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Short-Term *vs.* Long-Term Effects of Understory Removal on Nitrogen and Mobile Carbohydrates in Overstory Trees

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Abstract: Understory management in forest ecosystems has been applied to improve the wood production for hundreds of years worldwide. The carbon-physiological mechanisms underlying these positive effects of understory management on the growth of overstory trees have received less attention. We studied the non-structural carbohydrate (NSC) and total nitrogen (N) concentrations in tissues (needles, stem sapwood, and fine roots) of three tree species (two evergreen and one deciduous species) grown in the presence or absence (understory cut) of understory shrubs in plantations in southwestern China, to test whether understories affect the carbon and nitrogen status in the overstory trees. The concentrations of N, NSC (= soluble sugars + starch) in overstory trees varied significantly with understory treatments during the dry season rather than the wet season. Trees grown without understory shrubs had higher levels of N and NSC compared to trees grown with understories. The present study provides insight to explain the functional mechanisms for understory effects on growth of overstory trees, and indicates that the nitrogen and carbon status in overstory trees may be more strongly negatively affected by understory in stressful conditions rather than in optimal growth conditions. Moreover, the present study provides ecophysiology-based knowledge for dealing with understory vegetation management in forest ecosystems.

Keywords: carbon; competition; mobile carbohydrates; nitrogen; plant-plant interactions; soluble sugars; starch; tree-shrub interactions; understory-overstory interactions.

1. Introduction

Plant-plant interaction plays a key role in regulating and determining the composition, functioning and productivity of plant communities and ecosystems [1,2]. The co-existing plants (e.g., overstory trees growing with understory plants) may interact and compete for light, nutrients, water, and space,

but, at the same time, protect one another from stress such as the impacts of herbivores, potential competitors or extreme environmental fluctuations, and/or provide additional resources through canopy leaching, microbial enhancement, decomposition, and mycorrhizal networks.

Natural forests, old growth stands, and unmanaged forest plantations are normally multilayer structures with tall- and medium-size trees, shrubs, climbing vines, and ground vegetation. Compared to communities with a single layer canopy structure, an overstory-understory interaction exists in such a multilayer plant community [3]. Plants that occupy the overstory layer have a clear advantage in the competition for light, and thus have a nurse or competition effect on the understory layers including diversity and composition [4,5], germination and recruitment [6–8], leaf gas exchange and seedling water status [9,10], and levels of leaf nutrients and carbohydrates [9,11]. These overstory effects on understory were concluded by Hart and Chen [4] as results of changes in understory environment such as light availability, substrate, temperature, soil nutrients, and soil pH. On the other hand, possible effects of understory plants on the overstory growth and physiology have received less attention. The understory plants, due to their smaller size, are not able to compete for direct sunlight with the co-existing overstory trees, but they strongly compete for space, water and nutrients in soils [12–15]. To our knowledge, a few works, such as Miller *et al.* [16] and Kume *et al.* [17], have studied the effects of understory plants on the physiology (gas exchange, water use efficiency = WUE) in overstory trees, and found that understories significantly affected the overstory photosynthesis and WUE.

Photosynthesis is only one aspect, but what is more important is the carbon availability and carbon balance between carbon gain (photosynthesis) and utilization (respiration) within a tree [18–20]. This balance (= carbon gain – carbon consumption) represents a tree's capital for survival, vegetative and reproductive growth, and defense [18,20–22]. The status of carbon balance in a plant is commonly described using the concentration of non-structural carbohydrates (NSC) in tissues [18–24]. To better understand the functional mechanisms underlying the positive effects of understory management on overstory growth [25], we studied the concentrations of nitrogen (N) and NSC in overstory trees (two evergreen and one deciduous tree plantations) grown in the presence and absence of understory shrubs for both the dry and wet season in 2009, using understory removal experiments in southwestern China. We hypothesized that (1) understory cut increases the concentrations of N and NSC in overstory tissues due to reduced competition and enhanced availability of soil resources, and (2) this enrichment is more pronounced under stressful conditions such as in dry season rather than in wet season.

2. Material and Methods

2.1. Site Description

This study site is located in the An-Zi-He Forest Farm (103°07'–103°27' E, 30°42'–30°53' N; 1200–1400 m a.s.l.), Chongzhou, southwestern China. The climate of the region (data collected from 1970 to 2004 in Chongzhou Weather Station) is mid-subtropical monsoon with a mean annual precipitation of 1300–1450 mm (75%–80% of which falls in the rainy season from June to October). The mean annual temperature was 10.5–12.0 °C with July as the warmest month and January as the coldest month. The soil belongs to mountain gray-brown soil developed from sandstones.

2.2. Experimental Design

Two evergreen coniferous species, *Cryptomeria fortunei* Hooibrenk and *Taiwania flousiana* Gaussen, and a deciduous conifer, *Metasequoia glyptostroboides* Hu et Cheng, were widely planted in the region during 1980s due to their high growth rates and high productivities. The *C. fortunei* plantation and *T. flousiana* plantation were established in 1993, and *M. glyptostroboides* plantation was established in 1980, using seedlings of a local provenance each (H. Zhang, personal communication). Part of the plantations of each species has been continuously managed to cut the understory shrubs every 2 to 3 years (H. Zhang, personal communication), and thus this part of the plantations had almost no understory shrubs (LUR = long-term understory removal), whereas other part of the plantations

of each species has not been managed, and then this part had an overstory tree-understory shrub structure (OUS) (H. Zhang, personal communication). The aboveground biomass of understories cut was often but not always left on the forest floor (H. Zhang, personal communication). To avoid pseudoreplication, we selected three spatially separated stands ($n = 3$) with both LUR and OUS for each species in the present study.

In each stand, one 20 m \times 20 m plot in LUR, and two 20 m \times 20 m plots in OUS were established for each species in May 2008. One of the two plots in OUS was randomly selected as control with intact understory shrubs (CTR = control), and the other one was used to remove the understory shrubs in May 2008, to test the effects of short-term understory removal (SUR = short-term understory removal) on overstory NSC and N. LUR was used to test the effects of long-term understory removal on overstory tissue NSC and N. Each plot had 15 to 30 trees depending on the overstory species. Detailed information on stands, plots and sample trees are given in Table 1.

Table 1. Characteristics of the study stands (1200–1400 m a.s.l.), plots and sampled trees in An-Zi-He Forest Farm (103°07′–103°27′ E, 30°42′–30°53′ N), Chongzhou, Sichuan, in the southwest of China.

