

Impact of coastal wetland cultivation on microbial biomass, ammonia-oxidizing bacteria, gross N transformation and N₂O and NO potential production

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Abstract A ¹⁵N dilution experiment was carried out to investigate effects of cultivation on the gross N transformation rate in coastal wetland zone. Microbial community composition was estimated by phospholipid fatty acid (PLFA) analysis and abundance of soil ammonia-oxidizing bacteria (AOB) was quantified by real-time polymerase chain reaction (PCR). Soil salinity decreased significantly, while total N increased after coastal wetland was cultivated. Microbial biomass (total PLFA), bacterial biomass, fungal biomass, and actinomycete biomass of the native coastal wetland soils were significantly ($p < 0.05$) lower than those of the cultivated soils whereas AOB population size also significantly increased after coastal wetland cultivation. Multiple regression analysis showed that total PLFA biomass and soil total N (TN) explained 97% of the variation of gross N mineralization rate in the studied soils (gross mineralization rate = $0.179 \text{ total PLFA biomass} + 5.828 \text{ TN} - 2.505$, $n = 16$, $p < 0.01$). Gross nitrification rate increased by increasing the soil AOB population size and gross mineralization rate (M) (gross nitrification rate = $3.39 \text{ AOB} + 0.18 \text{ M} - 0.075$, $R^2 = 0.98$, $n = 16$, $p < 0.01$). Management of salt discharge and mineral N fertilization during the cultivation of wetland soils might have changed composition of soil microflora and AOB population size, thus influencing mineralization and nitrification. Probably,

the cultivation of coastal wetland soils increased the risk of N losses from soil through nitrate leaching and gas emission (e.g., N₂O and NO).

Keywords Coastal wetland · Gross mineralization rate · Gross nitrification rate · Phospholipid fatty acid · Soil ammonia-oxidizing bacteria (AOB) · N loss

Introduction

Coastal wetland, which is an important transitional ecosystem between terrestrial and ocean ecosystem, plays a crucial role in balancing and sequestering C, N and P in the ecosystem and protecting biodiversity. However, human disturbance (mainly drainage and cultivation) along the coast has destroyed >80% of the region's coastal wetland area in Jiangsu province in China and thus increased concentration of reactive N in soil due to fertilization and changes in the soil N cycling. While net mineralization and net nitrification rates can provide an index of plant available N, they do not provide an understanding of the respective processes (Hart et al. 1994). Gross N transformation rates can provide important information on the mechanisms and dynamics of the internal N cycle, since they quantify the process rates. The ¹⁵N dilution technique is the most widely used method to quantify gross N transformation rates in soil (Stark 2000) and it is based on the enrichment of the ammonium and nitrate pool with the stable isotope ¹⁵N. However, few studies have been carried out to study the effects of cultivation on the gross N transformation rate in the coastal wetland zone.

Because soil N transformations are directly driven by microbial activity (Martens 1995), the production and consumption of inorganic N is a function of microbial

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biomass, microbial activity and exoenzyme production (Schimel and Bennett 2004). Determination of community level PLFA profiles can be useful in detecting changes of the composition of soil microbial communities to a variety of land uses or disturbances (Hedrick et al. 2000; Harris 2003), and some PLFA marker can be used as indicators of some soil microbial functional groups (Zak et al. 1994; Zelles 1997; Zogg et al. 1997). Ammonia oxidation is thought to be the rate-limiting reaction of nitrification in most ecosystems (Kowalchuk and Stephen 2001). In arable soils, most of the ammonia oxidization is carried out by autotrophic ammonia-oxidizing bacteria (AOB) (Barraclough and Puri 1995) and thus it is important to monitor the size and activity of AOB population under different soil N conditions. Real-time polymerase chain reaction (PCR), targeting 16S rRNA gene (Hermansson and Lindgren 2001) and amplification of ammonia monooxygenase gene *amoA* (Okano et al. 2004) have been successfully used to quantify AOB population size in soil. Changes in land use and intensive cropping can alter N cycling in soil, resulting in increased N losses (NO, N₂O emission, NO₃⁻ leaching) (Meng et al. 2005; Ding et al. 2010). It is hypothesized that land use/management changes of the coast wetland can change composition, activity and size of soil microbial communities, including AOB community, with effects on soil N transformations.

