



Organic nitrogen components in soils from southeast China^{*}

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Abstract: Objective: To investigate the amounts of extractable organic nitrogen (EON), and the relationships between EON and total extractable nitrogen (TEN), especially the amino acids (AAs) adsorbed by soils, and a series of other hydrolyzed soil nitrogen indices in typical land use soil types from southeast China. Under traditional agricultural planting conditions, the functions of EON, especially AAs in the rhizosphere and in bulk soil zones were also investigated. Methods: Pot experiments were conducted using plants of pakchoi (*Brassica chinensis* L.) and rice (*Oryza sativa* L.). In the rhizosphere and bulk soil zone studies, organic nitrogen components were extracted with either distilled water, 0.5 mol/L K₂SO₄ or acid hydrolysis. Results: K₂SO₄-EON constituted more than 30% of TEN pools. K₂SO₄-extractable AAs accounted for 25% of EON pools and nearly 10% of TEN pools in rhizosphere soils. Overall, both K₂SO₄-EON and extractable AAs contents had positive correlations with TEN pools. Conclusions: EON represented a major component of TEN pools in garden and paddy soils under traditional planting conditions. Although only a small proportion of the EON was present in the form of water-extractable and K₂SO₄-extractable AAs, the release of AAs from soil exchangeable sites might be an important source of organic nitrogen (N) for plant growth. Our findings suggest that the content of most organic forms of N was significantly greater in rhizosphere than in bulk soil zone samples. However, it was also apparent that the TEN pool content was lower in rhizosphere than in bulk soil samples without added N.

Key words: Extractable organic nitrogen, Amino acids, Rhizosphere, Bulk soil

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1 Introduction

Nitrogen (N) is the factor most limiting the growth of almost all crops in temperate terrestrial ecosystems. The dynamics of inorganic nitrogen (NH₄⁺, NO₃⁻) has been intensively studied in soils, and increasingly research has been focused on the dynamics of soil organic nitrogen in agricultural soils.

Extractable organic N (EON) is the organic N present in different soil extracts (e.g., extracted with water or K₂SO₄) or that can be hydrolyzed (e.g., by acid hydrolysis) or solubilized (Ros *et al.*, 2009). EON contents of soils can be less than 5% of the total N that can be extracted by mild salt solutions, or may be more than 50% of the total extracted by acid hydrolysis methods (Stevenson, 1994; Matsumoto and Ae, 2004; Ros *et al.*, 2009). Plant roots can absorb not only inorganic N but also EON in the form of amino acids (AAs) from soil and solutions (Wu *et al.*, 2005). Hydrolyzed soil organic matter contains high organic N, of which 30%–50% is present in the form of AAs.

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The rhizosphere is the soil zone in which microbial activity is influenced by plant roots, distinguishing it from 'bulk' soil (Russell, 1982; Herman *et al.*, 2006). Active interaction occurs among plant roots, soil, and microbes in rhizosphere soils (Herman *et al.*, 2006). The interaction results in increase in soil nitrogen mineralization, which correspondingly increases net plant nitrogen assimilation (Bregliani *et al.*, 2010).

Several studies have attempted to measure the size and characteristics of EON pools in soils (Ros *et al.*, 2009). In contrast, very few have attempted to determine the distribution of EON either in the rhizosphere or in bulk soil of traditional agricultural soils. While it seems reasonable to assume that organic N pools would be larger in the rhizosphere than in bulk soil (DeAngelis *et al.*, 2008; Näsholm *et al.*, 2009), there are also compelling reasons why this may not be the case under fertilizer application or may depend on the plant species grown (monocotyledonous or dicotyledonous). Vegetables are essential to our life, and the area of soils used for vegetable production is increasing each year in China. Meanwhile, rice is a very important and widely spread terrestrial plant (Ding *et al.*, 2008). China is the largest rice producing and consuming country. Moreover, Chinese rice planting area accounts for 20% of the world's rice area. Because of the long-term and hydro-agricultural management under seasonal submergence for rice growth, paddy soil in China has been recognized as an anthropogenic soil type (Zheng and Zhang, 2011). Garden and paddy soils are the typical land use types in China and they differ in their soil chemical properties, crop types, textures, and agricultural management practices. Those differences have caused the two traditional soils to have different forms of N pools.

In this paper, monocotyledonous rice (*Oryza sativa* L.) and dicotyledonous pakchoi (*Brassica chinensis* L.) were planted in typical land use soil types (paddy soil and garden soil) from southeast

China with or without added N (urea) in a conventional fertilizer application. Laboratory analyses were used to determine the sizes of EON pools and the relationships between EON and total extractable N (TEN), especially the AAs adsorbed by soils, and a series of other hydrolyzed soil nitrogen indices. The functions of EON, especially adsorbed AAs in the rhizosphere and in bulk soils under traditional agricultural planting conditions, were also investigated.