	Plots (n)	Slope exposure	Soil humidity	Age (year)	Height		Diameter		Treatments
					H (m)	m/year	dhb (cm)	cm/year	
<i>Cryptomeria fortunei</i> plantation in the Gan-Gou Valley									
LUR	3	W	Mesic	16	10.9 ± 2.6	0.68	14.4 ± 3.0	0.90	Long-term understory removal
SUR	3	W	Mesic	16	8.9 ± 2.5	0.56	10.6 ± 3.5	0.66	Short-term understory removal
CUR	3	W	Mesic	16	9.8 ± 2.1	0.61	11.8 ± 3.3	0.74	Understory intact
<i>Taiwania flousiana</i> plantation in the Gan-Gou Valley									
LUR	3	W	Mesic	16	8.2 ± 1.6	0.51	14.1 ± 3.3	0.88	Long-term understory removal
SUR	3	W	Mesic	16	8.0 ± 3.5	0.50	10.5 ± 3.9	0.66	Short-term understory removal
CUR	3	W	Mesic	16	8.0 ± 2.7	0.50	10.8 ± 3.2	0.68	Understory intact
<i>Metasequoia glyptostroboides</i> plantation in the Dou-Peng Valley									
LUR	3	NW	Mesic	29	19.8 ± 2.7	0.68	27.9 ± 5.2	0.96	Long-term understory removal
SUR	3	NW	Mesic	29	22.6 ± 1.3	0.78	32.3 ± 4.8	1.11	Short-term understory removal
CUR	3	NW	Mesic	29	21.3 ± 2.8	0.73	30.6 ± 5.9	1.06	Understory intact

2.3. Tissue Sampling

We sampled tree tissues twice in 2009 (*i.e.*, 1 year after the understory treatment for SUR). We did not take samples twice from the same trees to avoid any damages in the sample trees. For each sampling date, four healthy, dominant and undamaged canopy trees were randomly selected as sample trees in the center of each plot. The first set of tissue samples was collected at the end of the dry season (between 8 and 10 May 2009), and the second set was collected at the end of the wet season (between 24 and 27 October 2009). Because the levels of mobile carbohydrates in needles fluctuate daily in response to photosynthetic activity [26], samples were always taken around noon, to ensure that the effects of sunshine and needle surface temperature on carbohydrates were kept constant.

For the two evergreen tree species (*C. fortunei*, *T. flousiana*), current-year needles (C-needles) and a mixed sample of previous year needles (P-needles) were separately collected. For the deciduous *M. glyptostroboides* trees, only current-year needles (C-needles) existed and were sampled. All needle samples were collected from non-shaded leading branches originating from the bottom one third of the crown segment in the downslope crown side in each sample tree. Two cores from each sample tree were taken using a 5-mm-diameter corer on two opposite stem sides (parallel to the slope) at the stem height of 40 cm above the ground. The outer ~7 cm of stem sapwood (xylem tissue only) of the two cores was cut down and mixed to obtain the minimum materials necessary for chemical analysis. Fine roots (<5 mm in diameter, with bark) were collected within the top 20-cm soil layer for each sample

tree. All samples were immediately stored in a cool box and dried in a microwave oven (40 s at 600 W) to stop the physiological activities, and dried to a constant weight at 65 °C [21,22].

2.4. Total Nitrogen and NSC (Soluble Sugars and Starch) Analysis

The total nitrogen concentration (% d.m.) was determined in finely ground oven-dried samples by the micro Kjeldahl procedure, using CuSO_4 , K_2SO_4 , and H_2SO_4 for digestion, and NH_3 was determined on a Technicon Auto-Analyzer, using the indophenol-blue colorimetric method [21,22].

Dried plant material was ground to pass a 1-mm sieve. The powdered material (0.1 g) was placed in a 10-mL centrifuge tube and 5 mL of 80% ethanol added. The mixture was incubated at 80 °C for 30 min, and then centrifuged at 5000 g for 5 min. The pellets were re-extracted twice with 80% ethanol. The pellet was saved for starch analysis and the combined supernatants were stored at −20 °C until analysis of soluble sugars using the anthrone method. Glucose was used as a standard. Soluble sugar concentration was calculated on a dry matter basis (% d.m.) [21,22].

Starch was extracted from the ethanol-insoluble pellet after removing the ethanol by evaporation. Starch in the residue was released by boiling in 2 mL of distilled water for 15 min. After cooling to room temperature, 2 mL of 9.2 M HClO_4 was added and the starch was hydrolyzed for 15 min. Four mL of distilled water were added and the mixture centrifuged at 5000 g for 10 min. The pellet was re-extracted with 2 mL of 4.6 M HClO_4 . The combined supernatants were adjusted with distilled water to 20 mL. Starch concentration was measured spectrophotometrically at 620 nm using anthrone reagent, and calculated by multiplying the glucose concentration by the conversion factor of 0.9. Glucose was used as a standard. The starch concentration was expressed on a dry matter basis (% d.m.).

2.5. Data Analysis

NSC is defined as the sum of the starch plus the total soluble sugars for each sample taken at each sampling date. The ratio of NSC to total N concentration (NSC-N ratio) was calculated for each sample [21,22]. Mean values for each plot were used for statistical analyses ($n = 3$ plots). Data were confirmed for normality by Kolmogorov–Smirnov Tests prior to statistical analysis.

A pre-test (four-way ANOVA) with species (3 species), sampling date (dry season *vs.* rainy season), stand type (LUR, SUR, CTR), and tissues (C-needles, stem sapwood, and fine roots) as factors showed significant effects of the four factors, and significant two-way and three-way interactions on the parameters studied (Table S1). Hence, two-way ANOVAs were performed for each species to test the effects of sampling date, stand types, and their interaction on the concentrations of tissue N, NSC, and NSC-N ratios. Effects of understory treatment on overstory N, NSC, NSC-N ratio in each tissue type for each species at each sampling time were tested using single factor ANOVAs, and followed, if significant, by Tukey's HSD test at a level of $\alpha = 0.05$ to evaluate whether difference between any two stand types is significant. All statistical analyses are performed using SPSS statistics 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Understory significantly affected the levels of the parameters studied in the overstory trees of the two evergreen species (*C. fortunei* and *T. flousiana*), with few exceptions (Table 2). The concentrations of tissue N and carbohydrates tended to decrease following the order of LUR > SUR > CTR across time, except for soluble sugars in some cases (Figure 1a–c, Figure 2a–c).