The objectives of this study were i) to compare the gross mineralization, nitrification and immobilization rates and the emissions of N₂O and NO between cultivated coastal wetland soils and native coastal wetland soils and ii) to investigate the effects of coastal wetland cultivation on soil gross N transformation and N₂O and NO production. Microbial community composition was estimated by phospholipid fatty acid (PLFA) analysis and the population size of AOB was quantified by real-time PCR.

Materials and methods

Site description

The study sites are located in Jiangsu province, China (33°24′–34°06′ N, 119°59′–120°33′ E), which covers an area of 2,795 km² with a typical monsoon climate. The mean annual precipitation is 1,032 mm (30-year average), about 65% of which occurs from June to September. The mean annual temperature is 13.8°C (30-year average).

Sites and experimental plan

We chosen two cultivated soils: one was cropped continuously with *Brassica rapa chinensis* for about 9 years (B) and was annually fertilized with about

150 kg Nha⁻¹ a⁻¹; the other was cropped continuously with *Hordeum uhulgare* L for 14 years (H) and was annually fertilized with about 150 kg Nha⁻¹ a⁻¹. Two native coastal wetland soils: one with *Spartina anglica* (S) as the dominating vegetation, whereas the other had a dominating vegetation *Phragmites australis* (P). The cultivated and wetland soils were adjacent (within 1,000 m) and had similar altitude. Before wetland was cultivated, the dominating vegetations were *S. anglica* (S) and *P. australis* (P). Soil samples were collected in April 2010. In each site, a representative 500 m×500 m plot was selected. Then, four grids (each about 10 m×10 m, the distance among grids was not shorter than 200 m, so four soil samples were independently taken from each site) were randomly selected. From each grid, ten soil cores (dia. 5 cm) were taken (0–10 cm), pooled together, sieved (<3 mm), and stored at 4°C for less than 1 month before the experiment.

There were two treatments (each with three replicates): in one, the ammonium pool was labeled with (¹⁵NH₄)₂SO₄ (20 at.% excess); in the other, the nitrate pool was labeled with K¹⁵NO₃ (20 at.% excess). Fresh soil (30 g oven-dry basis) was put in a 250-ml Erlenmeyer flask. Two milliliters of (¹⁵NH₄)₂SO₄ or K¹⁵NO₃ solution was added to each flask at a rate of 50 μg NH₄⁺-N g⁻¹ soil or 50 μg NO₃⁻-N g⁻¹ soil. The soil was adjusted to 50% water filled pore space and incubated for 24 h at 25°C, which corresponds to the temperature at soil sampling. The flasks were sealed with silicone rubber stoppers. Soils were extracted at 0.5 and 24 h after fertilizer application for the determination of concentration and isotopic composition of NH₄⁺ and NO₃⁻. Rates of N mineralization, nitrification and immobilization were calculated using the equations by Kirkham and Bartholomew (1954).

N₂O and NO emission rates

Six incubation bottles with fresh soil (30 g oven-dry basis) were used to measure the N₂O and NO emission rates at 6, 12, 24, 48, 96 and 144 h after the onset of incubation. Two milliliters of NH₄NO₃ solution was added to each flask at a rate of 50 μg NH₄⁺-N g⁻¹ soil and 50 μg NO₃⁻-N g⁻¹ soil. The bottles were allowed to aerate for approximately 30 min prior to being capped with a butyl rubber stopper. Three bottles treated with C₂H₂ (10 Pa) to inhibit autotrophic nitrification and the others (no C₂H₂) were used to measure the total N₂O and NO emission. The incubation bottles were sealed and incubated for 4 h and the headspace gas was sampled using a syringe. Each gas sample was spitted with two subsamples, one for NO measurement and the other for N₂O measurement. Prior to NO determination, the 20-ml gas samples were diluted to 1,000 ml using highly purified N₂ (>99.9%). The sample

NO concentration was measured using an EC 9841 T NO_x analyzer (ECOTECH, chemiluminescence detector). The N₂O concentration was determined by a gas chromatograph (Agilent 7890) equipped with an electron capture detector set at 300°C. Gas was passed through a 3-m long analytical column (i.d. 2 mm) packed with Porapak Q (80/100 mesh). The column temperature was maintained at 40°C and the carrier gas was argon–methane (5%) at a flow rate of 30 ml/min.

