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Stable carbon isotope ratio and nutrient contents of the *Kandelia candel* mangrove populations of different growth forms

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Abstract. *Kandelia candel* (L.) Druce, the dominant mangrove species on the west coast of northern Taiwan, has three distinct growth forms. To elucidate whether the growth of the dwarf form of *K. candel* is related to nutrient and water availability, we analyzed nutrient contents (N, P, K, Ca, Mg, Na) and carbon isotope ratios ($\delta^{13}C$) of leaves of four *K. candel* populations differing in canopy height. The objectives of the study were to investigate whether the dwarf *K. candel* populations have higher long-term water use efficiency and whether nutrient limitation is related to the growth of the dwarf form of mangrove plants. Leaves of dwarf mangrove populations had significantly higher $\delta^{13}C$ values (1–1.5‰ higher) and thus higher long-term integrated water use efficiency than the taller mangrove populations. N and P contents were significantly lower in leaves and soils of the dwarf mangrove populations than in those of medium height and tall mangrove populations. In contrast, no significant difference was found in K, Na, Ca and Mg contents among leaves of the four mangrove populations. It is proposed that water and nutrient availability, especially of nitrogen and phosphorus, limit the growth of *K. candel*.

Keywords: *Kandelia candel*; Mangrove; Nitrogen content; Phosphorus content; Stable carbon isotope ratio; Water use efficiency.

Introduction

Mangrove are a group of trees or shrubs that grow in the tidal waters of tropical and subtropical coastlines. Though mangrove are able to colonize saline habitats, factors such as high salinity, poor aeration (Davis, 1940), waterlogging (Egler, 1952), compacted peat (Craighead, 1971), and nutrient limitations (Feller, 1995; Lugo and Snedaker, 1974) have been thought to limit their growth. In addition, Lin and Sternberg (Lin and Sternberg, 1992a, b), using stable isotope techniques to compare the ecophysiology of scrub and fringe mangroves in Florida, USA, concluded that water availability and fluctuations in salinity could also limit the growth of mangrove plants.

Most of the above studies were conducted on *Rhizophora mangle* (Feller, 1995; Lin and Sternberg, 1992a,b; Lugo and Snedaker, 1974), *Avicennia* spp. (Lin and Sternberg, 1992a; Popp, 1984), *Aegialitis annulata* (Popp, 1984), and *Languncularia* spp. (Lin and Sternberg, 1992a). *Kandelia candel* is the dominant mangrove species in the west coast of northern Taiwan (Liu, 1982). *Kandelia candel* in the Chu-wei area of northern Taiwan exhibits three distinct growth forms: The average tree height of dwarf populations is about 1 m; the tall population is 4 m or greater; and the medium height population is 2 to 3 m. Factors responsible for the different growth forms of this mangrove species have not been studied. Huang (1983) found significant differences in soil nutrient content and salinity at six locations within the Chu-wei area. We also observed that dwarf form populations are located at higher elevations where there is less flooding. These observations suggest that the dwarf form of *K. candel* might be related to nutrient and/or water availability. In the present study, we focused on these two factors.

We thus conducted a survey to compare the long-term water use efficiency (WUE) and nutrient characteristics of leaves of *K. candel* plants from populations of different growth forms. Because there is a negative linear relationship between leaf stable carbon isotope ratio and the ratio of intercellular to ambient CO_2 concentration (C_i/C_a) (Farquhar et al., 1982), stable carbon isotope ratios are often used to indicate the long-term integrated WUE of plants (Farquhar et al., 1989). Hence, we used the technique analyzing leaf carbon isotope ratio to determine the long-term WUE of the mangrove plants in these populations. In addition, we compared nutrients, N, P, K, Ca, Na, and Mg of leaves of the mangrove populations with different growth forms. The concentrations of inorganic nitrogen (ammonia-N and nitrate-N) and available phosphorus of the corresponding soil samples were also analyzed. There are two specific objectives of this study: (1) to investigate whether dwarf form *Kandelia candel* populations have higher long-term water use efficiency; and (2) to evaluate whether nutrient limitations can explain variations in size among mangrove populations in the study site.

