates ($\Delta E/E$) and sample size in A_{S_stand} and J_S estimates (calculated using Eq. (5)). This figure appears in color in the online version of *Tree Physiology*.

ent VPD conditions. Our results showed that the optimal sample sizes did not change in the different VPD classes, although the potential error in lower VPD conditions (< 10 hPa) was larger than that in higher VPD conditions (> 10 hPa).

In most cases, the optimal sample sizes were larger for J_S than for A_{S_stand} , and the potential errors for J_S at the optimal sample sizes were larger than those for A_{S_stand} (Table 2). This suggests that J_S variations in tree-specific F_d could be a greater source of variability in scaling to stand scale. However, note that the potential errors for J_S varied with different J_S values. In this study, Monte Carlo sampling was used to estimate the relationships between sample size and SD and CV of J_S . The SD was low when J_S was low and was high when J_S was high. On the other hand, CV was high at night and on cloudy days when J_S values were low under low VPD conditions, because SD was high compared with the low J_S . Therefore, the contribution of errors in J_S to total errors in E could change with J_S . Thus, the

procedure for estimating A_{S_stand} is more important in conditions of low J_S than in conditions of high J_S .

At this site, the allometric regression of Kumagai et al. (2005a) successfully reproduced A_{S_stand} (Figure 2), suggesting that A_{S_stand} estimates from the allometric regression can substitute for the site-specific allometric regression. The estimates using the allometric regression based on the A_{S_tree} measurements in 1226 Japanese cypress trees resulted in a 6.7% error, which is nearly identical to the potential error derived from the sample size of 10 ($= 4.1$ – 6.0%) (Table 2). Furthermore, we should determine whether the procedures for A_{S_tree} measurements can be omitted in Japanese cypress plantations.

In this study, characteristics of tree-to-tree variability differed among plots. Oren et al. (1998a, 1998b) reported that the tree-to-tree variability can result from competition and exposure to the atmosphere depending on the canopy positions of individuals. As well, individuals with a higher stem density can have low F_d and individuals with a lower stem density can have a higher F_d . This implies that large spatial variations in stem density within a forest stand can cause large tree-to-tree variations in F_d and that sap flux measurements in small plots do not account for spatial variations in stem density in the stand. Here, DBH was strongly related to the surrounding stem density, that is, DBH decreases with increasing stem density (Tadaki 1969), suggesting that the individuals with a lower DBH have a lower F_d . However, the relationships between F_d and DBH were unclear at this site (Figure 4). Furthermore, the relationship between F_d and stem density in these subplots was unclear (data not shown). Further research is required to understand the mechanisms underlying tree-to-tree variability of F_d at this and other sites, including the measurements of canopy position, leaf area and their spatial distribution.

In previous studies, spatial variations in F_d were more significant in higher VPD conditions, because of spatial variations in the sensitivity of stomatal conductance to VPD (Adelman et al. 2008, Loranty et al. 2008). This suggests that optimal sample sizes could increase in high VPD conditions.

In this study, we measured the sap flow in an even-aged, single-species stand. This may be one reason why our results differed from those reported in other studies, in which the sap flux measurements were conducted on more than 100 individuals of different sizes and three or four different species. The high water availability in our stand may be another reason. The research plot was located in a riparian area in which the groundwater table was located at 20–50-cm soil depth throughout the year. The roots of Japanese cypress trees at this site reached this depth (data not shown). Tree-to-tree variations in transpiration could be more significant during dry periods than during periods of ample water supply, because of the different water use among the individuals (Čermák et al. 1995). Differences in tree transpiration were observed among plots at different positions on slopes; generally, there was a lower water availability at plots on upper slopes than at plots on lower slopes (Tromp-van Meerveld and McDonnell 2006). Furthermore, unusually severe drought conditions resulting from inter-annual variations in rainfall have been reported in this region (Komatsu et al. 2007). Therefore, optimal sample sizes could change in response to environmental conditions, depending on the topographic position within a watershed, or as a result of inter-annual variability of rainfall.

