Int. J. Environ. Sci. Technol. (2014) 11:2009–2014
DOI 10.1007/s13762-013-0397-7

ORIGINAL PAPER

Effects of nitrogen fertilization on soil labile carbon fractions of freshwater marsh soil in Northeast China

J. Y. Huang · H. Richard · S. F. Zheng

Received: 30 August 2013 / Accepted: 9 October 2013 / Published online: 5 November 2013

Abstract During the past 50 years, the amount of agricultural fertilizer used in Northern China increased from about 7.5 kg ha^{-1} in the 1950s to approximately 348 kg ha^{-1} in the 1990s. Given that little is known about the effects of nitrogen fertilization on soil labile carbon fraction in Northern China, this paper evaluated such effects in terms of microbial biomass and dissolved organic carbon in the Sanjiang Plain located in Northeast China. Soils with different cultivation time and undisturbed marsh with *Deyeuxia angustifolia* were selected to study the effects of nitrogen fertilization on the soil labile organic fractions microbial C (biomass C, microbial quotient, and basal respiration) and to estimate the contributions of nitrogen input on the dynamics of soil labile carbon. Continuous nitrogen application decreased total organic and dissolved organic carbon concentrations significantly, leading to the lack of carbon source for microbes. Therefore, continuous nitrogen fertilizer application induced negative effects on measured soil microbiological properties. However, a moderate nitrogen application rate (60 kg N ha^{-1}) stimulated soil microbial activity in the short term (about 2 months), whereas a high nitrogen application rate (150 kg N ha^{-1}) inhibited measured soil microbiological properties in the same period.

Keywords Dissolved organic carbon · Nitrogen fertilization · Northeast China · Soil labile carbon fraction

Introduction

During the past 50 years, the application of agricultural fertilizers has resulted in increased amounts of nitrogen (N) in the terrestrial ecosystem, influencing carbon (C) cycle processes and carbon sequestration and reallocation (Aber 1992; Foereid and Hogh-Jensen 2004). Restoration of atmospheric carbon in soils has principal many good effects (Brahim et al. 2011). N addition has been observed to reduce organic C decomposition and suppress soil respiration resulting in an increase in SOC (Foereid and Hogh-Jensen 2004). High nitrogen fertilizers application rates may increase the potential groundwater pollution (Mahvi et al. 2005). Nadelhoffer et al. (1999) proposed that N fertilization in northern temperate zones could lead to an annual increase in soil C storage by 0.3–0.5 pg C.

Labile fractions of organic matter such as dissolved organic C (DOC), microbial biomass C (MBC) can respond rapidly to changes in C supply. Such components have therefore been suggested as early indicators of the effects of N inputs on soil quality (Gregorich et al. 2000). DOC is an important labile fraction since it is the main energy source for soil microorganisms, a primary source of mineralizable N, P, and S, and it influences the availability of metal ions in soils by forming soluble complexes. MBC plays a key role in the energy flows, nutrient transformations, and element cycles in the environment (Tate 2000).

J. Y. Huang · S. F. Zheng (✉)
School of Government Administration, Heilongjiang University, No. 74 Xufu Road, Nangan District, Harbin 150080, People's Republic of China
e-mail: zsf7415@163.com

J. Y. Huang
Ministry of Education Key Laboratories of Integrated Regulation and Resource Development on Shallow Lakes, Hohai University, Nanjing 210098, People's Republic of China

H. Richard
Cooperative Wildlife Research Laboratory, Southern Illinois University, Carbondale, IL 62901, USA

Soil microbiological properties have the potential to be early and sensitive indicators of soil stress or productivity changes, and there are considerable evidences that they can be used to evaluate the influence of management on soils (Saggar et al. 2001). Recently, there has been increased interest in the importance of microbiological properties as indicators of change in soil quality (Saggar et al. 2001). Some studies on soil labile carbon fraction have been carried out in forests and agricultural lands (Joergensen and Scheu 1999; Ghani et al. 2003; Lee and Jose 2003). However, little is known about the effects of nitrogen fertilization on soil labile carbon fraction in freshwater marsh soil in Northeast China. Geoelectrical resistivity, hydrogeochemical and soil properties analysis methods were used for chemical fertilizer monitoring in sandy soil at a palm oil plantation in Machang, Malaysia (Islami et al. 2011). The present study has focused on the response of the ecosystem to nitrogen input on soil labile carbon fraction in the Sanjiang Plain.