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Study Site

The study was conducted at the Chuwei swamp (25°9' N, 121°26' E) located along the Tamsui River in Taipei County, Taiwan. The mean annual temperature and precipitation of this area is 22°C and 2,100 mm, respectively (Climatological data annual report, Central Weather Bureau, R. O. C.). The tidal condition in this region is semidiurnal. The average highest tide measured in 1994 was 122.3 cm, and the lowest was 95 cm below sea level (Liu, 1996). The tidal salinity ranges between 10 and 36 parts per thousand (Huang, 1983). The area has about 50 hectares of pure *K. candel* forest. In addition to the *K. candel* forest, *Clerodendrum inerme* (L.) Gaertn., *Pluchea indica* (L.) Less, *Wedelia biflora* (L.) DC., *Ipomoea pes-caprae* subsp. *brasiliensis* Oostat, *Suaeda nudiflora* (Willd.) Moq., *Phragmites communis* (L.) Trin. and *Imperata cyclindrica* (L.) are other common species growing in the area (Huang, 1983).

Materials and Methods

Four transects of *K. candel* populations with different growth forms were chosen for the study (Figure 1). Each transects was about 20 m × 20 m. Tree height of five individuals of *K. candel* in each transect were recorded. Individuals of each transect were sampled at least 2 m away from each other. To determine whether the tree age of *K. candel* can be estimated by counting its rings, three to four trees in each transect were cut, and the wood was polished with sand paper. Two to three pairs of the most recent fully expanded leaves (the second or third pair of leaves) from five individuals of each transect were collected on 8 June 1995. Leaf samples were then dried in an oven at 70°C for at least 48 h and ground to a fine powder with a mortar and pestle. The samples were then used for the following carbon isotope ratio and nutrient content analyses. Total nitrogen and carbon contents of leaf samples were determined with an elemental analyzer (NA 1500, Fisons, Italy).

About 2 mg of leaf material was sealed with 1 g of CuO wire (0.65 mm × 3 mm, Merck) and a piece of silver foil (3 mm × 10 mm, Johnson Matthey) under vacuum in a 6 mm quartz tube and heated to 850°C for 4 h. The tubes were then allowed to cool slowly to room temperature. The CO_2 produced from the foregoing procedure was purified cryogenically, and measured on an isotope ratio mass spectrometer (delta S, Finnigan Mat, Germany). Carbon isotope ratios were reported in δ units relative to the standard PDB (PeeDee Belemnite) as $\delta^{13}C$ (‰) = $[(R_{sample} / R_{PDB}) - 1] \times 1000$, where $R = {}^{13}C/{}^{12}C$. We then calculated the ratio of intercellular CO_2 concentration to ambient CO_2 concentration (C_i/C_a) from the leaf carbon isotope ratio ($\delta^{13}C_p$) according to the model of Farquhar et al. (1982), $\delta^{13}C_p = \delta^{13}C_a - a - (b - a) \times (C_i/C_a)$, where a = 4.4‰ and b = 27‰. The $\delta^{13}C$ of atmospheric CO_2 ($\delta^{13}C_a$) was assumed to be -8‰.

HCl (6 M) extracts were prepared from the ground leaf materials for Na, K, Ca, Mg, and P analyses (Lambert, 1976). Potassium and Na, and Ca and Mg contents were subsequently analyzed with a flame photometer (Model 410, Corning) and an atomic absorption spectrophotometer (Model 2380, Perkin-Elmer), respectively. Total leaf phosphorus was analyzed by the vanadomolybdophosphoric yellow color method (Kitson and Mellon, 1944).

