Introduction of a leguminous shrub to a rubber plantation changed the soil carbon and nitrogen fractions and ameliorated soil environments

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The conversion of monoculture rubber (Hevea brasiliensis) plantations into rubber-based agroforestry systems has become a common trend in forestry management in the past few decades. Rubber–Flemingia macrophylla (a leguminous shrub) systems are popular in southwestern China’s Xishuangbanna region. The biogeochemical cycles of soil carbon and nitrogen in forests are mainly affected by their fractions. This study investigated the effect of introducing Flemingia macrophylla to rubber plantations of different ages on soil carbon and nitrogen fractions. The experimental treatments included R1 (young rubber plantation), RF1 (young rubber–Flemingia macrophylla system), R2 (mature rubber plantation) and RF2 (mature rubber–Flemingia macrophylla system). The results showed that the introduction of Flemingia macrophylla to rubber plantations of different ages significantly changed soil carbon and nitrogen fractions, improved soil labile organic carbon and nitrogen contents, and ameliorated soil environments. The average soil microbial biomass organic carbon, nitrogen and nitrate-nitrogen in the 0–10 cm soil layer during the experimental period was 38.9%, 55.5%, and 214.7% higher in RF1 than R1, respectively, and 22.1%, 22.2%, and 652.2% higher in RF2 than R2, respectively. Therefore, Flemingia macrophylla can be used as an alternative interplanted tree species within rubber plantations in similar environments of southeastern Asia.

Monoculture rubber (Hevea brasiliensis) plantations have rapidly expanded in the last few decades in southeastern Asia1,2. Approximately 90% of global natural rubber production is derived from plantations in this region (http://www.rubberstudy.com), accounted for an estimated 84% of the total global rubber plantation area in 20121,3. Rapid growth in the Chinese economy has increased demand for natural rubber. In response to this demand, the natural tropical forests of southwestern China’s Xishuangbanna region were deforested and replaced with more than 470,000 ha of rubber plantations, which equates to more than 24% of the total land area of the region4. The expansion of these rubber plantations has led to water loss and soil erosion5,6, environmental degradation7, and threatened environmental biodiversity8. At present, rubber-based agroforestry systems are considered the best way to resolve the environmental problems associated with rubber monoculture. In recent years, the local government of Xishuangbanna proposed the development of environmentally friendly rubber plantations to reduce the water and soil losses and increase environmental biodiversity9,10.

Legume plants could greatly enhance ecosystem services. Lucerne (Medicago sativa) and erect milkvetch (Astragalus adsurgens) have the potential to phytoextract rhenium from coal fly ash-amended alkaline soils11. Alfalfa (Medicago sativa L.) can improve its phosphorus acquisition by increasing specific root length and exuding carboxylates into the rhizosphere in phosphorus-deficient environments12. A combination of legumes grass species can enhance soil C and N storage, productivity, and diversity in semi-arid grasslands13. Flemingia macrophylla is used in traditional medicine for various therapeutic uses and is widely planted in the Xishuangbanna area of China. Flemingia macrophylla is a perennial leguminous leafy shrub with strong biological nitrogen fixation and...
Soil carbon fractions. The SOC content in the 0–10 or 10–30 cm soil layers did not differ between the plantation treatments (R1, R2, RF1, RF2) for the duration of the study (April 2014 to January 2017) (Table 1). However, SOC content in the 0–10 cm soil layers was significantly higher than the 10–30 cm soil layers in each plantation type. In the 0–10 cm soil layer, RF1 had consistently higher WSOC content than R1 from June 2015 to January 2017, which differed significantly in August 2016 and January 2017. The WSOC content between R2 and RF2 did not differ significantly for the duration of the experiment (Table 2). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on WSOC content.

The objectives of this study were to examine: (1) soil carbon and nitrogen fractions, and (2) the relationships between soil carbon and nitrogen fractions in rubber and rubber–Flemingia macrophylla plantations of different ages.

**Results**

**Soil carbon fractions.** The SOC content in the 0–10 or 10–30 cm soil layers did not differ between the plantation treatments (R1, R2, RF1, RF2) for the duration of the study (April 2014 to January 2017) (Table 1). However, SOC content in the 0–10 cm soil layers was significantly higher than the 10–30 cm soil layers in each plantation type. In the 0–10 cm soil layer, RF1 had consistently higher WSOC content than R1 from June 2015 to January 2017, which differed significantly in August 2016 and January 2017. The WSOC content between R2 and RF2 did not differ significantly for the duration of the experiment (Table 2). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on WSOC content.

