forests

MDPI

Article

Biomass and Carbon Sequestration by *Juglans regia* Plantations in the Karst Regions of Southwest China

Hao Zhang [1,2,3], Kelin Wang [1,2], Zhaoxia Zeng [1,2], Hu Du [1,2] and Fuping Zeng [1,2,*]

1 Key Laboratory of Agro-Ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, China; zhanghao@isa.ac.cn (H.Z.); kelin@isa.ac.cn (K.W.); elizeberth@163.com (Z.Z.); hudu@isa.ac.cn (H.D.)
2 Huanjiang Observation and Research Station for Karst Ecosystem, Chinese Academy of Sciences, Huanjiang 547100, China
3 State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangxi University, Nanning 530004, China
* Correspondence: fpzeng@isa.ac.cn

Academic Editors: Phillip G. Comeau and Timothy A. Martin
Received: 23 December 2016; Accepted: 24 March 2017; Published: 29 March 2017

Abstract: To better understand carbon (C) sequestration by *Juglans regia* L. plantations in karst regions of southwest China, this study examined biomass increment and C storage in four different-aged *J. regia* stands, as well as the distribution of carbon stock among the various ecosystem components. Tree and ecosystem biomass increased with stand age. Aboveground biomass (AGB) represented 64.79% of the total biomass, belowground tree biomass comprised 22.73%, and shrubs and herbs totaled 11.38%, whereas only a small amount (1.11%) was associated with soil litter. Soil organic C (SOC) content in the top soil of plantations aged 1, 5, 9, and 13 years was 33.37, 53.15, 33.56, and 49.78 Mg ha^{-1}, respectively. SOC content decreased continually with increasing soil depth. Ecosystem C storage amounted to 33.49, 54.21, 46.40, and 65.34 Mg ha^{-1} for the 1-, 5-, 9-, and 13-year old plantations, respectively, with most (86.55%) of the ecosystem C being in the soil. Our results suggest that large-scale planting of *J. regia* has potential for not only vegetation restoration but also high C fixation capacity in the karst region of southwest China.

Keywords: afforestation; carbon storage; *Juglans regia*; karst region; stand age

1. Introduction

Land use change is often considered to be an important factor influencing the global carbon (C) cycle because it changes the rate of soil erosion and vegetation biomass [1,2]. Over the past 200 years, the conversion of forest and grassland to cropland has contributed to elevated atmospheric CO_2 concentrations and a warming global climate [3–5]. To improve land quality and prevent further soil erosion, the government of the People's Republic of China implemented the nationwide Grain for Green Program (GGP) to convert cropland to grassland and forest in 1999 [6]. In practice, as the most ambitious ecosystem preservation program in China, the GGP has helped return steep farmlands to forests and grass, which greatly improved ecological quality in mountainous areas. The Department of Chinese Forestry has reported that 23,344,000 ha of lands, including 15,076,000 ha of barren lands and 8,268,000 ha of croplands, has been afforested from 1999 to 2010 [7]. In addition, the GGP also contributed to increased accumulation of C in both vegetation and soil in degraded ecosystems, which has received increasing attention from researchers [8–10].

As one of the key national zones of the GGP, karst regions (covering 5.5×10^5 km^2) in Southwest (SW) China are considered fragile because of their geochemical properties, low environmental carrying capacity, and sensitivity to disturbance [11]. Moreover, karst regions of SW China have extensive areas of steep, eroded barren land and degraded cropland. Most of the farmland is on slopes ≥6°, whereas 20% of the total farmland area is on slopes ≥25° [12,13]. In the last 50 years, forests in karst regions have been negatively affected by fast-growing populations, excessive logging, and steep-slope farming [14]. Under the GGP, tree plantations were widely used to restore degraded ecosystems and maintain C balance [15,16]. For instance, by the end of 2011, 3.3×10^6 ha of land had been planted in SW China under the GGP, which accounted for 4.97% of total GGP lands [7]. Therefore, the implementation of GGP in the karst area may play a significant role in attempts to develop extensive new forests and ultimately reduce atmospheric CO_2 over the long term [11].

Juglans regia L. belongs to the family Juglandaceae and is cultivated extensively for nut production. It is widely disseminated in Asia, North and South America, Europe, South Africa, Australia, and New Zealand [17]. The nuts are a rich source of polyunsaturated fats, and the leaf, bark, and kernel tissues have a rich diversity of phenolics, which play unique roles in the human diet and the biology of the tree [18]. Under the GGP, multi-million hectare plantations of *J. regia* have been planted to reverse desertification in the karst region because of its drought tolerance and ability to grow rapidly under conditions of calcerous soils and in poor habitats [8]. The fruit of the *J. regia* tree can be an important source of income for farmers in impoverished karst regions of SW China [19]. Because of their economic and ecological benefits, *J. regia* plantations in Southwest China currently cover more than 3,000,000 ha, and their chemical and biological characteristics have been characterized [20]. For these large plantations in SW China, it is necessary to understand the importance of *J. regia* stands in the regional C budget. Although there is abundant research on genome sequence, food chemistry, plant disease, and nutrient cycling in *J. regia* plantations [18,19,21], the biomass and carbon sequestration by *J. regia* plantations in the karst regions is still unknown. Therefore, a better understanding of C sequestration in *J. regia* plantations at a regional scale is fundamentally important for forest managers, policy makers, and researchers wishing to enhance forest C sequestration.

Although our previous study demonstrated relationships between biomass and soil nutrients in *J. regia* stands of different ages in depressions between karst hills [21], the uncertainty regarding C and biomass allocation of this species along different stand ages represents a major information gap that hampers efforts to estimate C pool dynamics in karst regions. Thus, the present study examined changes in biomass and carbon storage in four different *J. regia* plantations of different ages under the GGP in Fengshan County, Guangxi Province. The aims of this research were to (1) estimate the biomass components of a plantation ecosystem across a chronosequence of four *J. regia* stands (1-, 5-, 9-, and 13-years old); (2) determine the changes in the C concentrations and C stocks of ecosystem components with increasing stand age in karst regions of SW China.

