S1-13

Depth of water uptake and flowering frequency of dipterocarp trees

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Abstract Irregular drought has been reported to trigger general flowering (GF) in lowland tropical rain forests in Southeast Asia. We hypothesized that the interspecific differences in water uptake depth may influence the flowering frequency and timing in GF among tree species. We compared the flowering peaks between high- and low-frequency flowering trees based on the phenology data during 1993–2011. As expected, the flowering peak of high-frequency flowering species preceded that of low-frequency species. The soil water sources of the dipterocarp trees were assessed using oxygen stable isotope composition ($\delta^{18}O$) with snap- and sequence-shot methods. Although both an unclear vertical profile of soil water $\delta^{18}O$ and the short sampling term made it difficult to estimate the water uptake depth using either method, $\delta^{18}O$ of dipterocarp trees is likely influenced by flowering frequency. However, an accurate estimation of vertical pattern of soil water use by tropical trees will be required to fully test this hypothesis.

Keywords Flowering phenology, Stable isotope, Tropical rain forest, Water use pattern

Introduction

Southeast Asian lowland dipterocarp forests are characterized by a community-wide masting phenomenon, termed general flowering (GF), during which large flower and seed productions occur unpredictably at irregular intervals of 1 to 10 years (Sakai et al. 1999, 2006). During GF, many dipterocarps and plants from various taxonomic groups bloom by turns within a ca. 3-month period. The magnitude of flowering (i.e., the percentage of flowering individuals) fluctuates among GF events (Sakai et al. 2006), and flowering frequency appears to differ among tree species (Sakai et al. 1999). Patterns of flowering phenology overlap with each other, whereas the flowering peak is observed to be shifted slightly among taxonomic groups and the flowering order seems to be stable (Sakai et al. 2006). Although many researchers have investigated the ultimate and proximate causes of GF and its impact on other organisms in the forest ecosystem, the determinants of flowering frequency and timing remain unclear (Sakai 2001).

Recent studies have indicated that GF is likely triggered by drought (30-day rainfall < 40 mm) rather than temperature drop (Sakai et al. 2006; Kobayashi et al. 2013). Considering that shallow soil is more likely to dry quickly (Oliveira et al. 2005) and that root systems and soil water acquisition strategies vary vertically among tree species (Baillie and Mamit 1983; Flanagan et al. 1992; Becker et al. 1999), the depth of soil water uptake for a given species may explain that species' flowering frequency and flowering timing during a GF event. In this scenario, trees that extract water from shallower soil layers that may dry more frequently and more quickly are expected to flower more frequently and more quickly during a GF compared with tree species that extract water at deeper soil layers.

The development of stable isotope techniques has provided insights into the patterns of soil water uptake by plants. Because fractionation does not occur between stable hydrogen and oxygen isotope composition during water uptake from roots, and water near the soil surface becomes enriched in the heavier isotopes as a result of evaporative fractionation, soil water pool or depth of water uptake can be estimated by analyzing stable isotopic forms of water in xylem and soil (Dawson and Ehleringer 1991; Ehleringer and Dawson 1992; Meinzer et al. 1999). Using this snap-shot method, seasonal changes in water resource use and interspecific partitioning of water source have been reported in mostly arid/semi-arid regions due to a clear vertical profile of stable isotope composition in soil water under dry conditions (Flanagan et al. 1992; Ohte et al. 2003; Yang et al. 2011). Recently, water sources have also been determined in humid regions using temporal variations in stable hydrogen and oxygen isotope compositions in soil and xylem water (Yamanaka et al. 2006; Kubota et al. 2012). Such a sequence-shot method could be applied within a humid tropical forest without disturbing the natural environment via a labelling experiment with deuterated water (Moreira et al. 2000).

The aim of this study was to examine the relationship between flowering phenology and depth of water uptake in a Bornean tropical rain forest. Specifically, we investigated the following hypotheses: (1) Trees with lower flowering frequency bloom later in a GF than those with higher flowering frequency do; and (2) Trees with higher flowering frequency are dependent on water in a shallower soil layer.