Soil analyses

Soil properties were determined as described by Zhang et al. (2009). Soil pH was measured in a 1:2.5 (v/v) soil to water ratio using a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China). Soil organic C (SOC) was analyzed by wet digestion with H₂SO₄–K₂Cr₂O₇ and total N was determined by semimicro Kjeldahl digestion using Se, CuSO₄ and K₂SO₄ as catalysts. Ammonium and NO₃[–] were extracted with 2 M KCl at a soil/solution ratio of 1:5 on a mechanical shaker for 60 min at 300 rpm at 25°C. The extract was filtered through a filter paper and the concentrations of NH₄⁺ and NO₃[–] were determined with a continuous flow analyzer (Skalar, Breda, Netherlands). Salinity was measured by simple electrical conductivity (EC) using a soil extract (1:5, soil: water). Isotopic composition of the NH₄⁺, NO₃[–], and soil organic N was measured by mass spectrometry (Integra C N mass spectrometer). Ammonium and nitrate were separated for ¹⁵N measurements by distillation with magnesium oxide and Devarda's alloy.

PLFA analyses

Microbial biomass was estimated as total extractable PLFA; PLFA profiles were analyzed to determine microbial community composition. Briefly, lipids were extracted from 0.5 g soil as already reported (Bligh and Dyer 1959; Yao et al. 2006). Fatty acids extracted from soil were automatically quantified and identified by the MIDI Sherlock software (MIDI, Newark, DE, USA). The abundance of individual fatty acid methyl esters was expressed as mole percentage. Nomenclature of fatty acids followed that used by Frostegård

et al. (1993). The fatty acids i12:0, i13:0, i14:0, i15:0, a15:0, i16:0, i17:0, 17:0cy, 18:1m7c and 19:0cy were used as indicators of bacterial PLFAs. The unsaturated PLFAs 18:1ω9, 18:2 ω6,9 and 18:3ω6 were used as indicators of fungal biomass. The monoenoic and cyclopropane unsaturated PLFAs 10Me16:0, 10Me17:0, 10Me18:0, i12:0, i13:0, i14:0, i15:0, a15:0, i16:0, a16:0, i17:0 and a17:0 were chosen to represent gram-positive bacteria (G⁺). Finally, the branched, saturated PLFAs 3OH 11:0, 3OH i11:0, 12:0, 3OH 12:0, 3OH 13:0, 14:0, 3OH 15:0, 3OH i15:0, 16:0, 2OH 16:1, 3OH i16:0, 16:1 ω5, 16:1 ω7, 17:0, 3OH i17:0, 18:1 ω5, 18:1 ω6, 18:1 ω7, cy17:0, cy19:0 and 11Me18:1ω7 were chosen to represent gram-negative bacteria (G[–]).

Real-time PCR

Soil total DNA extraction and purification has been described before (Chu et al. 2008). The primer pair *amoA*-1 F (forward) and *amoA*-2R (reverse) were used for PCR amplification targeting the gene *amoA* (Rotthauwe et al. 1997). The reaction mixture contained 25 μl of Platinum[®] SYBR[®] Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA, USA), 0.2 μM of each primer, 1 μl of ROX Reference Dye and 1 μl of 10-time diluted DNA template in a final volume of 50 μl. Real-time PCR was conducted using an ABI7000 (Applied Biosystem, Foster City, CA, USA) and the following thermal profile for amplification: 2 min at 50°C, 2 min at 95°C, 40 cycles of 15 s at 95°C and 30 s at 60°C. A standard curve based on known *amoA* copy numbers (10–10⁶ copies μl⁻¹) was generated using a purified PCR product obtained from the reference strain *Nitrosospora multiformis* ATCC25196. AOB population size was estimated by dividing the *amoA* copy number by 2.5, based on the fact that each AOB cell carries two or three nearly identical copies of the *amoA* gene (Norton et al. 2002).

