Pedosphere ISSN 1002-0160/CN 32-1315/P doi:10.1016/S1002-0160(18)60032-3

The Driving Factors of Soluble Organic Nitrogen Dynamics in Paddy Soils: Structure Equation Model Analysis

YANG Jing[1], YANG Wenhao[1,2], WANG Fan[3], ZHANG Liming[1,2], ZHOU Biqing[1,2], Rubab SARFRAZ [1], XING Shihe[1,2,*]

[1]*College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou 350002 (China)*
[2]*University Key Laboratory of Soil Ecosystem Health and Regulation in Fujian, Fuzhou 350002 (China)*
[3]*School of Environment and Energy, Shenzhen Graduate School, Peking University, Shenzhen, 518000 (China)*

ABSTRACT

Soil soluble organic nitrogen (SON) is one of the most active components in soil nitrogen pools, yet limited information is available with regard to its impact factors as well as their pathways and degrees of influence. In this study, a structural equation modeling (SEM) was used to analyze the driving factors, their significance and pathways which affected SON dynamics in a waterlogged experiment of two typical paddy soils incubated for 80 days after green manure application. Soil pH, Eh, microbial biomass, enzyme activity and SON dynamic were used to construct the structural equation model. The results showed that soil microbial biomass carbon (SMBC), protease, glutamine and initial organic matter directly affected soil SON significantly with path coefficients corresponded to 0.405, 0.547, 0.523 and -0.623 (p<0.01), respectively. SMBC and initial organic matter affected the SON dynamics indirectly through protease and glutamine activity. In addition, pH indirectly affected SON dynamics by glutamine activity. It is implied that SMBC, protease, glutamine and initial organic matter are the key factors affecting SON dynamics in the waterlogged paddy soils after green manure application. Our research indicated that the structural equation modeling could provide an effective method to clearly recognize the impact, significance and pathways of multiple factors on soluble organic nitrogen dynamics in the soil.

Key Words: *enzyme activities; green manure; impact factors; influence pathways; soil microbial biomass*

INTRODUCTION

Soluble organic nitrogen (SON) is generally defined as organic form of nitrogen that can be extracted by water or salt solutions ($CaCl_2$, KCl, K_2SO_4, etc.), which plays an important role in soil N cycling (Murphy et al., 2000). SON in soil can be used by crops directly (Yang et al. 2007; Wei et al. 2013). For example, free amino acids can be absorbed directly without decomposition by microorganisms under certain conditions, such as low temperature or low soil nitrogen content (Näsholm et al., 2009). In addition, SON can also be absorbed after being transformed to mineral N (Yang et al. 2007; Wei et al. 2013). On the other hand, due to its mobility, SON is easy to mobilize with runoff or leach down to the ground water, maybe leading to eutrophication of receiving water bodies (Quan et al., 2015). Recent studies have shown that SON may rival mineral N in quantity in

*Corresponding author. E-mail: E-mail: fafuxsh@126.com.

agricultural soil systems (Okamoto et al, 2008). As an important component of soil organic nitrogen, the SON pools in terrestrial ecosystems were affected comprehensively by lots of impact factors. Numerous studies have focused on the effects of soil properties on the dynamics and fate of dissolved organic nitrogen, including soil pH (Long et al., 2015), temperature (Jiang et al., 2016), clay (Filep et al., 2011), organic matter contents (Filep et al., 2011) and microbial biomass and activity (Schmidt et al, 2011; Su et al, 2016). In addition, a number of studies have also shown that agricultural management such as land use type (Christoua et al., 2005), irrigation (Yang et al., 2015), fertilization (Ros et al., 2009), etc. can also significantly affect soil SON pools. However, most of them were merely studied as independent impact factors on SON and the interaction of the factors has been little considered. In fact, the relationships between SON and its impact factors were very complicated. Some factors, i.e. organic matter, can directly affect the SON pools and also affect the enzyme and microbial activity, so that soil organic matter may also indirectly impact SON through the enzyme and microbial activity. Therefore, it is important to consider both direct and indirect cause–effect structural relationships to better understand the factors contributing to dynamics of SON. However, to date, a simple linear correlation model is always used to analyze the correlation between SON and the impact factor (Khalili et al., 2012; Huang et al, 2008), but the results can only reveal the close relationship between a single factor and SON. The intrinsic relationships between the dynamics of soil SON and its impact factors could not be fully explained. Structural equation modeling (SEM), defined as "the use of two or more structural [cause-effect] equations to model multivariate relationships", allows for an intuitive graphical representation of complex networks of relationships (Grace and Bollen, 2008; Eisenhauer et al, 2015). Compared with other statistical techniques, SEM has a prominent advantage that SEM allows for both the direct and indirect theoretical causal relationships between inter-correlated variables to be tested, and for potential multivariate relationships to be identified (Grace, 2006). At present, this model has been successfully applied in psychology, behavioral science and econometrics etc., and has recently gained increasing research interests in soil science, ecology and other fields (Deng et al., 2013; Gama-Rodrigues et al., 2014; Sarstedt, et al., 2014; Liu et al., 2016; Yuan et al., 2016). However, to our knowledge, SEM has never been used in studying the impact factors on the dynamics of soil SON.

Paddy ecosystems occupy a large area in China with diverse material types of soil which represent various soil conditions and N cycle performance. Therefore, in this study, two different types of paddy soils characterized with distinct organic matter content, total N, available N and physical clay content were chosen to answer the following two hypothesis: (1) Different factors are able to affect SON dynamics in paddy soils by various influence pathways with great differences of direct, indirect and total impact effects. (2) SEM is an effective method to clearly recognize the impact of multiple factors on soil SON dynamics. The chosen soils were Aquandic Endoaquepts and Typic Endoaquepts, which represented two typical paddy soils in subtropical China. To address the above hypothesis, waterlogged incubation experiments of the two paddy soils were conducted to simulate SON turnover after green manure (Chinese milk vetch) application at 15 ^{o}C and 25 ^{o}C. Based on this experiment, an SEM was then established to analyze the impact pathway and significance of multiple factors which affect SON directly or indirectly, as well as the key factors influencing SON dynamic pattern in subtropical paddy soil.