Materials and methods

Sampling sites

The study site is located in the Sanjiang Plain, the eastern part of Heilongjiang Province, Northeast China, and is bordered by Russia. It covers an area of 10.89 Mha, mostly dominated by marshes in 1893, and is presently the second largest marsh in China (Zhao 1999; Liu and Ma 2000). Drainage and the use of marshes for agricultural fields have occurred in the past 50 years along with population growth, resulting in increase in cultivated land from about 0.79 Mha in 1949 to 4.57 Mha in 1994 (Liu and Ma 2000). The average altitude is 51.3–75.5 m, and the mean annual temperature is 1.9 °C, with an average frost-free period of 125 days. The mean annual precipitation is 550–560 mm, with more than 65 % of the total precipitation occurring in July and August.

The study was set up at the Sanjiang Mire Wetland Experimental Station, Chinese Academy of Sciences, Sanjiang Plain, China, approximately 47°35′ N, 133°31′ E, originally dominated by *Deyeuxia angustifolia*. The sites were annually cultivated for 1, 3, 5, 9, and 15 years. All fields were fertilized with approximately 60 kg N ha^{-1} $year^{-1}$ and plowed to depths of 15–20 cm annually by machine. The parent material was Quaternary Period sediment, which was classified as Albaquic Paleudalfs with silty clay texture. A total of 3 plots (40 m × 40 m) were established in each field. For each plot, 20 cores with depths of 0–10 cm were taken before spring cultivation.

A field cultivated for 9 years was used for a short-term study of the effect of nitrogen fertilizer levels on soil microbiological properties and dissolved organic carbon. In this field, 9 plots (19.5 m^2) were established, and 3 treatments (0, 60, and 150 kg N ha^{-1}, conducted three repetitions) were chosen. For each plot, 3 cores with depths of 0–10 cm were taken once every 10 days for 2 months. Field cores were pooled, sieved (<2 mm), and stored at 4 °C.

The effects of ammonia nitrogen in horizontal subsurface flow and the performances of nutrients removal efficiency were different in the planted or the unplanted wetlands (Mesquita et al. 2013; Cheng et al. 2011). An undisturbed marsh with *Deyeuxia angustifolia* was also selected to study the effect of nitrogen fertilizer levels on dissolved organic carbon in the short term. A total of 6 plots (1 m^2) were established, and 2 treatments (0 and 240 kg N ha^{-1}, respectively, with three repetitions) were chosen to monitor the effect of nitrogen fertilizer levels on dissolved organic carbon in undisturbed marshland soil. Dissolved organic carbon concentration was determined by sampling pore water in situ at 3 specific depths of 5, 10, and 15 cm, respectively. Stainless steel tubes (3.0 mm outer diameter, 2.0 mm inner diameter, and with 4 lateral ports at the bottom), equipped with silicon tubes, a 3-way valve, and a silicon septum, were installed prior to flux measurement. These tubes were installed at depths of 5, 10, and 15 cm, respectively. Accumulated pore water was collected using 20/50 ml syringes every 10 days, injected into plastic bottles, and stored in a cooling box (about 4 °C) in the field. After being transported to the station, the samples in vials were frozen (−20 °C) in the refrigerator for subsequent analysis of dissolved organic carbon concentration.

Sample analysis

Soil bulk density was measured in situ using the core method (Culley 1993). Soil organic carbon was determined through the wet oxidation–redox titration method, after which total soil nitrogen was determined using the semi-micro-Kjeldahl digestion (Lu 2000).

Field-moist subsamples (25 g equivalent oven-dry soil) of each treatment were placed in beakers. These were incubated in 1-L glass jars containing vials with 10 mL CO_2-free water to avoid water loss during incubation. The jars were tightly sealed and incubated at 25 °C. Jars without soils were used as background references. The CO_2 production measured by HP 4890 gas chromatography after a day was considered the basal respiration (BR). Substrate-induced respiration (SIR) was measured according to the methods reported by Hofman et al. (2003).