Table 2. Results of two-way ANOVAs for total nitrogen, non-structural carbohydrate (NSC) and its components (soluble sugars, starch), NSC-Nitrogen(N) ratios in tissues of *Cryptomeria fortunei*, *Taiwania flousiana*, and *Metasequoia glyptostroboides* trees grown in three forest types T (LUR = long-term understory removal, SUR = short-term understory removal, and CUR = understory intact control) for two sampling seasons (dry season *vs.* wet season). F- and *p*-values are given. ns = non-significant; C-needles = Current year needles; P-needles = previous year needles.

		df	N		NSC		Sugars		Starch		NSC-N ratio		
			F	p	F	p	F	p	F	p	F	p	
<i>Cryptomeria fortunei</i>													
C-needles	Season (S)	1	155.07	0.000	194.34	0.000	35.82	0.000	304.66	0.000	144.93	0.000	
	Type (T)	2	16.66	0.000	40.89	0.000	15.14	0.001	13.30	0.001	17.66	0.000	
	S*T	2	1.06	ns	0.21	ns	6.36	0.013	3.65	ns	1.17	ns	
P-needles	Season (S)	1	101.14	0.000	44.07	0.000	1.22	ns	274.53	0.000	138.89	0.000	
	Type (T)	2	17.26	0.000	36.49	0.000	19.15	0.000	28.77	0.000	19.33	0.000	
	S*T	2	0.23	ns	1.73	ns	1.24	ns	1.56	ns	2.43	ns	
Sapwood	Season (S)	1	74.10	0.000	37.79	0.000	13.57	0.003	45.78	0.000	73.81	0.000	
	Type (T)	2	40.65	0.000	5.67	0.019	0.35	ns	12.59	0.001	32.90	0.000	
	S*T	2	0.68	ns	0.08	ns	7.46	0.008	2.70	ns	4.61	0.033	
Fine roots	Season (S)	1	24.03	0.000	34.92	0.000	5.31	0.040	28.20	0.000	37.01	0.000	
	Type (T)	2	38.11	0.000	11.01	0.002	3.21	ns	26.55	0.000	58.81	0.000	
	S*T	2	5.82	0.017	2.18	ns	2.52	ns	8.56	0.005	13.56	0.001	
<i>Taiwania flousiana</i>													
C-needles	Season (S)	1	213.07	0.000	188.48	0.000	38.82	0.000	257.10	0.000	217.90	0.000	
	Type (T)	2	18.59	0.000	6.95	0.010	7.36	0.008	12.74	0.001	28.43	0.000	
	S*T	2	7.50	0.008	2.58	ns	0.63	ns	6.38	0.013	0.52	ns	
P-needles	Season (S)	1	1.64	ns	520.93	0.000	80.50	0.000	188.84	0.000	0.57	ns	
	Type (T)	2	10.82	0.002	17.09	0.000	2.79	ns	9.29	0.004	12.83	0.001	
	S*T	2	0.37	ns	7.94	0.006	1.08	ns	8.56	0.005	0.55	ns	
Sapwood	Season (S)	1	290.04	0.000	1.78	ns	15.31	0.002	15.73	0.002	266.28	0.000	
	Type (T)	2	50.65	0.000	15.01	0.001	0.16	ns	27.64	0.000	42.39	0.000	
	S*T	2	2.13	ns	7.49	0.008	7.85	0.007	26.30	0.000	7.26	0.009	
Roots	Season (S)	1	211.91	0.000	0.65	ns	48.07	0.000	24.67	0.000	651.56	0.000	
	Type (T)	2	42.62	0.000	4.37	0.038	0.87	ns	10.52	0.002	124.74	0.000	
	S*T	2	3.64	0.058	0.46	ns	0.01	ns	0.73	ns	15.70	0.000	
<i>Metasequoia glyptostroboides</i>													
C-needles	Season (S)	1	131.27	0.000	552.87	0.000	325.95	0.000	64.29	0.000	145.54	0.000	
	Type (T)	2	12.56	0.001	11.33	0.002	11.13	0.002	0.25	ns	7.82	0.007	
	S*T	2	0.95	ns	1.11	ns	4.61	0.033	4.58	0.033	0.74	ns	
Sapwood	Season (S)	1	647.68	0.000	3.99	ns	5.90	0.032	1.40	ns	915.76	0.000	
	Type (T)	2	44.42	0.000	2.86	ns	2.93	ns	9.01	0.004	112.53	0.000	
	S*T	2	0.01	ns	0.59	ns	3.08	ns	2.32	ns	34.42	0.000	
Roots	Season (S)	1	0.10	ns	27.53	0.000	4.28	ns	32.27	0.000	1.12	ns	
	Type (T)	2	13.49	0.001	0.40	ns	1.47	ns	0.10	ns	9.54	0.003	
	S*T	2	5.71	0.018	0.24	ns	0.28	ns	0.93	ns	5.21	0.024	

