

Contribution of *Miscanthus transmorrisonensis* to soil organic carbon in a mountain grassland: Estimated from stable carbon isotope ratio

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Abstract. To estimate the contribution of *Miscanthus transmorrisonensis* to soil organic matter in a high mountain grassland at Tartarhia Anpu (altitude of 2,600 m) in Taiwan, I analyzed the stable carbon isotope ratios ($\delta^{13}\text{C}$) of dominant plant species and soil organic matter. The $\delta^{13}\text{C}$ analysis reveals that among the five dominant plant species *M. transmorrisonensis* ($\delta^{13}\text{C}$ of -12.37‰) is the only species that uses C_4 photosynthetic pathway. In contrast, the $\delta^{13}\text{C}$ values of other plants species ranged from -29.21 to -23.3 ‰ (average of -26.25‰), typical values for terrestrial plants with C_3 photosynthetic pathway. The average $\delta^{13}\text{C}$ value of soil samples collected from ten locations over the grassland was -18.56 ± 0.36 ‰ again indicating that the vegetation contains a mixture of C_3 and C_4 type plants. Using two-end member linear mixing models, I then estimated that about 55% of soil organic carbon in this grassland came from *M. transmorrisonensis*.

Keywords: C_3 and C_4 photosynthetic pathways; Grassland; *Miscanthus transmorrisonensis*; Soil organic carbon; Stable carbon isotopes.

Introduction

The concentration of greenhouse gasses, mainly carbon dioxide, methane, and nitrous oxide in the atmosphere has been increasing since the Industrial Revolution. As a consequence, the earth warms up and climates change (Houghton et al., 1990). It has been suggested that C_4 plants evolved as an adaptation to low CO_2 concentration in Late Tertiary (Ehleringer et al., 1991). The CO_2 concentrating mechanism of C_4 pathway confers a higher productivity, better water use efficiency, and greater temperature tolerance as compared with C_3 plants, however, the advantages may be diminished at elevated CO_2 . Thus, concern about the potential threat posed to plants containing the C_4 photosynthetic pathway by increasing atmospheric carbon dioxide has been raised (Henderson et al., 1994). To evaluate the potential effect of changes in climate on the distribution of C_4 plants, it is hence essential to study the current distribution of C_4 plants in the various ecosystems.

Stable carbon isotopes have been used in agricultural and ecological research for decades (Tieszen and Boutton, 1989). The two stable carbon isotopes are ^{12}C and ^{13}C , which comprise 98.89 and 1.11%, respectively, of all carbon in nature (Ehleringer and Rundel, 1989). Currently, the carbon isotope ratio ($\delta^{13}\text{C}$) of atmospheric CO_2 is about -7.5‰ (Keeling et al., 1984). Plants contain less ^{13}C than the atmosphere because the physical and chemical pro-

cesses involved in CO_2 uptake discriminate against the isotope (Berry, 1989). During photosynthetic uptake of CO_2 , the carboxylation enzyme ribulose bisphosphate carboxylase discriminates against ^{13}C more than phosphoenol pyruvate carboxylase (O'Leary, 1981; Roeske and O'Leary, 1984). As a consequence, C_3 plants have less ^{13}C than C_4 plants. For example, $\delta^{13}\text{C}$ values of C_3 plants vary from -23 to -34 ‰, whereas C_4 plants have values of approximately -10 to -15 ‰ (O'Leary, 1988). Therefore, ^{13}C analysis has become an accepted method for determining the pathway of CO_2 fixation of terrestrial plants.