2. Material and Methods

2.1. Experimental Sites

The four plantation sites are located in Fengshan County (106°42′ E–107°17′ E, 24°15′ N–24°49′ N) of Guangxi Province, Southwest China. The total area of this county is 1700 km^2, of which 70.1% belongs to the karst. The undulating land has an elevation of 600–900 m, and is characterized by broken terrain, sparse vegetation, eroded soil, and exposed bedrock. This county has a subtropical monsoon climate with an average annual temperature of 17.6 °C. The area receives an annual average of 2200 h of sunshine and 269 frost-free days, whereas the active accumulated temperature (≥10 °C) is 6426 °C. The mean precipitation is 1550 mm $year^{-1}$, with 65% falling during May to August. Evapotranspiration is approximately 1130 mm $year^{-1}$ [21].

Vegetation coverage has increased under the GGP in Fengshan County, from 24.8% in 2001 to 60.3% in 2008. Indeed, this county, with its extensive cover of *J. regia*, has become an example of how desertification can be controlled through the planting of forests under the GGP in Guangxi Province and SW China. The area of the plantation forest was approximately 5200 ha in 2015, 2100 ha of which was covered by *J. regia* plantations.

2.2. Field Sampling and Measurements

We applied a chronosequence approach using *J. regia* plantation stands that were 1, 5, 9, and 13 years old (containing 350 to 450 trees per ha). Four plantation sites for all age classes were randomly selected in this study. Based on a following site inspection, three 20 m × 50 m plots for each age class site were established in August 2015 (Table 1). Buffers were maintained between the plots and established plots for each stand age. To reduce the effects of environmental factors, all plots within each location were adjacent to each other (no more than 200 m) and characterized by similar terrain, topography, understory vegetation, and soil. All plots belonged to the GGP regions and had the same organic amendments, fertilizer trials, tillage, and weed control treatments before plantation. According to the standards of the Centre for Tropical Forest Science [22], we established the plots and divided them into ten 10 m × 10 m subplots. Tree density, height (H), diameter at breast height (DBH), and shrub and herb parameters (density, type, biomass) were recorded for each entire plot and all three subplots. Plant density was estimated according to the plant number per subplot. H and DBH were measured by hypsometer and tape measure, respectively.

Table 1. Site characteristics and stand characteristics of four age classes of *J. regia* plantations (n = 3).

Characteristics Parameters	1-Year-Old	5-Year-Old	9-Year-Old	13-Year-Old
Site characteristics				
Altitude (m)	605a ± 12	750b ± 19	790c ± 14	755b ± 20
Slope (°)	27a ± 3.6	29a ± 4.1	25a ± 3.4	26a ± 3.9
Soil depth (cm)	68a ± 5.9	65a ± 6.2	74a ± 6.0	70a ± 4.5
Soil pH	7.0a ± 0.2	6.8a ± 0.3	7.1a ± 0.3	7.2a ± 0.2
Soil texture	Silt	Silt	Silt	Silt
Cation exchange capacity (cmol/kg)	31.3a ± 3.2	32.5a ± 2.6	29.9a ± 2.9	32.1a ± 2.5
Bulk density (g/cm^3)	1.26a ± 0.20	1.72a ± 0.29	1.31a ± 0.22	1.22a ± 0.19
Coarse rock fragment (%)	20.1a ± 3.8	16.8a ± 3.3	13.7a ± 4.2	16.2a ± 3.2
Percentage of covered soil (%)	57.8ab ± 3.0	60.1b ± 2.2	51.4a ± 3.8	68.9c ± 3.4
Stand characteristics				
Mean DBH (cm)	1.8a ± 0.2	6.4b ± 1.1	13.8c ± 2.4	17.5c ± 2.9
Mean height (m)	1.7a ± 0.4	3.7b ± 1.2	5.2bc ± 1.5	7.9c ± 1.8
Stand density (plant/ha)	380a ± 11	378a ± 13	370a ± 10	325b ± 15

DBH, diameter at breast height. Data show the mean ± S.E. and different lower-case letters indicate significant difference at $p < 0.05$ (one-way ANOVA and LSD test).

Three trees of average size (i.e., height, stem, and crown diameter), located near the middle of each plot and as close to each other as possible, were selected for destructive sampling to determine tree biomass. Each selected tree was separated into foliage, stem, branch, root, and fruit [4,23]. The coarse root (>2 mm) of each tree in a radius between 1.5 m and 3.5 m (depending on tree size) was excavated and washed, and roots were separated by hand into size classes based on diameter (≤2 mm and >2 mm). The biomass of the coarse root per hectare was estimated by multiplying the mean weight of the coarse root volume by the number of trees in each hectare. Fine roots (≤2 mm) around 24 selected trees (in a radius of 5 m) at different depths (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, and 50–100 cm) were sampled using a soil corer (90 mm inside diameter). The fine root biomass per hectare was estimated by multiplying the weights of the fine roots per core 0.006392 m^2 by 1,564,455.57. Total root biomass per hectare was estimated by adding the biomass of the coarse root per hectare to the fine root biomass per hectare. The fresh weights of ecosystem components (foliage, stem, branch, root, and fruit) were measured in situ, and six subsamples of each component from each collected plant were dried at 65 °C to estimate dry biomass density (Mg ha^{-1}) and C concentration.

The biomass of the understory vegetation, dominated by *Cipadessa cinerascens* (Pell.) Hand-Mazz, *Vitex negundo* Linn, *Imperata cylindrical*, *Ageratum conyzoides*, and *Apluda mutica*, was weighed by destructive harvesting of shrubs and herbs within three 2 × 2 m microplots within each 20 m × 50 m plot. We also collected all litter from three 1 × 1 m herbaceous quadrats in each 20 m × 50 m plot.

Additionally, the aboveground biomass of understory vegetation and litter were taken back to the laboratory for drying and reweighing.

