Bot. Bull. Acad. Sin. (1996) **37:** 191-196

Nitrogen nutritional status and fate of applied N in mangrove soils

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(Received December 19, 1995; Accepted March 19, 1996)

Abstract. Fluctuation of inorganic N content in mangrove soils of the Tamshui estuary, northwestern Taiwan, showed that ammonium concentrations ranged from 0.15 to 17.10 mg N kg^{-1} soil, while nitrate ranged from trace amounts to 2.54 mg N kg^{-1} soil. Geographic and edaphic factors caused the difference of inorganic N between the sites. These levels were much higher than values reported elsewhere. Pot experiments showed the added ^{15}N labeled ammonium (6 mg N per pot) disappeared rapidly. The ^{15}N residues remaining in the soil were mostly in the organic form. N uptake by *Kandelia candel* was 13.1% after one month and 19.6% after three months. Recoveries of applied N after three months were 40.3% in planted treatment and 32.6% in unplanted treatment. Most of the N loss, occurring in the first month, can be attributed to denitrification. The large N loss suggests a high potential for mangrove soils to remove high input of N from the river through denitrification.

Keywords: Denitrification; *Kandelia candel*; Mangrove; ^{15}N.

Introduction

Nitrogen (N) has been indicated as a major factor limiting the growth of halophytes in intertidal areas (Stewart et al., 1979). Higher productivity of various kinds of halophytes seems to be induced by an additional supply of N (Tyler, 1967; Valiela and Teal, 1974). Evidence suggests that mangrove forests are generally nutrient limited with N (Onuf et al., 1977; Boto and Wellington, 1983; Boto and Wellington, 1984). Positive growth responses to added N were found in mangrove *Avicennia marina* (Boto et al., 1985; Naidoo, 1987). N was also considered a limiting factor for microbial activity in the mangrove swamp of the Indus Delta (Kristensen et al., 1992).

On the other hand, natural and artificial wetlands have been used to control and remove N in contaminated wastewaters discharges (Brodrick et al., 1988). Biological nitrification-denitrification reaction is important as an N removal mechanism in freshwater marshes (Reddy et al., 1989; Lindau and DeLaune, 1991), and in estuarine sediments (Jenkins and Kemp, 1984). It has been suggested that the denitrification in mangrove sediment, together with the assimilation of N by plants, might improve water quality in the eutrofication river (Nedwell, 1975).

However, information is limited about the effect and impact of nutrient enrichment on mangrove ecosystems. Previous research has shown that the mangrove forest of the Tamshui estuary in northwestern Taiwan, is polluted by municipal sewage (Chiu and Chou, 1991). The objectives of this study are to determine the status of N flux in this polluted mangrove swamp throughout the year and to evaluate the extent of the N loss through denitrification of mangrove soil.

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Materials and Methods

Field Site and Sampling

The Tamshui estuary is one of the most important preserves of mangrove in Taiwan. It includes three major areas (Figure 1). Chuwei, the largest site of all, can be divided into two areas, Tidal mangrove and Dwarf mangrove, depending on the elevation and the vegetation. Details of the ecological and edaphic information of Chuwei have been previously discussed (Chiu and Chou, 1991). Pali, located at about 1 km from rivermouth, was the outermost downstream site of all mangroves. A great number of bird nests were found in this area. Kuantu was the mangrove furthest from the shore, closest to the source of polluted sewage from metropolitan Taipei.

Surface soil (0 to 20 cm) samples were periodically collected from those sites from November 1992 to March 1994. The samples were kept in an ice box and brought back to the laboratory for chemical analysis.

Vertical change of redox potential (Eh) of soils were measured during ebb tide by a portable pH/mV meter (Jenco 6009) with Pt-electrode on May 25 and May 26, 1992. The calibration standard used for Eh was equimolar (M/300) solutions of potassium ferricyanide and potassium ferrocyanide in 0.1 M KCl potassim chloride. The system has an Eh of 0.430 mV at 25°C (Zobell, 1946). Five replicates were measured at each site.