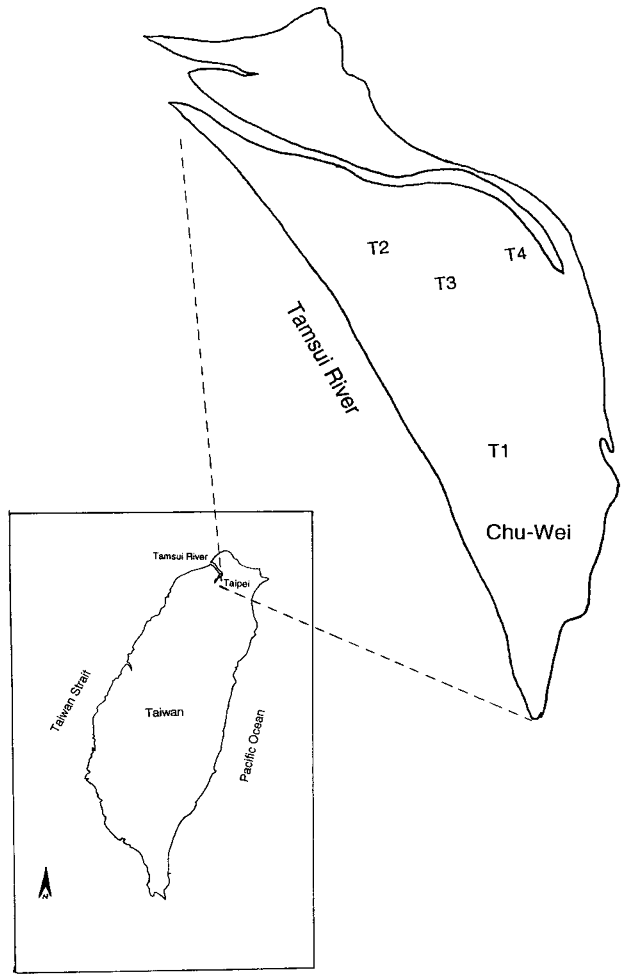

Figure 1. Location of four sampling transects in the Chu-wei swamp of the Tamsui River, Taipei County, Taiwan.

Five to six soil samples from the depth of 15–20 cm in each transect were also collected and sealed in plastic bags until analysis. The salinity analysis was based on electrical conductivity (EC). The electrical conductivity and pH value of an aqueous solution extracted from 1:5 soil:deionized water mixture were determined using a potential meter and a pH meter, respectively. Although a more standard procedure for salinity determination is the "soil paste" method, the 1:5 ratio method we used is recommended as a simpler technique to determine relative salinity (Rhoades, 1982) and is suitable for the purposes of this study (i.e. standardized comparison of relative salinity among transects). After measurements of electrical conductivity and pH value, soil samples were air-dried and the following analyses were conducted. The concentration of Na in the soil was determined using the same method as previously described for leaf samples. The available phosphorus of the soil was determined by the Bray-1 method (Bray and Kurtz, 1945). The concentrations of ammonia-nitrogen and nitrate-nitrogen of soil were analyzed using the indophenol (Scheinger, 1976) and Cd reducing methods (Keeney and Nelson, 1982), respectively.

Mean values among four populations were compared by Tukey's HSD test.

Results

Mean tree height of mangrove populations in Transects 1 and 2 (the dwarf populations) was significantly lower than that of mangrove populations in Transects 3 (medium high population) and 4 (tall population) (Table 1) ($n = 5$, $p < 0.05$).

The leaf carbon isotope ratio measured for *K. candel* (ranging from -28.55 to -25.59 ‰) was within the range of most C_3 plants (O'Leary, 1981). Leaves of plants in the dwarf mangrove populations have significantly higher $\delta^{13}C$ values than in medium high and tall mangrove populations (Table 1). No significant difference in carbon isotope ratios was found between leaves of medium high and tall mangrove trees ($n = 5$, $p > 0.05$). The estimated C_i/C_a over long-term carbon assimilation of the dwarf population (0.62 ± 0.01, mean ± S.E.) was significantly lower than that in the medium high and tall population, 0.66 ± 0.01 ($n = 5$, $P < 0.05$).

Phosphorus and nitrogen content was significantly higher in leaves of medium and tall mangrove trees than in those of dwarf mangrove trees (Table 1). In other words, there was a significant correlation between tree height and leaf total nitrogen content (Figure 2a) and between tree height and leaf phosphorus concentration (Figure 3a). In contrast, no significant difference was found in K, Na, Ca, or Mg content in leaves of the four mangrove populations ($p > 0.05$).

Table 1. Differences in tree heights (m), carbon isotope ratio ($\delta^{13}C$) and nutrient concentrations (mg g^{-1}) of leaves of *Kandelia candel* in four transects (mean ± standard error, n = 5).

	Transect 1	Transect 2	Transect 3	Transect 4
Tree height	1.40 ± 0.06^{a}	1.27 ± 0.06^{a}	2.45 ± 0.07^{b}	4.19 ± 0.07^{c}
$\delta^{13}C$ (‰)	-26.41 ± 0.26^{a}	-26.34 ± 0.22^{a}	-27.34 ± 0.31^{b}	-27.38 ± 0.13^{b}
N	13.2 ± 0.6^{ab}	12.9 ± 0.6^{a}	14.9 ± 0.6^{bc}	18.2 ± 0.7^{d}
P	1.15 ± 0.07^{a}	1.19 ± 0.08^{a}	1.35 ± 0.09^{ab}	1.56 ± 0.12^{b}
K	6.59 ± 0.40^{a}	6.97 ± 0.36^{a}	7.08 ± 0.32^{a}	6.45 ± 0.62^{a}
Ca	6.72 ± 1.05^{a}	6.62 ± 1.37^{a}	8.70 ± 1.27^{a}	7.51 ± 1.56^{a}
Na	15.08 ± 0.75^{a}	17.24 ± 1.11^{a}	17.79 ± 0.62^{a}	15.60 ± 1.07^{a}
Mg	4.23 ± 0.40^{a}	4.18 ± 0.64^{a}	3.91 ± 0.48^{a}	3.95 ± 0.54^{a}