The 0–10 cm soil layer had significantly higher LFOC content than the 10–30 cm soil layer in each plantation type. In the 0–10 cm soil layer, RF1 had consistently higher LFOC content than R1 from June 2015 to January 2017.

**Table 1.** Soil organic carbon (SOC) (g kg$^{-1}$) in rubber and rubber–Flemingia macrophylla plantations from April 2014 to January 2017 (mean ± SD, n = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: Flemingia macrophylla introduced to R1 in 2010; RF2: Flemingia macrophylla introduced to R2 in 2010. Values within a column followed by the same letter (lower case) or within the same row (upper case) do not differ significantly at $P \leq 0.05$.

| Soil depth (cm) | Treatments | Apr 2014 | Jun 2015 | Jan 2016 | Aug 2016 | Jan 2017 |
|----------------|------------|----------|----------|----------|----------|----------|
| 0–10 cm        | R1         | 12.50 ± 0.47abA | 12.45 ± 0.31aA | 12.64 ± 0.79abA | 12.50 ± 0.34aA | 12.77 ± 0.46abA |
|                | RF1        | 12.77 ± 2.53aA | 13.04 ± 1.23aA | 13.22 ± 1.42aA | 13.59 ± 1.77aA | 13.45 ± 1.65aA |
|                | R2         | 13.79 ± 0.31aA | 13.53 ± 0.60aA | 14.40 ± 1.02aA | 14.11 ± 1.35aA | 14.20 ± 0.41aA |
|                | RF2        | 13.16 ± 0.90aA | 12.74 ± 0.97aA | 13.04 ± 0.68abA | 12.76 ± 1.36aA | 13.24 ± 1.88aA |
| 10–30 cm       | R1         | 9.89 ± 0.71cA | 9.65 ± 0.06bA | 9.73 ± 0.94aA | 9.73 ± 0.43aA | 9.98 ± 0.63cA |
|                | RF1        | 10.49 ± 0.31cA | 10.61 ± 1.02bA | 10.25 ± 2.43aA | 9.84 ± 1.85bA | 10.08 ± 0.69cA |
|                | R2         | 10.22 ± 0.33cA | 10.90 ± 0.18bA | 11.00 ± 0.49bcA | 10.24 ± 0.42bA | 10.67 ± 0.64cA |
|                | RF2        | 10.84 ± 1.05bcA | 10.51 ± 0.49bA | 10.20 ± 0.37cA | 11.16 ± 1.34bA | 10.92 ± 1.32bcA |

**Table 2.** Soil water-soluble organic carbon (WSOC) (mg kg$^{-1}$) in rubber and rubber–Flemingia macrophylla plantations from June 2015 to January 2017 (mean ± SD, n = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: Flemingia macrophylla introduced to R1 in 2010; RF2: Flemingia macrophylla introduced to R2 in 2010. Values within a column followed by the same letter do not differ significantly at $P \leq 0.05$.

| Soil depth (cm) | Treatments | Jun 2015 | Jan 2016 | Aug 2016 | Jan 2017 |
|----------------|------------|----------|----------|----------|----------|
| 0–10 cm        | R1         | 70.7 ± 3.7b | 78.3 ± 1.0a | 67.5 ± 2.2b | 67.7 ± 6.7b |
|                | RF1        | 72.3 ± 1.9b | 80.4 ± 5.5a | 79.3 ± 7.0a | 80.5 ± 7.8a |
|                | R2         | 84.5 ± 6.1a | 86.6 ± 10.1a | 75.1 ± 5.0ab | 50.1 ± 4.4d |
|                | RF2        | 82.5 ± 1.7a | 80.5 ± 2.7a | 76.9 ± 6.2ab | 57.7 ± 8.0cd |
| 10–30 cm       | R1         | 71.2 ± 5.5b | 80.7 ± 8.4a | 72.8 ± 8.0b | 66.1 ± 1.5b |
|                | RF1        | 70.7 ± 7.3b | 82.5 ± 4.2a | 75.6 ± 7.1ab | 63.2 ± 8.7bc |
|                | R2         | 88.8 ± 3.4a | 82.3 ± 8.2a | 75.3 ± 4.6ab | 50.4 ± 6.0d |
|                | RF2        | 85.9 ± 2.3a | 87.8 ± 14.8a | 70.6 ± 7.7b | 55.0 ± 12.2c |