MATERIALS AND METHODS

Plant material

The green manure used in this study is Chinese milk vetch (CMV), which is widely planted in paddy fields in Southern China, and its variety was Minzi No.7 bred by Soil and Fertilizer Institute, Fujian Academy of Agricultural Sciences, sown in the field in October 2014, maintained with proper soil moisture and pest control strategy and harvested in March 2015 on the flowering stage. During harvest, the integrity of the root, stem and leaf were carefully preserved. The harvested plants were stored at 4 ^{o}C in a refrigerator. Subsamples of CMV were dried and crushed for subsequent characterization and incubation experiments. The typical chemical composition of the vetch residue was as follow: Total N, P and K: 32.7 g kg^{-1}, 1.2 g kg^{-1} and 20.3 g kg^{-1}, respectively.

Soils

Two soils used in this study were sampled from the topsoil (0-15 cm) of selected field sites in Minhou and Lianjiang County, Fujian province, respectively. The soil types were Aquandic Endoaquepts and Typic Endoaquepts, respectively (Soil Survey Staff, 1999), both of which are widely distributed throughout Fujian province in Southeastern China. Soil samples were air-dried and sieved (<5 mm) and plant debris were separated from samples. All samples were stored at 4 ℃ before subsequent analysis and incubation experiments. Basic soil properties are given in Table Ⅰ.

TABLE Ⅰ
Chemical and physical properties of Aquandic Endoaquepts and Typic Endoaquepts

Soil types	pH	Available N	Organic Matter	Total N	Physical clay content 1
		mg kg^{-1}	g kg^{-1}	g kg^{-1}	%
Aquandic Endoaquepts	6.5	70.66	10.98	0.78	9.0
Typic Endoaquepts	6.4	186.59	34.74	2.18	32.5

Incubation experiments

Waterlogged incubation experiments were conducted at temperatures of 15 ^{o}C and 25 ^{o}C which were determined on the basis of annual mean maximum and minimum temperature in Minhou and Lianjiang. 1 g CMV was added to 50 g soils (equivalent to field fertilization rate of 45000 kg hm^{-2}) and mixed thoroughly. Soils without addition of CMV were used for control experiments. Three replicates were used in each treatment. The mixtures of soil and CMV placed in 100 mL beakers and incubated in the darkness in an incubator at 15 ^{o}C or 25 ^{o}C. During the 80 days incubation period, distilled water was periodically added to control the consistency of waterlogging condition. Soils were subsampled at 5, 10, 15, 20, 25, 30, 40, 60 and 80 days (50 g of soil samples were set up for each sampling period and only one of them was taken out of each sampling period.) after addition of the CMV for basic soil properties, soil microbial biomass carbon (SMBC), soil microbial biomass nitrogen (SMBN) and enzyme activity analyses.

Measurements of pH, Eh, TN, clay content, organic matter content, available N. Soil samples were taken from the incubator, stirred for 2 mins, and settled for 30 mins. Then pH was measured using a pH meter (PHS-3C). Soil Eh values were measured by an ORP composite electrode (PHSJ-4A), which was directly put into the soil, and then showed the mv value after stabilization. soil total N (TN) and organic matter were analyzed using an Elemental Analyser (LECO TruMac, USA). Clay content was measured by Laser Particle Analyzer (Mastersizer 3000, UK), and soil available N was determined by the alkali permanganate method.

Analysis of soluble organic N. Soil SON was analyzed using the water extraction methods described by Chen et al. (2005). In brief, the samples taken from the incubator were subsequently shaken for 60 mins on an end-to-end shaker and filtered through a Whatman 42 paper followed by a 0.45 mm filter membrane. Total soluble N (TSN) in the extracts was analyzed by the high-temperature catalytic oxidation method using a TOC analyzer (Shimadzu, TOC-L, Japan). Concentrations of SIN (NH_4^+–N, NO_3^-–N and NO_2^-–N) in the extracts were measured using a continuous-flow analyzer (Systea, Flowsys, Italy). The concentrations of SON were calculated as the differences between TSN and the sum of SIN in the extracts.

Analysis of soil microbial biomass. Soil microbial biomass C (SMBC) and N (SMBN) were measured by the chloroform fumigation extraction-water bath method as described by Chen et al. (2006). Briefly, 700 μL liquid chloroform was directly added to 10 g of the waterlogged soil to be tested, which was fumigated for 24 h at 25 ^{o}C under normal atmospheric pressure in darkness, and then extracted with 40 mL 0.5 mol L^{-1} K_2SO_4 solution. The K_2SO_4 extractions were carried out in a 100 ^{o}C water bath for 60 mins to completely remove chloroform residues before measuring TOC. Non-fumigation controls were extracted directly with 0.5 mol L^{-1} K_2SO_4 solution. SMBC and SMBN were calculated using a conversion factor for C (Ec) of 2.64 (Vance et al., 1987) and for N (En) of

2.22 (Brookes et al., 1985).

Analysis of soil enzyme activities. Soil urease activity was estimated by indophenol blue colorimetry (Guan, 1986). Briefly, 1 mL of toluene and 1 mL of deionized water were added to 5 g soil, and 15 mins later, 10 mL of 10% urea and 20 mL of citrate buffer (pH 6.7) were added to the soil solution and incubated at 37 ºC for 24 h. After incubation, the mixture was taken out and filtered, then 800 μL phenol–sodium solution and 600 μL sodium hypochlorite were added to 3 mL of the filtrate. The mixture was incubated for another 20 mins and the developed blue color was measured at the wavelength of 578 nm in an enzyme marked instrument (BioTek, Epoch2, America). The mean urease activity was calculated as urea hydrolysis and expressed as mg NH_4^+ g^{-1} soil d^{-1}.

Soil protease activity was estimated by using Folin method (Guan, 1986). About 0.5 mL of toluene and 5 mL of tris-HCl (pH 8.1) were added to 5 g of soil. After 15 mins, 5 mL 1% caseinate-buffer solution was added, and incubated at 50 ºC for 2 h. After incubation, the reaction was stopped with 5 mL of 15% trichloroacetic acid. The mixture was centrifuged for 10 mins, and then 1 mL of supernatant was removed and added to 5.0 mL of 0.4 M Na_2CO_3 and 1 mL of threefold diluted Folin & Ciocalteu’s phenol reagent (BDH). Finally, the mixture was measured at the wavelength 680 nm in an enzyme marked instrument (BioTek, Epoch2, America). The protease activity was expressed as mg tyrosine g^{-1} soil $(2h)^{-1}$.