*Means within rows followed by different superscripts are different at P = 0.05 (Tukey's test).

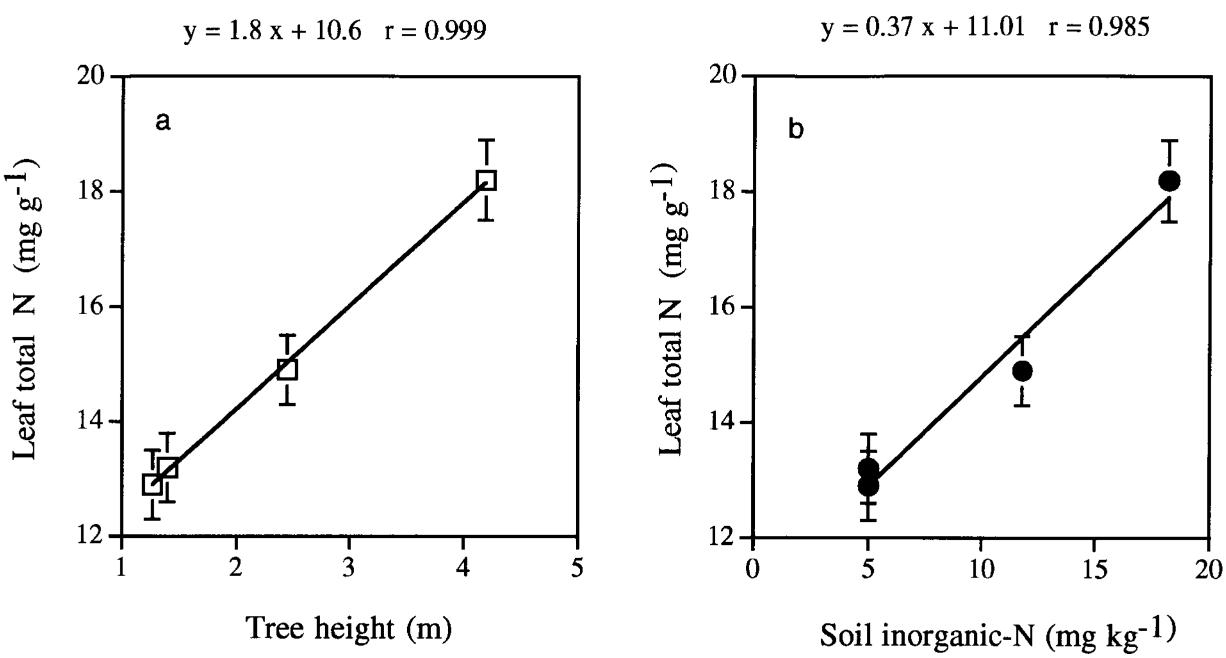

Figure 2. Correlation between tree height and leaf total nitrogen content (a) and that between soil inorganic nitrogen and leaf total nitrogen content (b) of *K. candel* plants from four mangrove populations.