Precise and accurate estimations of carbon and nitrogen levels in forest soil are important for understanding biogeochemical cycles. Some studies have indicated that soil organic carbon (SOC) has been depleted in rubber plantations. Rubber plantations have 15% lower annual surface soil CO$_2$ fluxes than natural forests, because they have lower soil respiration during the dry season. The conversion of tropical rainforests into rubber plantations has increased N$_2$O emissions, which may potentially enhance local climate warming trends. Rubber plantations have lower mean CH$_4$ uptake rates than secondary and tropical forests. These transformations and biogeochemical cycles of soil carbon and nitrogen are mainly affected by their fractions that play essential roles in the turnover of nutrients in soil, including water-soluble organic carbon (WSOC), light fraction of organic C (LFOC), microbial biomass organic C (MBC), ammonium N (AN), nitrate N (NN), light fraction of organic N (LFON), and microbial biomass organic N (MBN). These fractions are often used to study the impacts of land management and ecological succession. Rubber-based agroforestry systems have higher SOC and nitrogen levels and lower carbon and nitrogen losses than rubber plantations due to improved soil macroaggregates. However, little is known about the effect of these systems on soil carbon and nitrogen fractions.
and introduced to R2 in 2010. Values within a column followed by the same letter do not differ significantly at P ≤ 0.05.

Table 3. Light fraction organic carbon (LFOC) (g kg⁻¹) in rubber and rubber–Flemingia macrophylla plantations from June 2015 to January 2017 (mean ± SD, n = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: Flemingia macrophylla introduced to R1 in 2010; RF2: Flemingia macrophylla introduced to R2 in 2010. Values within a column followed by the same letter do not differ significantly at P ≤ 0.05.

| Soil depth (cm) | Treatments | Jun 2015 | Jan 2016 | Aug 2016 | Jan 2017 |
|----------------|------------|----------|----------|----------|----------|
| 0–10 cm        | R1         | 1.21 ± 0.15b | 1.13 ± 0.15b | 1.14 ± 0.25c | 1.03 ± 0.06b |
|                | RF1        | 1.19 ± 0.10b | 1.36 ± 0.21b | 2.38 ± 0.23a | 1.34 ± 0.11a |
|                | R2         | 1.56 ± 0.13a | 1.75 ± 0.30a | 1.61 ± 0.26b | 1.45 ± 0.14a |
|                | RF2        | 1.45 ± 0.09a | 1.31 ± 0.09b | 1.51 ± 0.13b | 1.00 ± 0.12b |
| 10–30 cm       | R1         | 0.45 ± 0.07c | 0.36 ± 0.02c | 0.54 ± 0.10d | 0.61 ± 0.01c |
|                | RF1        | 0.45 ± 0.07c | 0.50 ± 0.07c | 0.59 ± 0.06d | 0.51 ± 0.06c |
|                | R2         | 0.61 ± 0.09c | 0.59 ± 0.12c | 0.61 ± 0.11d | 0.57 ± 0.18c |
|                | RF2        | 0.54 ± 0.05c | 0.47 ± 0.08c | 0.60 ± 0.04d | 0.48 ± 0.11c |

Table 4. Soil microbial biomass carbon (MBC) (mg kg⁻¹) in rubber and rubber–Flemingia macrophylla plantations from June 2015 to January 2017 (mean ± SD, n = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: Flemingia macrophylla introduced to R1 in 2010; RF2: Flemingia macrophylla introduced to R2 in 2010. Values within a column followed by the same letter do not differ significantly at P ≤ 0.05.