Statistical analyses

ANOVA analysis was used to test the difference of soil properties and gross N reaction rate between native and cultivated coastal wetland soils. In order to determine

Table 1 Physical and chemical properties of studied soils

Dominating vegetation	SS dS m ⁻¹	pH	SOC g kg ⁻¹	TN g kg ⁻¹	C/N
S	1.59±0.35 ^c	8.28±0.06 ^a	10.82±0.71 ^a	0.64±0.07 ^a	16.96±2.71 ^b
P	1.31±0.36 ^c	8.24±0.16 ^a	11.09±1.65 ^a	0.66±0.08 ^a	16.77±1.64 ^b
B	0.43±0.12 ^b	8.21±0.08 ^a	12.33±0.49 ^a	1.16±0.11 ^b	10.63±0.77 ^a
H	0.28±0.07 ^a	7.94±0.18 ^a	13.64±1.06 ^a	1.42±0.17 ^c	9.96±0.61 ^a

S is soil under *Spartina anglica*, P is soil under *Phragmites australis*, B is soil under *Brassica rapa chinensis*, H is soil under *Hordeum uhulgare* L. SS is soil salinity, SOC is soil organic C, TN is total N. The same letter shows no statistical difference

Table 2 Total microbial biomass and biomasses of bacteria, fungi and actinomycetes estimated by the total extractable phospholipid fatty acids (PLFA)

Dominating vegetation	Total PLFAs nmol g ⁻¹	Bacteria nmol g ⁻¹	Fungi nmol g ⁻¹	Actinomycete nmol g ⁻¹
S	1.49±0.33 ^a	1.28±0.28 ^a	0.24±0.59 ^a	0.16±0.01 ^a
P	1.83±0.58 ^a	1.51±0.51 ^a	0.17±0.10 ^a	0.18±0.12 ^a
B	21.96±6.81 ^b	17.95±6.29 ^b	1.85±0.25 ^b	2.16±0.41 ^b
H	26.68±9.29 ^b	22.03±5.84 ^b	2.81±0.61 ^b	2.31±0.43 ^b

S is soil under *Spartina anglica*, P is soil under *Phragmites australis*, B is soil under *Brassica rapa chinensis*, H is soil under *Hordeum uhulgare* L. The same letter shows no statistical difference

the factors affecting soil mineral N production, we applied a multiple regression analysis using SPSS soft. We hypothesized that the various variables measured were correlated among themselves (multicollinearity). In order to reduce multicollinearity, a cross-correlation analysis was first applied. The colinearity issues were excluded, before multiple regression analysis was carried out. Analyses were conducted using SPSS 10.0 statistical package and differences were considered significant if $p < 0.05$.

Results

Soil properties

The soil salinity and the ratio of soil organic C to total N decreased significantly ($p < 0.05$), while the content of total N increased after coastal wetland was cultivated (Table 1). However, the soil pH and soil organic C contents did not change significantly after coastal wetland was cultivated.

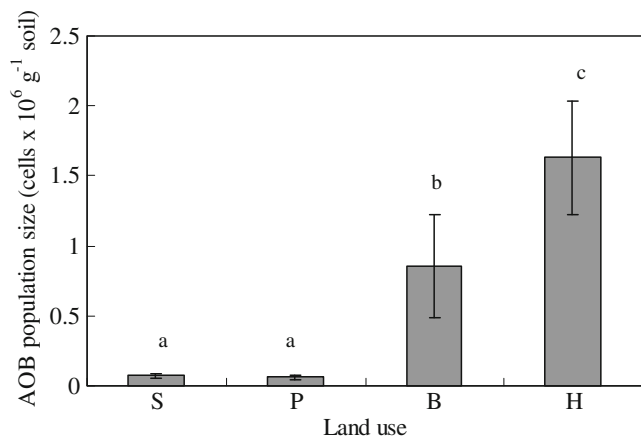


Fig. 1 AOB population size in different soils. S is soil under *Spartina anglica*, P is soil under *Phragmites australis*, B is soil under *Brassica rapa chinensis*, H is soil under *Hordeum uhulgare* L. The same letter shows no statistical difference

Soil microbial community and soil ammonia-oxidizing bacteria (AOB)

Total PLFA biomass (the sum of the 35 fatty acids that were identified in the samples), bacterial biomass, fungal biomass, and actinomycete biomass of the native coastal wetland soils were significantly lower than those of the cultivation soils ($p < 0.05$) (Table 2). However, the percentages of bacterial, fungal, and actinomycetes biomasses to total PLFA biomasses did not change significantly after cultivation of coastal wetland. The total PLFA biomass exponentially increased by decreasing soil salinity ($y = 44.1e^{-2.2x}$, $R^2 = 0.91$, $n = 16$, $p < 0.01$). The total PLFA biomass was positively correlated with soil total N content ($p < 0.01$).