Soil glutaminase activity was measured by nesslerization method (Guan,1986). Briefly, 0.5 mL of toluene, 5 mL of Tris buffer (pH=8.5) and 10 mL of 3% glutamine solution were added to 3 g of waterlogged soil and incubated at 37 ºC for 24 h. After incubation, 30 mL of 1 M KCl was added. The mixture was shaken and filtered, then 500 μL of 5% NaOH, 400 uL of 50% potassium tartrate and 400 uL Nessler reagent were added to 1 mL dilution. And finally, the mixture was measured at 420 nm in an enzyme marked instrument (BioTek, Epoch2, America). The glutaminase activity was expressed as NH_4^+ g^{-1} soil d^{-1}.

Analysis of structure equation model

SEM is referred to as covariance structural analysis that includes confirmatory factor analysis and regression or path analysis (Liu et al., 2016; Soliman et al., 2016). This analysis method is used to address complex interrelations for more than one independent variables or one or more dependent variables. The SEM variables are classified into two major groups: observable and latent variables. The former group is based on direct measurement, while the latter group is identified only by its effects on the observable variables (Gama-Rodrigues et al., 2014). We conducted the SEM analysis to analyze the main impact factors and their pathways affecting SON in paddy soil. In our study, eight observable variables (pH, Eh, initial OM, SMBC, SMBN, urease, protease and glutamine) were considered as independents, while the dynamics of soil SON in paddy soil after CMV application were treated as the dependent. In a SEM figure, latent variables are normally represented by circles or ovals; observable variables are represented by rectangles or squares; relationship between different variables are represented by two symbols: single-headed arrow, which represents a causal relationship such that the variable at the tail of the arrow is believed to be a direct cause of the variable at the head, while a double-headed arrow indicates an unresolved correlation between two variables (Gama-Rodrigues et al., 2014).

In this study, the SPSS 18.0 reliability test was used to test the consistency between soil factors and SON. Since both SMBC and SMBN represented the change of soil microbial biomass, the most commonly used SMBC was selected to represent both SMBC and SMBN in order to simplify the model. Based on the results of the correlation analysis (Table Ⅱ) and the prior theoretical knowledge, some hypothesis could be made as follows, a) pH, initial OM, SMBC, urease, protease and glutamine have a direct impact on SON, b) Soil Eh has an indirect effect on SON by affecting SMBC and urease, protease, c) Soil pH has an indirect effect on SON by affecting glutamine, d) Initial OM has an indirect effect on SON by affecting SMBC, urease, protease and glutamine, e) SMBC had an indirect effect on paddy soil SON by affecting urease, protease and glutamine. According to the influence path of hypothesis, the conceptual model of SON and its impact factors was established through Amos 21.0 software (Fig. 1a). The SEM was implemented using the software AMOS 21.0. We used the method of Maximum Likelihood to estimate the parameters, and *P*-values and chi-square test (χ^2) to assess the general model fit., because the respective *P*-values (*P*-values > 0.05) associated with the model chi-square are used to judge the fit between model and data (Eisenhauer et al., 2015). Several indices are also used to evaluate the ideal model, including the goodness-of-fit index (GFI), root mean square error of approximation (RMSEA), normed fit index (NFI), tucker-lewis index (TLI); comparative fit Index (CFI) and incremental fit index (IFI). Except for RMSEA which is less than 0.05, the value of these indices close to 1 indicates a good fit (Grace, 2006; Gama-Rodrigues et al., 2014; Eisenhauer et al., 2015).

TABLE Ⅱ

Correlation analysis of SON and its potential impact factors

	SON	Initial OM	pH	Eh	SMBC	SMBN	Urease	Protease	Glutaminase
SON	1								
Initial OM	0.503**	1							
pH	-0.368*	-0.143	1						
Eh	-0.105	-0.255	-0.667**	1					
SMBC	0.883**	0.640**	-0.171	-0.368*	1				
SMBN	0.841**	0.617**	-0.116	-0.378*	0.966**	1			
Urease	0.805**	0.769**	-0.092	-0.497**	0.950**	0.923**	1		
Protease	0.762**	0.873**	-0.206	-0.486**	0.806**	0.736**	0.867**	1	
Glutaminase	0.858**	0.742**	-0.415*	-0.189	0.849**	0.842**	0.836**	0.836**	1

* and ** significant at 0.05 or 0.01 probability levels, respectively.

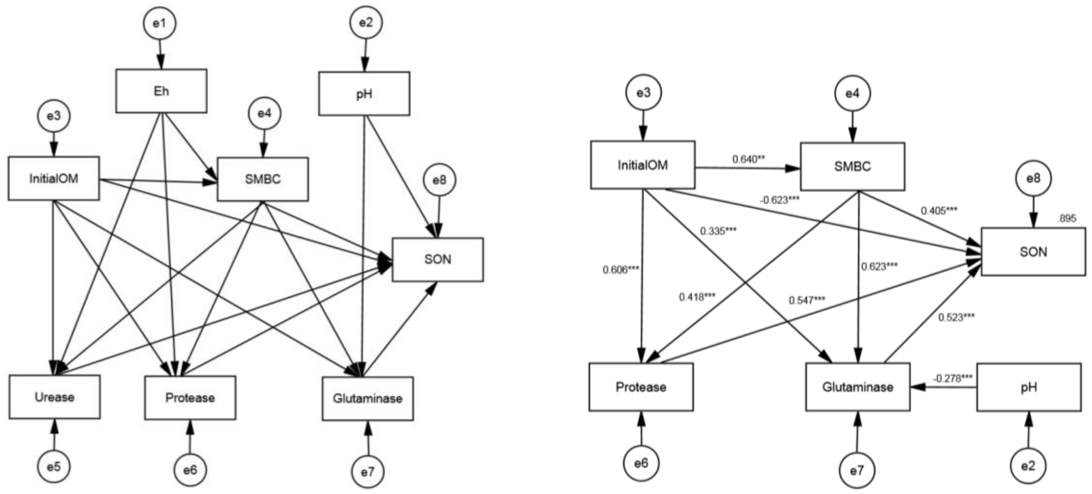

Fig. 1 SON and its impact factors conceptual model (a) and modified model (b) in paddy soil after application CMV. The value next to the arrow are standard path coefficients (also known as regression coefficients), P value represents the significance level, *** significant at 0.001 probability level

Statistical analysis

All statistical analysis and correlation analysis were performed using SPSS 18.0 or Excel 2003. Analysis of variance (ANOVA) and Least significant difference (Duncan, $p < 0.05$) analysis were used to separate the means with significant differences. Data plots were obtained from SigmaPlot 12.5. Error bars in data represented standard errors.