For our even-aged, single-species stand in the 20×20 m plot, a sample size of at least 10 for A_{S_stand} estimates and 15 for J_S estimates was necessary to account for tree-to-tree variability, but was not necessarily more than 10 and 15 trees for A_{S_stand} and J_S estimates, respectively. The accuracy of E estimates based on these sample sizes with potential errors of 16–21% (Table 2 and Figure 6) is reasonable compared with other methods for estimating E such as the eddy covariance technique (e.g., Goulden et al. 1996), the local water balance method (Oren et al. 1998b), the catchment water balance method (Ford et al. 2007) and the aggregated estimates of evapotranspiration to the landscape scale (Mackay et al. 2002). However, these methods have their own specific sources of uncertainty, such as energy balance of closure in the eddy covariance method (e.g., Kosugi and Katsuyama 2007), throughfall and discharge estimates in the local water balance method (Oren et al. 1998b), deep percolation estimates in the catchment water balance method (Uchida et al. 2003, Oda et al. 2009) and significant stand-to-stand variations in E in catchment- and landscape-scale estimates (Mackay et al. 2002, Ford et al. 2007). Thus, cross-check studies based on multiple measurements (e.g., Wilson et al. 2001) are required to understand the accuracy of measurements and the mechanisms of water balance in forested ecosystems.

Normally, artificial plantation forests contain fewer species than natural forests. In natural forests such as coniferous, broad-leaved and mixed forests, specific relationships between A_{S_tree} and DBH could be found for each species (Vertessy et al. 1995, Kumagai et al. 2005a). Also, more significant tree-to-tree variations in F_d are likely to be found in natural forests because of species-specific physiological

characteristics, such as stomatal responses (e.g., Eschenbach et al. 1998) and hydraulic architecture (e.g., Phillips et al. 1996, Clearwater et al. 1999). Therefore, larger sample sizes might be required for stand-scale transpiration estimates in natural forests. This idea is supported by Wilson et al. (2001). In that study, F_d values were measured in 15 sample trees in a natural forest with large species diversity. Their results showed that there were consistent discrepancies when the sap flux-based transpiration estimates were compared with the transpiration estimates from catchment water balance and eddy covariance techniques. However, few studies have reported the impacts of tree-to-tree variations in F_d on stand-scale transpiration estimates in natural forests (Hatton et al. 1995, Ewers et al., 2002). Overall, further research should be focused on tree-to-tree variations in F_d in natural forests and their temporal and spatial changes in both natural and man-made forests.

Extrapolation to catchment-scale estimates

This study showed the impacts of tree-to-tree variations in F_d on stand-scale transpiration estimates in a Japanese cypress plantation. To examine water use in forested watersheds, stand-scale estimation should be extrapolated to the catchment scale; this is comparable to evapotranspiration estimated from the catchment water balance method (Ford et al. 2007). Kumagai et al. (2008) examined the importance of plot-to-plot variations in J_S resulting from different positions on slopes and the impacts of such variations on catchment-scale estimations. Also, the differences in slope orientation might cause some differences in J_S because of the spatial heterogeneity of radiation and temperature environments (Tajchman et al. 1988, 1997). Thus, scaling from stand scale to catchment scale requires multi-plot measurements for sap flux within a watershed (Ford et al. 2007, Kumagai et al. 2007, 2008). The sample sizes for stand-scale transpiration estimates derived in this study could be used to design the sampling methods for each plot to enable optimal and efficient estimates of catchment-scale transpiration. Previously, various types of sap flux sensors were used to measure sap flux, e.g., Granier-type, heat pulse-type and Kucera-type sensors (e.g., Wullshleger et al. 1998). The Kucera-type sensors provide whole-tree sap flux estimates, while spatial variations in F_d in individuals must be measured across the stem sectional area using multi-sensors when Granier-type and heat pulse-type sensors are used for new species (e.g., Oren et al. 1998a, Ewers et al. 2002). To optimize the use of resources, studies on the water use of forests should have a balanced design considering the types of sap flux measurements as well as the optimal sample sizes.

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