2 Materials and methods

2.1 Soils

Soils were collected from the experimental farm of Zhejiang University at the Huajiachi Campus located in northern Zhejiang Province, southeast China (30°16' N, 120°12' E). The site has a humid subtropical climate with a mean annual precipitation of 1453 mm and an average annual temperature of 16.2 °C. Soil A is a garden soil, and was used for vegetable production. Soil B is a paddy soil, and was used for rice production. The soils were taken from surface 0–15 cm horizons, air-dried, and sieved to pass a 2-mm screen before being used for laboratory experiments. Soil properties are listed in Table 1.

2.2 Experimental design

Pot experiments were conducted in a naturally lit glasshouse at the Huajiachi Campus of Zhejiang University in 2008. The pots were laid out in a completely randomized design.

2.2.1 Vegetable experiment

Pots (17 cm in height, 19 cm in diameter) were each filled with 2.5 kg of air-dried soil. Three nylon mesh bags (10 cm in height, 6 cm in diameter), each filled with 200 g of air-dried soil, were buried evenly in each pot in a way that the soil surface in the bags was level with the soil surface in the pot. The mesh bags were used to separate the rhizosphere from the

Table 1 Selected properties of the experiment soils

Soil property	pH	Organic matter (g/kg)	Total N (g/kg)	Alkali-hydrolysable N (mg/kg)	Available P (mg/kg)	Extractable K (mg/kg)	CEC (cmol/kg)
Soil A	7.3±0.01	15.7±0.01	2.5±0.2	85.1±0.1	58.3±1.5	104.0±3.0	9.0±0.03
Soil B	5.6±0.10	21.7±0.50	2.8±0.1	136.0±2.7	98.5±2.0	104.5±5.4	8.4±0.10

Values represent the mean±standard error of the mean (SEM) ($n=3$). CEC: cation exchange capacity. Soil A: loamy mixed active thermic aeric humaquepts soil; Soil B: loamy mixed active thermic aeric endoaquepts soil

bulk soil: the soil in the bags was considered as the rhizosphere, and that outside the bags the bulk soil (Lu *et al.*, 2000). The bag had a mesh size of 24 μm , which allowed soil solutions to pass through but roots were unable to penetrate. The uptake of nutrients was not seriously restricted in this situation (Lu *et al.*, 2000). Therefore, the mesh bags could be used to study the localized N availability in the rhizosphere and in bulk soils.

The experiment had two N rates applied as urea (0 and 121.9 mg N/kg soil). K_2SO_4 was incorporated into the soil at a rate of 119.4 mg K_2O /kg soil. $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ was incorporated into the soil at a rate of 93.8 mg P_2O_5 /kg soil (Ke *et al.*, 2005). All fertilizers were thoroughly mixed with soil before planting. Treatments without N application were used as experimental controls (CK). Three pakchoi (*Brassica chinensis* L.) seeds were directly planted in the center of each nylon mesh bag on Nov. 15, 2008. After one week, only one healthy plant was allowed to grow in each bag. Plants were harvested on Dec. 31. The pots were watered regularly to prevent moisture stress to the plants. The experiment had seven replications for each treatment.

2.2.2 Rice experiment

The rice (*Oryza sativa* L.) variety used in the experiment was the japonica rice Bing 98110. Rice seeds were surface-sterilized in 1.5% H_2O_2 for 10 min, rinsed thoroughly with deionized water, soaked in deionized water at room temperature overnight, and germinated in moist quartz sand. Seedlings were grown for 21 d in a climate-controlled room with day and night temperatures of 30 and 25 $^\circ\text{C}$, respectively, and a relative humidity of 60%–70%. The photoperiod in the climate-controlled room was 14 h at a photon fluence rate of $(200 \pm 25) \mu\text{mol}/(\text{m}^2 \cdot \text{s})$ (photosynthetically active radiation (PAR) 400–700 nm). Uniform 21-d-old seedlings were used for experiments.

Pots (22 cm in height, 21 cm in diameter) were each filled with 3.5 kg air-dried soil. Three nylon mesh bags (15 cm in height, 8 cm in diameter), each filled with 600 g air-dried soil, were buried in each pot as in the vegetable experiment. Two N rates, 0 and 133 mg N/kg soil as urea, were applied to the treatment soils. All treatment soils in pots were mixed with 88 mg K_2O /kg soil as K_2SO_4 and 88 mg P_2O_5 /kg

soil as $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ before seedlings were transplanted into the pots (Bai and Xiao, 1998). Seedlings were transplanted on Aug. 3 with one seedling in each mesh bag and harvested on Dec. 8. A water depth of 3–5 cm above the soil surface in each pot was established and manually maintained throughout the experiment. Each treatment had eight replications.