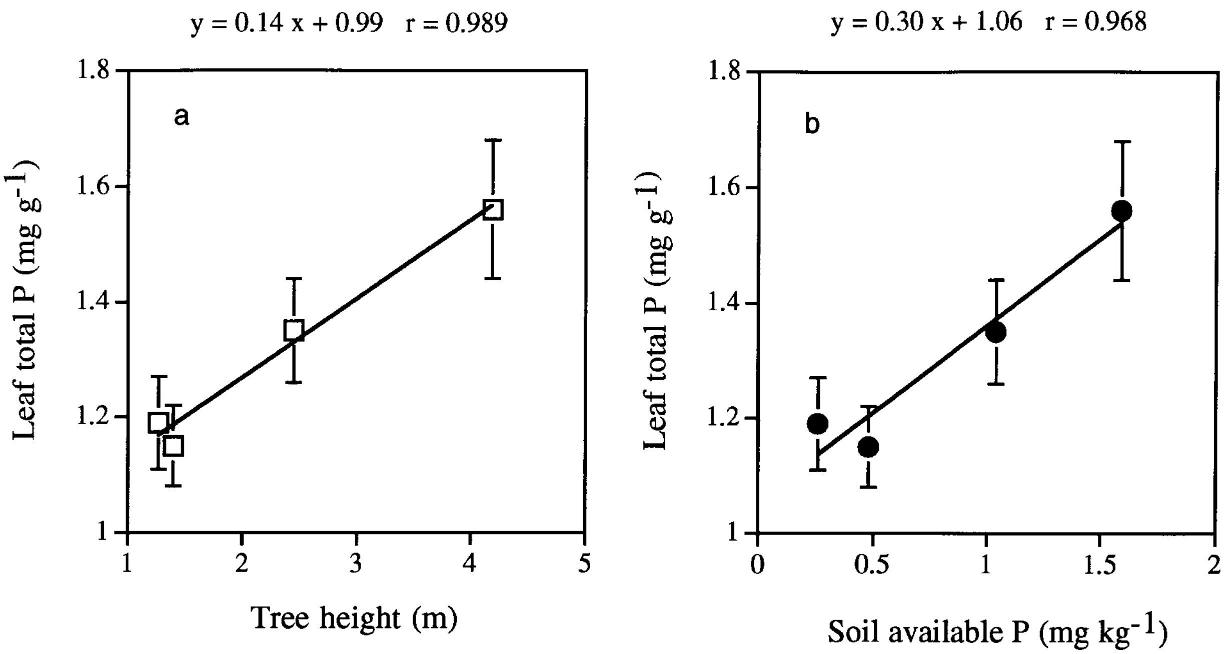

Figure 3. Correlation between tree height and leaf total phosphorus content (a) and that between soil available phosphorus and leaf total phosphorus content (b) of *K. candel* plants from four mangrove populations.

Total N, inorganic N, total C, and available P contents were lower, but electrical conductivity and Na concentration were higher in soil from the dwarf mangrove transects than in the medium and tall mangrove transects (Table 2). When leaf total N content was plotted with the corresponding inorganic nitrogen content of soil, a significantly positive correlation was found (Figure 2b, $r = 0.985$. $p<0.01$). Similarly, a positive linear relationship ($r = 0.968$, $p<0.01$) between leaf total phosphorus contents and the corresponding soil available phosphorus contents was also measured (Figure 3b).

Discussion

We were unable to separate the growth rings of wood to determine age of *K. candel* trees. This is consistent with most previous findings that datable growth rings do not exist in mangrove wood (e.g., Tomlinson, 1986). Therefore, no indirect measure of tree age was possible in this study. If different growth forms result from different age, we would expect within the dwarf stands trees of different height. However, the tree height of *K. candel* was quite homogeneous within dwarf populations (Table 1). In addition, not only were tree heights different, the dwarf populations also had shorter internodes (personal observation) and lower population density (Huang, personal communication) than the tall and medium populations. We thus concluded that the different growth forms of *K. candel* could not be due to age.

It has been shown that electrical conductivity is highly correlated with salinity, hence electrical conductivity is often used to represent relative salinity (Rhoades, 1982; Walker and Sinclair, 1992; Sandquist and Ehleringer, 1995). The variation in electrical conductivity of soil was consistent with variation in the soil Na contents, indicating that the soils from the dwarf mangrove populations have the highest salinity (Table 2). Although the Na content of soil from the dwarf mangrove populations is about twofold that of soil from the taller mangrove populations, we did not find significant differences in Na content of leaves among the four mangrove populations (Table 2). This result suggests that leaves of *K. candel* were able to

Table 2. Soil characteristic of four tansects of the *K. candel* mangrove forest.

	Transect 1	Transect 2	Transect 3	Transect 4
EC (mS cm^{-1})	11.07	13.18	5.56	7.59
pH	6.85	7.10	5.97	5.46
C (mg g^{-1})	10.6	6.3	26.0	34.3
Total N (mg g^{-1})	0.5	0.5	1.3	2.0
Ammonia-N (mg kg^{-1})	3.5	3.5	7.6	17.6
Nitrate-N (mg kg^{-1})	1.5	1.5	4.2	0.6
Na (mg g^{-1})	87.2	103.9	42.7	58.3
P (mg kg^{-1})	0.48	0.26	1.04	1.59

maintain a fairly constant amount of Na despite twofold increases in Na concentration of the habitats. It also indicates that higher salinity in the soil did not directly limit the growth of the dwarf mangrove populations. However, we can not rule out the possibility that a higher cost associated with salt exclusion and/or of maintaining a constant non-damaging Na concentration in the cell could have potentially limited the growth of *K. candel* in high saline environments such as in Transects 1 and 2. Further investigation on the cost of salt exclusion is needed to evaluate this possibility.