| Soil depth (cm) | Treatments | Jun 2015 | Jan 2016 | Aug 2016 | Jan 2017 |
|----------------|------------|----------|----------|----------|----------|
| 0–10 cm        | R1         | 122.0 ± 7.5e | 203.9 ± 23.9de | 126.5 ± 16.5de | 115.6 ± 17.7c |
|                | RF1        | 141.8 ± 15.0e | 256.3 ± 24.3bc | 245.0 ± 30.4c | 145.9 ± 20.3c |
|                | R2         | 174.3 ± 23.0d | 281.3 ± 21.0ab | 341.8 ± 25.7b | 179.7 ± 17.6b |
|                | RF2        | 236.1 ± 11.1b | 314.0 ± 31.6a | 420.7 ± 65.2a | 222.4 ± 31.5a |
| 10–30 cm       | R1         | 47.4 ± 5.9f | 182.2 ± 17.3e | 72.1 ± 4.2e | 68.2 ± 13.4d |
|                | RF1        | 54.9 ± 11.8f | 234.7 ± 18.1cd | 164.4 ± 27.8d | 70.3 ± 7.9d |
|                | R2         | 199.7 ± 18.1c | 136.9 ± 14.6f | 310.6 ± 39.6bc | 138.1 ± 17.9c |
|                | RF2        | 280.9 ± 3.6a | 192.8 ± 19.0e | 354.3 ± 61.8ab | 194.5 ± 7.2ab |

2017, which differed significantly in August 2016 and January 2017. In the same layer, RF2 had consistently lower LFOC content than R2, with significant differences observed in January 2016 and 2017 (Table 3). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on LFON content but increased MBC content and the ratios of MBC/SOC in the 0–10 and 10–30 cm soil layers (Table 4 and Fig. 1). In the 0–10 cm soil layer, RF1 had consistently higher ratios of LFOC/SOC than R1 from January 2016 to January 2017, but no significant differences were observed between the RF2 and R2 for the duration of the study (Fig. 2). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on the ratios of LFOC/SOC.

Soil nitrogen fractions. The TN content in the 0–10 or 10–30 cm soil layers did not differ between the plantation treatments (R1, R2, RF1, RF2) for the duration of the study (April 2014 to January 2017) (Table 5). However, the 0–10 cm soil layer had significantly higher TN contents than the 10–30 cm soil layer in each plantation type. The introduction of Flemingia macrophylla to the rubber plantations generally decreased AN content and increased NN content in the 0–10 and 10–30 cm soil layers (Fig. 3). In the 0–10 cm soil layer, RF1 had consistently higher LFON content than R1 from June 2015 to January 2017, with significant differences observed from January 2016 to January 2017. In the same layer, no significant differences in LFON content were observed between R2 and RF2, except for January 2017 when the LFON content was significantly higher in R2 than RF2 (Table 6). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on LFON content. The 0–10 cm soil layer had significantly higher LFON contents than the 10–30 cm soil layer in each plantation type.

The introduction of Flemingia macrophylla to the rubber plantations increased MBN content and the ratios of MBN/TN in the 0–10 and 10–30 cm soil layers (Table 7 and Fig. 1). In the 0–10 cm soil layer, RF1 had consistently higher ratios of LFON/TN than R1 from January 2016 to January 2017, but no significant differences were observed between the RF2 and R2 for the duration of the study (Fig. 2). In the 10–30 cm soil layer, the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on the ratios of LFON/TN. The introduction of Flemingia macrophylla to the rubber plantations decreased the ratios of LFOC/LFON in the 0–10 and 10–30 cm soil layers (Fig. 4).
Relationships between soil carbon and nitrogen fractions. MBC and MBN had significant positive correlations with LFOC, LFON, and the ratios of MBC/SOC, LFOC/SOC, MBN/TN and LFON/TN (Table 8). MBC and MBN also had negative correlations with the ratio of LFOC/LFON. AN and NN had significant positive correlations with LFOC and LFON, and the ratios of LFOC/SOC and LFON/TN. Furthermore, AN and NN had significant negative correlations with LFOC/LFON.