RESULTS

Dynamics of soil SON pools

The dynamics of soil SON in the process of CMV decomposition were shown in Fig. 2. The concentrations of SON varied between 1.42 mg L^{-1} and 5.15 mg L^{-1} for the Aquandic Endoaquepts and between 1.39 mg L^{-1} and 11.07 mg L^{-1} for the Typic Endoaquepts after the application of CMV. Addition of CMV increased SON up to 199.22% for the Aquandic Endoaquepts and 249.36% for the Typic Endoaquepts than the control. In the treatment adding Chinese milk vetch, the contents of SON in the soil solution increase slowly in the first 15 days, then decline, and finally tend to stabilization gradually at 15 °C. The maximum SON contents were 4.60 mg L^{-1} and 7.82 mg L^{-1} for the Aquandic Endoaquepts and the Typic Endoaquepts, respectively. At 25 °C, the contents of SON showed similar temporal variations to those at 15 °C, but its peak time was a bit earlier than at 15 °C. The maximum SON contents were 5.15 mg L^{-1} and 11.08 mg L^{-1} for the Aquandic Endoaquepts and the Typic Endoaquepts, respectively.

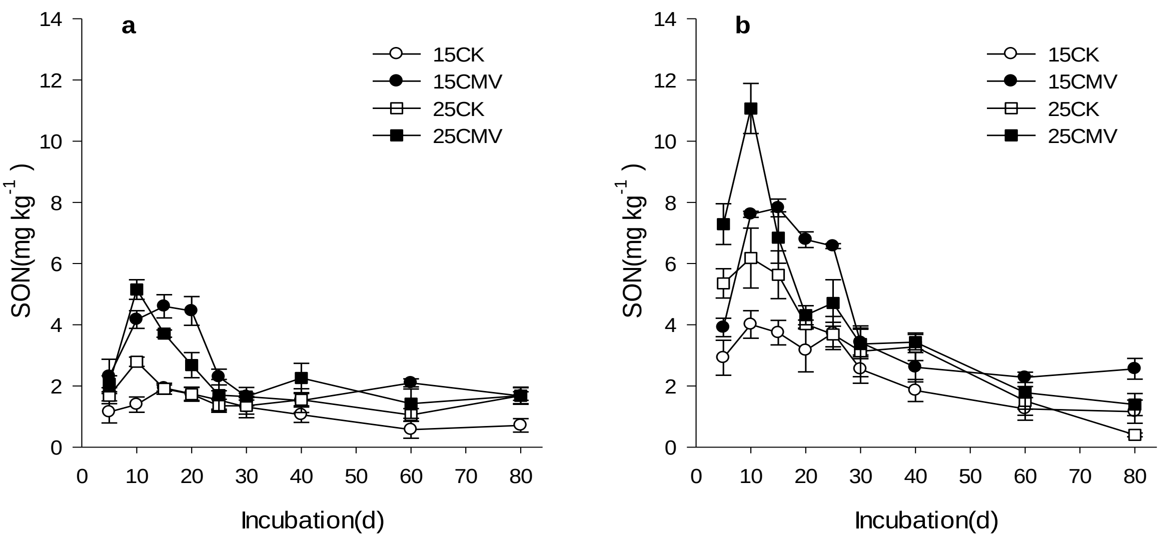

Fig. 2 SON contents of Aquandic Endoaquepts (a) and Typic Endoaquepts (b). Vertical error bars are the standard error of means of three replicates. 15CK, 15 °C without CMV; 15CMV, 15 °C with CMV; 25CK, 25 °C without CMV; 25CMV, 25 °C with CMV.

Impact factors of soil SON pools

Numerous studies have shown that pH, Eh, initial OM, SMBC, SMBN, urease, protease and glutamine are important factors affecting soil SON content. Therefore, this study measured the pH, Eh, initial OM, SMBC, SMBN, urease, protease and glutamine indicators, and the data were shown in Fig. 3. The results of correlation analysis revealed that soil SON was significantly correlated with initial OM, pH, SMBC, SMBN, urease, protease and glutamine, while significant positive correlations among many influential factors of SON were also observed (Table Ⅱ). However, Eh was negatively correlated with SMBC, SMBN, urease and protease (r = -0.368, -0.378, -0.497, -0.486, respectively). These results showed that SON is affected by many factors, and these factors are interdependent on each other.