We collected ten composite samples of soil in the middle of the subplots (10 m × 10 m) of each plot (20 m × 50 m). Soil samples at depths of 0–10, 10–20, 20–30, 30–50, and 50–100 cm were collected using a soil auger corer (70 mm inside diameter) to calculate soil C storage. Three soil cores per subplot were taken and thoroughly mixed into one composite sample. Because the soil depth in the karst region is lower than that in the non-karst region [16], the soil samples at depths of 50–100 cm depended on the soil depth in different plots. For each plot, 50 soil composite samples (10 composite samples per layer × five layers) were collected. Additionally, to determine soil bulk density at different depths (0–10 cm, 10–20 cm, 20–30 cm, 30–50 cm, and 50–100 cm) in each location, we collected six soil samples at each depth using a 70 mm height × 52 mm diameter ring. After removal of rocks and plant residues from the samples, the bulk density at each soil depth was determined by weighing the samples after drying at 105 °C. In the laboratory, 200 g of soil per auger sample was sieved with a 2-mm sieve and mixed. To determine soil organic carbon (SOC) concentration, 0.2 g of sieved soil per auger sample was measured in a KCr_2O_7-H_2SO_4 oil bath using a vario macro elemental analyzer (Elementar Analysensysteme GmbH, Berlin, Germany). To eliminate the influence of rocks on the soil C storage measurement in the karst region, the percentage of covered soil was also measured by the method described above [16]. In addition, the site characteristics (e.g., elevation, slope, and soil depth, cation exchange capacity, and coarse rock fragments) were determined by the methods described by Du et al. [16] and Song et al. [24].

2.3. Data Analysis

The effects of stand age on C concentration, C storage, and biomass accumulation of ecosystem components were compared using one-way ANOVA, followed by Fisher's LSD method for testing the null hypothesis. α = 0.05 was set as the significance level for all statistical analyses. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS 12.0, Chicago, IL, USA). In addition, the C storage in ecosystem components, such as herbaceous plants, shrubs, trees, and litter, was calculated by multiplying the C concentration of each component by its mass per hectare. The C storage in each soil layer (CS, Mg ha^{-1}) was determined according to SOC concentration (Cc, g kg^{-1}), sampled depth (D, cm), bulk density (BD, g cm^{-3}), and percentage of covered soil (PCS, %) using the equation: $CS = Cc \times D \times BD \times PCS/10$. The SOC stock was the sum of each soil layer.

3. Results

3.1. Biomass Distribution in Ecosystem Components

In plantations of *J. regia*, belowground biomass (BGB), aboveground biomass (AGB), and total tree biomass (TTB) increased significantly with stand age (Table 2). BGB and AGB increased from 0.08 and 0.18 Mg ha^{-1}, respectively, in the 1-year-old stand to 5.63 and 21.01 Mg ha^{-1}, respectively, in the 13-year-old stand. Similarly, the biomass of tree components also changed with stand age (Table 2). For the 1-year-old stand, biomass increased in the following order: foliage < root < stem. For the 13-year old stand, the increase in biomass production was in the following order: foliage < fruit < root < branch < stem. The ratio of root biomass to TTB in the 1-year-old stand was higher than that in the 5-, 9-, and 13-year-old stands.

There were no shrubs or herbs found in the understory of 1- and 5-year old *J. regia* plantations because both are usually removed by farmers. The biomass of shrub, herb, and litter in the 9-year old stand was not significantly different than that of the 13-year old stand. The overall biomass of the ecosystem also increased markedly with stand age.

Table 2. Biomass (Mg ha^{-1}) in trees, understory vegetation, and litter in four age classes of *J. regia* plantations (*n* = 3).

Component	1-Year-Old	5-Year-Old	9-Year-Old	13-Year-Old
Tree				
Foliage	0.07a ± 0.02	0.26b ± 0.09	1.63c ± 0.17	2.28d ± 0.24
Branch	-	0.39a ± 0.12	4.95b ± 1.02	6.57b ± 1.54
Stem	0.11a ± 0.03	1.03b ± 0.27	6.46c ± 1.79	9.25c ± 1.95
Fruit	-	-	0.87a ± 0.18	2.91b ± 0.33
Aboveground biomass (AGB)	0.18a ± 0.06	1.68b ± 0.49	13.91c ± 2.49	21.01d ± 4.04
Roots (BGB)	0.08a ± 0.03	0.61b ± 0.12	4.24c ± 1.06	5.63c ± 1.19
BGB/AGB	0.44	0.36	0.30	0.27
Total tree biomass (TTB)	0.26a ± 0.07	2.29b ± 0.62	18.15c ± 3.52	26.64c ± 5.28
Understory vegetation				
Shrub	-	-	2.94a ± 0.43	2.48a ± 0.51
Herb	-	-	4.33a ± 1.40	3.92a ± 1.23
Total understory vegetation	-	-	7.27a ± 1.53	6.40a ± 1.75
Litter	-	-	0.59a ± 0.23	0.72a ± 0.38
Total biomass	0.26a ± 0.07	2.29b ± 0.62	26.01c ± 5.25	33.76c ± 7.12

BGB, belowground biomass. Note: Data show the mean ± S.E. and different lower-case letters indicate significant difference at $p < 0.05$ (one-way ANOVA and LSD test). There are no shrubs and herbs in the understory of 1-, and 5-year old *J. regia* plantations due to their removal by local farmers.

3.2. Carbon Concentration and Accumulation in Biomass

Carbon concentration in trees, shrubs, herbs, and litter was age-independent (Table 3). On average, the C concentration in fruit was the highest among all ecosystem components, whereas that in the branch fraction was the lowest. Among all tree components, fruit showed the highest C concentration, with a mean value of 486.4 g kg^{-1}. In contrast, the C concentration in the tree branches had the lowest values. The mean C concentrations of foliage, stems, and roots were 464.7, 461.3, and 452.3 g kg^{-1}, respectively. The mean C concentration of litter was 476.2 g kg^{-1}.

Table 3. Carbon concentration (g kg^{-1}) in trees, understory vegetation, and litter for 1-, 5-, 9-, and 13-year-old *J. regia* plantations (*n* = 3).

Component	1-Year-Old	5-Year-Old	9-Year-Old	13-Year-Old	Mean
Tree					
Foliage	457.8ab ± 9.5	476.3b ± 7.6	439.0a ± 9.3	485.7b ± 8.2	464.7
Branch	-	430.7a ± 10.2	459.2b ± 8.4	421.6a ± 11.5	437.2
Stem	449.5a ± 7.3	478.1b ± 12.1	464.6ab ± 10.7	452.9a ± 9.5	461.3
Fruit	-	-	478.7a ± 8.1	494.1a ± 9.3	486.4
Roots	448.3a ± 9.3	461.6a ± 10.2	442.9a ± 9.6	456.7a ± 9.9	452.3
Understory vegetation					
Shrub	-	-	434.9a ± 10.4	484.2b ± 11.5	459.6
Herb	-	-	456.3a ± 9.8	492.3b ± 13.2	474.3
Litter	-	-	469.5a ± 12.3	482.9a ± 9.8	476.2

Note: Data show the mean ± S.E. and different lower-case letters indicate significant difference at $p < 0.05$ (one-way ANOVA and LSD test).