AOB population sizes in the cultivated soils (B, and H) were 14, and 27 times, respectively, higher than those of the native coastal wetland soils (Fig. 1). The AOB population size also exponentially increased by decreasing soil salinity ($y = 2.03e^{-2.2x}$, $R^2 = 0.82$, $n = 16$, $p < 0.01$).

Gross N reactions

The gross N mineralization rates were 1.71 ± 0.36 , 1.48 ± 0.37 , 8.25 ± 0.14 , and 10.56 ± 1.82 mg kg⁻¹ d⁻¹ for soil S, P, B, and H, respectively (Table 3). The cultivation of coastal wetland significantly ($p < 0.05$) increased the gross N mineralization rate. The multiple regression analysis showed that total PLFA biomass and soil total N (TN) explained 97% of the variation of gross N mineralization rate in the studied soils (gross N mineralization rate = 0.179 total PLFA biomass + 5.828 TN - 2.505 , $n = 16$, $p < 0.01$). The standardized coefficients indicated that the total PLFA biomass (0.508) played a more important role in controlling N mineralization than TN (0.493). The gross ammonium immobilization rate also increased significantly after cultivation of native coast wetland soil ($p < 0.01$) and was positively correlated with the gross N mineralization ($p < 0.01$).

Similar to N mineralization, the cultivation of coastal wetland also significantly ($p < 0.05$) increased the gross nitrification rates, which were 0.51 ± 0.11 , 0.51 ± 0.17 ,

Table 3 Mean (±SD) gross N transformation rate (μg Ng⁻¹ d⁻¹)

Dominating vegetation	M	I _{NH4}	N	I _{NO3}
S	1.71±0.36 ^a	0.89±0.42 ^a	0.51±0.11 ^a	0.06±0.22 ^a
P	1.48±0.37 ^a	1.07±0.32 ^a	0.51±0.17 ^a	-0.08±0.13 ^a
B	8.25±1.14 ^b	6.67±1.34 ^b	4.13±0.67 ^b	-0.17±0.27 ^a
H	10.56±1.82 ^c	8.11±1.62 ^b	7.37±1.86 ^c	-0.15±0.31 ^a

S is soil under *Spartina anglica*, P is soil under *Phragmites australis*, B is soil under *Brassica rapa chinensis*, H is soil under *Hordeum uhulgare* L., M gross mineralization of organic-N, I_{NH4}, immobilization of NH₄⁺, N gross nitrification, I_{NO3} immobilization of NO₃⁻. The same letter shows no statistical difference

4.13±0.67, and 7.37±1.86 mg kg⁻¹ d⁻¹ for S, P, B, and H, respectively. The multiple regression analysis showed that the gross nitrification rate increased by increasing the soil AOB population size and gross mineralization rate (M) (gross nitrification rate=3.39AOB+0.18M-0.075, R²=0.98, n=16, p<0.01). The standardized coefficients indicated that the soil AOB population size (0.763) played a more important role in affecting nitrification than gross N mineralization rate (0.247). However, gross immobilization of nitrate rate did not change after coastal wetland was cultivated.

N₂O and NO emission rate

The comparison of results between control and the inhibition with 10 Pa C₂H₂ indicated that the emission of N₂O and NO in the present investigation was mainly produced via autotrophic nitrification (Table 4). The cultivation of coast wetland significantly (p<0.05) increased the emission of N₂O and NO from soil. The N₂O emission rate logarithmically increased by increasing the gross nitrification rate (p<0.01); similarly, NO emission rate linearly increased by increasing the gross nitrification rate (p<0.01).