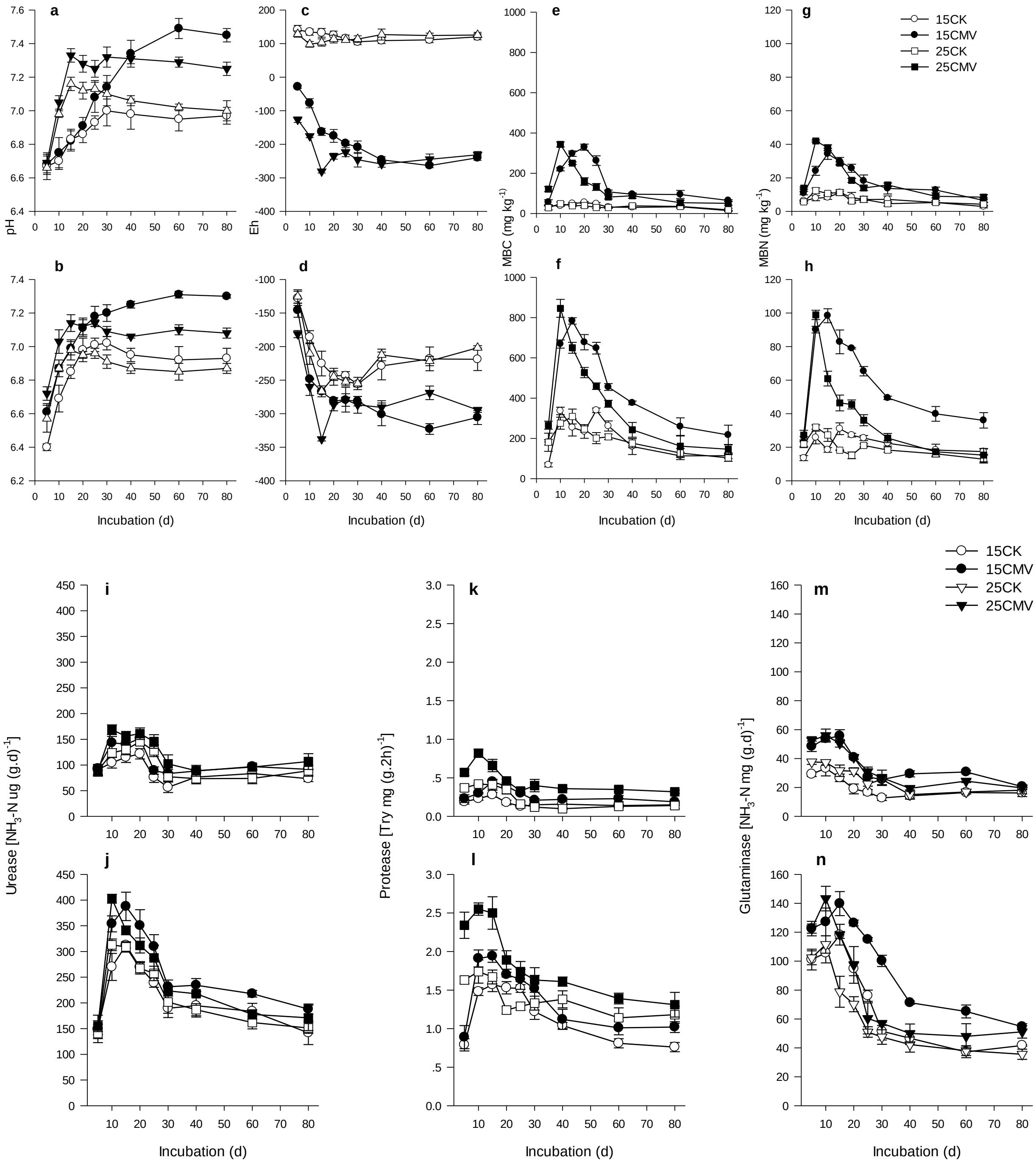

Fig. 3 Soil pH, Eh, SMBC, SMBN, urease activities, protease activities and glutaminase activities of Aquandic Endoaquepts (a, c, e, g, i, k, m) and Typic Endoaquepts soil (b, d, f, h, j, l, n). Vertical error bars are the standard error and from three replicates per site. 15CK, 15 °C without CMV; 15CMV, 15 °C with CMV; 25CK, 25 °C without CMV; 25CMV, 25 °C with CMV.

Structural equation model analysis of soil factors on SON pools

The result of reliability analysis of SON and its impact factors (initial OM, pH, Eh, SMBC, urease, protease, glutamine) showed that Cronbach's Alpha coefficient was 0.752 (n = 8), which indicated that the internal consistency of the selected factors is ideal. The first fittings of SON and its impact factors are shown in Fig. 1a. The results of first fitting coefficients showed that the *P* values of

Eh to SMBC, protease and urease, pH to SON were 0.896, 0.970, and 0.893, 0.190 (> 0.05), respectively, which did not pass the test of significance (Table Ⅲ). Thus, the second fitting was validated by releasing some pathways, including the effect of Eh on SMBC, protease and urease, and the effect of pH on SON, according to the actual meaning of the model and revised indices given by the model.

TABLE Ⅲ

Estimates of first fitting coefficients for SON and its impact factors

			Estimate [a)]	S.E. [b)]	C.R [c)]	P [d)]
SMBC	<---	Initial OM	11.774	2.914	4.041	***
SMBC	<---	Eh	-0.068	0.520	-0.131	0.896
Protease	<---	Initial OM	0.037	0.006	6.744	***
Protease	<---	SMBC	0.001	0.000	5.176	***
Glutaminase	<---	SMBC	0.103	0.014	7.146	***
Urease	<---	Initial OM	1.789	0.433	4.131	***
Urease	<---	SMBC	0.329	0.021	15.856	***
Glutaminase	<---	Initial OM	1.034	0.268	3.857	***
Urease	<---	Eh	-0.126	0.064	-1.968	0.049
Protease	<---	Eh	0.000	0.001	0.038	0.970
Glutaminase	<---	pH	-46.072	11.219	-4.107	***
SON	<---	SMBC	0.005	0.002	2.179	0.029
SON	<---	Protease	1.645	0.452	3.637	***
SON	<---	Glutaminase	0.025	0.008	3.025	0.002
SON	<---	Urease	-0.001	0.006	-.135	0.893
SON	<---	InitialOM	-0.108	0.025	-4.383	***
SON	<---	pH	-0.902	0.688	-1.310	0.190

a): Non standardized regression coefficients of estimate.

b):Standard error of estimate.

c): critical ratio is the t values of T-test, C.R.>1.96 represents significant at 0.05 probability levels.

d): P value represents the significance level, ***significant at 0.001 probability levels, P > 0.05 implied causal hypothetical pathways were false.

As shown in Table Ⅳ, the reliability coefficients of the second fitting fall within an acceptable range, and the overall fit indices for the proposed model were acceptable, with χ^2/ df = 0.866, GFI = 1.000, SRMR = 0, NFI = 0.982, TLI = 1.009, CFI = 1.000, IFI = 1.003. All of these fit indices for the initial model indicated an acceptable fit. The structural equation modeling results showed that initial OM, SMBC, glutaminase, protease and pH can explain 89.5% of the variations in SON contents under different temperature conditions (Fig. 1b).

TABLE Ⅳ

Fitting coefficients of the SON and its impact factors model

Indices name	χ^2/ df	GFI	RMSEA	NFI	TLI	CFI	IFI
Evaluation criterion	<3	close to 1	<0.05	close to 1	close to 1	close to 1	close to 1
Results	0.866	1	0	0.982	1.009	1	1.003

a):chi-square test (χ^2), b): goodness-of-fit index, c): root mean square error of approximation, d): normed fit index, e): tucker-lewis index, f): comparative fit Index, g): incremental fit index

The detailed path coefficients of the proposed model were shown in Table Ⅴ. The pathways from each variable to soil SON content were significant. The results (Table Ⅴ, Fig. 1a) showed that initial OM, SMBC, glutaminase and protease had direct impacts on SON pools with the standardized path coefficients of -0.648, 0.406, 0.500 and 0.594, respectively. Meanwhile, initial OM had an indirect impact on the SON pools by affecting soil protease, glutaminase and SMBC, with the standardized indirect effect of 1.153, and SMBC had an indirect impact on the SON pools by affecting soil protease, glutaminase, with the standardized indirect effect of 0.549. In addition, pH had an indirect impact by affecting soil glutaminase with the standardized indirect effect of -0.145.