The average value of C stored in the AGB was 0.08 Mg ha^{-1} for the 1-year-old plantation, and 0.78, 6.42, and 9.51 Mg ha^{-1} for the 5-, 9-, and 13-year-old plantations, respectively (Table 4). Root biomass C storage steadily increased from 0.04 Mg ha^{-1} in the 1-year-old plot to 0.28, 1.88, and 2.57 Mg ha^{-1} in the 5-, 9-, and 13-year-old plots, respectively, representing 33.3%, 26.4%, 20.2%, and 21.3%, respectively, of the total tree biomass (TTB) C storage in the four stand age classes (Figure 1). Most of the carbon in the biomass was stored in the TTB, shrubs, and herbs, whereas only a small amount was stored in litter (Figure 1). Biomass C content in fruit also increased with stand age. The contribution of fruit

C storage to total biomass C content varied from 0 for the 1- and 5-year-old stands to 9.25% for the 13-year-old stand (Figure 1). Moreover, the C storage in shrub, herb, and litter was not age-dependent. The total biomass C in trees, understory vegetation, and litter increased significantly with stand age.

Table 4. Biomass carbon (Mg ha^{-1}) in trees, understory vegetation, and litter in 1-, 5-, 9-, and 13-year-old *J. regia* plantations (n = 3).

Component	1-Year-Old	5-Year-Old	9-Year-Old	13-Year-Old
Tree				
Foliage	0.03 a ± 0.01	0.12b ± 0.04	0.72c ± 0.27	1.11c ± 0.25
Branch	-	0.17a ± 0.07	2.27b ± 0.42	2.77b ± 0.54
Stem	0.05a ± 0.02	0.49b ± 0.13	3.01c ± 0.89	4.19c ± 1.05
Fruit	-	-	0.42a ± 0.08	1.44b ± 0.16
Aboveground biomass (AGB)	0.08a ± 0.03	0.78b ± 0.29	6.42c ± 1.30	9.51c ± 2.21
Roots (BGB)	0.04a ± 0.02	0.28b ± 0.06	1.88c ± 0.54	2.57c ± 0.58
BGB/AGB	0.50	0.36	0.29	0.27
Total tree biomass (TTB)	0.12a ± 0.04	1.06b ± 0.36	9.30c ± 1.75	12.08c ± 2.78
Understory vegetation				
Shrub	-	-	1.28a ± 0.25	1.20a ± 0.27
Herb	-	-	1.98a ± 0.77	1.93a ± 0.63
Total understory vegetation	-	-	3.26a ± 0.78	3.13a ± 0.81
Litter	-	-	0.28a ± 0.11	0.35a ± 0.16
Total biomass	0.12a ± 0.04	1.06b ± 0.36	12.84c ± 2.67	15.56c ± 3.51

Note: Data show the mean ± S.E. and different lower-case letters indicate significant difference at $p < 0.05$ (one-way ANOVA and LSD test).

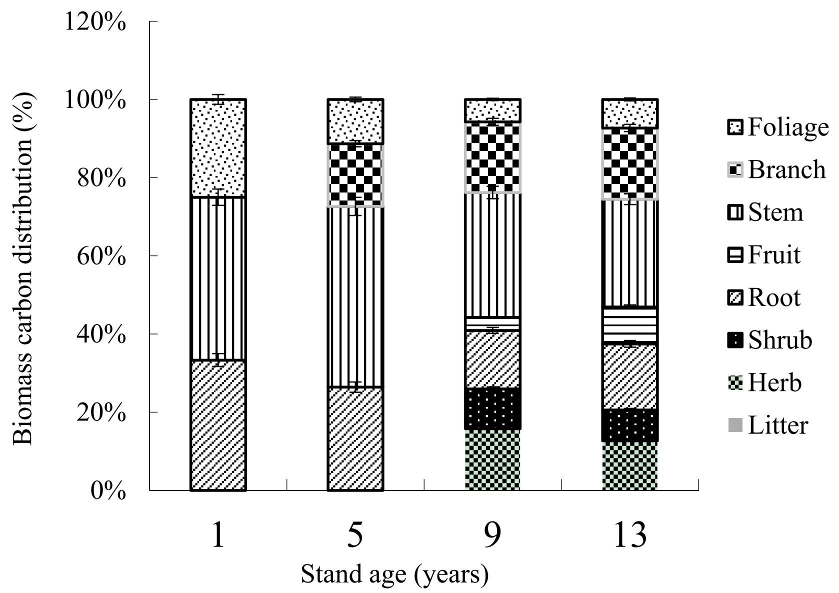

Figure 1. Percentage distribution of biomass C storage in *J. regia* plantations of four different ages.

3.3. Carbon Concentration and Storage in Soil

Statistically significant differences were observed for SOC values between different aged stands at different soil depths (Figure 2; Table 5). SOC concentration decreased with soil depth irrespective of stand age (Figure 2). The SOC concentration at 0–10 cm ranged from 16.96 g/kg to 26.46 g/kg, which was much higher than that at other soil depths. The C concentration of the soil at 0–10 cm, 10–20 cm, and 20–30 cm increased significantly with stand age, whereas the C concentration at 30–50 cm and 50–100 cm did not. In the karst area, SOC storage in *J. regia* plantations did not increase with stand age because soil depth, soil bulk density, coarse rock fragments, and rocks in the soil can affect the total mass of the soil. The mean SOC storage in the top soil (less than 100 cm in the karst soil) in the 1-, 5-, 9-, and 13-year-old stands was 33.37, 53.15, 33.56, and 49.78 Mg ha^{-1}, respectively. More than 79.5% of SOC was stored within the upper 0.5 m of the soil profile from each stand (Table 5).