In natural ecosystems, soil organic carbon is derived predominantly from the residues of native vegetation, hence the stable carbon isotope ratio of the vegetation has a direct impact on the $\delta^{13}\text{C}$ of the soil organic matter ($\delta^{13}\text{C}_{\text{som}}$). It has been shown that the natural isotopic abundance of soil organic matter corresponds closely to that of the vegetation cover from which it originated (Nadelhoffer and Fry, 1988). Thus, the characteristic $\delta^{13}\text{C}$ of the vegetation serves as a marker to indicate the origin of soil organic matter (Balesdent et al., 1987; Rao et al., 1994; Kessel et al., 1994). For example, mass-balance calculations based on the $\delta^{13}\text{C}$ values of a geological sample and assumed end-member $\delta^{13}\text{C}$ values for C_3 and C_4 plants have been used to estimate the relative proportion of C_3 - and C_4 - derived carbon in a sample of organic carbon (Bird et al., 1994).

The objective of this study was to analyze the carbon isotopes ratio of soil organic carbon to estimate the relative contribution of C_3 and C_4 plants to soil organic carbon in a mountain grassland.

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Study Site

Tartarchia Anpu grassland (altitude of 2,600 m) is located in Yushan National Park (23°29' N, 120°48' E), Nantou county, in central Taiwan. It is classified as a mountain temperate grassland. The mean monthly air temperature ranges from 6°C in December to 14°C in July (Climatological data annual report, Central Weather Bureau, ROC). The mean annual precipitation is approximately 4,000 mm. A visual examination of the vegetation indicated that *Miscanthus transmorrisonensis*, *Baeothryon subcapitatum* (Thwaites) T. Koyama, *Pinus taiwanensis* Hayata, *Yushania nittakayamensis*, and *Lycopodium cernuum* Linn. are the five most dominant plant species of the vegetation composition.

Materials and Methods

Leaf materials were collected from five individuals of each of the five dominant species. Individuals of each species were sampled at least 1 m away from each other. Leaves were dried at 70°C for at least 48 h and then ground to a fine powder using a mortar and pestle. Ten soil sampling points were chosen over the grassland, the distance between each sampling points was at least 1 m. To minimize the effect of progressive modification of isotopic composition of soil organic matter by microbial degradation (Nadelhoffer and Fry, 1988), at each sample point the top 5 cm of soil of a 0.02 m plot was collected. Soil samples were air-dried, after removal of coarse litter and roots, and sieved through a 0.5 mm mesh (Der Shuenn, Taiwan). The pH value of the soil was determined using a pH meter (Suntex TS-2, Taiwan) by mixing soil with distilled water in a proportion of 1 to 10. Total carbon contents were determined with an elemental analyzer (NA1500, Fisons, Italy).

Inorganic carbon was removed from soil samples prior to isotopic analysis by pre treatment with 1 M HCl at room temperature overnight, rinsed with distilled water, then oven dried for 12 h.

Two to 3 mg of dried plant material or 10 to 15 mg of soil sample were sealed with 1 g of copper oxide wire (Merck) and 1 piece of silver foil (2 mm × 10 mm) under vacuum in a 6 mm quartz tube and heated to 850°C for 4 h (Ehleringer and Osmond, 1989). The tubes were then allowed to cool slowly for 10 h. After combustion, the sealed tube contained CO₂, H₂O, and N₂. The tube was then cracked under vacuum, and the gasses were separated by passing them through an ethanol-dry ice trap to remove H₂O and a liquid nitrogen trap to collect CO₂ (Ehleringer and Osmond, 1989). The carbon isotope ratio of the purified CO₂ was determined on an isotope ratio mass spectrometer (SIRA10, VG Instruments, Oxford, UK). The carbon isotope ratio of the organic matter was expressed as

$$\delta^{13}\text{C} (\text{‰}) = [(R_{\text{sample}}/R_{\text{PDB}}) - 1] \times 1000, \text{ where } R = {}^{13}\text{C}/{}^{12}\text{C}.$$

Thus, $\delta^{13}\text{C}$ is the difference in carbon isotope ratios between a sample (R_{sample}) and the PDB standard (R_{PDB}), in

thousandths (‰) of the isotope ratio in the standard, and PDB refers to the belemnite carbonate standard of the Peedee Formation, South Carolina, USA, which is accepted as the international standard. Measurements of organic standards were reproducible to $\pm 0.05\text{‰}$.