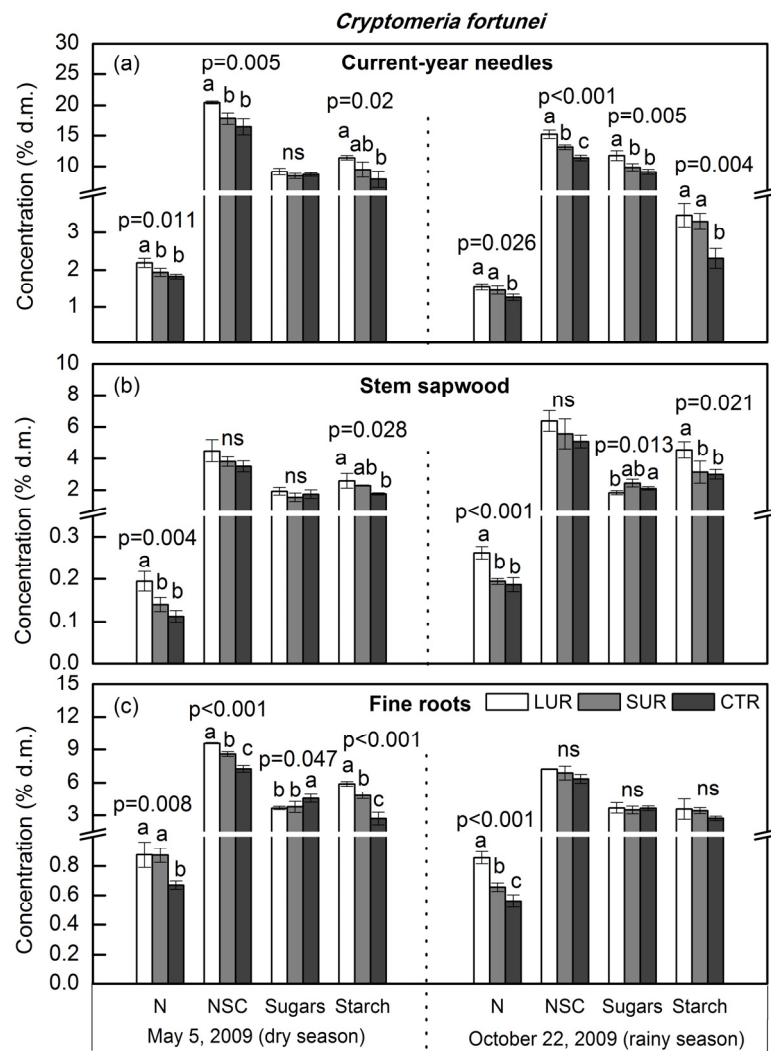


Figure 1. Concentration (% d.m., mean values \pm 1 SD, $n = 3$) of total nitrogen (N), non-structural carbohydrate (NSC) and its components (soluble sugars, starch) in (a) current-year needles, (b) stem sapwood, and (c) fine roots of *Cryptomeria fortunei* trees grown in the absence (LUR = long-term understory removal, SUR = short-term understory removal) and presence of understory shrubs (CTR = understory intact control) on a gentle W-facing slope, Chongzhou, SW China. Statistical differences were tested using one-way ANOVAs, and followed, if significant, by Tukey's HSD test. Different letters indicate significant differences at $p < 0.05$; ns indicates non-significant difference; SW indicates southwest.

Sampling date (*i.e.*, season) also significantly affected the levels of the parameters studied in the overstory trees of the two evergreen species, with a few exceptions (Table 2). The understory effects on carbohydrates in overstory trees were more pronounced during the dry season than the rainy season (Figure 1a–c, Figure 2a–c), especially for *T. flousiana* (Figure 2a–c).

For both evergreen species, the needles and fine roots tended to have higher N, NSC, and starch concentrations in trees within each stand type in the dry season than in the rainy season (Figure 1a,c, Figure 2a,c), whereas the stem sapwood had higher concentrations of N, NSC, sugars, and starch in the rainy season than in the dry season (Figures 1b and 2b), except for NSC and starch in *T. flousiana* trees in LUR (Figure 2b). The tissue NSC–N ratios varied significantly with season and understory treatment (Table 2).

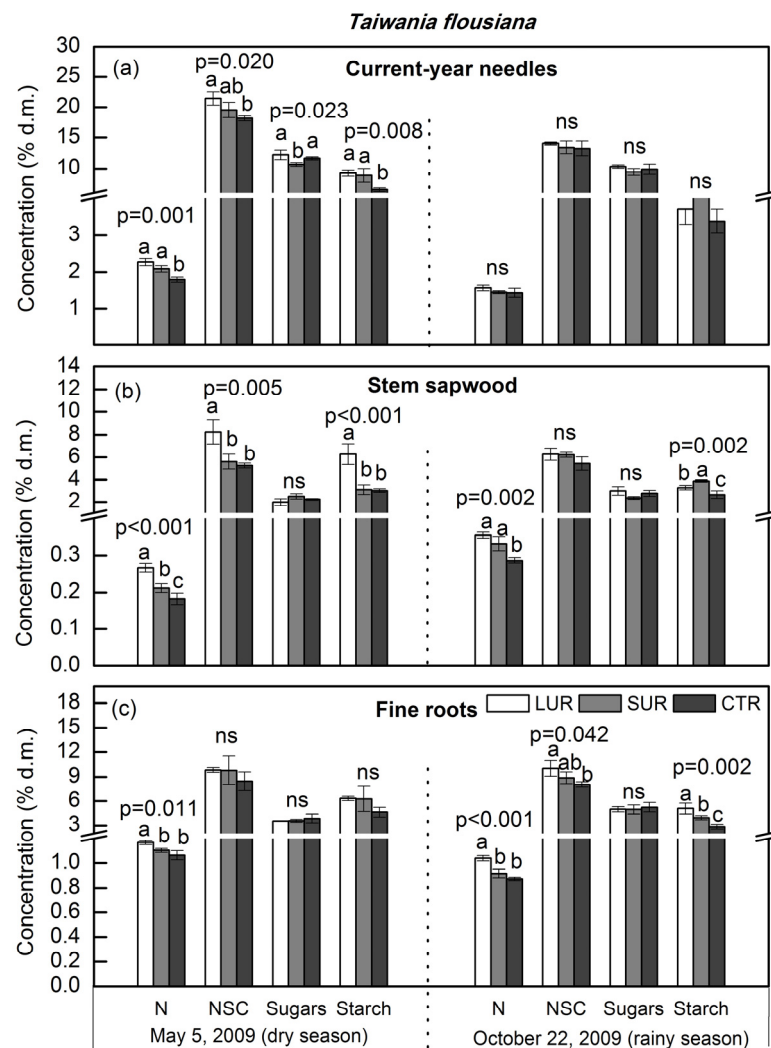


Figure 2. Concentration (% d.m., mean values \pm 1 SD, $n = 3$) of total nitrogen, non-structural carbohydrate (NSC) and its components (soluble sugars, starch) in (a) current-year needles, (b) stem sapwood, and (c) fine roots of *Taiwania flousiana* trees grown in the absence (LUR = long-term understory removal, SUR = short-term understory removal) and presence of understory shrubs (CTR = understory intact control) on a gentle W-facing slope, Chongzhou, SW China. Statistical differences were tested using one-way ANOVAs, and followed, if significant, by Tukey's HSD test. Different letters indicate significant differences at $p < 0.05$; ns indicates non-significant difference; SW indicates southwest.