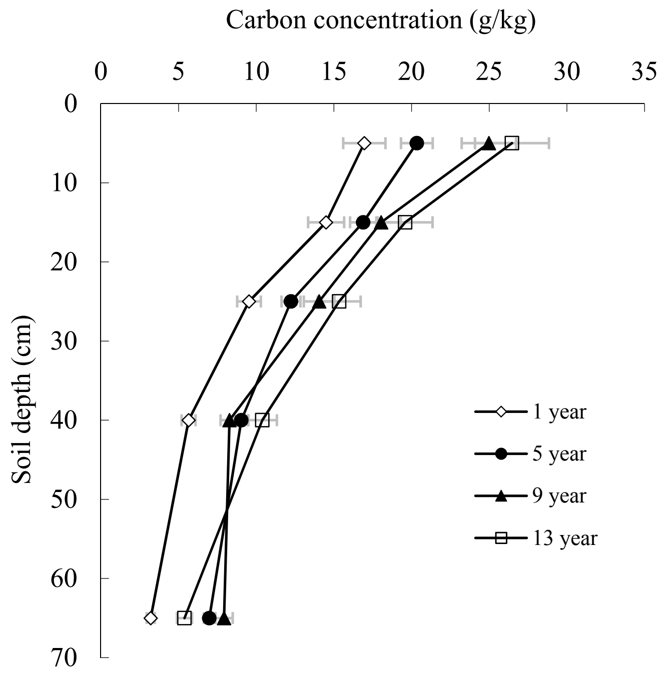

Figure 2. Soil carbon content in different depths in *J. regia* plantations of four different ages. Note: Error bar indicates S.E.

Table 5. Soil carbon storage (Mg ha^{-1}) in different depths in 1-, 5-, 9-, and 13-year-old *J. regia* plantations (n = 30).

Soil	Depth (cm)	1-Year-Old	5-Year-Old	9-Year-Old	13-Year-Old
	0–10	9.75a ± 1.12	13.85b ± 2.07	9.05a ± 1.52	14.17b ± 2.38
	10–20	8.33ab ± 0.81	11.50b ± 2.34	6.53a ± 1.08	10.49b ± 2.63
Carbon storage (Mg ha^{-1})	20–30	5.48a ± 1.20	8.34b ± 1.12	5.09a ± 1.06	8.21b ± 1.65
	30–50	6.49a ± 1.04	12.33b ± 1.43	6.00a ± 1.21	11.14b ± 1.06
	50–100	3.32a ± 0.59	7.13c ± 0.68	6.89c ± 0.70	5.77b ± 0.54
	0–100	33.37a ± 4.18	53.15b ± 6.46	33.56a ± 6.39	49.78b ± 5.81

Note: Data show the mean ± S.E and different lower-case letters indicate significant difference at $p < 0.05$ (one-way ANOVA and LSD test).

3.4. *Carbon Storage in the* J. regia *Plantation Ecosystem*

Ecosystem C storage amounted to 33.49, 54.21, 46.40, and 65.34 Mg ha^{-1} for the 1-, 5-, 9- and 13-year old plantations, respectively. In stands of all ages, most of the ecosystem C accumulated in the soil rather than in the biomass (Figure 3). In the 1-year-old plantation, 99.64% of the ecosystem C was stored in the soil, and this proportion decreased to 76.19% in the 13-year-old plantation. Carbon accumulation in biomass increased with stand age. The contribution of biomass C to ecosystem C increased with stand age from 0.36% for the 1-year-old stand to 23.81% for the 13-year-old stand (Figure 3).

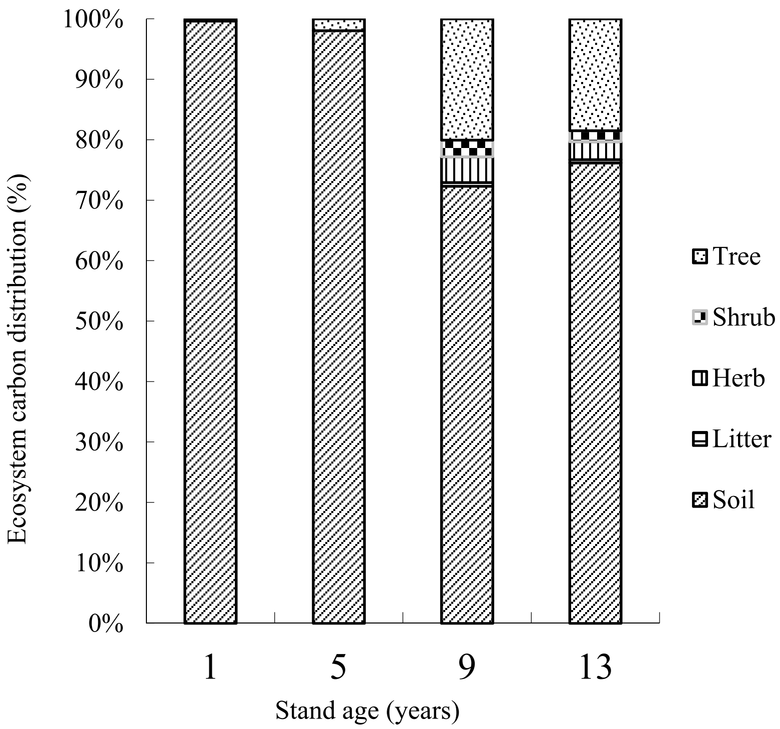

Figure 3. Percentage distribution of ecosystem C storage in *J. regia* plantations of four different ages.

4. Discussion

4.1. Biomass of J. regia Plantations of Different Ages

In this study, the biomass of *J. regia* plantations across the chronosequence indicated that tree biomass increased with stand age. This pattern was similar to that of many previous reports [25–27], and is consistent with expected patterns of biomass accumulation. Our results revealed a total tree biomass (TTB) of 0.26 Mg ha^{-1} for the 1-year-old stand and 26.01 Mg ha^{-1} for the 9-year-old stand of *J. regia* (Table 2), which was higher than that previously reported for other tree species. For example, Cheng et al. measured a TTB of 0.05 Mg ha^{-1} for a 1-year-old stand and 16.70 Mg ha^{-1} for 10-year-old stands within *Zanthoxylum bungeanum* plantations [13]. Similarly, the TTB for a 10-year-old mixed forest of *Betula luminifera* and *Populus euramevicana* was only 4.03 Mg ha^{-1}, and even less (1.12 Mg ha^{-1}) for a plantation of *Cupressus funebris* [28,29]. These comparisons demonstrate that afforestation of the karst region with *J. regia* under the GGP has greater potential to accumulate biomass than afforestation with these other species.

