BRIEF COMMUNICATION

Foliar δ^{13}C values, photosynthetic pathway and functional type for 238 common species on farm land and disturbed forest in Laos

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Abstract

Despite the elimination of the original forest and frequent cultivation using slash and burn, a large spread in leaf δ^{13}C was recorded in weeds, crops, and bush fallow species, reflecting a forest environment rather than a broken canopy or open environment.

Additional key words: C_3 and C_4 plants; coppice; tropical forest; tropical weeds.

Stable carbon isotope ratios (δ^{13}C) are widely used to survey the distribution of C_3 and C_4 photosynthetic pathways in plant communities and to gain insights into fundamental physiological processes such as water use, growth rates, and the fate of intercellular CO_2. In plant communities, δ^{13}C varies among co-occurring species and possibly among genotypes within the species. Environmental factors are known to cause variations in C_3 plants, but less in C_4 plants (Evans *et al*. 1986), *e.g*. the δ^{13}C of plants decreases with light availability in the habitat (Ehleringer *et al*. 1987) and increases with water stress (Caldeira *et al*. 2001) and CO_2 concentration (Farquhar *et al*. 1982). This study analysed species at a single site which had the advantage to limit environmental effects because of the unity of climate and soil type. The aim of this study was to obtain δ^{13}C values of the flora in fields, thickets, and secondary forest. For many species, although common and widespread in tropical Asia, these were first recordings of foliar δ^{13}C. Secondly, using the comparison of traits, we discussed the effect of the functional type on δ^{13}C values.

The study site was Houay Pano, northern Laos (19°51'00"–51'45"N, 102°09'50"–10'20"E), representative for large areas in humid tropical Asia where very little original forest persists. The site is a long-term observatory for effects of land-use change on hydrology, flora, and soils. Climate is characterized by the summer monsoon with mean annual air temperature and precipitation of 25.3°C and 1,403 mm, respectively (Valentin *et al*. 2008). Altitudes are between 425 m and 718 m a.s.l and deep (>2 m) to moderately deep (>0.5 m) loamy clay Alfisols dominate (MSEC 1999). The original vegetation is a lowland forest. Farmers clear the vegetation with slash and burn techniques and plant rain-fed rice (*Oryza sativa* L.), maize (*Zea mays* L.), or Job's tears (*Coix lacryma-jobi*, L.), then allow the field to revert back to thicket or low forest. Farmers often recultivate the land after 2–6 years (Huon *et al*. 2013). Abandoned fields consist of open or closed canopies; they are often mixed with teak or fruit trees, and used by the local population for firewood, food, medicine, construction wood, and hunting.

A floristic survey was carried out (2001–2003) comprising full species lists in 199 plots of 18 m^2 each, 98 in cultivated fields, and 101 in natural regrowth. A total of 459 different species were recorded including crops. From species occurring 3 times or more (n = 238) leaf samples were taken from growing plants. Leaf material was dried and ground for determination of δ^{13}C by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS, *Elemental Analyser NA1500*, *Carlo Erba*, Milan, Italy). Carbon isotope ratios were calculated as deviations of the C isotope ratio (C^{13}/C^{12}; R) from the international standard (Vienna Pee Dee Belemnite, V-PDB).

$$\delta^{13}C = [(R_{sample}/R_{standard}) - 1] \times 1{,}000$$

The 238 plant species were categorized into growth types: trees, bamboo, large lianas, shrubs, small lianas, forbs, vines, ferns, grasses, and sedges; and into strategy types: evergreen *vs*. deciduous, Leguminosae *vs*. non-Leguminosae. In the field, we checked regeneration by resprouting from stumps or root stocks and when no such structures were visible, the plant had apparently established from seed. The Student *t*-test (two-tailed) was

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Table 1. Total flora of fields and disturbed forest, Houay Pano, Laos and fraction of flora analysed with mean leaf $\delta^{13}C$ ± 1SE across growth forms. Sample size in parentheses, NP – growth form not present. [a] all species recorded in 199 plots of 18 m^2, [b] species occurring ≥ 3 times in the 199 plots.