Similar to the results for the two evergreen species, the levels of tissue N, NSC, sugar, and starch concentrations for the deciduous *M. glyptostroboides* trees tended to decrease following the order of LUR > SUR > CTR across time, except for sugars in the stem sapwood (Figure 3a–c), although the understory effects in many cases were not statistically significant (Table 2, Figure 3a–c).

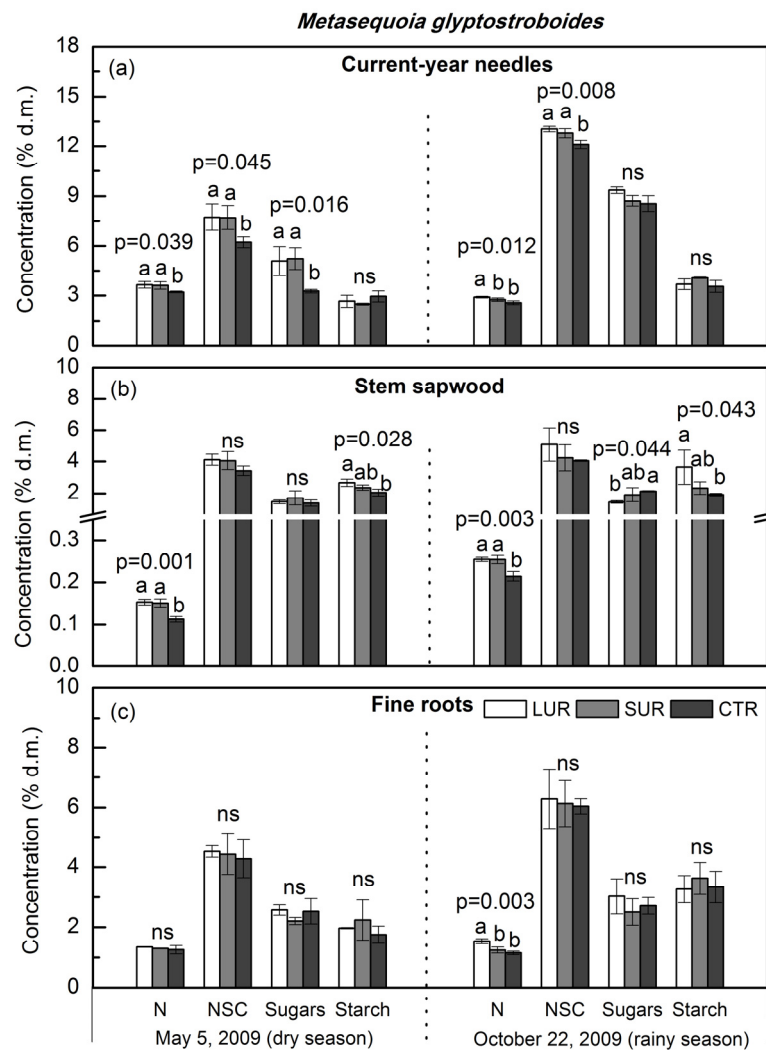


Figure 3. Concentration (% d.m., mean values \pm 1 SD, $n = 3$) of total nitrogen, non-structural carbohydrate (NSC) and its components (soluble sugars, starch) in (a) current-year needles, (b) stem sapwood, and (c) fine roots of *Metasequoia glyptostroboides* trees grown in the absence (LUR = long-term understory removal, SUR = short-term understory removal) and presence of understory shrubs (CTR = understory intact control) on a gentle NW-facing slope, Chongzhou, SW China. Statistical differences were tested using one-way ANOVAs, and followed, if significant, by Tukey's HSD test. Different letters indicate significant differences at $p < 0.05$; ns indicates non-significant difference; SW indicates southwest.

There were no interactions between species and stand type on overstory N (Table S1), showing that tissue N concentrations decreased following an order of LUR > SUR > CTR for the three species consistently (Figures 1–3). Species interacted with stand type to affect the overstory carbohydrates (Table S1), and thus the concentrations of tissue carbohydrates did not show any consistent trend for the three species with stand type (Figures 1–3). The season effects on overstory N and carbohydrates significantly varied with species (Table S1). The deciduous *M. glyptostroboides* trees seemed to have higher N and NSC levels in the rainy season than in the dry season for all tissue types within each stand type (Figure 3a–c), although this seasonal effect on the parameters in sapwood and roots were statistically weak (Table 2). An overall trend indicated that the deciduous species was less sensitive to understory shrubs than the evergreen species (Table 2, Table S1, Figures 1–3).

4. Discussion

4.1. Tissue Nitrogen and Mobile Carbohydrates in Overstory Trees

The tissue N concentrations in overstory trees were found to vary significantly with stand types associated with understory treatments (Table 2). Consistent with our hypothesis 1 that understory cut increases the N concentrations in overstory tissues (see Introduction), our results showed a general trend that trees grown in the absence of understories had higher tissue N concentrations compared to trees grown in the presence of understories (Figures 1–3). Similar results have been published. Foliar N concentrations in *Pinus radiata* trees increased progressively as the width of weed-free strip surrounding trees increased from 0, 1, 2, to 3 m [27]. Foliar N concentration was 2.11% in *P. radiata* trees grown in 100% weed-free treatment and 1.53% in trees grown with weeds [27]. Kume *et al.* [17] found that the tissue N concentrations of a managed *Pinus densiflora* stand (understory shrubs removed) were significantly higher than those of the unmanaged stands grown in the presence of the understory shrubs. Matsushima and Chang [28] also found that understory removal increased needle N concentrations of white spruce, which implies that understory compete for N with the overstory spruce. Chang and Preston [29] found that understory vegetation removal increased the N fertilizer-use efficiency of trees by limiting understory competition for N [30,31]. Woods *et al.* [32] reported that there was a close relationship between N uptake by trees and the weed-free soil area accessible to trees. All these results indicated that understories or weeds compete directly with the overstory for available N. We, hence, conclude that understory removal can result in increases in overstory tissue N concentrations, and these enhanced N concentrations are a direct result of reduced competition for soil N due to understory removal. Moreover, in comparison with previous studies, the present study tested the treatment time effects of understory removal, *i.e.*, we compared the long-term effects with the short-term effects of understory removal on the tissue N concentrations in overstory trees, and found that these concentrations increased with treatment time (Figures 1–3).