TABLE Ⅴ

Total effects of factors contributing to the variability of SON content

	Direct Effects[a)]	Indirect Effects[b)]	Total Effects[c)]
SON <--- Initial OM	-0.623	1.121	0.498
SON <--- SMBC	0.405	0.554	0.959
SON<--- Glutaminase	0.523	0.000	0.523
SON <--- Protease	0.547	0.000	0.547
SON <--- pH	0.000	-0.145	-0.145

a): Standard path coefficients
b): The product of the direct effects path coefficients
c): The sum of direct effects and indirect effects
The absolute value of the path coefficient represents the size of the correlation, "-" represents a negative correlation

DISCUSSION

Dynamics pattern of soil SON

The content of SON in soil has a certain temporal dynamic after CMV application. In this study, SON contents significantly increased in the first 10--15 days, then decreased, and finally became stable, which may be due to that the decomposition of soil organic material has two consecutive stages. The first is the nutrition-controlling stage, during which organic matter decomposition and N mineralization are processed rapidly. Thereafter, decomposition rate slows down and stays at a low level, which is called the lignose-controlling stage (Berg, 1986). In our experiment, SON concentrations doubled in 10-15 days of incubation after CMV application, suggesting a rapid turnover of CMV. This is probably due to that the CMV used in our study was rich in nitrogen compounds with the C-to-N ratio of 14 and thus, can significantly increase the SON contents in the short term. This is consistent with the negative correlation between the carbon to nitrogen ratio (C:N) of soluble organic matter and its decomposition rate as revealed by Filep and Rékási (2011) and Long et al. (2015) . This study showed that the time when SON contents obtained the peak value at 25 ^{o}C was earlier than at 15 ^{o}C, suggesting temperature is an important factor affecting the decomposition of organic matter through the increase of microorganisms and enzyme activities (Wang, D. et al., 2016).

Dynamics mechanism of soil SON

In this study, we constructed a flooded environment to explore the influence of soil pH, Eh, microbial biomass and enzyme activity on soil soluble organic nitrogen. A significant correlation exists between SON and SMBC, protease, glutamine, initial OM and pH, which was proven in this study by using the SEM analysis.

Soil organic matter (SOM), an essential source of available C and N for soil microorganisms and plants (Ghani et al., 2013), plays a pivotal role in controlling soil function and quality (Zhao et al., 2016). Our study showed that initial OM is the important factor that influences SON after applying CMV, with the total effect of SEM 0.498. This might be due to the fact that high SOM contents after CMV addition can increase the number and activity of soil microorganisms to promote the decomposition and transformation of soil organic matter. This is consistent with Kieloaho et al. (2016) that after initial OM decomposition, the released organic N forms including proteinaceous material can be degraded into smaller units that can be utilized by the majority of soil organisms and plants. The results of SEM showed that the direct effect of initial OM on soil SON is -0.623, which can be attributed to the negative priming effect of SOM decomposition. In brief, the decomposition rate of initial OM decreased caused by the fresh organic matter (CMV) added to the soil. Similar results were observed after the addition of maize residue and fresh biochars (Qiu et al., 2016; Wang, J.Y. et al., 2016). Meanwhile, the results of SEM showed that initial OM can indirectly affect soil SON via enzymes activity, with the path coefficients of 1.121, which may be due to soil enzyme-catalyzed depolymerization of organic matter resulting in the production of dissolved organic compounds (Tian et al., 2010).

Soil microbes play an important role in nitrogen cycle by mediating many nitrogen transformations to influence available N pools (Tang et al., 2014; Yang et al., 2016). Our study showed that SMBC and SMBN increased significantly after application of CMV, which is similar to the previous study that turning over CMV can create good soil conditions for soil microorganisms to promote microbial growth and significantly increase soil SMBC (Zhang et al., 2012). Microbial decomposition of soil organic matter is considered to be a major factor controlling the amount of soluble organic matter retained in soil (Qualls et al., 2002). The results of our study demonstrated that SMBC had an important influence on the soil SON contents, with the total effect of SEM 0.959, which suggested that soil microbes are responsible for the decomposition of soil organic matter in the course of soil SON formation. Furthermore, the results of SEM showed that the indirect effect of SMBC through enzyme activity on soil SON is 0.554, suggesting soil microorganisms exuded enzymes (protease, glutaminase) into the soil to decompose macromolecular compounds into smaller, available resources, which indirectly affect the formation of SON (Min et al., 2014). In conclusion, soil microbial biomass increase is one of the important reasons for increasing of SON contents after CMV application.

Soil enzyme activities is the key impact factor of biological processes that link the quality of SOM (e.g. the relative availability of C and N) with the ability of microbes to assimilate nutrients, and changes in the activities of N-degrading enzymes will affect the soil N availability (Kotroczó et al., 2014; Cenini et al., 2016). It has been found that green manure can release enzymes into soil after application, meanwhile provide energy and nutrients for soil microorganisms, resulting in obvious increases in soil enzyme activities (Tang et al., 2014). Similarly, in our study soil protease and glutaminase activity increased significantly after application of CMV. SEM analysis showed that protease and glutamine had an important influence on the soil SON contents, with the path coefficients of 0.547, 0.523, respectively. This is mainly because protease and glutamine are involved in soil nitrogen metabolism. For example, protease plays an important role in the hydrolysis of organic nitrogen to the amino acids, and glutaminase can hydrolyze glutamine to glutamate and nitrogen in the soil. Generally, CMV application can release a large number of organic components (e.g. organic carbon fractions and organic nitrogen fractions), most of which, however, are large molecules and cannot be immediately bioavailable, so they need to be depolymerized by enzymes to release simple organic N, such as amino acids and amino sugars, which are bioavailable for plant N uptake (Yang et al., 2014). Our results also indicated that enzyme activities were critical factors that influence SON in the paddy field ecosystem.