Through analysis of leaf carbon isotope ratios, an indicator of integrated long-term water use efficiency in C_3 plants, we found that leaves of dwarf mangrove populations have higher water use efficiency (a more positive $\delta^{13}C$ value, thus a lower C_i/C_a). Lin and Sternberg (1992a) have also shown that individuals from dwarf mangrove forests have more positive $\delta^{13}C$ values and hence higher water use efficiency than those from tall mangrove forests in Florida. The authors suggested that due to less availability of water, dwarf mangroves maintain a higher water use efficiency by reducing stomatal conductance. Lower stomatal conductance would result in lower CO_2 uptake which could reduce photosynthetic rates. Therefore, there may be a tradeoff between water conservation and photosynthesis. However, because only a slight difference (about 7%) in C_i/C_a exists between leaves of the dwarf and the tall populations, the limitation of stomatal conductance in carbon assimilation cannot fully account for the dwarf form of the *K. candel* mangrove populations.

Results from soil analysis reveal that soil from Transects 1 and 2, where the dwarf populations of *K. candel* occur, has lower N and P availability compared to that from Transects 3 and 4 (Table 2). Nutrient levels in soil are highly dynamic, especially in swamp areas. Thus this result could not represent a long-term integrated nutrient status of the soil. However, a similar result was found in an independent study by Huang (Huang, 1983). In addition, the differences in soil nutrient content among transects were also reflected in the leaf nutrient contents of *K. candel* populations growing at each transect. Among the nutrients analyzed, both nitrogen and phosphorus contents were significantly higher in leaves of medium high and tall populations than in dwarf *K. candel* populations (Table 1). Soil available nitrogen showed the same trend with the increasing leaf nitrogen resulting in a positive linear relationship between leaf N content and soil available nitrogen (Figure 2b). A similar relationship was also found between leaf phosphorus contents and soil available phosphorus concentration (Figure 3b). It is evident that soil nitrogen and phosphorus limitation is an important factor contributing to the dwarf growth form of the *K. candel* populations in this study. We thus hypothesize that nitrogen and phosphorus availability limit the growth of dwarf populations of the *K. candel*. Nutrient deficiency has been suggested to be responsible for the dwarf plant form of red mangrove (Feller, 1995; Lugo and Snedaker, 1974). Bradley and Morris (1991) reported that the growth of salt marsh plant *Spartina alterniflora* was principally limited by an environment induced inhibition of nutrient uptake. In this study, we were not able to distinguish whether nutrient limitation of growth of dwarf *K. candel* populations is a function of nutrient availability or a result of the environment induced inhibition of the kinetics of nutrient uptake. Further studies on the effect of nutrient fertilization on the growth of mangrove forests are needed to evaluate these two factors.

It is not clear yet why soils from Transects 1 and 2 have less N and P than those from Transects 3 and 4. We suggest that one edaphic factor might account for the differences. Tidal waters distribute nutrients through the swamp systems, with the nutrient inputs being greatest in frequently flooded areas (Ball, 1988; Boto and Wellington, 1983; Boto and Wellington, 1984; Valiela and Teal, 1974). We observed that dwarf mangrove populations are located at transects of higher elevations and they are less flooded than the medium high and tall mangrove transects. According to Boto and Wellington (1983, 1984) due to the depletion of oxygen, the major form of combined inorganic nitrogen in the sediments at frequent tidal flooding is ammonia. The fact that soil from the taller mangrove transects have higher ammonia-nitrogen (Table 2) provides the evidence that the transects where the taller mangrove populations occur are more frequently flooded than the transects where dwarf mangrove populations grow.

In conclusion, we found that leaves of dwarf *K. candel* tree have higher water use efficiency and lower nitrogen and phosphorus contents than those of taller *K. candel* trees. Accordingly, we propose that water and nutrient availability, especially of nitrogen and phosphorus, limit the growth of dwarf populations of the *K. candel*.

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