Concentrations of tissue NSC in trees varied significantly with stand types associated with understory treatments (Table 2), and this result supports our hypothesis 1 (see Introduction). An overall trend is that trees grown in the absence of understory had higher levels of NSC, sugars, and starch compared to trees grown in the presence of understories (Figures 1–3). Shainsky and Rose [11] reported that concentrations of starch and NSC in needles of *Pseudotsuga menziesii* (Mirb.) Franco trees decreased significantly in mixture with *Alnus rubra* Bong. compared to monoculture. Their findings, in line with our present results, indicate that the co-existing species can alter or affect the levels of mobile carbohydrates in the target trees. To our knowledge, the present study, for the first time, investigated the effects of understory management on overstory carbohydrates, and provided carbon-physiological evidence for the positive growth effects of overstory trees after understory removal.

As mentioned above, the plant tissue NSC concentrations measured reflect the carbon balance between photosynthesis and carbon consumption [18–20]. Some studies measured photosynthesis of trees grown in the absence compared to the presence of understory vegetation. Kume *et al.* [17] found that the maximum net photosynthesis of overstory *P. densiflora* grown with understory shrubs was significantly lower than that of trees grown in the absence of understory shrubs. Nambiar and Sands [27] reported that two-year-old *P. radiata* trees grown in a weed-free circular area of 1.5 m radius had significant higher net photosynthesis compared to trees grown with weeds. An increase in photosynthesis could be partially associated with a decrease in light competition, in particular at the lower crown level, and an increase in soil water availability, in particular in water-limited areas [33,34].

Binkley *et al.* [35] found that the light-saturated rates of photosynthesis in *Eucalyptus saligna* increased linearly with increasing N concentrations in leaves. Experimental studies have also revealed that leaf photosynthetic rates of various plants/trees were positively correlated with leaf N contents [36–39]. The present study indicated that understory removal increased overstory tissue N concentrations (Figures 1–3), which may imply increases in photosynthesis of trees in LUR and SUR. On the other hand, understory removal may increase not only the availability of soil N but also the

availability of water and other resources. The overstory trees are thus able to (1) acquire a greater proportion of the soil resources, (2) use resources more efficiently for producing biomass, and (3) allocate assimilates in ways that maximize the growth capacity.

4.2. Dry Season vs. Wet Season

Concentration levels of N and NSC differed significantly between the dry and wet season (Table 2). The understory effects on overstory tissue N and NSC were more pronounced during the dry season than the wet season (Figures 1–3). These results support our hypothesis 2 (see Introduction) that expected marked understory effects on overstory trees in stressful conditions. Petersen *et al.* [40] found that water stress was significantly stronger in Douglas fir trees grown in the presence of understories (*Ceanothus velutinus* Dougl. and forbs) than in trees with no vegetation around them during the dry late summer. During the dry season, understories compete with the overstory trees for the limited water resource in soils, directly leading to decreases in resource uptake and thus photosynthetic rates in overstory trees.

Many weed control/removal experiments carried out in tree plantations and agroforestry systems showed that dense understory vegetation (weeds) led to increased water stress in crop trees [27,40–42]. Needle water potential of juvenile *P. radiata* trees decreased when weeds are present [43,44], and a negatively linear relationship between percentage cover of weeds and *P. radiata* needle water potential has also been reported [45].

Tall trees that occupy the overstory layer have a clear advantage in the competition for light, but they may not have advantages in the acquisition of water and nutrients from soils when the forest floor is covered by dense understory vegetation [17]. Spatial and temporal segregation of roots and shoots by understory and overstory plants may be an effective strategy for reducing competition for space and resources [46]. For instance, shallow-rooted herbaceous species as understory plants utilize water and other resources in topsoils, and deep-rooted woody plants as overstory plants acquire separate resources from deeper soil horizons [26,46,47]. However, the overstory trees having roots in the deep soil layers may also suffer from water deficit caused by dense understories which colonize the upper horizon, and thus, lead to an almost complete withdrawal of soil water in the upper soil layer and to a gradual drying of the deeper soil layers [46], especially in dry conditions.

Hence, the understory may be most likely to affect the overstory through below ground competition for soil resources [48]. For example, dense understory vegetation can significantly decrease the soil temperature, net N mineralization, net nitrification rates, and soil water availability [12]. Removal experiments showed that understory removal can lead to significant increases in soil N [49,50] and soil water availability [12,51], and consequently, result in increased growth rate of the overstory trees [6,51]. The higher sensitivity of N and NSC in dry season and their insensitivity in the wet season (Figures 1–3) suggest that the most important effects of understories occur in regions with stressful growth conditions such as dry areas.

5. Conclusions

Previous experimental studies have found that removal of understory plants led to significant increase in soil N content [49,50] and soil water availability [12,51], and consequently, resulted in increased growth rate of the overstory trees [6,51]. The present study provided insight to explain the functional mechanisms underlying the positive effects of understory-removal on overstory growth. The most pronounced effects of understories on the overstory layer occurred during the dry season, suggesting that these effects may result mainly from the understory competition for soil water. This may also further indicate that the environmental conditions play an important role in determining the understory-overstory relationships. Moreover, the present study provided basic ecophysiological data to help us develop ecophysiology-based management strategies for dealing with understory vegetation in forest ecosystems.

Supplementary Materials: <http://www.mdpi.com/1999-4907/7/3/67/s1>. Table S1. Results of four-way ANOVA analysis using species (three species), stand types (LUR, SUR, CUR), season (dry and wet season), and tissue types (current-year needles, stem sapwood, fine roots) as factors. F and p values are given.