Discussion

Soil carbon and nitrogen fractions in the rubber and rubber–Flemingia macrophylla plantation systems. Plantations with suitable native, broad-leaved species (for example, Alnus subcordata C. A. Mey.) along with planned forestation management could potentially rehabilitate the degraded natural forests of northern Iran11. Mo and Sha22 reported that adding carbon-sink plants into rubber plantations increased the soil organic carbon storage. The presence of legumes in semi-arid grasslands increased soil C and N storage by increasing the above- and below-ground biomass, litter biomass, plant species richness, and diversity13. In 2014 in the present study, biomass of Flemingia macrophylla accumulated in the young and mature rubber plantations at 23.02 and 0.55 t ha$^{-1}$, respectively14 but had no significant effect on SOC or TN contents. There are four possible explanations for this response: (1) the rubber–Flemingia macrophylla system had only been established for seven years, and it is unlikely that soil C and N storage would have changed significantly in such a short time; (2) the high-temperature and high-humidity environments in the rainy season would promote the decomposition of plant residues and nutrient leaching, which is not conducive to C and N storage; (3) the introduction of Flemingia macrophylla to the rubber plantations reduced plant species richness and diversity (Fig. 5) and the input of litter biomass of other species; (4) most rubber plantations in this area have been planted on sloping land20. In our study, the rubber and rubber–Flemingia macrophylla plantations were planted on sloping land between 47 and 58%, and the nutrients of the litter decomposition and some un-decomposed litter of Flemingia macrophylla would have been lost due to runoff.

While the introduction of Flemingia macrophylla to the rubber plantations had no significant effect on the C and N contents in the soil, the fractions of C and N changed significantly. The introduction of Flemingia macrophylla in the young rubber plantations was propitious for improving the labile organic C (WSOC and LFOC) and LFON content in the 0–10 cm soil layer due to the input of large amounts of litter from Flemingia macrophylla each year (Fig. 5C). The light fraction of the soil mainly consisted of plant residues, small animals, and microorganisms in various stages of decomposition. It served as a readily decomposable substrate for soil microorganisms and a

Figure 1. Ratio of soil microbial biomass carbon (MBC) to soil organic carbon (SOC) (MBC/SOC); and ratio of soil microbial biomass nitrogen (MBN) to soil total nitrogen (TN) (MBN/TN) in the rubber and rubber–Flemingia macrophylla plantations from June 2015 to January 2017. R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: Flemingia macrophylla introduced to R1 in 2010; RF2: Flemingia macrophylla introduced to R2 in 2010. Vertical bars are LSD at $P \leq 0.05$. 
short-term reservoir for plant nutrients. It potentially served as an early indicator of the effects of the management practices\textsuperscript{33,34}. The WSOC also played a dominant role in several soil processes and was more sensitive for land use than the total SOC pool\textsuperscript{28,35}. The WSOC indicated that the introduction of \textit{Flemingia macrophylla} to the young rubber plantations could change the soil C fractions in the 0–10 cm soil layer within a short time. However, the introduction of \textit{Flemingia macrophylla} into the mature rubber plantations had no significant effect on WSOC, LFOC, or LFON content in the short term, due to the lower input levels of \textit{Flemingia macrophylla} litter each year.

In this study, the introduction of \textit{Flemingia macrophylla} to the rubber plantations decreased AN content and increased NN content, which may have been due to the increased rate of nitrification from improved microbial activity in the rubber–\textit{Flemingia macrophylla} systems. The NN content in soil is important for the growth of rubber trees, as well as rubber production\textsuperscript{36}. In this region, soil NN content in rubber plantations is generally <1 mg kg\textsuperscript{-1}\textsuperscript{37}. In our study, soil NN content in the rubber plantations ranged from 0.02–0.93 mg kg\textsuperscript{-1}. These