	Flora [a]	Analysed [b]	Erect	Climbing
Woody – canopy				
Trees & large lianas	170	79	–30.7‰ ± 0.3 (50)	–30.5‰ ± 0.3 (29)
Bamboos	5	4	–30.8‰ ± 0.9 (4)	NP
Woody – understorey				
Shrubs & small lianas	71	34	–31.3‰ ± 0.4 (26)	–31.6‰ ± 0.9 (8)
Perennial herbs				
Forbs & vines	78	32	–31.4‰ ± 0.7 (12)	–29.6‰ ± 0.4 (20)
Ferns	16	11	–31.0‰ ± 0.7 (8)	–31.9‰ ± 3.1 (3)
C_3 grasses & sedges	13	9	–30.7‰ ± 0.5 (8)	–29.2‰ (1)
C_4 grasses & sedges	14	14	–12.3‰ ± 0.3 (14)	NP
Annual herbs				
Forbs & vines	65	36	–30.9‰ ± 0.4 (30)	–31.3‰ ± 0.5 (6)
C_3 grasses & sedges	7	4	–31.6‰ ± 0.7 (4)	NP
C_4 forbs	5	3	–13.6‰ ± 0.2 (3)	NP
C_4 grasses & sedges	15	12	–11.8‰ ± 0.4 (12)	NP

used to determine differences in leaf-$\delta^{13}C$ between pairs of species groups.

Table 1S (*supplementary material available online*) shows $\delta^{13}C$, photosynthetic pathway, growth form, and resprouter or seedling type of the species. Mean values and SE of C_3 pathway species (n = 209) were: –30.8‰ ± 0.14, minimum –37.7‰, maximum –25.5‰; C_4 species (n = 29): –12.3‰ ± 0.24, minimum –15.2‰, maximum –10.3‰. There were no CAM species. Canopy trees and canopy lianas shared the same habitat and had similar mean $\delta^{13}C$ values (Table 1). This was also observed by Zhu and Cao (2010) in trees (–30.4‰) and lianas (–29.3‰), and by Cai *et al*. (2009) in evergreen trees (–31.2‰) and evergreen lianas (–30.1‰). Unlike temperate forests, where evergreenness is linked to xeric conditions (De Lillis *et al*. 2004), it is associated with moisture in the humid tropics (Zheng and Shangguan 2007, Hasselquist *et al*. 2010) and in this study. In Houay Pano, significant differences were reported in shrubs (evergreen: –32.0‰, deciduous: –30.3‰, t_{16} = 2.63, 0.025>p>0.010) and in large lianas (evergreen: –31.2‰, deciduous: –29.6‰, t_{22} = 2.41, 0.05>p>0.025), but not in trees and herbs. The trend of Leguminosae being less depleted than non-Leguminosae, known from literature, was not apparent in any of our species groups. Higher $\delta^{13}C$ in non-Leguminosae can be explained by the ability of Leguminosae to colonize harsh habitats more easily (Zheng and Shangguan 2007). In this study, Leguminosae and non-Leguminosae share the same climate and soil and show the same range of $\delta^{13}C$ values. Species regenerating from seed being mostly weeds were expected to have less negative $\delta^{13}C$ compared to the group of woody resprouting plants due to their smaller root system and their association with a more open environment. This proved not that the case for both types of C_3 species gave similar mean values: resprouting species: –30.9‰ ± 0.17, seedlings: –30.5‰ ± 0.24, (t_{207}, 0.4>p>0.2), possibly because both types grow mixed in fields and disturbed forest and thus share the same environment. Both values match the average leaf $\delta^{13}C$ of closed forest habitats (–30.9‰), rather than those of intermediate (–29.3‰) and open canopy habitats (–27.1‰, South China, Ehleringer *et al*. 1986) or those of disturbed habitats in Thailand (–29.2‰, wet season, Yoneyama *et al*. 2010).

Literature leaf $\delta^{13}C$ values were available for 28 plant species analysed in this study. In 19 species, mostly pantropical weeds, literature values were more enriched, 2.1‰ on average, possibly because the material was collected in drier habitats (Diels *et al*. 2001, Yoneyama *et al*. 2010). Largest differences were found in *Celastrus paniculatus* (–4.5‰), *Mimosa diplotricha*. (–4.4‰), and *Vernonia cinerea* (4.4‰ and 3.1‰). Cassava leaves, collected in Thailand from open fields ranging from –29.7 to –26.1‰ (Yoneyama *et al*. 2010), were more enriched than our value of –30.2‰. In Houay Pano, cassava grows freely in dense vegetation where it is kept for planting material (stem) and not for the production of tubers. This would agree with the experiment of Makoi *et al*. (2010) who observed a significant lowering of leaf $\delta^{13}C$ in dense or mixed stands compared to low planting densities or monocropping. Wild varieties of yams were more depleted in Houay Pano compared to wild varieties in Benin (–3.1‰) and both were more depleted than cultivated yams (Cornet *et al*. 2007). This can be explained by the drier climate in Benin and the drier environment of fields compared to natural vegetation.