2.3 Sampling and analysis

When the plants were harvested, soil samples were collected for analysis. Roots were removed carefully by hand from the soil samples. The soil outside the mesh bag was taken as the bulk soil, while soil inside the mesh bag was taken as the rhizosphere soil. The soil samples were air-dried, passed through a 2-mm sieve, and stored in glass bottles until analysis.

2.3.1 Water or K_2SO_4 -extractable nitrogen

Water or K_2SO_4 -extractable N was measured using air-dried soil samples (<2 mm). Four-gram soil was added to 40 ml of deionized water or 0.5 mol/L K_2SO_4 solution in 50-ml centrifuge tubes. The mixtures were shaken with a reciprocating shaker for 1 h, and then centrifuged at 4000 r/min for 15 min. The supernatant was filtered through Whatman No. 42 filter paper. The extracts were immediately stored at -20°C until analyzed. The extraction process was repeated five times for each treated soil sample.

2.3.2 Acid-hydrolysable nitrogen

Acid hydrolysis is the most widely used chemical method for characterizing soil organic nitrogen (Kelley and Stevenson, 1985). Acid-hydrolysable N was measured using air-dried soil (0.149 mm) as described by Bremner (Stevenson, 1982). The air-dried soils were hydrolyzed with 6 mol/L HCl at 110 $^\circ\text{C}$ for 24 h.

2.4 Chemical analysis

The amounts of NH_4^+ -N and NO_3^- -N in the water and K_2SO_4 extracts were determined using standard colorimetric procedures (Keeney and Nelson, 1982). TEN in the extracts was determined using persulfate to oxidize organic N and NH_4^+ -N to NO_3^- -N, as described by Cabrera and Beare (1993). The amounts of EON in the extracts were calculated by subtracting extractable inorganic N

(EIN= $\text{NH}_4^+\text{-N} + \text{NO}_3^-\text{-N}$) from TEN. The AA contents of the extracts were determined using the method described by Joergensen and Brookes (1990). Leucine was used as a calibration standard.

The amount of hydrolyzable N in the soils was determined by a procedure described by Bremner (1965). This involved estimation of the total hydrolyzable N, AA N, amino sugar N, ammonia N, and hydrolyzable unknown N (HUN). Correction factors were applied to the ammonia N and amino sugar N fractions to account for partial deamination of amino sugars during hydrolysis. HUN was estimated by the difference (Bremner, 1965).

pH was determined using 1:2.5 (w/v) soil:H₂O extracts. Total soil N was determined by the Kjeldahl digest method and the organic matter by the dichromate oxidation method (Keeney, 1982). Alkali-hydrolysable N and cation exchange capacity (CEC) were determined by the method of Lu (1999). Available phosphorus was measured using extracts of 0.5 mol/L NaHCO₃ (pH 8.5) or HCl-NH₄F (Bray and Kurtz, 1945; Olsen *et al.*, 1954). Extractable potassium was determined by extracting the soil with 1 mol/L CH₃COONH₄ and then measuring by flame photometry. All extractions were performed in triplicate. The analysis values from the triplicate extractions were averaged before statistical analysis was performed.

2.5 Statistical analysis

All statistical analyses were performed using STATISTICA (V. 5.5). Data were subjected to analysis of variance (ANOVA) and the differences in mean values were tested using Duncan's multiple range method ($P < 0.05$).

3 Results

3.1 Water-extractable N in rhizosphere and bulk soils

The amounts of water-extractable inorganic and organic N in the soils are shown in Fig. 1a. The concentration of water-extractable $\text{NH}_4^+\text{-N}$ was inversely correlated with the concentration of water-extractable $\text{NO}_3^-\text{-N}$ in paddy soil (correlation coefficient $r = -0.46$, $P < 0.05$), but for the garden soil, the

relationship was not significant.

The amounts of water-EON in soils are shown in Fig. 1a. Water-EON contents were not significantly affected by N addition (Table 2, $P < 0.05$). In garden soil, there was a significant increase of water-EON content in rhizosphere soil after N addition compared with that in bulk soil (Table 2, $P < 0.05$). On average, water-EON constituted 50% of the water-TEN pools in garden soil. Rhizosphere soil without N addition contributed the most (72.3%) and bulk soil with N addition the least (32.3%) to water-TEN.