Soil microbial biomass carbon (MBC) was determined using a fumigation–extraction method (Zhang et al. 2007). Fumigated and non-fumigated soils were extracted with 0.5 M K_2SO_4 for 30 min (1:5 soil/extractant ratio), after which the extracts were analyzed for carbon using a total organic carbon analyzer (TOC VCPH, Shimadzu). The carbon obtained from the fumigated samples minus that from non-fumigated samples was assumed to represent the microbial-C flush. This was then converted to MBC using the relationship: microbial biomass C = microbial-C flush/0.38 (Lu 2000). The microbial quotient was calculated as MBC/TOC, whereas the metabolic quotient (qCO_2) was calculated as BR/MBC. Biomass-specific potential respiration was calculated as SIR/MBC (Hofman et al. 2003).

Soil samples (10 g equivalent oven-dry weight) were weighed into 40 mL polypropylene centrifuge tubes. These were extracted with 30 mL of distilled water for 30 min on an end-over-end shaker at 30 rpm. Then, the samples were centrifuged for 20 min at 8,000 rpm, after which the supernatant was passed through a 0.45 µm filter (Ghani et al. 2003). The extracts were analyzed for carbon using the TOC analyzer.

Data analysis

Statistical analysis was performed using SPSS software package for Windows. ANOVA was used to evaluate differences among the field sites. The level of significance was set at $P < 0.05$, for all statistical tests.

Results and discussion

Changes of measured soil microbiological properties

Microbial biomass C ranged from 229 to 1,154 mg kg^{-1} among studied soils, and it differed significantly among cultivated soils ($P < 0.05$). Microbial biomass C ($P < 0.05$) and BR ($P < 0.0001$) decreased with increasing cultivation time. Metabolic quotient value increased

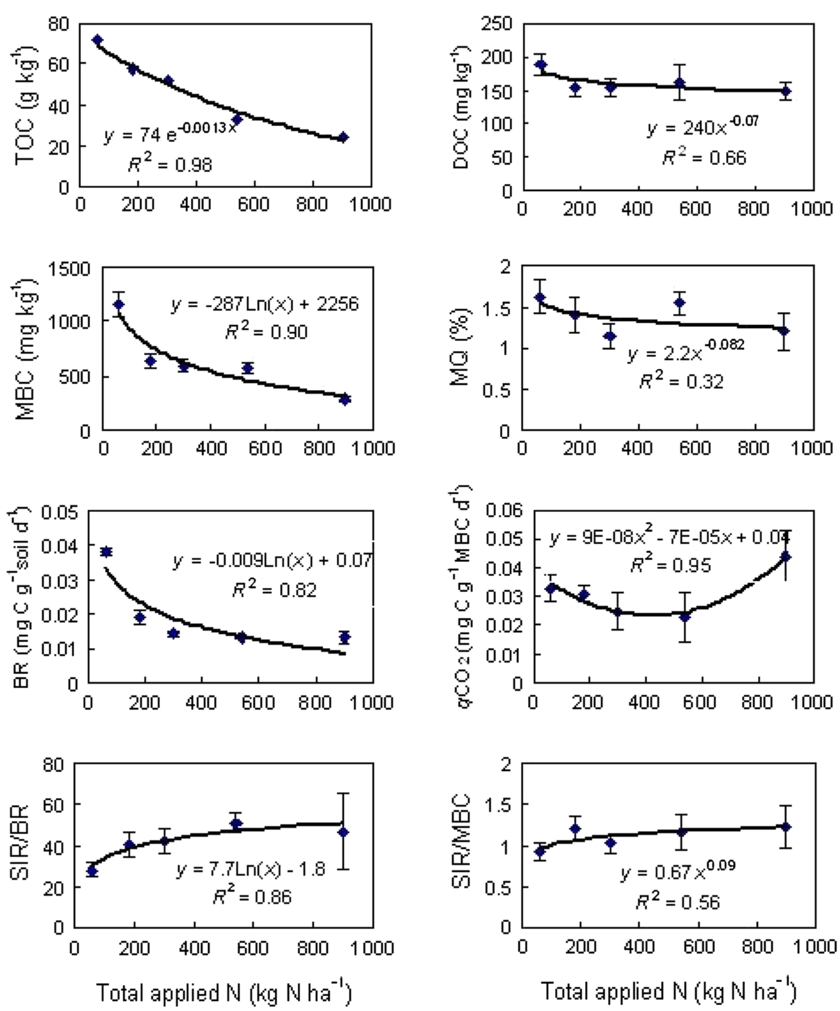

Fig. 1 Effects of continuous nitrogen application on measured soil microbiological properties and dissolved organic carbon in the Sanjiang Plain of Northeast China. Values are means and standard error. *TOC* total organic carbon, *DOC* dissolved organic carbon, *MBC* microbial biomass carbon, *MQ* microbial quotient, *BR* basal respiration, *qCO2* metabolic quotient, *SIR/BR* substrate-induced respiration to basal respiration ratio, *SIR/MBC* substrate-induced respiration to microbial biomass carbon ratio