The ratio of belowground to aboveground biomass decreased with stand age, which was consistent with the results of our previous study [30]. In the karst region, shallow soil and the soil-rock mixture from lime soil may be responsible for the smaller root biomass of adult trees [13]. Thus, these results may not be consistent with some reports of adult forests that have large amounts of root biomass in non-karst regions [29]. However, the large ratio of belowground (root) biomass to total biomass (0.27–0.50) of adult trees also highlights the importance of accounting for roots in the accurate estimation of the total biomass and carbon of plantation forests [21].

In the karst region, abundant rainfall, sunlight, and sparse tree cover may favor the growth of understory vegetation [13]. Here, our data showed that the understory biomass ranged from 6.40 Mg ha^{-1} in the 13-year-old stand to 7.27 Mg ha^{-1} in the 9-year-old stand. These values were higher than other previously reported values of 0.87–3.55 Mg ha^{-1} and 1.61–3.76 Mg ha^{-1} [26,27]. In addition, the understory biomass may not correspond to stand age. In fact, it may depend more on farming activities, soil conditions, stand-specific canopy, stand density, and forest types, which could affect nutrient availability, water, and light for the development of the understory [31].

4.2. Biomass C of J. regia Plantations of Different Ages

C concentration is usually regarded as a fixed value (0.5) to convert dry biomass into carbon storage [30]. In this study, the C concentration for components of *J. regia* ranged from 0.422 to 0.494 (Table 3). The mean value of 0.464 was similar to the C concentration previously observed for *Acacia auriculiformis* (0.466), but lower than that for *Masson pine* (0.577) and tea-oil *Camellia* (0.503) [32]. Previous studies showed that C concentrations of plantation forests are often influenced by geographical location, climate, soil conditions, wood type, and tree components [13,33]. Thus, all of these factors might be used to accurately calculate C stock when measuring C concentrations under different edaphic and climatic conditions.

The overall within-stand biomass C increased with stand age, from 0.12 Mg C ha^{-1} in the 1-year-old stand to 12.08 Mg C ha^{-1} in the 13-year-old stand (Table 4). The accumulation of C in the total tree biomass changed with stand age, and the pattern of change was similar to that of previous studies [10,13]. Accumulation of biomass C in younger stands was more rapid than that in older stands. In this study, the highest rate of C accumulation was found in 5–9-year-old stands (Table 4). This indicates that *J. regia* is well able to adapt to the water deficiency stress that is common in karst regions [21]. The *J. regia* plantations in the present study stored more biomass C than other plantations in similar karst regions. For example, the tree C stock of *Z. bungeanum* increased from 0.02 to 7.56 Mg C ha^{-1} in the 1–10 stand ages [13], which is less than that in the present study.

Previous research showed that the C stock of understory vegetation decreased with increasing stand age [34]. However, the C stock of the understory vegetation in this study was not significantly affected by stand age. This discrepancy might be explained by farmer activity that removed both herbs and shrubs in the understory of the 1- and 5-year-old *J. regia* plantations. Interestingly, we found that herbs dominated the understory vegetation C pool in 9- and 13-year old *J. regia* stands, and the proportion of herb C relative to the total understory vegetation C was approximately 60%. In addition, the understory vegetation C in the four stands ranged from 0 to 3.26 Mg ha^{-1}, which is a smaller amount than that previously reported for a same-aged *Eucalyptus* plantation [16]. Litter C is highly susceptible to human disturbances, decomposition rate, and litter input [35], which may explain why there was no association between litter C and stand age in this study.

4.3. Soil Organic Carbon (SOC) Storage in J. regia Plantations

In this study, SOC storage did not increase with stand age (Table 5). Previous research on C storage in mineral soils has been inconsistent. Many studies have reported no significant increase in SOC stocks with stand age [36,37], whereas some studies showed increasing SOC stocks in the early period after forestation [38]. This discrepancy may be due to differences in the C balance between fine root production and soil respiration rates, which could overshadow the effect of stand age [4]. Additionally, the SOC stocks in our study were considerably lower than those previously reported in non-karst regions (16, 27). In karst soils under *J. regia* plantations, over 80% of ecosystem C is stored in the top 50 cm of the soil. This means that, although it is vulnerable to human disturbance and soil erosion, the top soil in the karst area is a major C pool. In practice, implementation of good plantation management practices in the GGP is necessary to promote carbon sequestration [13].

We found that the soil C concentration at 0–30 cm increased with stand age (Figure 2), which was consistent with other studies [23,26]. The soil C concentrations in the top soil layers increase as the stands age, resulting from an increase in slow decomposition and litter input [39]. Therefore, our results suggest that destruction of topsoil from human disturbances is detrimental to C sequestration in the karst region.

4.4. Influence of GGP on C Sequestration in the Karst Region

Our studies suggest that the GGP will enhance ecosystem carbon sequestration due to afforestation in plantations. Biomass C storage in *J. regia* plantations can rapidly increase from 0.12 Mg ha^{-1} for the 1-year-old stand to 12.08 Mg ha^{-1} for the 13-year-old stand, which is similar to that found in other studies [13,14,40,41]. If *J. regia* was planted in all karst region under the GGP, more than 3.3×10^7 Mg C could have been sequestered between 1999 and 2010. In the long term, the conversion of farmland or abandoned land to *J. regia* plantations in the karst region would increase farmer incomes, protect fragile karst region ecology, and offset greenhouse gas emissions. However, we also note that pseudoreplication, the failure to establish a control plot, and limitations of root biomass data may seriously affect the reliability of the present results. Collection of additional data in other similar plantations is necessary to provide support for these observations. Moreover, we are also aware that the cultivation of a single species might reduce biodiversity and increase vulnerability of plantations to diseases and pests. Thus, the ecological issues related to pure forest, mixed forest, and agroforestry in the GGP also should be considered in future research.

5. Conclusions

The results presented here demonstrate changes in biomass components for the plantation ecosystem across a chronosequence of four *Juglans regia* stands and illustrate the changes in the C concentration and C stock of ecosystem components with increasing stand age. The biomass in trees and ecosystems increased with stand age, whereas an increase in the biomass of shrubs, herbs, and litter was not observed. Most of the carbon in the biomass was stored in the aboveground tree biomass, tree roots, shrubs, and herbs, whereas only a small amount was associated with soil litter. Soil organic C (SOC) in the top soil of plantations was not age-dependent. SOC content decreased continually with increasing soil depth. Ecosystem C storage was dominated by the SOC, with this component representing between 72.33% and 99.64% of ecosystem carbon. Therefore, planting of *J. regia* has potential for not only vegetation restoration but also high C fixation capacity in the karst region of Southwest China.