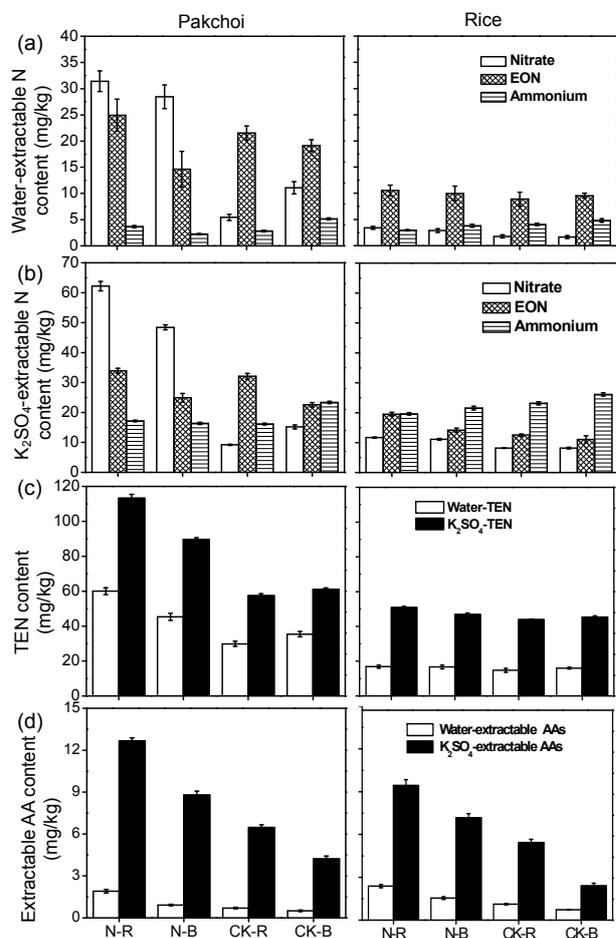


Fig. 1 Nitrogen distributions in rhizosphere and bulk soil samples in relation to plant types and N addition

Values are expressed as mean \pm SEM ($n_{\text{Pakchoi}}=7$; $n_{\text{Rice}}=8$). (a) Water-extractable N (nitrate, EON, and ammonium) content per unit soil mass. (b) K_2SO_4 -extractable N (nitrate, EON, and ammonium) per unit soil mass. (c) Extractable TEN content (water and K_2SO_4 -TEN) per unit soil mass. (d) Extractable AA content (water and K_2SO_4 -extractable AAs) per unit soil mass. N-R: rhizosphere soil with N addition; N-B: bulk soil with N addition; CK-R: rhizosphere soil without N addition; CK-B: bulk soil without N addition

Table 2 *F*-statistics from two-way ANOVA of the effects of N addition (no N versus urea added) and soil zone (rhizosphere versus bulk) on water-EON, TEN, and AAs in soils planted with pakchoi or rice

Plant	Source of variation	EON		TEN		AA	
		<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>
Pakchoi	N addition	1, 24	0.05	1, 24	129.00*	1, 24	122.00*
	Rhizosphere effect	1, 24	6.78*	1, 24	6.68*	1, 24	65.30*
	N×rhizosphere	1, 24	2.59	1, 24	32.90*	1, 24	28.70*
Rice	N addition	1, 28	2.28	1, 28	0.86	1, 28	187.00*
	Rhizosphere effect	1, 28	0.29	1, 28	0.00	1, 28	63.90*
	N×rhizosphere	1, 28	0.60	1, 28	0.29	1, 28	8.96*

* $P < 0.05$. *df*: degree freedom

Water-EON constituted 60.6% of the water-TEN pools in paddy soil. However, the amount of water-EON as a proportion of the water-TEN pool showed no significant difference between rhizosphere or bulk paddy soil.

The concentrations of water-extractable AAs in rhizosphere and bulk soils are shown in Fig. 1d. The water-extractable AAs concentrations were low (<3 mg/kg) in samples from both soils. There was a significant increase in the water-extractable AA content in rhizosphere soil after N addition compared with that in bulk soil (Table 2, $P < 0.05$). Generally, the highest concentrations were observed in rhizosphere soil with N addition and the lowest in bulk soil without N addition. In garden soil, water-extractable AAs constituted 5% of the water-EON pool and 2.2% of the water-TEN pool on average. Significant positive relationships were found between the concentration of water-extractable AAs and both the water-EON ($r = 0.39$) and the water-TEN ($r = 0.83$). In paddy soil, water-extractable AAs constituted 14.6% of the water-EON pool and 8.9% of the water-TEN pool.

3.2 K_2SO_4 -extractable N in rhizosphere and bulk soils

K_2SO_4 -extractable N had a much larger N pool than water-extractable N (Figs. 1a and 1b). The concentration of K_2SO_4 -extractable NH_4^+ -N was inversely correlated with K_2SO_4 -extractable NO_3^- -N in paddy soil ($r = -0.74$). On average, the K_2SO_4 -EIN content was about double that of water-EIN in garden soil. Similarly, the K_2SO_4 -EIN content was more than four times that of water-EIN in paddy soil.

Generally, significant amounts of K_2SO_4 -EON were found in samples from both soils (Fig. 1b, $P < 0.05$). The K_2SO_4 -EON content was not significantly affected by N addition in garden soil, but was greater in rhizosphere than in bulk soil (Table 3, $P < 0.05$). In contrast, K_2SO_4 -EON content was significantly increased with N addition in paddy soil (Table 3, $P < 0.05$). However, K_2SO_4 -EON content did not show a rhizosphere effect when N was not applied.