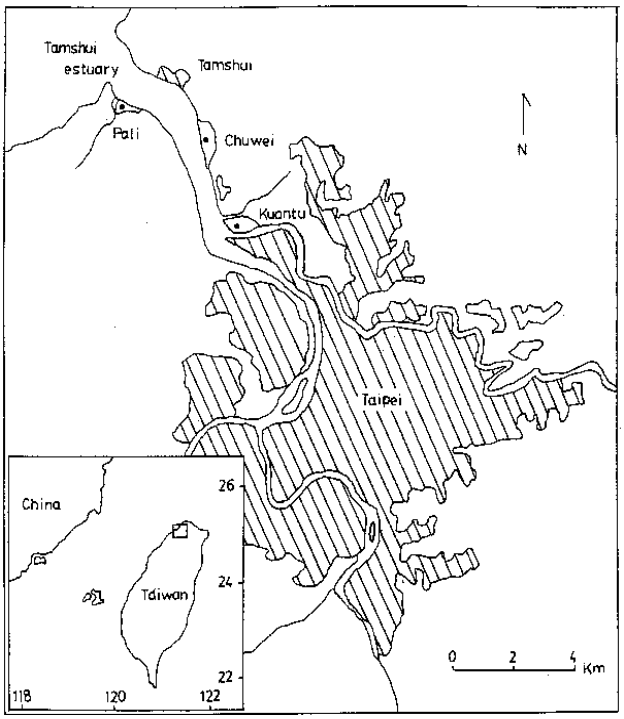

Figure 1. Location of mangroves in Tamshui estuary. Shaded areas represent residential districts.

Pot Experiment

Fresh surface soil collected from Chuwei Tidal mangrove was used for a pot experiment. Root and visible plant detritus were removed carefully from the soil. The soil (2.5% organic C, 0.21% total N, 48% clay, and 5.7 pH) was completely mixed by hand, and 2.5 kg of the fresh soil was put in plastic pots 12.5 cm in diameter. In May 1991, mature viviparous seedlings of *Kandelia candel*, with an average oven-dried weight of 3.8 g, were planted in 10 pots, 6 of which were kept unplanted. One single seedling was in each planted pot. All pots remained in greenhouse conditions. Water in each pot was maintained 1 cm above the soil surface throughout the experiment. The surface of each pot was covered with black plastic film to inhibit the growth of algae.

^{15}N labeled ammonium sulfate, enriched with 71.8 atom% ^{15}N (purchased from Shoko Co. Ltd., Tokyo) was applied, 6 mg N per pot, when the third pair of leaves had emerged from the seedling, seven weeks after the beginning of the experiment. Using a 5 ml hypodermic syringe with an 11 cm length of needle, a solution of ^{15}N labeled ammonium sulfate was injected into different areas of the soil to evenly distribute the applied N in the pot. The pots were harvested at one month and three months, respectively, after applying ^{15}N labeled fertilizer. Plants were dried at 70°C and ground with Udy Mill after washing with tap water.

N Analysis

Inorganic N was extracted from fresh soil with 2 M KCl. For the field soil samples, ammonium was determined by the indophenol blue colorimetric method, and nitrate was determined by the Griess-Ilosvay method, followed by passing the extract through a Cd-Cu reducing column (Keeney and Nelson, 1982).

Inorganic N in the soil extracts from the ^{15}N labeled pot experiment were determinated by using steam distillation with magnesium oxide-Devarda's alloy (Keeney and Nelson, 1982). Moist soil samples were digested by a modified Kjeldahl method with permanganate-reduced iron (Bremner and Shaw, 1958). Plant samples were Kjeldahl digested with salicylic-concentrated sulfuric acid. The digestion was processed in a heating aluminum block. N content was determined with steam distilution (Bremner and Mulvaney, 1982). Content of N in plant and soil samples was converted into an oven dried basis.

^{15}N Analysis

Distillates obtained after the titration of inorganic N and total N in the soil were collected by beakers containing 0.1 M HCl and concentrated on a hot plate under 90°C. Ammonium in the concentrated distillates was kept by Chromosorb G/AW (Merck) in tin capsules, while ground plant samples were directly put in tin capsules for the ^{15}N analysis. ^{15}N abundance in the soil and plant samples was determined with the Automatic Nitrogen and Carbon Analyzer-Mass Spectrometry (MS) system (Europa Scientific Limited, UK), comprising a sample preparation unit (autosampler to GC), a capillary interface, and an MS system. Terminology and calculation of recovery by ^{15}N technique basically followed those described in Rennie et al. (1978).