Discussion

The gross N mineralization rate significantly increased after coast wetland was cultivated, probably due to the increase in soil microbial biomass (i.e., total PLFA biomass) and soil

total N. Nitrogen mineralization is mainly due to soil microbes (Martens 1995) and this may explain the positive and significant correlation between soil microbial biomass and N mineralization. The significant and negative relationship between soil microbial biomass (i.e., total PLFA biomass) and soil salinity suggests that salt management of soil is important in affecting N cycling in the studied soils. The reduction of soil salinity can provide a suitable environment for soil microbes (Setia et al. 2010). The soil organic N pool being the direct source of mineralized N can be the predictive of N mineralization (Booth et al. 2005). The increase in mineral N fertilization in arable soils can increase plant growth and thus input of crop residues and roots to soil with the increased soil organic N content.

In arable soils, most of the ammonia oxidization may be carried out by autotrophic ammonia-oxidizing bacteria (AOB) (Barraclough and Puri 1995). The present investigation demonstrated that AOB population size significantly increased after coastal wetland was cultivated (Fig. 1), probably because cultivation reduced the soil salinity, providing also a suitable environment for ammonia-oxidizing bacteria. The other important factor affecting soil AOB population size was mineral N fertilization in arable soil. The increased AOB population size by N fertilization has been observed in both laboratory (Okano et al. 2004) and field experiments (Mendum et al. 1999; Chu et al. 2008). In addition, the increase in N mineralization provided more substrate for ammonia-oxidizing bacteria. Since ammonia oxidation is the rate-limiting step of nitrifica-

Table 4 N₂O and NO emission rate

Dominating vegetation	N ₂ O		NO	
	μg kg ⁻¹ d ⁻¹		μg kg ⁻¹ d ⁻¹	
	-C ₂ H ₂	+C ₂ H ₂ (10 Pa)	-C ₂ H ₂	+C ₂ H ₂ (10 Pa)
S	12.44±2.41 ^a	1.44±1.21 ^a	27.25±1.32 ^a	0.41±0.17 ^a
P	14.12±4.40 ^a	0.91±0.85 ^a	30.94±0.69 ^a	0.22±0.21 ^a
B	38.89±6.81 ^b	2.45±2.53 ^a	51.04±5.57 ^b	0.45±0.52 ^a
H	45.21±1.38 ^b	1.51±0.39 ^a	71.37±10.98 ^b	0.53±0.38 ^a

S is soil under *Spartina anglica*, P is soil under *Phragmites australis*, B is soil under *Brassica rapa chinensis*, H is soil under *Hordeum uhulgare* L. The same letter shows no statistical difference

tion and most of its activity is carried out by AOB in arable soils (Killham 1986; Barraclough and Puri 1995), it can be assumed that the nitrification was regulated by AOB in the aerobic soils. Gross nitrification rate increased by increasing the soil AOB population size and gross N mineralization rate (M) (gross nitrification rate = $3.39\text{AOB} + 0.18M - 0.075$, $R^2 = 0.98$, $n = 16$, $p < 0.01$). The gross nitrification rate, however, did not affect nitrate immobilization, which was very low. Therefore, the cultivation of coastal wetland can increase the risk of N losses through nitrate leaching.

NO and N₂O emissions, important N losses, are produced in soils by nitrification and denitrification. Probably in our studies both N₂O and NO were mainly produced by nitrification, since the N₂O and NO emission rate increased by increasing the gross nitrification rate. In addition, our results indicated that fertilization increased NO and N₂O emission compared to unfertilized soils (Smith et al. 1998; Smart et al. 1999; Meng et al. 2005; Ding et al. 2010).

Conclusion

The cultivation of coastal wetland significantly increased gross N mineralization, nitrification, and N₂O and NO production rates. Management of salt discharge and mineral N fertilization during the cultivation of wetland soils might have affected soil microbial biomass and AOB population size with effects on N mineralization and nitrification after cultivation of coastal wetland soils. Therefore, the cultivation of coastal wetland soils can increase the risk of N losses from soil through nitrate leaching and gas emission (e.g., N₂O and NO). Future studies are needed to measure the field water dynamics, denitrification rate, nitrification rate and N immobilization in situ and to better understand the underlying mechanisms of the effects of cultivation of coastal wetland on N cycle.

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