On average, K_2SO_4 -EON constituted 37.6% of the K_2SO_4 -TEN pool in garden soil, with the highest proportion (55.9%) seen in rhizosphere soil without N addition and the lowest (27.7%) seen in bulk soil with N addition. K_2SO_4 -EON constituted 30.4% of the K_2SO_4 -TEN pool in paddy soil, with the highest proportion (38.4%) seen in rhizosphere soil with N addition and the lowest (24.4%) seen in bulk soil without N addition. A significant positive relationship between the concentrations of K_2SO_4 -EON and K_2SO_4 -TEN was found in both garden (Fig. 2a, $r = 0.36$) and paddy soils (Fig. 2a, $r = 0.88$). In contrast, the concentration of EON was inversely correlated with NH_4^+ -N in garden ($r = -0.57$) and paddy soils ($r = -0.79$). Generally, the K_2SO_4 -EON content was about 1.4 times that of water-EON in both soils.

The contents of K_2SO_4 -extractable AAs in rhizosphere and bulk soils are shown in Fig. 1d. The results indicate that addition of N significantly increased K_2SO_4 -extractable AAs content in both soils (Table 3, $P < 0.05$). The K_2SO_4 -extractable AA content was significantly greater in rhizosphere soil than in bulk soil (Table 3, $P < 0.05$).

In garden soil, on average, K_2SO_4 -extractable AAs constituted 27.9% of the K_2SO_4 -EON pool and