Acknowledgments: This research was supported by the National Key Research and Development Program (2016YFC0502505); Major State Basic Research Development Program of China (2015CB452703); Science & Technology Server (STS) Program of Chinese Academy of Sciences (KFJ-EW-STS-092); Joint Program of Guizhou Province, Bijie City and the Chinese Academy of Sciences (2015-05), Strategic Priority Research Program-Climate Change: Carbon Budget and Related Issues of the Chinese Academy of Sciences (XDA05070404 and XDA05050205), the "100 talents program" of the Chinese Academy of Sciences (2060299, Y251101111); the Western Light Program of Talent Cultivation of the Chinese Academy of Sciences, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources and Guangxi Provincial Program of Distinguished Expert in China.

Author Contributions: Hao Zhang conducted the literature search, the field investigation, and the statistical analysis for the manuscript and wrote and edited the majority of the manuscript. Kelin Wang organized the research collaboration, conducted the field investigation, critically reviewed the manuscript, and contributed to the writing. Zhaoxia Zeng and Hu Du contributed to the writing and editing of the manuscript. Fuping Zeng designed the field survey method.

Conflicts of Interest: The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Fang, J.Y.; Chen, A.P.; Peng, C.H.; Zhao, S.Q.; Ci, L.J. Changes in forest biomass carbon storage in China between 1949 and 1998. *Science* **2001**, *292*, 2320–2322. [CrossRef] [PubMed]
2. Lal, R. Soil carbon sequestration in China through agricultural intensification, and restoration of degraded and desertified ecosystems. *Land Degrad. Dev.* **2002**, *13*, 469–478. [CrossRef]

3. Pacaldo, R.S.; Volk, T.A.; Briggs, R.D. Greenhouse gas (GHG) potentials of shrub willow biomass crop (*Salix* x *dasyclados*) based on the above- and belowground biomass production across a 19-year chronosequence. *BioEnergy Res.* **2013**, *6*, 252–262. [CrossRef]
4. Pacaldo, R.S.; Volk, T.A.; Briggs, R.D. No differences in soil organic carbon in short rotation willow (*Salix* x *dasyclados*) along a 19-year chronosequence. *Biomass Bioenergy* **2013**. [CrossRef]
5. Pacaldo, R.S.; Volk, T.A.; Briggs, R.D. Carbon sequestration in fine roots and foliage offsets soil CO_2 effluxes along a 19-year chronosequence of Shrub Willow Biomass Crops. *BioEnergy Res.* **2014**, *7*, 769–776. [CrossRef]
6. Chang, R.Y.; Fu, B.J.; Liu, G.H.; Liu, S.G. Soil carbon sequestration potential for 'Grain for Green' Project in Loess Plateau, China. *Environ. Manag.* **2011**, *48*, 1158–1172. [CrossRef] [PubMed]
7. State Forestry Administration. *China Forestry Statistical Yearbook 1999–2011*; China Forestry Publishing House: Beijing, China, 2012.
8. Chen, X.G.; Zhang, X.Q.; Zhang, Y.P.; Wan, C.B. Carbon sequestration potential of the stands under the grain for green program in Yunnan Province, China. *For. Ecol. Manag.* **2009**, *258*, 199–206. [CrossRef]
9. Zhang, H.; Song, T.Q.; Wang, K.L.; Du, H.; Yue, Y.M.; Wang, G.X.; Zeng, F.P. Biomass and carbon storage in an age-sequence of Cyclobalanopsis glauca plantations in southwest China. *Ecol. Eng.* **2014**, *73*, 184–191. [CrossRef]
10. Zhang, H.; Song, T.Q.; Wang, K.L.; Yang, H.; Yue, Y.M.; Zeng, Z.X.; Peng, W.X.; Zeng, F.P. Influences of stand characteristics and environmental factors on forest biomass and root-shoot allocation in southwest China. *Ecol. Eng.* **2016**, *91*, 7–15. [CrossRef]
11. Yuan, D.X. The geology environment and hydro-ecological problem of karst region. *Land Res. South China* **2003**, *1*, 21–25.
12. Anderson, K.J.; Teuber, S.S.; Gobeille, A.; Cremin, P.; Waterhouse, A.L.; Steinberg, F.M. Walnut polyphenolics inhibit in vitro human plasma and LDL oxidation. *J. Nutr.* **2001**, *131*, 2837–2842. [PubMed]
13. Cheng, J.Z.; Lee, X.Q.; Theng, B.K.G.; Zhang, L.K.; Fang, B.; Li, F.S. Biomass accumulation and carbon sequestration in an age-sequence of *Zanthoxylum bungeanum* plantations under the Grain for Green Program in karst regions, Guizhou province. *Agric. For. Meteorol.* **2015**, *203*, 88–95. [CrossRef]
14. Xiong, Y.J.; Qiu, G.Y.; Mo, D.K.; Lin, H.; Sun, H.; Wang, Q.X.; Zhao, S.H.; Yin, J. Rocky desertification and its causes in karst areas: A case study in Yongshun county, Hunan province, China. *Environ. Geol.* **2009**, *57*, 1481–1488. [CrossRef]
15. Peng, W.X.; Wang, K.L.; Song, T.Q.; Zeng, F.P.; Wang, J.R. Controlling and restoration models of complex degradation vulnerable Karst ecosystem. *Acta Ecol. Sin.* **2008**, *28*, 811–820.
16. Du, H.; Wang, K.L.; Peng, W.X.; Zeng, F.P.; Song, T.Q.; Zhang, H.; Lu, S.Y. Spatial heterogeneity of soil mineral oxide components in depression between karst hills, Southwest China. *Chin. Geogr. Sci.* **2014**, *24*, 163–179. [CrossRef]
17. Crews, C.; Hough, P.; Godward, J.; Brereton, P.; Lees, M.; Guiet, S.; Winkelmann, W. Study of the main constituents of some authentic walnut oils. *J. Agric. Food Chem.* **2005**, *53*, 4853–4860. [CrossRef] [PubMed]
18. Labuckas, D.O.; Maestri, D.M.; Perello, M.; Martınez, M.L.; Lamarque, A.L. Phenolics from walnut (*Juglans regia* L.) kernels: Antioxidant activity and interactions with proteins. *Food Chem.* **2008**, *107*, 607–612. [CrossRef]
19. Xie, W.D.; Xie, X.P.; Jiang, G.X.; Lai, J.Y.; Wei, X.M. Vegetational diversity and biomass in *Juglans regia* forest under conversion cultivated land into forests model. *Guizhou Agric. Sci.* **2010**, *38*, 177–179.
20. Javid, A.D.; Somaiah, S. Effect of Land Use Change on Carbon Sequestration: A Case Study in Shahmirzad Walnut Orchad, Semnan, Iran. *Environ. Monit. Assess.* **2015**, *187*, 55.
21. Tan, Q.J.; Qin, Z.S.; Wang, X.Y.; Zhang, H.; Zeng, F.P.; Chen, H.S.; Wang, W.L. Relationships between biomass and soil nutrients in *Juglans regia* of different ages in depression between karst hills. *Carsol. Sin.* **2016**, *35*, 226–232.
22. Condit, R. Research in large, long-term tropical forest plots. *Trends Ecol. Evol.* **1995**, *10*, 18–22. [CrossRef]
23. Cote, L.; Brown, S.; Pare, D.; Fyles, J.; Bauhus, J. Dynamics of carbon acid nitrogen mineralization in relation to stand type, stand age and soil texture in the boreal mixedwood. *Soil Biol. Biochem.* **2000**, *32*, 1079–1090. [CrossRef]
24. Song, T.Q.; Peng, W.X.; Zeng, F.P.; Wang, K.L.; Ouyang, Z.W. Vegetation succession rule and regeneration strategies in disturbed karst area, northwest Guangxi. *J. Mt. Sci.* **2008**, *26*, 597–604.