Methods

Study site

The study was conducted in and around the Canopy Biology Plot (8 ha, 200 × 400 m) and Crane Plot (4 ha, 200 × 200 m) in Lambir Hills National Park, Sarawak, Malaysia (4°12'N, 114°02'E). The plots are covered with old-growth mixed dipterocarp lowland forests, and the average annual rainfall and temperature are 2600 mm and 25.8 °C, respectively (Kume et al. 2011). The region experiences irregular and short-term droughts, but no seasonally regular dry season occurs. The soils contain sandstone-derived humult ultisols and shale-derived clay-rich udult ultisols (Lee et al. 2002).

Flowering phenology

Reproductive phenology in and around the plots has been monitored using canopy observation systems (tree towers, aerial walkways, and a canopy crane) for nearly 500 plant species in various taxa since 1993 (Sakai et al. 1999, 2006). To compare the timing of flowering peaks, we selected five species of high- and five of low-frequency flowering trees based on the phenology data during

Table 1 Category of flowering frequency for each tree species and number of individuals analyzed in each study.

Family	Genus	Species	Flowering frequency	Time lag	Snap-shot method	Sequence-shot method
Dipterocarpaceae	*Dipterocarpus*	*globosus*	Mid		5	2
	Dryobalanops	*aromatica*	High	22	3	2
		lanceolata	High	4		2
	Shorea	*beccariana*	Mid		3	2
		biawak	Low	5		2
		laxa	Mid		5	
		ovata	High	3		
		smithiana	Low	9		2
Euphorbiaceae	*Clesitabthus*	*venosus*	High	3		
	Mallotus	*leucodermis*	High	5		
Ixonanthaceae	*Allantospermum*	*borneensis*	Low	6		
Leguminosae	*Callerya*	*vasta*	Low	3		
Sterculiaceae	*Scaphium*	*borneensis*	Low	3		

1993–2011 (Table 1). The flowering peaks with more than 10 % flowering individuals were targeted in this analysis. We calculated lag times between the highest flowering peak among high- and low-frequency flowering species for each GF event.

Field survey and measurement of oxygen stable isotope

We tried to estimate the water uptake depth for the dipterocarp trees using two experimental designs, snap- and sequence-shot methods. Dipterocarp trees of 16 and 12 individuals were selected from four and six species, including high-, mid-, and low-frequency flowering trees based on the phenology data for snap- and sequence-shot methods, respectively (Table 1).

For the snap-shot method, xylem tissues for each individual tree were collected once by extracting small cylinders of wood using an increment borer at a height of approximately 1.3 m. Near these sampled trees (within a 10-m radius), we also collected soil samples using an auger (DIK-100A) at depths of 0, 10, 20, 40, 60, 100, 140, and 200 cm. Sampling was conducted six times during July–August 2013.

For the sequence-shot method, xylem tissues for each individual tree were collected four to six times every 2–3 days in June (for 10 days) and July (13 days) 2014 using the methods described for the snap-shot method. To reduce the damage to trees, different individuals were used in the two sampling periods. Soil water samples were collected every other day at a fixed point using a soil water sampler (DIK-8392) at depths of 10, 30, 60, and 100 cm during the sampling period. When soil water was not available due to dry soil or instrument error, we sampled the soil using an auger. All samples for both methods were rapidly stored in screw-cap glass vials and kept frozen (or cold, for soil water) before being analyzed.

Table 2 Date of flowering peak and time lag between high- and low-frequency flowering species during GFs in 1996, 1998, 2001, 2005, and 2009.

	Date of flowering peak						Time lag (days)
	1996	1998	2001	2005	2009		Mean ± SD
High-frequency flowering species	25-Apr	20-Mar	27-Aug	4-Mar	22-Jun	30-Sep	
Low-frequency flowering species	5-Jun	27-May	20-Sep	4-Apr	31-Aug	26-Oct	
Time lag (days)	41	68	24	31	70	26	43.3 ± 20.7

In 2009, the flowering peak was observed twice.