Soil pH is one of the major factors affecting soil N transformation (Cheng et al., 2013). In this study, the result of SEM analysis showed that pH indirectly affected SON pools through the activity of enzymes in soil. This is in agreement with previous studies that pH was an important edaphic variable that influences SOM dynamics decomposition through affecting the activity of soil enzymes

(Yao et al., 2009; Min et al., 2014). A global-scale meta-analysis using data from 40 ecosystems has proved that soil pH is the primary controller of soil enzyme activity as evident by the fact that all the activities of tested soil enzymes were correlated with soil pH (Sinsabaugh et al., 2008). Our study showed that pH had a significant negative correlation with soil glutaminase, with the path coefficient of -0.278, which is possible because soil pH affects the activity of soil enzymes through its controls on microbial enzymatic production, ionization-induced conformational changes of enzymes, and/or availability of substrates and enzymatic co-factors (Tabatabai, 1994).

This study was based on indoor incubation experiment under strictly controlled soil, temperature, water and other environmental conditions, but the actual conditions in the field are more complex, and there are many controllable, uncontrollable and unknown factors which affect soil SON dynamics. Therefore, the impact of climate factors (temperature, precipitation, etc) and field management (mulching, irrigation and fertilization) should be taken into consideration in the field - study.

CONCLUSIONS

We proposed and analyzed conceptual model and modified model of SON in paddy soils by SEM. The results indicated that SMBC, protease, glutamine, initial OM and pH had stronger total effects on SON dynamics, showing that these five factors were key factors affecting the SON dynamics in paddy soils after adding green manure. The results also imply that SON dynamics were complex and influenced by sorts of factors with different pathways and direct, indirect and total effects.

Structural equation modeling proved to be an effective tool to clearly recognize different impacts of soil factors on the SON dynamics, indicating the degree to which the alterations in a factor level can affect the other factor and SON pool. Therefore, on the personal point of view SEM may be an effective way to explore complex factors in scientific research.

REFERENCES

Berg B, 1986. Nutrient release from litter and humus in coniferous forest soils—a mini review. *Scand J For Res*. **1:** 359--369.

Brookes P C, Landman A, Pruden G, Jenkinson D S. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem*. **17:** 837--842.

Cenini V L, Fornara D A, Mcmullan G. 2016. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. *Soil Biol Biochem*. **96:** 198--206.

Chen, C.R., Xu, Z.H., Zhang, S.L., Keay, P., 2005. Soluble organic nitrogen pools in forest soils of subtropical Australia. *Plant Soil*. **277:** 285--297.

Chen G, Liu Y Y, Yao H Y, Huang C Y. 2006. A method for measuring microbial biomass C in waterlogged soil: chloroform fumigation extraction-water bath method. *Acta Pedol Sin* (in Chinese). **6:** 981--988.

Cheng Y, Wang J, Mary B, Zhang J B, Caid Z C, Chang S X. 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. *Soil Biol Biochem*. **57:** 848--857.

Christoua M, Avramidesa E J, Robertsb J P, Jones D L. 2005. Dissolved organic nitrogen in contrasting agricultural ecosystems. *Soil Biol Biochem*. **37:** 1560--1563.

Deng P, Lu S, Xiao H. 2013. Evaluation of the relevance measure between ports and regional economy using structural equation modeling. *Transp Policy*. **27:** 123--133.

Eisenhauer N, Bowker M A, Grace J B, Powell J R. 2015. From patterns to causal understanding: Structural equation modeling (SEM) in soil ecology. *Pedobiologia*. **58:** 65--72.

Filep T, Rékási M. 2011. Factors controlling dissolved organic carbon (DOC), dissolved organic nitrogen (DON) and DOC/DON ratio in arable soils based on a dataset from Hungary. *Geoderma*. **162:** 312--318.

Gama-Rodrigues A C, Sales M V S, Silva P S D, Comerford N B, Cropper W P, Gama-Rodrigues E F.

2014, An exploratory analysis of phosphorus transformations in tropical soils using structural equation modeling. *Biogeochemistry*. **118:** 453--469.

Ghani A, Sarathchandra U, Ledgard S, Dexter M, Lindsey S, 2013. Microbial decomposition of leached or extracted dissolved organic carbon and nitrogen from pasture soils. *Biol Fer Soils*. **49:** 747--755.

Grace J B. 2006. Structural equation modeling and natural systems. Cambridge University Press, New York.

Grace J B, Bollen K A. 2008. Representing general theoretical concepts in structural equation models: the role of composite variables. *Environ Ecol Stat*. **15:** 191--213.

Guan S Y. 1986. Soil enzyme and research methods for soil enzyme. Agricultural Press, Beijing.

Huang Z Q, Xu Z H, Chen C R. 2008. Effect of mulching on labile soil organic matter pools, microbial community functional diversity and nitrogen transformations in two hardwood plantations of subtropical Australia. *Appl Soil Ecol*. **40:** 229--239.

Jiang L, Wang S, Luo C, Zhu X, Kardol P, Zhang Z, Li Y, Wang C, Wang Y, Jones D L. 2016. Effects of warming and grazing on dissolved organic nitrogen in a Tibetan alpine meadow ecosystem. *Soil Till Res*.**158:** 156--164.

Khalili B, Nourbakhsh F. 2012. Vertical distribution of soluble organic nitrogen mineralization, nitrification, and amidohydrolase activities in a manure-treated soil. *J Plant Nutr Soil Sci*. **175:** 265--272.

Kieloaho A J, Pihlatie M, Carrasco M D, Kanerva S, Parshintsev J, Riekkola M L, Pumpanen J, Heinonsalo J. 2016. Stimulation of soil organic nitrogen pool: The effect of plant and soil organic matter degrading enzymes. *Soil Biol Biochem*. **96:** 97--106.

Kotroczó Z, Veres Z, Fekete I. 2014. Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biol Biochem*. **70:** 237--243.

Liu Y, Pan X Z, Wang C K, Li Y L, Shi R J. 2016. Can subsurface soil salinity be predicted from surface spectral information? -From the perspective of structural equation modelling. *Biosyst Eng*. **152:** 1--10.

Long G Q, Jiang Y J, Sun B. 2015. Seasonal and inter-annual variation of leaching of dissolved organic carbon and nitrogen under long-term manure application in an acidic clay soil in subtropical China. *Soil Till Res*. **146:** 270--278.

Min K, Lehmeie C A, Ballantyne F. 2014. Differential effects of pH on temperature sensitivity of organic carbon and nitrogen decay. *Soil Biol Biochem*. **76:** 193--200.