Table 5. Soil total nitrogen (TN) (g kg\textsuperscript{-1}) in rubber and rubber–\textit{Flemingia macrophylla} plantations from April 2014 to January 2017 (mean ± SD, \textit{n} = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: \textit{Flemingia macrophylla} introduced to R1 in 2010; RF2: \textit{Flemingia macrophylla} introduced to R2 in 2010. Values within a column followed by the same letter (lower case) or within the same row (upper case) do not differ significantly at \textit{P} ≤ 0.05.
findings suggest that *Flemingia macrophylla* is important for improving NN content in the soil of the rubber plantations. Relationships between soil carbon and nitrogen fractions. The microbial biomass of soil controls organic matter sequestration and decomposition and is generally used as an ecological sensitivity indicator for changes in soil nutrients due to land use practices. For example, high MBC/SOC and MBN/TN ratios indicate that the organic matter decomposed quickly. In the current study, the introduction of *Flemingia macrophylla* to the rubber plantations increased MBC and MBN, along with the MBC/SOC and MBN/TN ratios in the 0–10 and 10–30 cm soil layers. These results agree with those of Wen et al., who suggested that the conversions of monocultures into mixed forests had a strong positive effect on soil microbial biomass, and increased the efficiency of microbes in soil carbon decomposition along the profile by improving the ratios of the MBC/SOC and MBN/TN. Interestingly, the introduction of *Flemingia macrophylla* to the rubber plantations in our study decreased the ratio...
Soil depth (cm) Treatments Jun 2015 Jan 2016 Aug 2016 Jan 2017  
0–10 cm  
R1 18.2 ± 1.3d 35.2 ± 14.1b 21.2 ± 3.2c 20.5 ± 4.6cd  
RF1 28.8 ± 4.6bc 42.5 ± 11.9ab 44.1 ± 10.5b 32.5 ± 5.4ab  
R2 24.0 ± 4.8c 54.7 ± 10.2a 38.6 ± 3.9b 34.0 ± 1.4ab  
RF2 34.0 ± 3.2b 55.2 ± 3.3a 59.3 ± 7.1a 36.1 ± 7.1a  
10–30 cm  
R1 5.3 ± 0.2e 32.7 ± 3.4b 17.1 ± 4.1c 17.0 ± 6.2de  
RF1 10.8 ± 3.3e 36.6 ± 3.9b 25.2 ± 1.7e 12.3 ± 3.9e  
R2 30.4 ± 2.1b 32.8 ± 5.9b 49.4 ± 15.5ab 27.1 ± 6.0bc  
RF2 37.1 ± 3.3a 35.8 ± 6.4b 45.8 ± 4.7b 32.2 ± 6.0ab  

Table 7. Soil microbial biomass nitrogen (MBN) (mg kg⁻¹) in rubber and rubber–*Flemingia macrophylla* plantations from June 2015 to January 2017 (mean ± SD, n = 3). R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: *Flemingia macrophylla* introduced to R1 in 2010; RF2: *Flemingia macrophylla* introduced to R2 in 2010. Values within a column followed by the same letter do not differ significantly at *P* ≤ 0.05.

| Measurement                | LFON/TN | LFOC/SOC | LFOC/LFON | MBC/TN | MBN/TN | NN   | AN   | LFON | MBC  | LFOC | MBC  |
|----------------------------|---------|----------|-----------|--------|--------|------|------|------|------|------|------|------|
| MBC                        | 0.45*   | 0.40*    | −0.47**   | 0.91***| 0.95***| 0.26 | 0.39*| 0.43*| 0.92***| 0.39**|
| LFOC                       | 0.97*** | 0.99***  | 0.69***   | 0.20   | 0.13   | 0.39*| 0.41*| 0.99***| 0.44* |
| MBN                        | 0.48**  | 0.44*    | −0.46**   | 0.96***| 0.85***| 0.28 | 0.20 | 0.48**|
| MBN                        | 0.99*** | 0.98***  | −0.75***  | 0.23   | 0.17   | 0.45**| 0.41*|
| AN                         | 0.43*   | 0.42*    | −0.45**   | 0.13   | 0.30   | 0.25 |
| NN                         | 0.47*** | 0.42*    | −0.55**   | 0.17   | 0.16   |
| MBC/SOC                    | 0.19    | 0.15     | 0.28      | 0.92***|
| MBN/TN                     | 0.25    | 0.21     | 0.28      |        |
| LFOC/LFON                  | 0.76*** | 0.67***  |           |        |        |
| LFOC/SOC                   | 0.98*** |         |           |        |

Table 8. Correlation coefficients (R) among different soil carbon and nitrogen fractions in rubber and rubber–*Flemingia macrophylla* plantations from June 2015 to January 2017. MBC: soil microbial biomass carbon; LFOC: light fraction organic carbon; MBN: soil microbial biomass nitrogen; LFON: light fraction organic nitrogen; AN: soil ammonium nitrogen; NN: soil nitrate-nitrogen; MBC/SOC: ratio of MBC to SOC; MBN/TN: ratio of MBN to TN; LFOC/LFON: ratio of LFOC to LFON; LFOC/SOC: ratio of LFOC to SOC; LFON/TN: ratio of LFON to TN. Statistical significance: *P* ≤ 0.05, **P* ≤ 0.01, ***P* ≤ 0.001.