Water was extracted from xylem tissues and soil samples using a cryogenic vacuum distillation method. The oxygen stable isotope ratio was expressed in standard delta notation (‰) relative to the Vienna Standard Mean Ocean Water (V-SMOW) standard as:

$$\delta^{18}O = (R_{sample}/R_{standard} - 1) \times 1000,$$

where R_{sample} and $R_{standard}$ represent the $^{18}O/^{16}O$ of the samples and standards, respectively. The $\delta^{18}O$ in water from xylem tissues ($\delta^{18}O_{stem}$) and soil ($\delta^{18}O_{soil}$) were analyzed using an isotope ratio mass spectrometer (Finnigan Delta V, Thermo Fisher Scientific). The $\delta^{18}O_{soil}$ of soil water during 2014 was analyzed using cavity ring-down spectroscopy (Picarro L2120-I with an A0211 vaporizer and auto sampler).

The $\delta^{18}O_{stem}$ and $\delta^{18}O_{soil}$ for the sequence-shot method were compared among categories of flowering frequency and soil depths, respectively, using Kruskal–Wallis tests and post hoc multi comparisons (Wilcoxon rank sum tests) with Bonferroni corrections.

Results

The flowering peaks of high-frequency flowering species were observed earlier than those of low-frequency flowering species in all GF events (Table 2). The time lag between high- and low-frequency flowering species ranged from 24 to 70 days, with a mean of 43 days.

The vertical profiles of $\delta^{18}O_{soil}$ based on the snap-shot method were all S-shaped graphs; the $\delta^{18}O_{soil}$ decreased from the soil surface to 10~40-cm depths, increased thereafter at 10~100-cm depths, and then decreased again below 100 cm (Fig. 1). Most values of $\delta^{18}O_{stem}$ did not overlap with the vertical profiles of $\delta^{18}O_{soil}$ or else overlapped at multiple points (Fig. 1).

For the sequence-shot method, the $\delta^{18}O_{soil}$ decreased along the soil depth (Fig. 2). We found interspecific variations in the $\delta^{18}O_{stem}$ during both June and July 2014 (Fig. 2). Pooling the data for the two sampling periods, the $\delta^{18}O_{soil}$ and $\delta^{18}O_{stem}$ were significantly affected by soil depth ($P < 0.001$) and flowering frequency ($P < 0.05$). The $\delta^{18}O_{soil}$ differed at each soil depth, except between 30- and 60-cm depths. The $\delta^{18}O_{stem}$ of high-frequency flowering species was higher than that of mid-frequency flowering species, whereas the $\delta^{18}O_{stem}$ of low-frequency flowering species did not differ from the others (Fig. 3). On the other hand, the $\delta^{18}O_{soil}$ for each soil depth and $\delta^{18}O_{stem}$ for each species showed similar variations, except for the $\delta^{18}O_{soil}$ at 30- and 60-cm depth in June 2014.