Results

Redox Potential (Eh) in the Soils

The highest value of Eh was found at Dwarf mangrove at Chuwei, while the lowest value was found in Pali (Figure 2). These results were attributed to the differences in geographic and edaphic conditions. The Dwarf mangrove

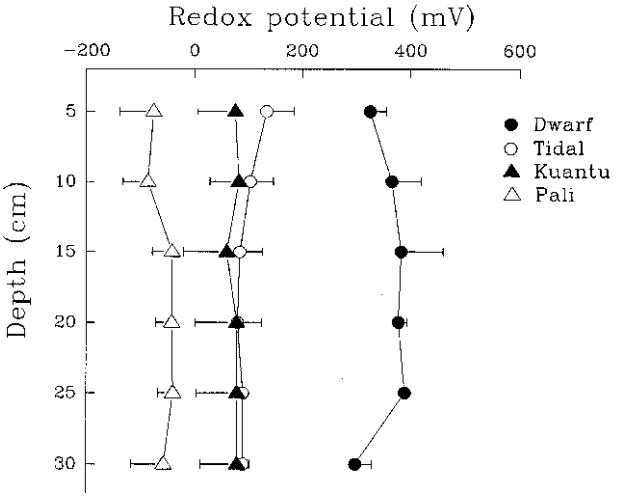

Figure 2. Vertical change of redox potential in mangrove soils. Horizontal bars indicate standard deviations from four replicates.

at Chuwei and the mangrove at Pali were the sites with the highest and lowest elevations, respectively. Frequency of tidal action brought not only the factor of waterlogging but the accumulation of organic matter, which might have reduced soil Eh during submergency. Since denitrification begins when the Eh falls to 421 mV after O_2 is depleted by aerobic respiration (Cho, 1982), all of the study sites seem to have the potential for denitrification. The Eh in each soil was not significantly related to the change of depth. It was due to the variation of soil texture and the heterogeneous distribution of rootlet.

Seasonal Change of Inorganic N in Mangrove Soil

Ammonium and nitrate content in the mangrove soils is shown in Figure 3. The results indicate the soil samples at Kuantu (1.68 to 17.10 mg kg^{-1} soil), and Pali (1.44 to 8.91 mg kg^{-1} soil) had a much higher amount of ammonium N than Chuwei Tidal (0.82 to 5.98 mg kg^{-1} soil) and Dwarf mangrove (0.15 to 1.08 mg kg^{-1} soil). Amounts of ammonium were the highest in summer and the lowest in winter at the Kuntu site.

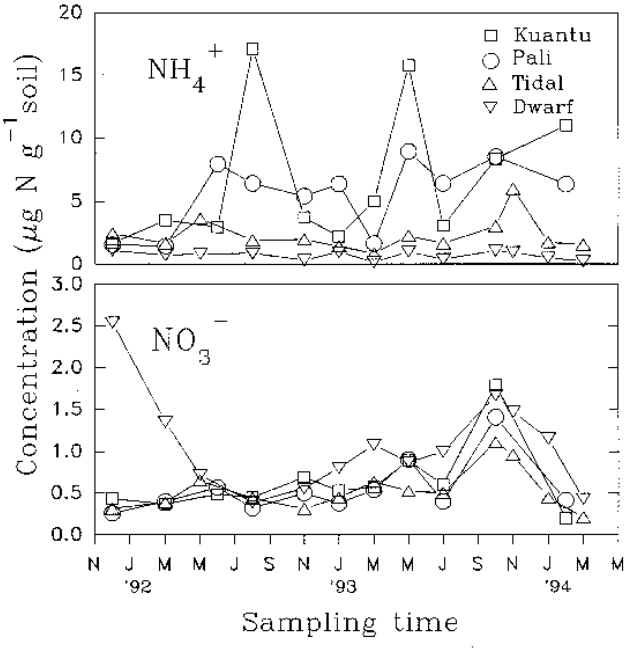

Figure 3. Seasonal change of ammonium and nitrate concentration in mangrove soils.

Amounts of nitrate were negligible, ranging from trace to 2.54 mg kg^{-1} soil, in most of the mangrove soils. Dwarf mangrove, with the highest elevation and consequently the highest Eh in the soil among all sites (Figure 2), seems to be the only exception. The higher amount of nitrate found in the cold season is possibly due to the reduction of denitrification activity and/or the lower plant uptake from the soil.