Murphy D V, Macdonald A J, Stockdale E A, Goulding K W T, Fortune S, Gaunt J L, Poulton P R, Wakefield J A, Webster C P, Wilmer W S, 2000. Soluble organic nitrogen in agriculture soils. *Biol Fert Soils*. **30:** 374--387.

Näsholm T, Kielland K, Ganeteg U. 2009. Uptake of organic nitrogen by plants. *New Phytol*. **182:** 31--48.

Okamoto M, Okada K.2008. Available organic nitrogen intemperate, subtropical, and tropical soils extracted with different solutions. *Biol Fert Soils*. **44:** 533--537.

Qiu Q Y, Wu L F, Zhu Q Y, Li B B, Xu S S, Gregorich E G. 2016. Priming effect of maize residue and urea N on soil organic matter changes with time. *Appl Soil Ecol*. **100:** 65--74.

Qualls R G, Haines B L, Swank W T, Tyler S W. 2002. Retention of soluble organic nutrients by a forested ecosystem. *Biogeochemistry*. **61:** 135--171.

Quan Z, Lu C Y, Shi Y, Chen X, Huang B, Wang Y Z, Zhao Y J, Ma J. 2015. Manure increase the leaching risk of soil extractable organic nitrogen in intensively irrigated greenhouse vegetable cropping systems. *Acta Agr Scand.B-S P*. **65:** 199--207.

Ros G H, Hoffland E, Kessel C V, Temminghoff E J M E. 2009. Extractable and dissolved soil organic nitrogen – A quantitative assessment. *Soil Biol Biochem*. **41:** 1029--1039.

Soil Survey Staff, 1999. Soil Taxonomy. A basic system of soil classification for making and interpreting soil surveys, 2nd edition. Agricultural Handbook 436, Natural Resources Conservation Service, USDA, Washington DC, USA

Sarstedt M, Ringle C M, Smith D, Reams R, Hair J F. 2014. Partial least squares structural equation modeling (PLS-SEM):A useful tool for family business researchers. *J Fam Bus Strat*. **5:** 105--115.

Schmidt B H M, Kalbitz K, Braun S, Fuß R, McDowell W H, Matzner E. 2011. Microbial immobilization and mineralization of dissolved organic nitrogen from forest floors. *Soil Biol Biochem*. **8:** 1742--1745.

Sinsabaugh R L, Lauber C L, Weintraub M N, Ahmed B, Allision S D, Crenshaw C, Contasta A R. 2008. Stoichiometry of soil enzyme activity at global scale. *Ecol Lett*. **11:** 1252--1264.

Soliman A, Bellaj T, Khelifa M. 2016. An integrative psychological model for radicalism: Evidence from structural equation modeling. *Pers Indiv Differ*. **95:** 127--133.

Su M, Zhang J, Huo S, Xi B, Hua F, Zan F, Qian G, Liu J. 2016. Microbial bioavailability of dissolved organic nitrogen (DON) in the sediments of Lake Shankou, Northeastern China. *J Environ Sci*. **42:** 79--88.

Tabatabai M A. 1994. Soil enzymes. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis. Part 2. Microbiological and Biochemical Properties. Soil Science Society of America, Madison, WI, pp. 775--833.

Tian L, Del E, Shi W. 2010. Chemical composition of dissolved organic matter in agroecosystems: Correlations with soil enzyme activity and carbon and nitrogen mineralization. *Appl Soil Eco*l. **46:** 426--435.

Tang H M, Xiao X P, Tang W G, Lin Y C, Wang K, Yang G L. 2014. Effects of Winter Cover Crops Residue Returning on Soil Enzyme Activities and Soil Microbial Community in Double-Cropping Rice Fields. *PloS ONE*. **9:** 1--8.

Vance E D, Brookes P C, Jenkinson D S. 1987. An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem*. **19:** 703--707.

Wang D, He N P, Wang Q, Wang Q F, Xu Z W, Zhu J J. 2016. Effects of Temperature and Moisture on Soil Organic Matter Decomposition Along Elevation Gradients on the Changbai Mountains, Northeast China. *Pedosphere*. **26:** 399--407.

Wang J Y, Dokohely M E, Xiong Z Q, Kuzyakov Y. 2016. Contrasting effects of aged and fresh biochars on glucose-induced priming and microbial activities in paddy soil. *J Soil Sediment*. **16:** 191--203.

Wei L L, Chen C R, Xu Z H, Näsholm T. 2013. Direct uptake and rapid decrease of organic nitrogen by Wollemia nobilis. *Biol Fert Soils*. **49:** 1247--1252.

Yang L J, Zhang L L, Geisseler D, Wu Z J, Gong P, Xue Y, Yu C X, Juan Y H, Horwath W R. 2016. Available C and N affect the utilization of glycine by soil microorganisms. Geoderma. 283: 32-38.

Yang R, Zhou J B, Zhao M X. 2007. Contents of soluble organic nitrogen in soil and its influencing factors. *Chin J Soil Sci* (in Chinese). **38:** 15--18.

Yang S H, Peng S Z, Xu J Z, He Y P, Wang Y J. 2015. Effects of water saving irrigation and controlled release nitrogen fertilizer managements on nitrogen losses from paddy fields. *Paddy Water Environ*. **1:** 71--80.

Yang Z P, Zheng S X, Nie J, Liao Y L, Xie J. 2014. Effects of long-term winter planted green manure on distribution and storage of organic carbon and nitrogen in water-stable aggregates of reddish paddy soil under a double-rice cropping system. *J Integr Agr*. **13:** 1772--1781.

Yao H Y, Bowman D, Rufty T. 2009. Interactions between N fertilization, grass clipping addition and pH in turf ecosystems: Implications for soil enzyme activities and organic matter decomposition. *Soil Biol Biochem*. **41:** 1425--1432.

Yuan K H, Wai C. 2016. Structural equation modeling with unknown population distributions: ridge generalized least squares. *Struct Equ Modeling*. **23:** 163--179.

Zhao S C, Li K J, Zhou S J. 2016. Changes in soil microbial community, enzyme activities and organic matter fractions under long-term straw return in north-central China. *Agr Ecosyst. Environ*. **216:** 82--88.

Zhang H, Zhao Z, Yi X, Lu Y, Cao L. 2012. Effect of fertilization on composition and spatial distribution of dissolved organic nitrogen in paddy soil-microbial systems. *Plant Soil Environ*. **58:** 128--134.