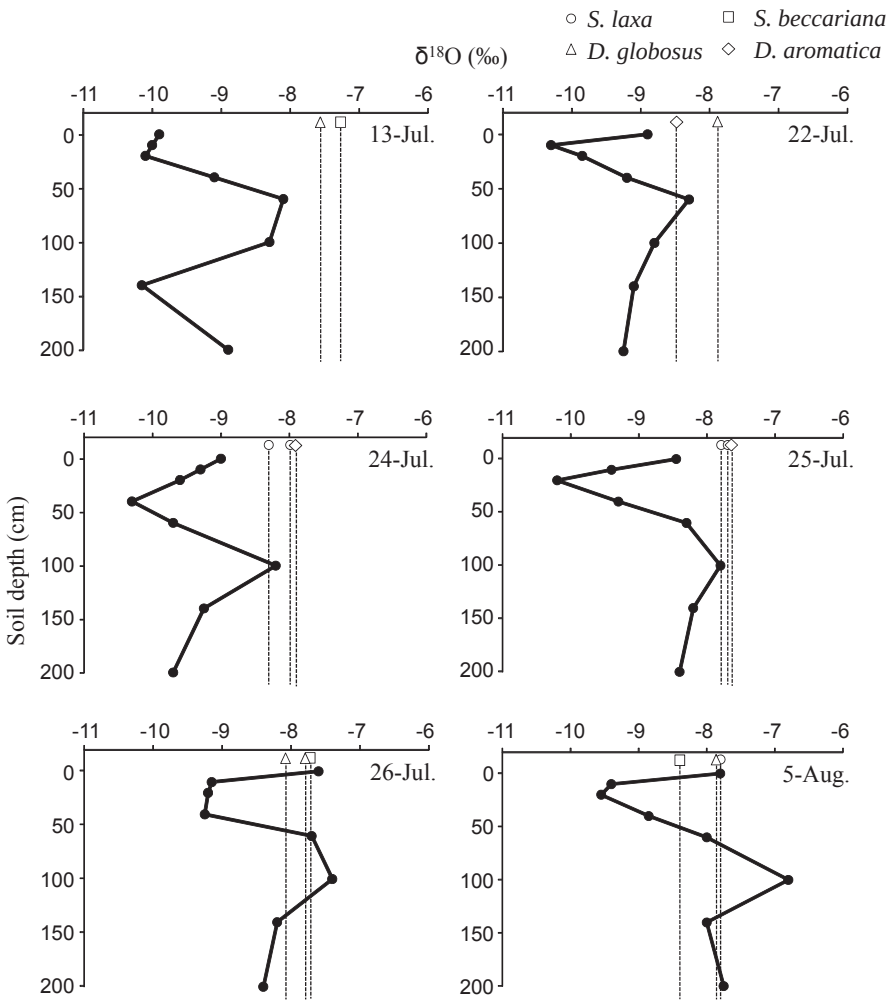

Fig. 1 Vertical profiles of $\delta^{18}O_{soil}$ using the snap-shot method. The $\delta^{18}O_{stem}$ values for four dipterocarp species are also shown by vertical dotted lines.

Discussion

We found that the flowering peak of high-frequency flowering species preceded that of low-frequency flowering species, which is consistent with our hypothesis that the interspecific differences in water uptake depth may influence the flowering frequency and timing in GF. The nearly three-fold variation in the time lag among GF events may reflect the inter-individual response to a flowering trigger due to the microhabitat environments, individual sizes, or available nutrients (Momose et al. 1998; Ichie and Nakagawa 2013).

The application of the snap-shot method to estimate water source was difficult in the tropical rain forest. As expected, a non-linear vertical $\delta^{18}O_{soil}$ profile was observed. Similar patterns have been detected by other studies in tropical regions under natural conditions (Moreira et al. 2000; Liu et al. 2014). Frequent precipitation events may disturb the construction of a clear vertical $\delta^{18}O_{soil}$ profile (Hsieh et al. 1998). Although we obtained soil samples within a 10-m radius from the targeted tree individuals, spatial heterogeneity in the vertical $\delta^{18}O_{soil}$ profile and root systems may have caused discrepancies between the values of $\delta^{18}O_{stem}$ and the $\delta^{18}O_{soil}$ profile for some dipterocarp trees.