The following equation was then used to calculate the proportion of the soil carbon from C₄ plants:

$$\delta^{13}\text{C}_{\text{som}} = \delta^{13}\text{C}_{\text{C}_4} \times f + \delta^{13}\text{C}_{\text{C}_3} \times (1-f) \quad (\text{eqn. 1})$$

where

$\delta^{13}\text{C}_{\text{som}}$ = carbon isotope ratio of the soil organic matter,

$\delta^{13}\text{C}_{\text{C}_3}$ = carbon isotope ratio of C₃ plants,

$\delta^{13}\text{C}_{\text{C}_4}$ = carbon isotope ratio of C₄ plants,

f = proportion of carbon from C₄ plants, and

1-f = proportion of carbon from C₃ plants.

Results and Discussion

$\delta^{13}\text{C}$ Values of Vegetation and Soil Organic Matter

Results of the $\delta^{13}\text{C}$ values of the five dominant species in the Tartarchia Anpu grassland are presented in Table 1. According to these values, the five dominant species can be divided into two groups. The $\delta^{13}\text{C}$ value of *M. transmorrisonensis* is within the range of typical C₄ plants ($\delta^{13}\text{C}$ of -14 to -10 ‰) indicating that they use the C₄ photosynthetic pathway. In contrast, *L. cernuum*, *P. taiwanensis*, *Y. nittakayamensis*, and *B. subcapitatum* have $\delta^{13}\text{C}$ values ranging from -29.21 to -23.33 ‰, which are typical of plant species with the C₃ photosynthetic pathway, indicating that these species are C₃ plants.

The average pH value and total carbon content of ten soil samples were 4.5 and 8.0 ± 0.7 (mean \pm S.E.), respectively. The $\delta^{13}\text{C}$ values of these samples range from -19.02 to -18.11 ‰ (Table 2). The $\delta^{13}\text{C}$ values of the soil samples were between that of C₃ plants and C₄ plants, implying that soil organic carbon of this grassland came from a mixture of C₃ and C₄ vegetation.

Estimate of Soil Carbon Derived from M. transmorrisonensis

The primary influence of $\delta^{13}\text{C}$ of soil organic matter is the relative contribution of C₃ versus C₄ plants to the total net primary productivity of the community under study. The distinct difference in $\delta^{13}\text{C}$ value of *M. transmorrisonensis* from other dominant plant species

Table 1. The $\delta^{13}\text{C}$ values (mean \pm S. E., n=5) of the dominant species in the Tartarchia Anpu grassland.

Species	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)
<i>Miscanthus transmorrisonensis</i>	-12.37 \pm 0.14
<i>Baeothryon subcapitatum</i> (Thwaites) T. Koyama	-23.33 \pm 0.26
<i>Pinus taiwanensis</i> Hayata	-26.03 \pm 0.22
<i>Yushania nittakayamensis</i>	-26.41 \pm 0.31
<i>Lycopodium cernuum</i> Linn.	-29.21 \pm 0.13

Table 2. $\delta^{13}\text{C}$ values (‰), total carbon content and pH of the soil organic matter and the calculated contribution of C_4 plants (according to eqn.1 and the values of Table 1) in the Tartachia Anpu grassland.