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References

1. Brooker, R.W. Plant-plant interactions and environmental change. *New Phytol.* **2006**, *171*, 271–284. [[CrossRef](#)] [[PubMed](#)]
2. Craine, J.M.; Dybzinski, R. Mechanisms of plant competition for nutrients, water and light. *Funct. Ecol.* **2013**, *27*, 833–840. [[CrossRef](#)]
3. Silvertown, J. Plant coexistence and the niche. *Trend. Ecol. Evol.* **2004**, *19*, 605–611. [[CrossRef](#)]
4. Hart, S.A.; Chen, H.Y.H. Understory vegetation dynamics of North American boreal forests. *Crit. Rev. Plant Sci.* **2006**, *25*, 381–397. [[CrossRef](#)]
5. Sagar, R.; Raghubanshi, A.S.; Singh, J.S. Comparison of community composition and species diversity of understorey and overstorey tree species in a dry tropical forest of northern India. *J. Environ. Manag.* **2008**, *88*, 1037–1046. [[CrossRef](#)] [[PubMed](#)]
6. Oren, R.; Waring, R.H.; Stafford, S.G.; Barrett, J.W. Twenty-four Years of Ponderosa Pine Growth in Relation to Canopy Leaf Area and Understory Competition. *For. Sci.* **1987**, *33*, 538–547.
7. Takahashi, K. Regeneration and coexistence of two subalpine conifer species in relation to dwarf bamboo in the understorey. *J. Veg. Sci.* **1997**, *8*, 529–536. [[CrossRef](#)]
8. O'Brien, M.J.; O'Hara, K.L.; Erbilgin, N.; Wood, D.L. Overstory and shrub effects on natural regeneration processes in native *Pinus radiata* stands. *For. Ecol. Manag.* **2007**, *240*, 178–185. [[CrossRef](#)]
9. Barnes, P.W.; Archer, S. Tree-shrub interactions in a subtropical savanna parkland: Competition or facilitation? *J. Veg. Sci.* **1999**, *10*, 525–536. [[CrossRef](#)]
10. Parker, W.C.; Dey, D.C. Influence of overstory density on ecophysiology of red oak (*Quercus rubra*) and sugar maple (*Acer saccharum*) seedlings in central Ontario shelterwoods. *Tree Physiol.* **2008**, *28*, 797–804. [[CrossRef](#)] [[PubMed](#)]
11. Shainsky, L.J.; Rose, C.L. Effects of competition on the foliar chemistry of young Douglas-fir in monoculture and mixed stands with young red alder. *Can. J. For. Res.* **1995**, *25*, 1969–1977. [[CrossRef](#)]
12. Matsushima, M.; Chang, S.X. Effects of understory removal, N fertilization, and litter layer removal on soil N cycling in a 13-year-old white spruce plantation infested with Canada bluejoint grass. *Plant Soil* **2007**, *292*, 243–258. [[CrossRef](#)]
13. Nilsson, M.C.; Wardle, D.A. Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Front Ecol. Environ.* **2005**, *3*, 421–428. [[CrossRef](#)]
14. Gilliam, F.S. The ecological significance of the herbaceous layer in temperate forest ecosystems. *BioScience* **2007**, *57*, 845–858. [[CrossRef](#)]
15. Elliott, K.J.; Vose, J.M.; Knoepp, J.D.; Clinton, B.D.; Kloeppel, B.D. Functional Role of the Herbaceous Layer in Eastern Deciduous Forest Ecosystems. *Ecosystems* **2015**, *18*, 221–236. [[CrossRef](#)]
16. Miller, B.J.; Clinton, P.W.; Buchan, G.D.; Robson, A.B. Transpiration rates and canopy conductance of *Pinus radiata* growing with different pasture understories in agroforestry systems. *Tree Physiol.* **1998**, *18*, 575–582. [[CrossRef](#)] [[PubMed](#)]
17. Kume, A.; Satomura, T.; Tsuboi, N.; Chiwa, M.; Hanba, Y.T.; Nakane, K.; Horikoshi, T.; Sakugawa, H. Effects of understory vegetation on the ecophysiological characteristics of an overstory pine, *Pinus densiflora*. *For. Ecol. Manag.* **2003**, *176*, 195–203. [[CrossRef](#)]