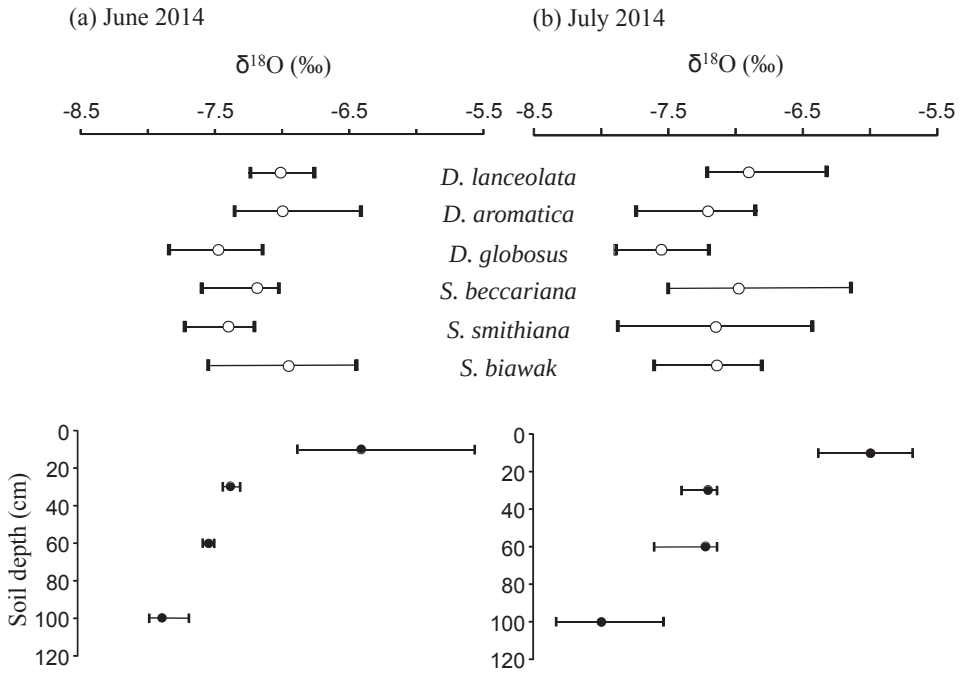

Fig. 2 The $\delta^{18}O_{stem}$ values for six dipterocarp species and the $\delta^{18}O_{soil}$ values for four soil depths in: (a) June and (b) July, 2014, using the sequence-shot method. Data are shown as the range with the mean (circles).

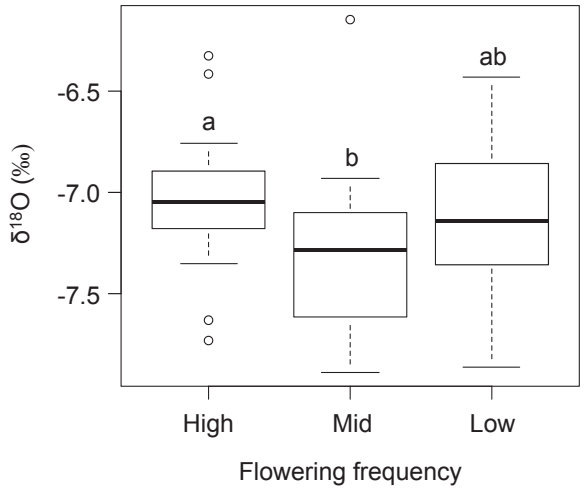

Fig. 3 Comparison of $\delta^{18}O_{soil}$ among categories of flowering frequency using the sequence-shot method. Different letters indicate significant differences between categories using multi-comparisons with Bonferroni correction ($P < 0.05$).

Although larger temporal variation in $\delta^{18}O_{soil}$ was expected for shallower relative to deeper soil layers in the sequence-shot method, such a pattern was not detected. Temporal variation in $\delta^{18}O_{stem}$ was of similar magnitude for all dipterocarp species. Therefore, it was difficult

to estimate the main source of water uptake using the extent of temporal variation in $\delta^{18}O$. Previous studies using the sequence-shot method were conducted across much longer periods (5 months by Yamanaka et al. 2006, 21 months by Kubota et al. 2012) than ours (23 days total). In northern Borneo, the $\delta^{18}O$ of rainfall has been reported to fluctuate during 30~60- or 30~90-day intervals associated with the Madden–Julian Oscillation or Borneo Vortex (Horikawa 2006; Kanamori and Yasunari 2013; Moerman et al. 2013). Thus, longer-term sampling, at least 30 days, may be essential to identify the depth of water uptake based on temporal variation in $\delta^{18}O$ using the sequence-shot method at the study site. However, we found that the $\delta^{18}O_{stem}$ of dipterocarp trees was likely influenced by flowering frequency, indicating that the vertical pattern of soil water use is related to flowering phenology. This conclusion requires further exploration based on a more accurate estimation of the depth of water uptake.

Acknowledgements

We thank the Forest Department Sarawak for permitting research in Lambir Hills National Park, local people for helping with the field survey, and researchers for assisting in measurement of oxygen stable isotope. This study was financially supported by Grants-in-Aid from the Japanese Ministry of Education, Science, and Culture (23255002 and 26660124).

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