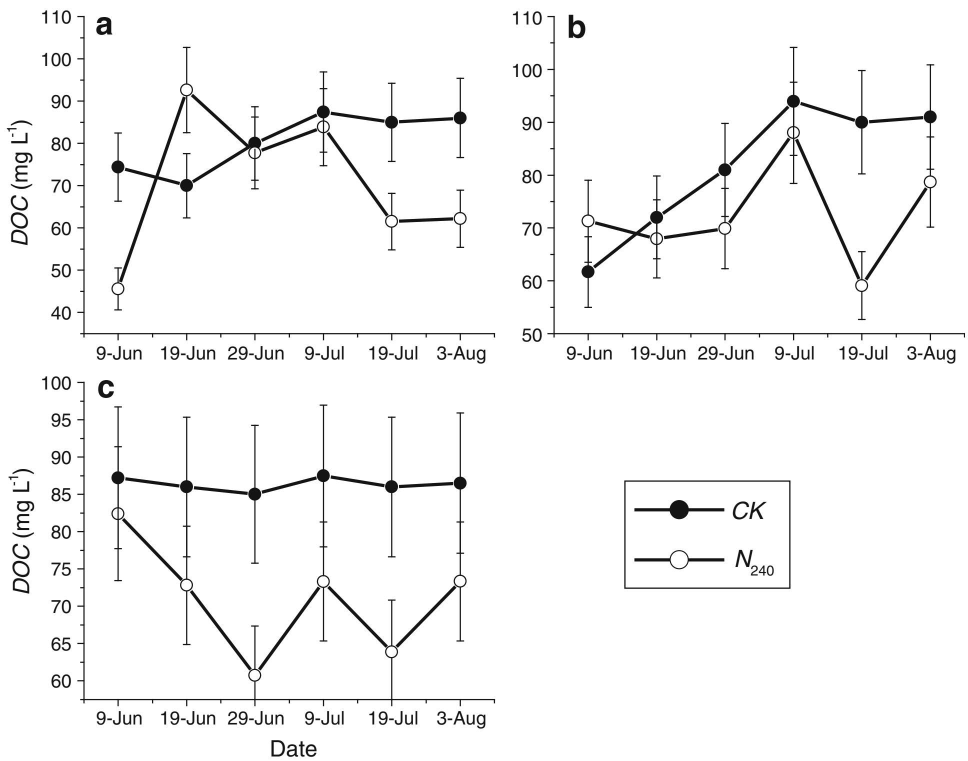

Fig. 2 Effects of nitrogen application on dissolved organic carbon at different depths of **a** 5 cm, **b** 10 cm, and **c** 15 cm below the ground in undisturbed marshland in the short term. Values are means and standard error. CK and N240 denote the sites applied with 0 and 240 kg N ha^{-1}, respectively

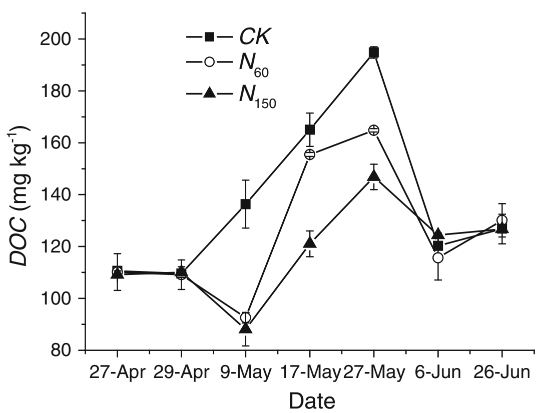

Fig. 3 Effects of applied nitrogen on dissolved organic carbon in cultivated soil in the short term. Values are means and standard error. CK, N60, and N150 denote the sites applied with 0, 60, and 150 kg N ha^{-1}, respectively