Wang (2006) found no evidence of C_4 endemic species in the original forests of Yunnan, South China. The same should account for Northern Laos with similar relief and flora as in our study area. C_4 weeds may invade farmland that is slashed and burnt too frequently (Ramakrishnan 1992). Although the original forest has been cut, high levels of bush cover were maintained in the study area due to the practice of alternating cropping and fallowing. This would produce a relatively humid climate in which the flora has more depleted leaf $\delta^{13}C$ than could be expected in cutover and cultivated land.

References

Cai Z.Q., Schnitzer S.A., Bongers F.: Seasonal differences in leaf-level physiology give lianas a competitive advantage over trees in a tropical seasonal forest. – Oecologia **161**: 25-33, 2009.

Caldeira M.C., Ryel R.J., Lawton J.H., Pereira J.S.: Mechanisms of positive biodiversity-production relationships: insights provided by $\delta^{13}C$ analysis in experimental Mediterranean grassland plots. – Ecol. Lett. **4**: 439-443, 2001.

Cornet D., Sierra J., Bonhomme R.: Characterization of the photosynthetic pathway of some tropical food yams (*Dioscorea* spp) using leaf natural ^{13}C abundance. – Photosynthetica **45**: 303-305, 2007.

De Lillis M., Matteucci G., Valentini R.: Carbon assimilation, nitrogen, and photochemical efficiency of different Himalayan tree species along an altitudinal gradient. – Photosynthetica **42**: 597-605, 2004.

Diels J., Vanlauwe B., Sanginga N. *et al*.: Temporal variations in plant $\delta^{13}C$ values and implications for using the ^{13}C technique in long-term soil organic matter studies. – Soil Biol. Biochem. **33**: 1245-1251, 2001.

Ehleringer J.R., Field C.B., Lin Z.F., Kuo C.Y.: Leaf carbon isotope and mineral composition in subtropical plants along an irradiance cline. – Oecologia **70**: 520-526, 1986.

Ehleringer J.R., Lin Z.F., Field C.B. *et al.*: Leaf carbon isotope ratios of plants from a subtropical monsoon forest. – Oecologia **72**: 109-114, 1987.

Evans J.R., Sharkey T.D., Berry J.A., Farquhar G.D.: Carbon isotope discrimination measured concurrently with gas exchange to investigate CO_2 diffusion in leaves of higher plants. – Aust. J. Plant Physiol. **13**: 281-292, 1986.

Farquhar G.D., O'Leary M.H., Berry J.A.: On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. – Aust. J. Plant Physiol. **9**: 121-137, 1982.

Hasselquist N.J., Allen M.F., Santiago L.S.: Water relations of evergreen and drought-deciduous trees along a seasonally dry tropical forest chronosequence. – Oecologia **164**: 881-890, 2010.

Huon S., de Rouw A., Bonté P. *et al*: Long-term soil carbon loss and accumulation in a catchment following the conversion of forest to arable land in northern Laos. – Agr. Ecosyst. Environ. **169**: 43-57, 2013.

Makoi J.H.J.R., Chimphango S.B.M., Dakora F.D.: Photosynthesis, water-use efficiency and δ13C of five cowpea genotypes grown in mixed culture and at different densities with sorghum. – Photosynthetica **48**: 143-155, 2010.

MSEC (Management of Soil Erosion Consortium): Biophysical and socio-economic inventories. Houay Pano catchment KM-10 village. – Soil Survey and Land Classification Center, National Agriculture and Forestry Research Institute, Lao PDR, 1999.

Ramakrisnan P.S.: Shifting Agriculture and Sustainable Development. An Interdisciplinary Study from North-Eastern India. Man Biosphere ser. Vol.10, UNESCO, Paris 1992.

Valentin C., Agus F., Alamban R. *et al.*: Runoff and sediment losses from 27 upland catchments in Southeast Asia: Impact of rapid land use changes and conservation practices. – Agr. Ecosyst. Environ. **128**: 225-238, 2008.

Wang R.Z.: The occurrence of C_4 photosynthesis in Yunnan province, a tropical region in South-western China. – Photosynthetica **44**: 286-292, 2006.

Yoneyama T., Okada H., Ando S.: Seasonal variations in natural ^{13}C abundances in C_3 and C_4 plants collected in Thailand and the Philippines. – Soil Sci. Plant Nutr. **56**: 422-426, 2010.

Zheng S.X., Shangguan, Z.P.: Foliar $\delta^{13}C$ values of nine dominant species in the Loess Plateau of China. – Photosynthetica **45**: 110-119, 2007.

Zhu S.D., Cao K.F.: Contrasting cost-benefit strategy between lianas and trees in a tropical seasonal rain forest in southwestern China. – Oecologia **163**: 591-599, 2010.