Changes of N in Soil of Pot Experiment

Most of the ^{15}N labeled N was not found in inorganic form one month after applying fertilizer to soil (Table 1), possibly due to immobilization, N loss, and/or rapid plant uptake. Recoveries of ^{15}N (Table 2), however, indicate that the major reason for the low recovery might be due to N loss during the early stage, as the recovery in the unplanted pots was only about 38% (Table 4) and 33% (Table 5) after one month and three months; respectively.

Much higher inorganic N concentrations were found in the soil of pot treatments (Table 1) than in those from the field (Figure 3). This may be due to the disturbance of the soil during preparation for planting. Collection and mixing of the soil may have induced release of inorganic N.

Translocation and Recovery of Applied N

Table 3 shows that the concentration of total N in the plant part and the uptake of fertilizer N was found in the leaves, indicating fast translocation during the development of the young plants.

Balance of the applied N and the total recovery in plants and soils for one and three months after applying ^{15}N labeled fertlizer are given in Table 4 and 5, respectively. Total recoveries of the planted treatment were almost the same during two stages, while the unplanted treatments declined from one to three months. The recoveries between planted and unplanted treatment were not significantly different ($p>0.05$).

Discussion

Results of the present study indicate much higher concentrations of dissolved inorganic N in mangrove soils than elsewhere, i.e., northern Australia (Boto and Wellington, 1984). Differences were also found in the pattern of seasonal change of inorganic N in the soil. Figure 1 showed

Table 1. Mineral nitrogen residue in mangrove soil at different stage after applying ^{15}N labeled ammonium sulfate.

Stage[a]	Treatment	Concentration[b] (mg N kg^{-1} dried soil)	^{15}N abundance (atom % ex)	Recovery (%)
1 month	Planted	32.3 ± 4.4[c]	0.824 ± 0.183	8.7 ± 2.6
	Control	41.3 ± 3.8	1.172 ± 0.098	15.4 ± 1.3
3 months	Planted	32.8 ± 9.1	0.464 ± 0.131	5.0 ± 1.9
	Control	56.0 ± 2.0	0.744 ± 0.009	13.3 ± 0.6

[a] ^{15}N labeled fertilizer was added 7 weeks after planting.
[b] Concentration was converted to an oven dried basis.
[c] Mean ± standard deviation. Six and four replicates were in planted and control treatment, respectively.

Table 2. Nitrogen residue in mangrove soil at different stage after applying ^{15}N labeled ammonium sulfate.

Sampling stage	Treatment	Concentration of total N (mg N g^{-1} dried soil)	^{15}N abundance (atom % ex)	Recovery (%)
1 month	Planted	2.10 ± 0.08	0.041 ± 0.004	27.3 ± 2.4
	Control	2.11 ± 0.12	0.056 ± 0.003	38.0 ± 3.5
3 months	Planted	2.01 ± 0.10	0.032 ± 0.003	20.7 ± 2.4
	Control	2.16 ± 0.01	0.047 ± 0.003	32.6 ± 1.8

See footnote in Table 1.

Table 3. Nirogen uptake by *K. candel* at different stage after applying ^{15}N labeled ammonium sulfate in mangrove soil under greenhouse conditions.

Stage	Plant part	Dried weight (g pot^{-1})	Concentration (mg N g^{-1})	^{15}N abundance (atom % ex)	Recovery (%)
1 month	Leaf	1.19 ± 0.15	21.73 ± 0.61	1.410 ± 0.149	8.5 ± 1.5
	Stem	2.59 ± 0.35	5.50 ± 0.86	0.468 ± 0.092	1.5 ± 0.3
	Root	1.41 ± 0.24	7.52 ± 0.66	1.257 ± 0.251	3.1 ± 0.6
3 months	Leaf	1.36 ± 0.27	23.80 ± 1.12	1.364 ± 0.263	10.4 ± 3.4
	Branch	0.57 ± 0.15	12.55 ± 2.20	1.220 ± 0.264	2.0 ± 0.7
	Stem	3.30 ± 0.35	6.27 ± 0.82	0.577 ± 0.165	2.7 ± 0.6
	Root	1.74 ± 0.29	11.21 ± 1.10	1.003 ± 0.179	4.5 ± 0.8

See footnote in Table 1.

Table 4. Nitrogen balance sheet in *K. candel* and mangrove soil 1 month after applying ^{15}N labeled ammonium sulfate under greenhouse conditions.