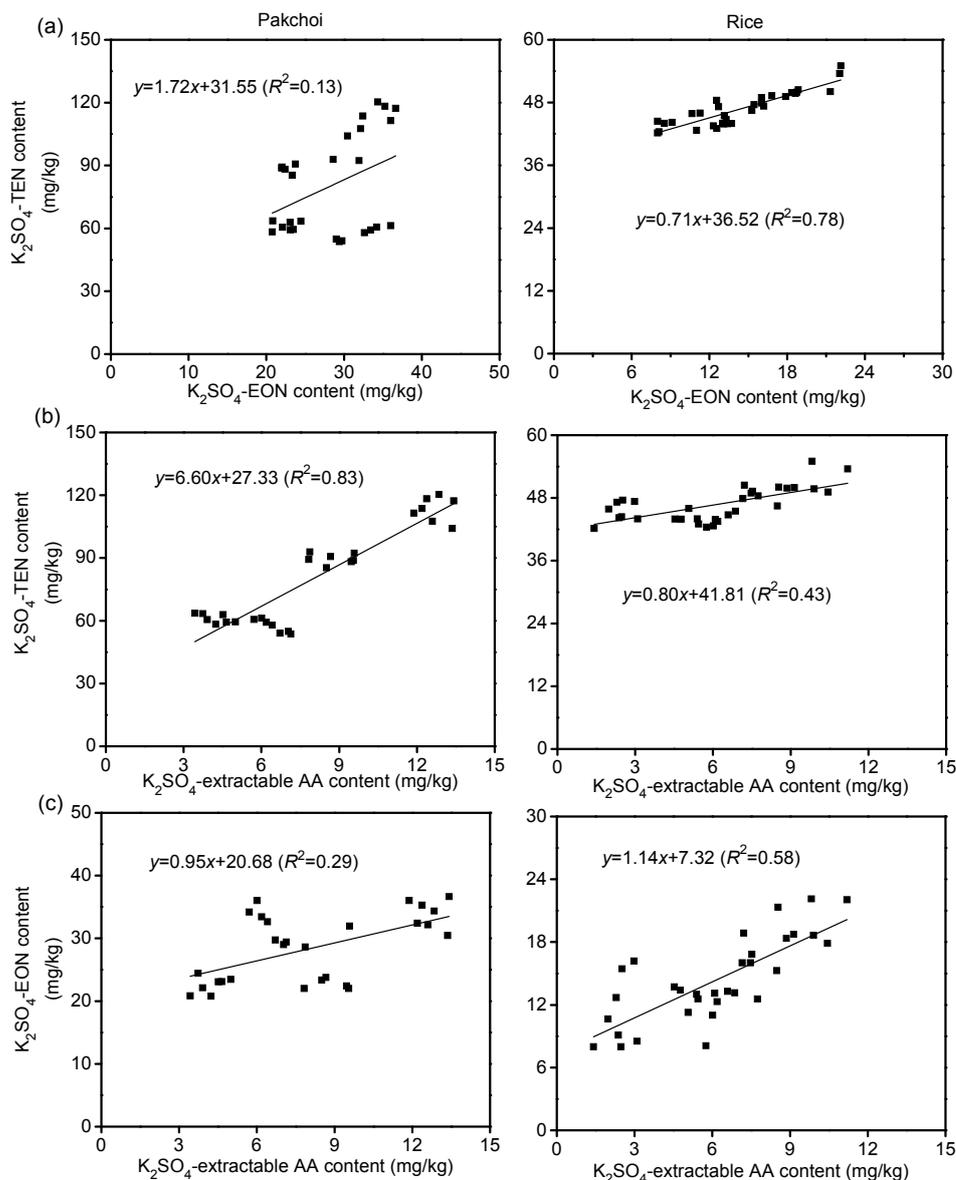


Fig. 2 Relationships among components of K_2SO_4 -extractable N (TEN, EON, and AAs) in soils planted with pakchoi or rice

(a) Relationship between K_2SO_4 -EON and K_2SO_4 -TEN; (b) Relationship between K_2SO_4 -extractable AA and K_2SO_4 -TEN; (c) Relationship between K_2SO_4 -extractable AA and K_2SO_4 -EON

Table 3 *F*-statistics from two-way ANOVA of the effects of N addition (no N versus urea added) and soil zone (rhizosphere versus bulk) on K_2SO_4 -EON, K_2SO_4 -TEN, and K_2SO_4 -extractable AAs in soils planted with pakchoi and rice

Source of variation		EON		TEN		AA	
		<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>	<i>df</i>	<i>F</i>
Pakchoi	N addition	1, 24	4.11	1, 24	881.00*	1, 24	538.00*
	Rhizosphere effect	1, 24	81.70*	1, 24	48.90*	1, 24	175.00*
	N×rhizosphere	1, 24	0.06	1, 24	92.00*	1, 24	12.40
Rice	N addition	1, 28	30.40*	1, 28	35.10*	1, 28	230.00*
	Rhizosphere effect	1, 28	14.00*	1, 28	3.10	1, 28	84.50*
	N×rhizosphere	1, 28	4.53*	1, 28	14.70*	1, 28	1.82

* $P < 0.05$

9.8% of the K₂SO₄-TEN pool. Significant positive relationships were found between the concentration of K₂SO₄-extractable AAs and both the K₂SO₄-EON (Fig. 2c, $r=0.76$) and the K₂SO₄-TEN (Fig. 2b, $r=0.91$). In paddy soil, K₂SO₄-extractable AAs constituted 41.0% of the K₂SO₄-EON pool and 12.9% of the K₂SO₄-TEN pools. Significant positive relationships were again found between the concentrations of K₂SO₄-extractable AAs and both the K₂SO₄-EON (Fig. 2c, $r=0.76$) and the K₂SO₄-TEN (Fig. 2b, $r=0.66$). Generally, the content of K₂SO₄-extractable AAs was about 3–9 times greater than that of water-extractable AAs in both soils.

3.3 Acid-hydrolysable N in rhizosphere and bulk soils

The results showed that addition of N significantly increased acid-hydrolysable AA content in both soils. Acid-hydrolysable AA content was significantly greater in rhizosphere soil than in bulk soil in both soil types (Tables 4 and 5). On average, acid-hydrolysable AAs constituted 10.7% of the total N pool in garden soil, and 19.1% of the total N pool in paddy soil.

Similarly, in garden soil, the amount of acid-hydrolysable ammonium significantly increased in the treatments with nitrogen addition. The acid-hydrolysable ammonium content was also

significantly greater in the rhizosphere compared to the bulk soil ($P<0.05$). However, acid-hydrolysable ammonium content was not affected by either N addition or the rhizosphere effect in paddy soil. Generally, acid-hydrolysable ammonium constituted 13.3% and 20.4% of the total N pool in garden and paddy soils, respectively.

In the N addition treatments, the contents of acid-hydrolysable amino sugar in both soils were significantly increased compared to those following treatments without N addition. Significantly higher acid-hydrolysable amino sugar content was also observed in rhizosphere samples than in bulk soil ($P<0.05$). On average, acid-hydrolysable amino sugar constituted about 3.0% of the total N pool in both soils. We were unable to identify the chemical properties of a portion of the organic N classified as ‘unknown N’ (Tables 4 and 5). The total amount of unknown N and AAs accounted for about 50.0% of the total organic N of the soils.