25. Guo, L.B.; Gifford, R.M. Soil carbon stocks and land use change: A meta-analysis. *Glob. Chang. Biol.* **2002**, *8*, 345–360. [CrossRef]
26. Miles, L.; Kapos, V. Reducing greenhouse gas emissions from deforestation and forest degradation: Global land-use implications. *Science* **2008**, *320*, 1454–1455. [CrossRef] [PubMed]
27. Fonseca, W.; Benayas, J.M.R.; Alice, F.E. Carbon accumulation in the biomass and soil of different aged secondary forests in the humid tropics of Costa Rica. *For. Ecol. Manag.* **2011**, *262*, 1400–1408. [CrossRef]
28. Tian, D.L.; Wang, X.K.; Fang, X.; Yan, W.D.; Ning, X.B.; Wang, G.J. Carbon storage and spatial distribution in different vegetation restoration patterns in karst areas, Guizhou province. *Sci. Silvae Sin.* **2011**, *47*, 7–14.
29. Wang, F.M.; Xu, X.; Zou, B.; Guo, Z.H.; Li, Z.A.; Zhu, W.X. Biomass accumulation and carbon sequestration in four different aged *Casuarina equisetifolia* coastal shelterbelt plantations in South China. *PLoS ONE* **2013**, *8*, e77449. [CrossRef] [PubMed]
30. Malhi, Y.; Baker, T.R.; Phillips, O.L.; Almeida, S.; Alvarez, E.; Arroyo, L.; Chave, J.; Czimczik, C.I.; Di Fiore, A.; Higuchi, N.; et al. The above-ground coarse wood productivity of 104 Neotropical forest plots. *Glob. Chang. Biol.* **2004**, *10*, 563–591. [CrossRef]
31. Redondo-Brenes, A. Growth, carbon sequestration, and management of native tree plantations in humid regions of Costa Rica. *New For.* **2007**, *34*, 253–268. [CrossRef]
32. Lasco, R.D.; Pulhin, F.B.; Visco, R.G.; Racelis, D.A.; Guillermo, I.Q.; Sales, R.F. Carbon stocks assessment of Philippine forest ecosystems. In Presented at the Science-Policy Workshop on Terrestrial Carbon Assessment for Possible Carbon Trading, Bogor, Indonesia, 28–29 February 2000.
33. Romberger, J.A.; Hejnowicz, Z.; Hill, J.F. *Plant Structure: Function and Development*; The Blackburn Press: Caldwel, NJ, USA, 2004.
34. Sartori, F.; Lal, R.; Ebinger, M.H.; Eaton, J.A. Changes in soil carbon and nutrient pools along a chronosequence of poplar plantations in the Columbia Plateau, Oregon, USA. *Agric. Ecosyst. Environ.* **2007**, *122*, 325–339. [CrossRef]
35. Peichl, M.; Arain, A.A. Above- and belowground ecosystem biomass and carbon pools in an age-sequence of temperate pine plantation forests. *Agric. For. Meteorol.* **2006**, *140*, 51–63. [CrossRef]
36. Farley, K.A.; Kelly, E.F.; Hofstede, R.G.M. Soil organic carbon and water retention following conversion of grasslands to pine plantations in the Ecuadoran Andes. *Ecosystems* **2004**, *7*, 729–739. [CrossRef]
37. Seely, B.; Welham, C.; Blanco, J.A. Towards the application of soil organic matter as an indicator of forest ecosystem productivity: Deriving thresholds, developing monitoring systems, and evaluating practices. *Ecol. Indic.* **2010**, *10*, 999–1008. [CrossRef]
38. Xu, W.; Yin, Y.; Zhou, S. Social and economic impacts of carbon sequestration and land use change on peasant households in rural China: A case study of Liping, Guizhou province. *J. Environ. Manag.* **2007**, *85*, 736–745. [CrossRef] [PubMed]
39. Schmid, I.; Kazda, M. Root distribution of Norway spruce in monospecific and mixed stands on different soils. *For. Ecol. Manag.* **2002**, *159*, 37–47. [CrossRef]
40. Kenzo, T.; Ichie, T.; Hattori, D.; Kendawang, J.J.; Sakurai, K.; Ninomiya, I. Changes in above- and belowground biomass in early successional tropical secondary forests after shifting cultivation in Sarawak, Malaysia. *For. Ecol. Manag.* **2010**, *260*, 875–882. [CrossRef]
41. Abdipour, M.; Hoseini, S.M.; Kaboli, H.; Kianian, M.K. Effect of Land Use Change on Carbon Sequestration: A Case Study in Shahmirzad Walnut Orchad, Semnan, Iran. *Res. J. Soil. Biol.* **2015**, *7*, 1–12.

© 2017 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).