Fraction		Recovery (%)	
		Planted	Control
Plant	Leaf	8.5 ± 1.5	
	Stem	1.5 ± 0.3	
	Root	3.1 ± 0.6	
	Sum	13.1 ± 2.1	
Soil		27.3 ± 2.4	38.0 ± 3.5
Total		40.4 ± 3.0	38.0 ± 3.5

See footnote in Table 1.

Table 5. Nitrogen balance sheet in *K. candel* and mangrove soil 3 months after applying ^{15}N labeled ammonium sulfate under greenhouse conditions.

Fraction		Recovery (%)	
		Planted	Control
Plant	Leaf	10.4 ± 3.4	
	Branch	2.0 ± 0.7	
	Stem	2.7 ± 0.6	
	Root	4.5 ± 0.8	
	Sum	19.6 ± 5.0	
Soil		20.7 ± 2.4	32.6 ± 1.8
Total		40.3 ± 6.4	32.6 ± 1.8

See footnote in Table 1.

that the amount of ammonium reached its peak during summer, contradicting results of Boto and Wellington (1984), in which ammonium was lower during periods of rapid plant growth. The difference between studies might be due to the input of pollutants, including inorganic N and N-rich suspended particles, carried from river, which induced the accumulation of inorganic N in present study sites.

Volatilization of ammonium was negligible in this experiment as the soil pH was 5.7. Denitrification seems to be the key pathway to N removal as it is in most wetlands (Howard-Williams and Downes, 1993).

Chiu and Chou (1993) suggest that *Kandelia candel* translocates O_2 to its roots to satisfy metabolic requirements and overcome the harassment of toxic substance produced under anaerobic conditions. A fraction of the O_2 diffuses from the roots into the adjacent reduced soil creating a thin, aerobic soil layer around the plant roots. The thin, oxidized boundaries promote nitrification. The presence of these two distinct soil zones gives access to the simultaneous occurrence of nitrification-denitrification in a flooded sediment (Jenkins and Kemp, 1984; Reddy et al., 1989).

Nevertheless, this experiment was unable to show the increase of soil N loss through denitrification caused by plant, as the recoveries in the planted treatment were not significantly different from those in the unplanted treatment (Tables 4 and 5). This was contradicted by our recent results revealing that denitrification potential was higher in rhizoshpere than in the non-rhizoshpere soil collected from mangrove (Chiu et al. paper in preparation). The discrepancy is due to the nitrate availability. The mobility of ammonium ion and the nitrification occurring in the boundry of the aerobic-anaerobic layer might be the prime limiting factor for N loss.

Part of the ^{15}N labeled ammonium sulfate solution might have been driven out, verified as visible slurry, to the surface soil and/or water through the channel created by the needle during the process of injection. Ammonium could have been oxidized to nitrate in this aerobic layer and con-

sequently caused N loss through denitrification when nitrate reached the anaerobic layer. The process was significant in the first month but ceased later on as little ^{15}N labeled ammonium remained in the surface layer (Table 4 and Table 5). On the other hand, ^{15}N labeled ammonium existed in the anaerobic layer, where O_2 did not penetrate, so it could hardly have advanced nitrification and the subsequent denitrification.

The mobility of ammonium in mangrove soil is affected by various factors. A high level of sodium in mangrove soil could displace ammonium in the cation exchange sites, resulting in the enhancement of ammonium mobility. However, the permeability of mangrove soils is generally quite low, particularly in soils with high clay content (Clough et al., 1983). Moreover, most of the ^{15}N labeled N remaining in the soil was immobilized (Table 1 and Table 2). Ammonium formed by subsequent mineralization, with or without further reaction of nitrification, could be utilized by plants. It eventually reduced the mineral N for denitrification. Applied fertilizer N underwent actively the simultaneous process of immobilization, nitrification, and denitrification in the mangrove soil. This was especially clear in the planted treatment, revealing that the increase of plant N uptake between the first and third month's samplings was equal to the difference of soil N at the same stage (Table 4 and Table 5).

Acknowledgements. The study was supported by the National Science Council, Taiwan (NSC- 83-0211-B-001-060 and NSC-84-2311-B-001-052). The authors appreciate the valuable review and criticism given by Prof. Willim Koskinen, Department of Soil, Water and Climate, University of Minnesota, USA.

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