4 Discussion

4.1 Organic N component pools in soils

Two of the main plant species cultivated in China were planted under traditional agricultural planting conditions in our research. The organic N

Table 4 Acid-hydrolysable N components in vegetable soil

Group	Concentration (mg/kg)					
	Total N	Ammonium	Amino sugar	Amino acid	Unknown N	Total acid-hydrolysable N
Rhizosphere (N [*])	1348.6±43.8 a	194.6±7.5 a	64.9±5.3 a	151.0±3.5 a	137.5±15.8 a	548.1±23.3 a
Bulk (N [*])	1145.7±25.9 b	164.1±7.0 b	44.9±3.5 b	130.1±2.1 b	100.9±13.3 b	440.0±13.8 b
Rhizosphere (CK)	1152.9±32.4 b	158.3±7.0 b	31.2±4.6 c	128.6±3.9 b	42.6±4.2 c	360.6±24.0 c
Bulk (CK)	1161.4±32.1 b	124.4±7.2 c	21.6±3.1 c	107.1±4.1 c	20.1±3.5 c	273.2±13.7 d

Values represent the mean±SEM ($n=7$). Different letters in each column indicate significant differences between treatments at the $P<0.05$ level (the treatments in a column compared with each other). N^{*}: urea-added treatment; CK: no urea-added treatment

Table 5 Acid-hydrolysable N components in paddy soil

Group	Concentration (mg/kg)					
	Total N	Ammonium	Amino sugar	Amino acid	Unknown N	Total acid-hydrolysable N
Rhizosphere (N [*])	911.3±16.7 a	188.9±15.5 a	45.9±2.3 a	208.1±13.4 a	196.4±12.5 a	639.4±12.2 a
Bulk (N [*])	955.0±28.1 a	183.8±18.2 a	31.2±2.4 b	163.1±8.9 b	138.3±7.5 b	516.4±13.7 b
Rhizosphere (CK)	922.5±20.3 a	196.6±11.4 a	23.1±1.2 c	160.5±8.2 b	71.1±10.3 c	451.3±17.1 c
Bulk (CK)	918.8±20.3 a	171.1±3.4 a	19.5±1.4 c	125.0±7.1 c	38.6±10.0 d	354.1±14.7 d

Values represent the mean±SEM ($n=8$). Different letters in each column indicate significant differences between treatments at the $P<0.05$ level (the treatments in a column compared with each other). N^{*}: urea-added treatment; CK: no urea-added treatment

component pools in rhizosphere and bulk soils of typical land use types from southeast China were investigated. N is heterogeneously distributed in soil (Hodge, 2001). Soil organic N serves both as an important mineralization substrate and as a direct source of N for a variety of plant species in arctic, boreal, temperate, Mediterranean, shrubland, and alpine ecosystems. Our results indicated that K₂SO₄-EON contents ranged from 11.1 to 33.9 mg/kg (dry weight basis) with an average of 21.3 mg/kg. This finding was within the range typically found in agricultural systems in Europe and China (Murphy *et al.*, 2000; Willett *et al.*, 2004; Yang *et al.*, 2007). Generally, K₂SO₄-EON contents were lower than K₂SO₄-EIN contents; however, they constituted more than 30% of the K₂SO₄-TEN pools in the soils. Results showed that EON might be a major component of N cycling in soils.

AAs account for the major constituents of the low molecular weight EON in soil solution (Jones *et al.*, 2005a). Although they occur at low concentrations in soil solutions (Raab *et al.*, 1999; Öhlund and Näsholm, 2001; Jones *et al.*, 2005b), given their fast turnover rate (1–12 h) and large net fluxes, many researchers have suggested that water-extractable AA pools in soil solution rival or exceed those of mineral N, particularly in cold-temperate forests (Rothstein, 2009), alpine tundra (Chapin *et al.*, 1993; Raab *et al.*, 1996), boreal forests (Kielland *et al.*, 2007), and agricultural fields (Ge *et al.*, 2009; Reeve *et al.*, 2009). Therefore, they might serve as an important N source for plants. Our results showed that acid-hydrolysable AAs contents ranged from 107.0 to 208.1 mg/kg (dry weight basis) with an average value of 146.7 mg/kg. Acid-hydrolysable AAs constituted more than 10.0% of the total N pool in soils but low concentrations of water-extractable AAs were found. The water-extractable AAs were present in low concentrations in soil solutions in comparison to inorganic NH₄⁺-N and NO₃⁻-N due to the combined effects of their slow production rates and their rapid decomposition by microorganisms (Christou *et al.*, 2006). In our study, although the concentrations of water-extractable AAs in soil solution were low, significant amounts of K₂SO₄-EON might immediately replenish the pool of free AAs after its absorption by plants and microorganisms (Fig. 1). AAs, peptides, EON, and NH₄⁺ can

be readily adsorbed on the soil's solid phase, and the tightly bound AAs are poorly extracted by water. The K₂SO₄-extractable AA contents were much higher than the water-extractable AA contents. It is possible that K₂SO₄ extractant may release AAs from soil exchangeable sites. In agricultural soils, Näsholm *et al.* (2009) found that water-extractable AAs accounted for <5.0% of EON pools. Our results showed that the K₂SO₄-extractable AAs constituted 25.0% of K₂SO₄-EON pools and more than 9.0% of TEN pools in soils. Therefore, determining the amount of N associated with both the aqueous and adsorbed N is important in assessing the amount of bioavailable N in soil.