Location	pH	C (%)	$\delta^{13}\text{C}_{\text{PDB}}$ (‰)	% of C_4
1	4.5	7.7	-18.23	57.8
2	4.3	12.2	-18.11	58.6
3	4.2	7.1	-18.07	58.9
4	4.4	9.3	-18.58	55.3
5	4.4	8.2	-19.02	52.1
6	4.5	9.4	-18.98	52.4
7	4.5	5.5	-18.78	53.8
8	4.6	6.2	-18.39	56.6
9	4.6	5.6	-18.94	52.7
10	4.5	9.1	-18.45	56.2
mean \pm S.E.	4.5 \pm 0.0	8.0 \pm 0.7	-18.56 \pm 0.36	55.4 \pm 0.8

(Table 1) serves as a marker to trace the contribution of this species to soil organic carbon. Thus using the mass-balance equation (eqn. 1) and assuming two end-members of -12.37 (the average $\delta^{13}\text{C}$ value of *M. transmorrisonensis*) and -26.26 ‰ (the average $\delta^{13}\text{C}$ value of dominant C_3 plants), it was estimated that *M. transmorrisonensis* contributes about 55% of the total soil organic carbon in this mountain grassland (Table 2).

In a steady-state system, $\delta^{13}\text{C}$ of the soil organic matter should be nearly identical to that of the plant community from which the organic matter was derived, unless the isotopic composition of plant tissue is altered during decomposition (Boutton, 1996). Direct measurements of five dominant plant species in a woodland indicate that $\delta^{13}\text{C}$ of plant tissue remains relatively constant during the early stages of decomposition (Boutton, 1996). Wedin et al. (1995) found small (0.6 to 1.5 ‰) shifts in the $\delta^{13}\text{C}$ of litter from four grass species. In addition, some indirect evidence also suggests that fractionation during decomposition is small (from < 1 to 2‰) especially in terrestrial ecosystems (Nadelhoffer and Fry, 1988; Boutton, 1996). The average $\delta^{13}\text{C}$ value of *M. transmorrisonensis* is about 14‰ higher than that of C_3 plants. Therefore, even if there is discrimination during decomposition, the isotopic discrimination would be very small compared to the large $\delta^{13}\text{C}$ difference in C_3 and C_4 plants. Though fractionation during decomposition was not measured in this study, the estimate of the relative contribution of *M. transmorrisonensis* versus other C_3 plants to soil organic carbon based on the analysis of carbon isotopic composition is still valid.

Chou et al. (1991) had previously studied the vegetation succession in the same grassland, finding that the coverage percentage of *M. transmorrisonensis* after a surface clearing varied in three measurements, from 3% to 22%. In addition, seasonal variation in contribution to primary production by C_3 and C_4 plants has been found in a mixed prairie (Ode et al., 1980). These results indicate that an estimate of the relative contribution of C_3 versus C_4 plants to primary production based on a one-time measurement of the vegetation coverage could result in an erroneous conclusion. In contrast, analysis of the $\delta^{13}\text{C}$ of the soil organic matter pool reflects the long-term integrated $\delta^{13}\text{C}$ of

the plant communities that contributed to the soil organic matter at a given site.

In conclusion, analysis of the stable carbon isotope ratio of soil organic carbon provides quantitative evidence of the relative contribution of C_3 versus C_4 plants to a community's net primary productivity.

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高山草原土壤有機碳之來源

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本文使用穩定性碳同位素技術追蹤塔塔加鞍部草原區土壤有機碳之主要來源。塔塔加鞍部草原區位於台灣中部玉山國家公園內，海拔高度約 2,600 公尺。分析本區五種主要植物種類（高山芒、玉山箭竹、過山龍、台灣二葉松、玉山針藜）之穩定性碳同位素比值 ($\delta^{13}C$)，發現高山芒是唯一的 C_4 型植物 ($\delta^{13}C = -12.3\text{‰}$)，其他四種均屬 C_3 型植物 ($\delta^{13}C$ 值從 -29.21 到 -23.3‰ 平均為 -26.25‰)；土壤有機碳之穩定性碳同位素比值平均為 -18.46‰，介於 C_4 型和 C_3 型植物之間。利用這些值，推算此草原區土壤有機碳約有 55% 來自高山芒，另外 45% 則來自其他 C_3 植物。

關鍵詞：高山草原；穩定性碳同位素；土壤有機碳；高山芒； C_4 型和 C_3 型植物。