sharply during the initial 1–3 years of cultivation and increased with cultivation time. The SIR/BR ($P < 0.05$) and SIR/MBC ($P < 0.001$) increased with cultivation time. MBC ($R^2 = 0.9$, $P < 0.05$) and BR ($R^2 = 0.82$, $P < 0.05$) were negatively correlated with the amount of nitrogen applied in the sites where nitrogen was continuously applied (Fig. 1). The microbial quotient was also negatively correlated with the amount of nitrogen applied, although the correlation was not as strong (Fig. 1) ($R^2 = 0.32$, $P = 0.31$). The metabolic quotient ($R^2 = 0.95$, $P < 0.05$), SIR/MBC ($R^2 = 0.56$, $P = 0.14$), and SIR/BR ($R^2 = 0.86$, $P < 0.05$) were positively correlated with the amount of nitrogen applied in the sites where nitrogen was continuously applied (Fig. 1).

However, in a short period, the highest MBC and microbial quotient were observed in the plot treated with 60 kg N ha^{-1}, while the lowest values were observed in the plot treated with 150 kg N ha^{-1}. In contrast, the highest metabolic quotient was measured in the plot treated with 0 kg N ha^{-1}, and the lowest was in the plot treated with 60 kg N ha^{-1}, indicating that a moderate nitrogen application rate (60 kg N ha^{-1}) stimulated soil microbial activity in the short term (about 2 months). A high nitrogen application rate (150 kg N ha^{-1}), however, inhibited measured soil microbiological properties in the short term.

Previous studies have reported mixed results of the effects of nitrogen addition on soil microbiological properties. For example, Joergensen and Scheu (1999) reported that nitrogen fertilization induced an increase in MBC content, while Ghani et al. (2003), Lee and Jose (2003) reported that nitrogen fertilization resulted in a decrease in MBC content. On the other hand, Galicia and Felipe (2004) reported no effects of nitrogen fertilization on MBC and microbial activity in the dry season; however, the effects of nitrogen fertilization on MBC and microbial activity are significant in the wet season. In the present study, MBC content, the microbial quotient, and BR were negatively correlated with the total amount of nitrogen application, whereas qCO_2, PR/MBC, and PR/BR were positively correlated with the total amount of nitrogen application, indicating that chronic nitrogen application affects soil microbiological properties negatively.

Dissolved organic carbon

Total organic C concentration and dissolved organic C ranged from 71.2 to 24.0 g kg^{-1} and 187.5 to 147.9 mg kg^{-1}, respectively, among studied soils and decreased with increasing cultivation time ($P < 0.05$). In the current study, TOC significantly decreased with the amount of nitrogen added to the sites where nitrogen was continuously applied ($r^2 = 0.98$, $P < 0.05$) (Fig. 1). Dissolved organic carbon concentrations decreased with the amount of nitrogen addition as well, although the effects were not as obvious (Fig. 1) ($r^2 = 0.66$, $P = 0.09$). The amount of dissolved organic carbon was reduced because most of the carbon was used in nitrogen fixation. Continuous nitrogen application resulted in a significant decrease in total organic and dissolved organic carbon concentrations that, in turn, induced the lack of carbon source for microbes. Therefore, continuous nitrogen application affected the measured soil microbiological properties negatively. Nitrogen application induced a significant decline in dissolved organic carbon concentration in the short term both in the undisturbed marshland and in cultivated soils (Figs. 2, 3). Nitrogen input stimulated soil microbial activity to immobilize carbon in microbial biomass, inducing the growth of microbial biomass in the short term (Rochette and Gregorich 1998). As arid crops have been converted to rice paddy in this area, this resulted in significant changes in the microbial communities and composition involved in removal of inorganic N into dinitrogen gas by anaerobic ammonium oxidation (Wang and Gu 2013).

Meanwhile, chronic nitrogen application resulted in the significant decrease in dissolved organic carbon concentrations that, in turn, induced the lack of carbon source for microbes. However, over the short-term period, moderate nitrogen application improved the immobilization of C or mobilization of C in microbial biomass and favored the growth of microbial biomass (Galicia and Felipe 2004).

Conclusion

Continuous nitrogen fertilizer application resulted in the lack of carbon for microorganisms, negatively affecting measured soil microbiological properties. However, a moderate nitrogen application rate (60 kg N ha^{-1}) stimulated soil microbial activity in the short term (about 2 months). Meanwhile, a high nitrogen application rate (150 kg N ha^{-1} in the present study) inhibited the measured soil microbiological properties in the same period. The continuous decrease in nitrogen fertilizer application can guarantee the sufficient carbon supply for microorganisms.