4.2 N application and rhizosphere effect

Our results suggested that N addition had positive effects on the K₂SO₄-TEN and extractable AA contents in soil solutions. As the application of inorganic N-fertilizer solubilises soil organic N because of its pH effect, the concentrations of organic N contents would increase in soils (Chantigny, 2003; Ros *et al.*, 2009). AAs might originate from microbial and root exudates (Haynes, 2005). The microbial community structures might be affected, and the microbial biomass may increase and reach a maximum value within a few days after inorganic N-fertilizer application. The enriched soil bacteria might decompose through auto digestion or attack by other types of microbes. Cell death might temporarily lead to large organic N contents in soils (Matsumoto and Ae, 2004; McDowell *et al.*, 2004; Jones *et al.*, 2005b; Ros *et al.*, 2009).

The rhizosphere is a zone of active interchange between plants and soil bacteria (DeAngelis *et al.*, 2008). Our results indicated that the rhizosphere had positive effects on K₂SO₄-EON and extractable AA contents in soils. Root exudates may contribute to EON in the rhizosphere. Although they are transient in the N pools of soils, the net flow of N would be from soil N to plant tissue as plants grow (DeAngelis *et al.*, 2008). Concentrations of AAs in soils are also affected by their uptake and release both by plant roots and various micro-organisms (Näsholm *et al.*, 2009). In addition, as root exudates could lead to rapid microbial biomass turnover, and microbes would preferentially decompose the organic acids and sugars produced by roots over AAs, the

rhizosphere effect may alter the balance of plant-microbe competition for AAs in soils (Lipson and Näsholm, 2001; Chen and Xu, 2008). Therefore, AA concentrations in the rhizosphere may be very different from those in bulk soil.

In contrast, it was found that TEN contents were lower in the rhizosphere than in bulk soil in both soil types without N addition (Fig. 1c). Root exudates play important roles in helping plants adapt to and resist environmental stress. Under N stress, root exudates could produce organic acids and affect the growth of micro-organisms by way of allelopathy. This may enhance the hydrolysis of solid organic N to dissolved N, and increase the uptake of N by plants, thereby enhancing stress resistance (Shi, 2004).

5 Conclusions

The results confirm that EON is a major contributor to TEN pools in typical land use soil types (garden and paddy soils) under traditional planting conditions in southeast China. Although only a small proportion of the EON was present in the form of water-extractable AAs, the results presented here indicate that K₂SO₄-extractable AAs accounted for 25.0% of the K₂SO₄-EON pool and nearly 10.0% of the TEN pools in both rhizosphere soils. K₂SO₄-extractable AAs and the release of AAs from soil exchangeable sites might be important sources of organic N for plant growth. Overall, both K₂SO₄-EON and AA contents had positive correlations with TEN pool contents. Our findings suggest that the contents of most forms of organic N were significantly greater in rhizosphere than in bulk soils. However, it was also apparent that, without N application, the TEN pool contents were lower in the rhizosphere than in bulk soils.

Compliance with ethics guidelines

Xian-you CHEN, Liang-huan WU, Xiao-chuang CAO, and Yuan-hong ZHU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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Changes of paired-pulse evoked responses during the development of epileptic activity in the hippocampus

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Abstract: Dysfunction of inhibitory synaptic transmission can destroy the balance between excitatory and inhibitory synaptic inputs in neurons, thereby inducing epileptic activity. The aim of the paper is to investigate the effects of successive excitatory inputs on the epileptic activity induced in the absence of inhibitions. Paired-pulse orthodromic and antidromic stimulations were used to test the changes in the evoked responses in the hippocampus. Picrotoxin (PTX), γ -aminobutyric acid (GABA) type A ($GABA_A$) receptor antagonist, was added to block the inhibitory synaptic transmission and to establish the epileptic model. Extracellular evoked population spike (PS) was recorded in the CA1 region of the hippocampus. The results showed that the application of PTX induced a biphasic change in the paired-pulse ratio of PS amplitude. A short latency increase of the second PS (PS2) was later followed by a reappearance of PS2 depression. This type of depression was observed in both orthodromic and antidromic paired-pulse responses, whereas the GABAergic PS2 depression [called paired-pulse depression (PPD)] during baseline recordings only appeared in orthodromic-evoked responses. In addition, the depression duration at approximately 100 ms was consistent with a relative silent period observed within spontaneous burst discharges induced by prolonged application of PTX. In conclusion, the neurons may ignore the excitatory inputs and intrinsically generate bursts during epileptic activity. The depolarization block could be the mechanisms underlying the PPD in the absence of $GABA_A$ inhibitions. The distinct neuronal responses to stimulations during different epileptic stages may implicate the different anti-epileptic effects of electrical stimulation.