**Figure 4.** Ratio of organic C to total N in the soil light fractions (LFOC/LFON) in the rubber and rubber–*Flemingia macrophylla* plantations from June 2015 to January 2017. R1: rubber plantations established in 2006; R2: rubber plantations established in 1994; RF1: *Flemingia macrophylla* introduced to R1 in 2010; RF2: *Flemingia macrophylla* introduced to R2 in 2010. Vertical bars are LSD at *P* ≤ 0.05.
It was observed that large amounts of leaves from the *Flemingia macrophylla* were incorporated into the soil each year, which tended to decrease the LFOC/LFON ratio in the soil due to the low C/N ratio in the leaves of *Flemingia macrophylla*. Generally speaking, the C/N ratio of *Flemingia macrophylla* is approximately 15:1. Chen and Huang reported that low C/N ratios in soil could increase microbial biomass, and accelerate the decomposition of soil organic matter. We also observed a significant negative correlation between the ratio of LFOC/LFON and MBC and MBN ($P \leq 0.01$). These results suggest that the decreased ratios of LFOC/LFON in the rubber–*Flemingia macrophylla* plantations enhanced microbial activity.

**Conclusions**

While the introduction of *Flemingia macrophylla* to the rubber plantations did not significantly increase total soil carbon or nitrogen levels over a short period, it changed the carbon and nitrogen fractions, improved labile organic carbon and nitrogen contents, and ameliorated the soil environment. We recommend that local governments and farmers in southeastern Asia use *Flemingia macrophylla* as alternative interplanted tree species within rubber plantations.

**Materials and Methods**

**Description of the study site.** This study was conducted in the Xishuangbanna region (21°33′N, 101°28′E; 880 to 900 m asl) of Yunnan Province in southwestern China. This region has a typical tropical monsoon climate, with an annual mean temperature of 21.8 °C. The area receives mean annual precipitation of ~1,500 mm, 80% of which occurs in the rainy season (May to October). Furthermore, Xishuangbanna contains the largest area of tropical rainforests in China. Its biodiversity is rich as it is part of the Indo-Burma world biodiversity hotspot. The soil has been classified as laterite (Oxisol), which developed from arenaceous shale sediment.

In 1991 and 2003, the tropical forests with slopes ranging from 47–58% were deforested. Sugarcane (*Saccharum officinarum* L.) was then planted annually. Rubber trees were planted on these sites in May 1994 and 2006 at a density of 450 rubber trees ha$^{-1}$, with 8 m spacing between adjacent rows. In accordance with the local practices for rubber trees less than three years of age, fertilizers were applied between the rubber trees at depths of 20 cm using spades at rates of 27.0 kg ha$^{-1}$ N, 5.9 kg ha$^{-1}$ P, and 11.2 kg ha$^{-1}$ K, which were split into two applications per year (May and October). Once the rubber trees were more than three years of age, the fertilizer application rates changed to 54.0 kg ha$^{-1}$ N, 11.8 kg ha$^{-1}$ P, and 22.4 kg ha$^{-1}$ K. The rubber plantation farmers generally sprayed sulfur powder at 30–60 kg ha$^{-1}$ yr$^{-1}$ to control powdery mildew on the rubber trees. Weeds in the plantations were cut using a sickle twice per year (April/May and November/December) and left on the ground.

**Figure 5.** Photographs of the rubber and rubber–*Flemingia macrophylla* plantations. (A) rubber plantation; (B) rubber–*Flemingia macrophylla* plantation; (C) *Flemingia macrophylla* after mowing in the rubber–*Flemingia macrophylla* plantation.
In July 2010, *Flemingia macrophylla* was introduced into the differently aged rubber plantations (4 and 16 years of age) at a density of 10,830 plants ha⁻¹. From 2011 onwards, the *Flemingia macrophylla* in the different rubber plantations was cut using a sickle in December each year and left as ground cover. From 2012 onwards, given the strong biological nitrogen fixation of *Flemingia macrophylla*, no additional N was applied in the rubber–*Flemingia macrophylla* plantations. The inputs of P, K, and S in the rubber–*Flemingia macrophylla* plantations remained the same as those in the adjacent rubber plantations.

**Experimental design, sampling, and measurements.** In this study, three replicate sites were selected within each rubber and rubber–*Flemingia macrophylla* plantation (Fig. 5). Each replication site consisted of 20 × 25 m² survey plots (four rows of rubber trees, and three 8-m wide hedgerows) containing nine sampling subplots (8 × 6 m²), with three located at each slope position (upper, middle, and lower slope).