18. Li, M.H.; Hoch, G.; Körner, C. Source/sink removal affects mobile carbohydrates in *Pinus cembra* at the Swiss treeline. *Trees-Struct. Funct.* **2002**, *16*, 331–337. [[CrossRef](#)]
19. Hoch, G.; Körner, C. The carbon charging of pines at the climatic treeline: Aglobal comparison. *Oecologia* **2003**, *135*, 10–21. [[CrossRef](#)] [[PubMed](#)]
20. Körner, C. Carbon limitation in trees. *J. Ecol.* **2003**, *91*, 4–17. [[CrossRef](#)]
21. Li, M.H.; Xiao, W.F.; Shi, P.L.; Wang, S.G.; Zhong, Y.D.; Liu, X.L.; Wang, X.D.; Cai, X.H.; Shi, Z.M. Nitrogen and carbon source-sink relationships in trees at the Himalayan treelines compared with lower elevations. *Plant Cell Environ.* **2008**, *31*, 1377–1387. [[CrossRef](#)] [[PubMed](#)]
22. Li, M.H.; Xiao, W.F.; Wang, S.G.; Cheng, G.W.; Cherubini, P.; Cai, X.H.; Liu, X.L.; Wang, X.D.; Zhu, W.Z. Mobile carbohydrates in Himalayan treeline trees I. Evidence for carbon gain limitation but not for growth limitation. *Tree Physiol.* **2008**, *28*, 1287–1296. [[CrossRef](#)] [[PubMed](#)]
23. Li, M.H.; Hoch, G.; Körner, C. Spatial variability of mobile carbohydrates within *Pinus cembra* trees at the alpine treeline. *Phyton-Ann. Rei Bot. A.* **2001**, *41*, 203–213.
24. Hoch, G.; Popp, M.; Körner, C. Altitudinal increase of mobile carbon pools in *Pinus cembra* suggests sink limitation of growth at the Swiss treeline. *Oikos* **2002**, *98*, 361–374. [[CrossRef](#)]
25. Li, M.H.; Du, Z.; Pan, H.; Yan, C.; Xiao, W.; Lei, J. Effects of neighboring woody plants on target trees with emphasis on effects of understorey shrubs on overstorey physiology in forest communities: a mini-review. *Community Ecol.* **2012**, *13*, 117–128. [[CrossRef](#)]
26. Graham, E.A.; Mulkey, S.S.; Kitajima, K.; Phillips, N.G.; Wright, S.J. Cloud cover limits net CO₂ uptake and growth of a rainforest tree during tropical rainy seasons. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 572–576. [[CrossRef](#)] [[PubMed](#)]
27. Nambiar, E.K.S.; Sands, R. Competition for Water and Nutrients in Forests. *Can. J. For. Res.* **1993**, *23*, 1955–1968. [[CrossRef](#)]
28. Matsushima, M.; Chang, S.X. Vector analysis of understory competition, N fertilization, and litter layer removal effects on white spruce growth and nutrition in a 13-year-old plantation. *For. Ecol. Manag.* **2006**, *236*, 332–341. [[CrossRef](#)]
29. Chang, S.X.; Preston, C.M. Understorey competition affects tree growth and fate of fertilizer-applied ¹⁵N in a coastal British Columbia plantation forest: 6-year results. *Can. J. For. Res.* **2000**, *30*, 1379–1388. [[CrossRef](#)]
30. Staples, T.E.; van Rees, K.C.J.; van Kessel, C. Nitrogen competition using ¹⁵N between early successional plants and planted white spruce seedlings. *Can. J. For. Res.* **1999**, *29*, 1282–1289. [[CrossRef](#)]
31. Hanks, R.D.; Knight, J.D.; van Rees, K.C.J. Nitrogen accumulation by conifer seedlings and competitor species from ¹⁵N-labeled controlled-release fertilizer. *Soil Sci. Soc. Am. J.* **2003**, *67*, 300–308. [[CrossRef](#)]
32. Woods, P.V.; Nambiar, E.K.S.; Smethurst, P.J. Effect of Annual Weeds on Water and Nitrogen Availability to *Pinus-Radiata* Trees in a Young Plantation. *For. Ecol. Manag.* **1992**, *48*, 145–163. [[CrossRef](#)]
33. Ginn, S.E.; Seiler, J.R.; Cazell, B.H.; Kreh, R.E. Physiological and Growth-Responses of 8-Year-Old Loblolly-Pine Stands to Thinning. *For. Sci.* **1991**, *37*, 1030–1040.
34. Groninger, J.W.; Seiler, J.R.; Peterson, J.A.; Kreh, R.E. Growth and photosynthetic responses of four Virginia Piedmont tree species to shade. *Tree Physiol.* **1996**, *16*, 773–778. [[CrossRef](#)] [[PubMed](#)]
35. Binkley, D.; Stape, J.L.; Ryan, M.G. Thinking about efficiency of resource use in forests. *For. Ecol. Manag.* **2004**, *193*, 5–16. [[CrossRef](#)]
36. Martindale, W.; Leegood, R.C. Acclimation of photosynthesis to low temperature in *Spinacia oleracea* L. 2. Effects of nitrogen supply. *J. Exp. Bot.* **1997**, *48*, 1873–1880. [[CrossRef](#)]
37. Davey, P.A.; Parsons, A.J.; Atkinson, L.; Wadge, K.; Long, S.P. Does photosynthetic acclimation to elevated CO₂ increase photosynthetic nitrogen-use efficiency? A study of three native UK grassland species in open-top chambers. *Funct. Ecol.* **1999**, *13*, 21–28. [[CrossRef](#)]
38. Kitajima, K.; Hogan, K.P. Increases of chlorophyll a/b ratios during acclimation of tropical woody seedlings to nitrogen limitation and high light. *Plant Cell Environ.* **2003**, *26*, 857–865. [[CrossRef](#)] [[PubMed](#)]
39. Diaz-Espejo, A.; Nicolas, E.; Fernandez, J.E. Seasonal evolution of diffusional limitations and photosynthetic capacity in olive under drought. *Plant Cell Environ.* **2007**, *30*, 922–933. [[CrossRef](#)] [[PubMed](#)]
40. Petersen, T.D.; Newton, M.; Zedaker, S.M. Influence of *Ceanothus velutinus* and Associated Forbs on the Water-Stress and Stemwood Production of Douglas-Fir. *For. Sci.* **1998**, *34*, 333–343.
41. Carter, G.A.; Miller, J.H.; Davis, D.E.; Patterson, R.M. Effect of Vegetative Competition on the Moisture and Nutrient Status of Loblolly-Pine. *Can. J. For. Res.* **1984**, *14*, 1–9. [[CrossRef](#)]

42. Elliott, K.J.; White, A.S. Competitive Effects of Various Grasses and Forbs on Ponderosa Pine-Seedlings. *For. Sci.* **1987**, *33*, 356–366.
43. Sands, R.; Nambiar, E.K.S. Water Relations of *Pinus radiata* in Competition with Weeds. *Can. J. For. Res.* **1984**, *14*, 233–237. [[CrossRef](#)]
44. Watt, M.S.; Whitehead, D.; Mason, E.G.; Richardson, B.; Kimberley, M.O. The influence of weed competition for light and water on growth and dry matter partitioning of young *Pinus radiata*, at a dryland site. *For. Ecol. Manag.* **2003**, *183*, 363–376. [[CrossRef](#)]
45. Nambiar, E.K.S.; Zed, P.G. Influence of Weeds on the Water Potential, Nutrient Content and Growth of Young Radiata Pine. *Aust. For. Res.* **1980**, *10*, 279–288.
46. Balandier, P.; Collet, C.; Miller, J.H.; Reynolds, P.E.; Zedaker, S.M. Designing forest vegetation management strategies based on the mechanisms and dynamics of crop tree competition by neighbouring vegetation. *Forestry* **2006**, *79*, 3–27. [[CrossRef](#)]
47. Casper, B.B.; Jackson, R.B. Plant competition underground. *Annu. Rev. Ecol. Syst.* **1997**, *28*, 545–570. [[CrossRef](#)]
48. Montgomery, R.A.; Reich, P.B.; Palik, B.J. Untangling positive and negative biotic interactions: views from above and below ground in a forest ecosystem. *Ecology* **2010**, *91*, 3641–3655. [[CrossRef](#)] [[PubMed](#)]
49. Tripathi, S.K.; Sumida, A.; Ono, K.; Shibata, H.; Uemura, S.; Takahashi, K.; Hara, T. The effects of understory dwarf bamboo (*Sasa kurilensis*) removal on soil fertility in a *Betula ermanii* forest of northern Japan. *Ecol. Res.* **2006**, *21*, 315–320. [[CrossRef](#)]
50. Tripathi, S.K.; Sumida, A.; Shibata, H.; Uemura, S.; Ono, K.; Hara, T. Growth and substrate quality of fine root and soil nitrogen availability in a young *Betula ermanii* forest of northern Japan: Effects of the removal of understory dwarf bamboo (*Sasa kurilensis*). *For. Ecol. Manag.* **2005**, *212*, 278–290. [[CrossRef](#)]
51. Takahashi, K.; Uemura, S.; Suzuki, J.I.; Hara, T. Effects of understory dwarf bamboo on soil water and the growth of overstory trees in a dense secondary *Betula ermanii* forest, northern Japan. *Ecol. Res.* **2003**, *18*, 767–774. [[CrossRef](#)]



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