Acknowledgments This work was supported by the Natural Science Foundation (51208177) and the Sanjiang Experimental Station of Wetland Ecology. In addition, we would like to thank the reviewers for their time and suggestions.

References

Aber JD (1992) Nitrogen cycling and nitrogen saturation in temperate forest ecosystems. Trends Ecol Evol 7:220–223

Brahim N, Blavet D, Gallali T, Bernoux M (2011) Application of structural equation modeling for assessing relationships between organic carbon and soil properties in semiarid Mediterranean region. Int J Environ Sci Tech 8(2):305–320

Cheng B, Hu CW, Zhao YJ (2011) Effects of plants development and pollutant loading on performance of vertical subsurface flow constructed wetlands. Int J Environ Sci Tech 8(1):177–186

Culley JLB (1993) Density and compressibility. In: Carter MR (ed) Soil sampling and methods of analysis. Lewis Publishers, Boca Raton, Ann Arbor, London, Tokyo, pp 529–539

Foereid B, Hogh-Jensen H (2004) Carbon sequestration potential of organic agriculture in northern Europe – a modelling approach. Nutr Cycl Agroecosyst 68:13–24

Galicia L, Felipe GO (2004) The effects of C, N and P additions on soil microbial activity under two remnant tree species in a tropical seasonal pasture. Appl Soil Eco 26:31–39

Ghani A, Dexter M, Perrott KW (2003) Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilization, grazing and cultivation. Soil Biol Biochem 35:1231–1243

Gregorich EG, Liang BC, Drury CF (2000) Elucidation of the source and turnover of water soluble and microbial biomass carbon in agricultural soils. Soil Biol Biochem 32:581–587

Hofman J, Bezchlebová J, Dušek L, Doležal L, Holoubek I, Anděl P, Ansorgová A, Malý S (2003) Novel approach to monitoring of the soil biological quality. Environ Int 28:771–778

Islami N, Taib S, Yusoff I, Abdul Ghani A (2011) Time lapse chemical fertilizer monitoring in agriculture sandy soil. Int J Environ Sci Tech 8(4):765–780

Joergensen RG, Scheu S (1999) Response of soil microorganisms to the addition of carbon, nitrogen and phosphorus in a forest Rendzina. Soil Biol Biochem 31:859–866

Lee KH, Jose S (2003) Soil respiration, fine root production, and microbial biomass in cottonwood and loblolly pine plantations along a nitrogen fertilization gradient. Forest Ecol Manag 185:263–273

Liu XT, Ma XH (2000) Effect of large-scale reclamation on natural environment and regional environmental protection in the Sanjiang Plain. Scienta Geographic Sinica 20:14–19

Lu RK (2000) Soil agrochemistry and analytical methods. Chinese Agricultural Science and Technology Press, Beijing (in Chinese)

Mahvi AH, Nouri J, Babaei AA, Nabizadeh R (2005) Agricultural activities impact on groundwater nitrate pollution. Int J Environ Sci Tech 2(1):41–47

Mesquita MC, Albuquerque A, Amaral L, Nogueira R (2013) Effect of vegetation on the performance of horizontal subsurface flow constructed wetlands with lightweight expanded clay aggregates. Int J Environ Sci Tech 10(3):433–442

Nadelhoffer KJ, Emmet BA, Gundersen P, Kjonaas OJ, Koopmans CJ, Schleppi P, Tietemal A, Wright RF (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398:145–148

Rochette P, Gregorich EG (1998) Dynamics of soil microbial biomass C, soluble organic C and CO2 evolution after three years of manure application. Can J Soil Sci 78:283–290

Saggar S, Yeates GW, Shepherd TG (2001) Cultivation effects on soil biological properties, microfauna and organic matter dynamics in Eutric Gleysol and Gleyic Luvisol soils in New Zealand. Soil Till Res 58:55–68

Tate RL (2000) Soil microbiology, 2nd ed. Wiley, New York

Wang J, Gu J-D (2013) Dominance of *Candidatus* Scalindua species in anammox community revealed in soils with different duration of rice paddy cultivation in Northeast China. Appl Microbiol Biotechnol 97:1785–1798

Zhang JB, Song CC, Yang WY (2007) Effects of cultivation on soil microbiological properties in a freshwater marsh soil in Northeast China. Soil Tillage Res 93:231–235

Zhao KY (1999) Mires in China. Science Press, Beijing