For each of the nine subplots, soil samples were collected using a soil auger, avoiding the fertilization holes, at two depths (0–10 cm and 10–30 cm) after carefully removing the litter-fall and/or grass layer. For each replicate site in the different rubber and rubber–*Flemingia macrophylla* plantations, soil core samples were collected in April 2014, June 2015, January and August 2016, and January 2017. The nine soil cores were combined into a composite sample, which were air-dried, ground, and sieved (<0.2 mm) for analysis of ammonium nitrogen (AN) and nitrate-nitrogen (NN), and the light fraction organic carbon (LFOC) and nitrogen (LFON). The sieved samples were sieved again (at <0.25 mm) for determination of soil organic carbon (SOC) and total nitrogen (TN). For soil water-soluble organic carbon (WSOC), microbial biomass carbon (MBC) and nitrogen (MBN) measurements, the samples were taken to the laboratory and stored at 4 °C for subsequent analyses.

The SOC and TN of the bulk soil were determined using a Vario MAX CN-Analysier (Elementar Analysensystems GmbH, Germany). A density fractionation scheme for light was used following the method described by Gregorich and Ellert. During fractionation, 25 g of air-dried soil was shaken with 50 mL of NaI solution (sp.Gr. = 1.70) for 60 min. After centrifugation, the supernatant was passed through a Millipore filter (0.45 μm) and the light fraction collected. The soil residue in the centrifuge was extracted again with NaI, and the additional light fraction collected. The light fraction was oven-dried at 60 °C for 72 h. The concentration of organic carbon and nitrogen was determined by dry combustion using a Vario MAX CN-Analysier (Elementar Analysensystems GmbH, Germany). Dried samples, each weighing 5 g, were added to 50 mL of 2 M KCl, shaken for one hour, and analyzed with an Auto Analyser 3 (SEAL Analytical GmbH, Germany) to determine AN and NN contents. Microbial biomass carbon (MBC) and nitrogen (MBN) in the soil were estimated using a fumigation-extraction method that included a purified CHCl₃ treatment, followed by a 0.5 M K₂SO₄ extraction of fumigated and unfumigated soil. After which, soil samples (equivalent to 25 g of dry soil weight) were fumigated for 24 h at 25 °C with CHCl₃ (ethanol-free). Following fumigant removal, the soil was extracted with 100 mL of 0.5 M K₂SO₄ by shaking for 1 h at 200 rpm, followed by filtering. The non-fumigated portions were extracted at similar time intervals. Following the extraction, MBC and MBN contents were measured by determining the C and N masses in the filtrate using a Vario TOC cube-Analysier (Elementar Analysensystems GmbH, Germany). Microbial biomass nitrogen was calculated as follows: MBN = (Corg(fum) − Corg(non))/0.38. Water-soluble organic carbon (WSOC) was extracted from field-moist samples within 24 h of sampling by shaking 15 g soil with 30 mL distilled water for 2 h at 25 °C, followed by centrifugation at 5000 r min⁻¹ at 4 °C for 15 min. The supernatant was filtered through a 0.45 μm carbon-free membrane. The filtrates were stored at 4 °C and analyzed within 24 h using a Vario TOC cube-Analysier (Elementar Analysensystems GmbH, Germany).

**Statistical analysis.** The data were subjected to analysis of variance (ANOVA) using SAS statistical analysis software version 8.0. One-factor ANOVA was deployed to compare treatment effects. The least significant difference (LSD at 0.05 level of probability) test was applied to assess the differences between means. Pearson’s coefficient analysis was used for correlation.

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Acknowledgements

We would like to acknowledge the Public Technology Service Center of XTBG, CAS, for their support with field measurements and soil analyses. This study was funded by the National Natural Science Foundation of China (31470639), the Natural Science Foundation of Yunnan Province (2017FB059 and 2016PA047), the Key Program of CAS (KFZD-SW-312) and the Project of Xishuangbanna Science and Technology Bureau (200915, 201116).
Author Contributions
C.A.L. conducted the experiments and wrote the manuscript; Y.N. analyzed the data assisted. J.W.T., K.H.M.S. and Y.M.Z. assisted with writing the manuscript.

Additional Information
Competing Interests: The authors declare no competing interests.

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